

Performing the best science: Pathways to validity and reliability and effective data management

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Center for Musculoskeletal Research

MEDICINE *of* THE HIGHEST ORDER



Evidence Based Science

- 1. Begley CG et al. Institutions must do their part for reproducibility. Nature 2015; 525:25**
- 2. McCoun R et al Hide results to find the truth. Nature 2015; 526:187**
- 3. Blumberg RS et al. Unravelling the autoimmune translational research process layer by layer. Nature Med 2012; 18(1):35**
- 4. Kilkenney C. The ARRIVE Guidelines. PLOS Biology 2010**



Overview

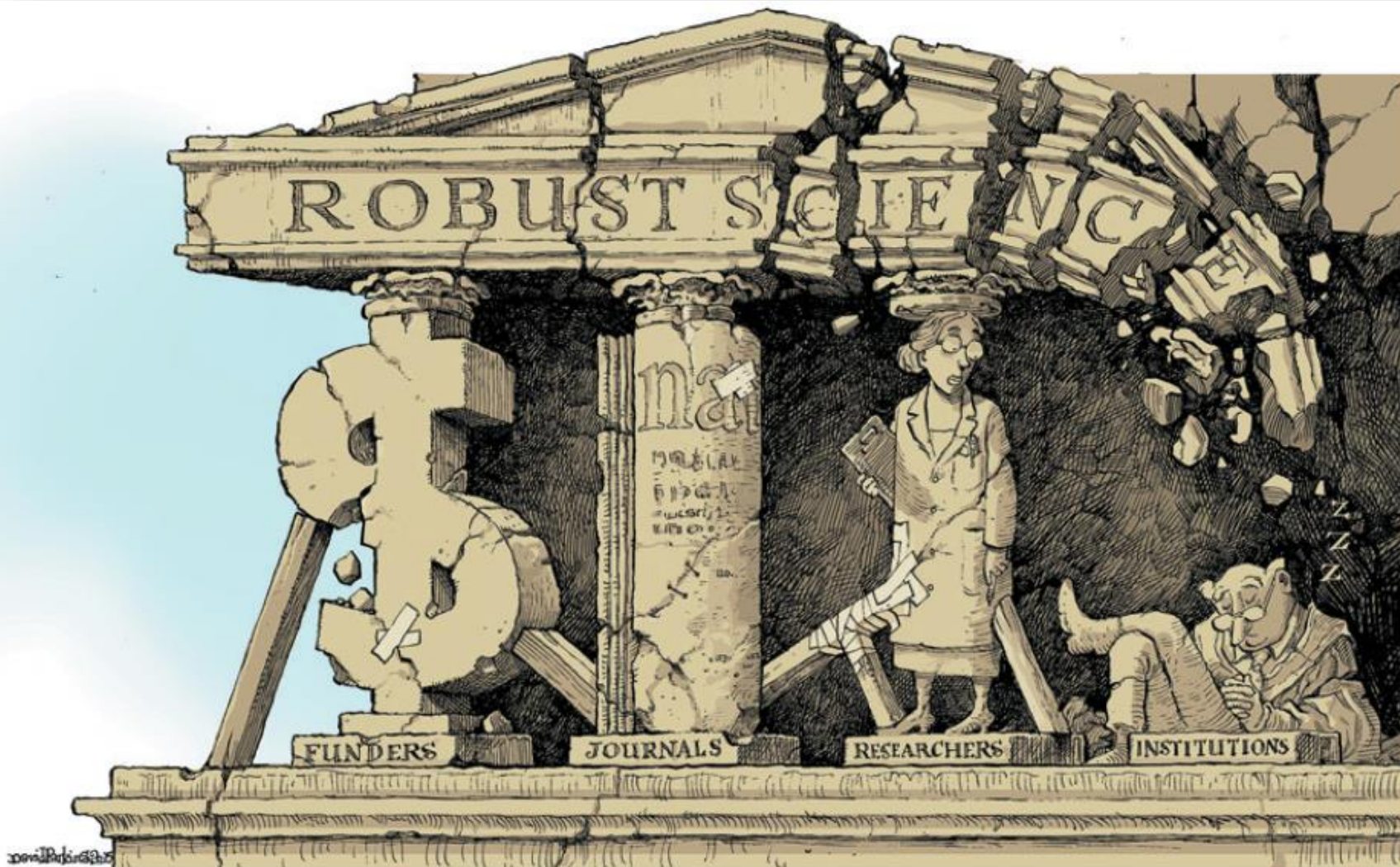
1. Reproducibility & Scientific Rigor

2. Data Collection and Storage

3. Data Analysis

Reproducibility and scientific rigor

Rigor and Reproducibility



**<25% high profile
science is reproducible**

**Journal Checklists
Biostatistician reviewers
NIH initiatives
No limit on methods
Stress quality over
quantity**

Begley G. Nature Sept 2015

Long Term Outcomes



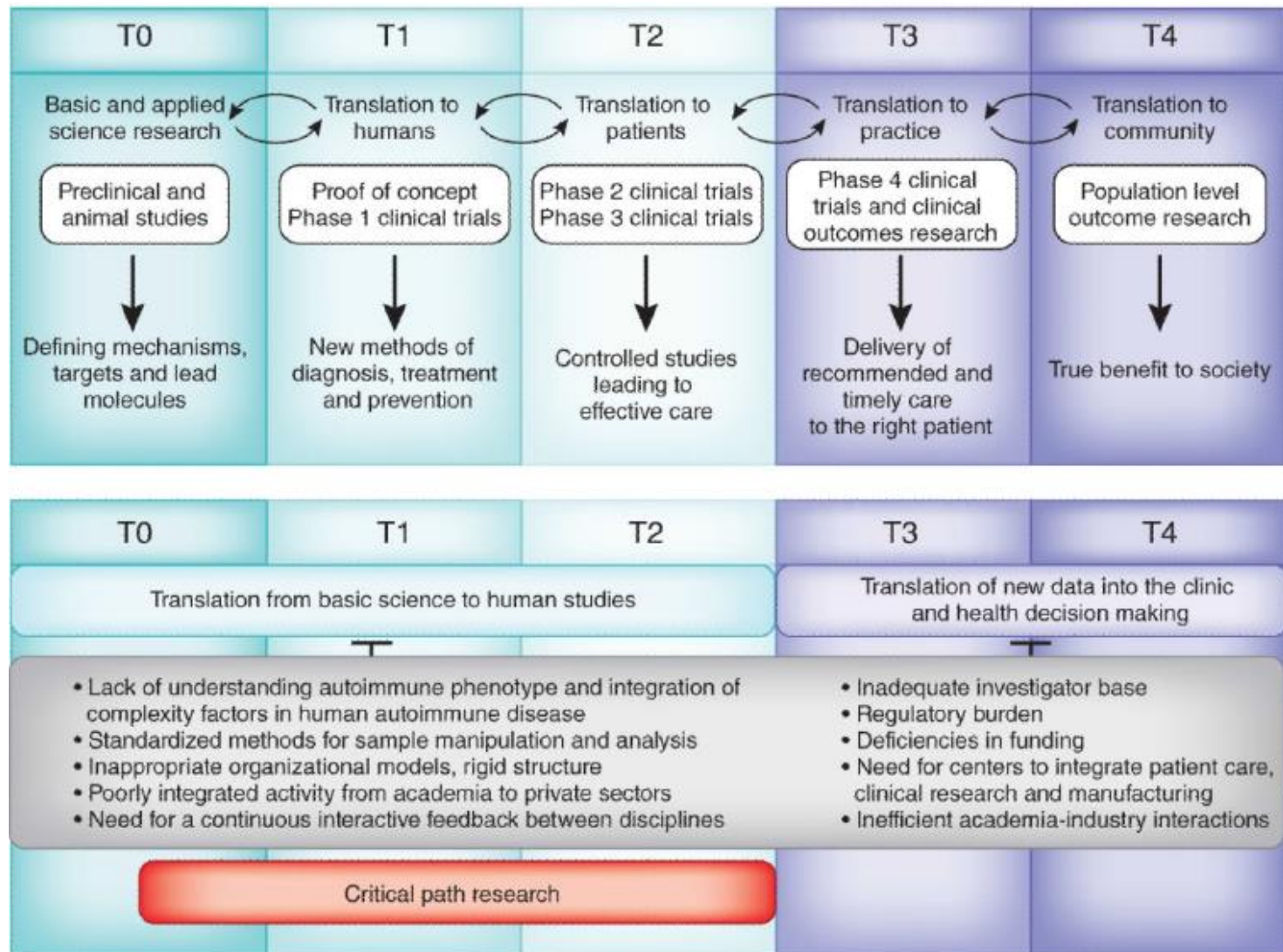
BIG DATA

Volume
Analytics
Unstructured
Structured
Data
People driven
Decision making
Zettabyte
Framework
Smart content
database
Text
Semantic
Concept extraction
Metadate
Useful
Metadate
Text analytics

Good Institutional Practice

- **Routine discussion of research methods**
- **Good reporting systems**
- **Training & standards**
- **Records & quality management**
- **Appropriate incentive & evaluation systems**

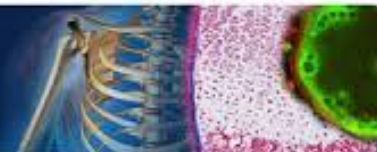
Operational challenges for translational medicine



Establish Good Research Practices

**Quality Research in Preclinical and
Translational Research Core**

**Data Management,
Bioinformatics and Analytics Core**



Standard Operating Procedures

Table 2. SOP Format and Content Description

Title: A specific name assigned to the document

Purpose: What the document will be used for

Scope: Describes boundaries of what procedure will / will not deliver

Responsibilities: Defines the roles and associated duties

Safety Precautions: Lists any specific safety precautions for procedure

Materials: Lists equipment, supplies and reagents with specifications

Specimens: Lists specimens, collection and storage requirements

Quality Control: Activities use to fulfill and verify quality requirements

Procedure: Sequence of steps defining what is to be done

Limitations: Items restricting execution when all steps are completed

Calculations: A process for transforming inputs into results

Interpretation: Information relating to the evaluation of results

Result reporting: Details how to report results

Training: Information of personnel roles and training requirements

References: Citations, user guides or other supporting SOPs

Standard Operating Procedures

https://www.urmc.rochester.edu/musculoskeletal- Histology - Core Services - ... x

File Edit View Favorites Tools Help Convert Select

Sutrayana Mahayana 2015 Google ScholarOne Manuscripts Web Slice Gallery

ROCHESTER MEDICAL CENTER

PATIENTS & FAMILIES EDUCATION RESEARCH COMMUNITY

Referring Physicians Find a Physician Departments & Centers About URMC Libraries Alumni Giving

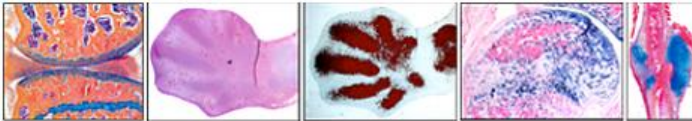
Center for Musculoskeletal Research

URMC » Center for Musculoskeletal Research » Core Services » Histology, Biochemistry, and Molecular Imaging (HBMI) Core

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- Research Labs
- Core Services
 - Histology, Biochemistry, and Molecular Imaging (HBMI) Core
 - Facilities
 - Protocols
 - Biomechanics and Multimodal Tissue Imaging (BMTI) Core
 - Kenneth DeHaven Arthroscopic Surgical Skills Laboratory
 - Administrative Core
- Programmatic Grants
- Pilot Funding
- Seminars
- Job Opportunities

Histology, Biochemistry, and Molecular Imaging (HBMI) Core



Mission Statement:

The primary mission of the HBMI Core is to provide efficient, high quality, and cost-effective histological, biochemical, cellular, and molecular services to investigators throughout the Center for Musculoskeletal Research using both tissue and cellular models. The HBMI Core also provides cutting-edge histomorphometric and molecular imaging and analysis services and resources as well as consultation and training in the use of primary cell culture models and small animal surgical models

Services include:

- » Paraffin and frozen tissue processing, embedding, and sectioning.
- » Histological staining.
- » Guidance and support for standardized Immunohistochemistry (IHC) and In Situ Hybridization (ISH).
- » Optimization of new IHC and ISH protocols.

News

- » [Study Finds Link Between Rare Genetic Mutation and Osteoporosis](#)
October 30, 2015
- » [Maquat Receiving Canada's Top Prize for Biomedical Research](#)
October 27, 2015
- » [Nanoparticle-mediated drug delivery could help prevent tendon adhesions](#)
October 16, 2015
- » [Center for Musculoskeletal Research helps young faculty hone grant proposals](#)
October 9, 2015
- » [Wilmot Cancer Institute's Research Director Wins \\$6.3M Outstanding Investigator Award](#)
September 17, 2015

1:54 PM 11/5/2015

Standard Operating Procedures

The screenshot shows a web browser window with the URL <https://www.urmc.rochester.edu/musculoskeletal-research/core-services/histology/documents/HBMICenterMeetingPresent...>. The page title is "Center for Musculoskeletal Research". The breadcrumb trail is "URMC » Center for Musculoskeletal Research » Core Services » Histology, Biochemistry, and Molecular Imaging (HBMI) Core". The page content is titled "Histology Forms and Protocols" and is divided into three sections: "Internal Forms:", "General Protocols:", and "Histology Staining Protocols:". The "Internal Forms:" section lists "Internal Work Order Form – Histology" and "Internal Justification Form – Histology". The "General Protocols:" section lists "Histology Guidelines Presentation", "Fixation of Skeletal Tissues – Paraffin", "Fixation of Skeletal Tissues – Frozen", "Preparation of Frozen Specimens", and "Slide Preparation". The "Histology Staining Protocols:" section lists "Hematoxylin and Eosin Stain", "Alcian Blue/Orange G Stains", "Safranin O/Fast Green", and "Toluidene Blue/Fast Green". On the right side, there is a "News" section with several articles, including "Study Finds Link Between Rare Genetic Mutation and Osteoporosis", "Maquat Receiving Canada's Top Prize for Biomedical Research", "Nanoparticle-mediated drug delivery could help prevent tendon adhesions", "Center for Musculoskeletal Research helps young faculty hone grant proposals", "Wilmot Cancer Institute's Research Director Wins \$6.3M Outstanding Investigator Award", and "Funding Bonanza at CMSR Shows Strategic Planning Pays Off". The browser window also shows a menu bar with "File", "Edit", "View", "Favorites", "Tools", and "Help", and a toolbar with "Convert" and "Select". The taskbar at the bottom shows the Windows logo, several application icons, and the system clock displaying "1:55 PM 10/25/2015".

Center for Musculoskeletal Research

URMC » Center for Musculoskeletal Research » Core Services » Histology, Biochemistry, and Molecular Imaging (HBMI) Core

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Seminars
Job Opportunities
Clinical Trials

Histology Forms and Protocols

Internal Forms:

- [Internal Work Order Form – Histology](#)
- [Internal Justification Form – Histology](#)

General Protocols:

- [Histology Guidelines Presentation](#)
- [Fixation of Skeletal Tissues – Paraffin](#)
- [Fixation of Skeletal Tissues – Frozen](#)
- [Preparation of Frozen Specimens](#)
- [Slide Preparation](#)

Histology Staining Protocols:

- [Hematoxylin and Eosin Stain](#)
- [Alcian Blue/Orange G Stains](#)
- [Safranin O/Fast Green](#)
- [Toluidene Blue/Fast Green](#)

News

- » [Study Finds Link Between Rare Genetic Mutation and Osteoporosis](#)
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September 17, 2015
- » [Funding Bonanza at CMSR Shows Strategic Planning Pays Off](#)
September 15, 2015

Quality Research Practices

Table 4: QPTR Core Checklist

Personnel Criteria

- Researcher has a training record on file ('Researcher' includes all technicians, students, postdocs, etc. Each needs to complete all of these points)
- Training record contains current CV
- Training record lists all completed trainings within appropriate timeframe
- Researcher has met with study design and biostatistics experts and has developed an approved study (see below)
- Researcher has demonstrated that documentation practices are satisfactory in both paper or online training
- Passed QA auditing of procedures
- For any techniques that require precise or tricky skills, researcher has mastered these skills exactly as stated in the SOP
- QA has agreed that this researcher has completed all required training both through OnBase as well as hands-on training as required
- No researcher on a study is currently 'not in good standing' with QA

Study Protocol Criteria

- The study protocol has been reviewed by a study design specialist and contains the following required elements:
 - Proper controls, adequate power, required gender balance of test subjects, blinding (feasible/required), randomization as necessary
- The methods planned are adequate to conduct the described hypothesis
- Methods for data analysis are outlined in advance, are feasible and have been established with other cores or services
- Plans exist for long term storage of both physical samples and data as needed
- The procedures in the study are contained in the list of approved procedures which can be conducted under the established quality system elements
- The relevant core has indicated current capacity to take on the outlined study
- Facilities listed in the study are all currently in good working order and have been cleared by QA as meeting the selected elements of the quality system
- All equipment to be used in this study has documented maintenance and calibration as appropriate

Table. Existing and New Strategies That *Annals* Uses to Guard against the Publication of Biased or Invalid Research

Strategy	Purpose
Prepublication	
Conflict disclosures	Alerts editors and reviewers to potential sources of bias
Peer review	Provides an opportunity for content experts to examine the work for potential threats to validity
Protocol submission	Enables editors to reconcile what researchers planned with what they report
Statistical review by an in-house team (may include requests for data and alternative analyses)	Enables independent statisticians to critique analysis and look for threats to validity
Trial registration	Permits public access to research plans; discourages suppression of unfavorable results
Publication	
Publication of conflicts of interest	Alerts readers to potential sources of bias
Publication of author contributions	Establishes accountability for specific components of the research process
Publication of detailed methods	Enables readers to critique methods and look for threats to validity
Postpublication	
Letters to the editor/rapid responses	Provides a venue for readers to air their concerns
New safeguards	
Publication of information about availability of protocol, statistical code, and data	Increases potential for reproducibility, allows greater scrutiny for potential threats to validity Permits confirmation of results by independent individuals

Data collection, storage and analysis

Biomarkers

Types

RNA

DNA

Serum

Epigenetic

Proteomics

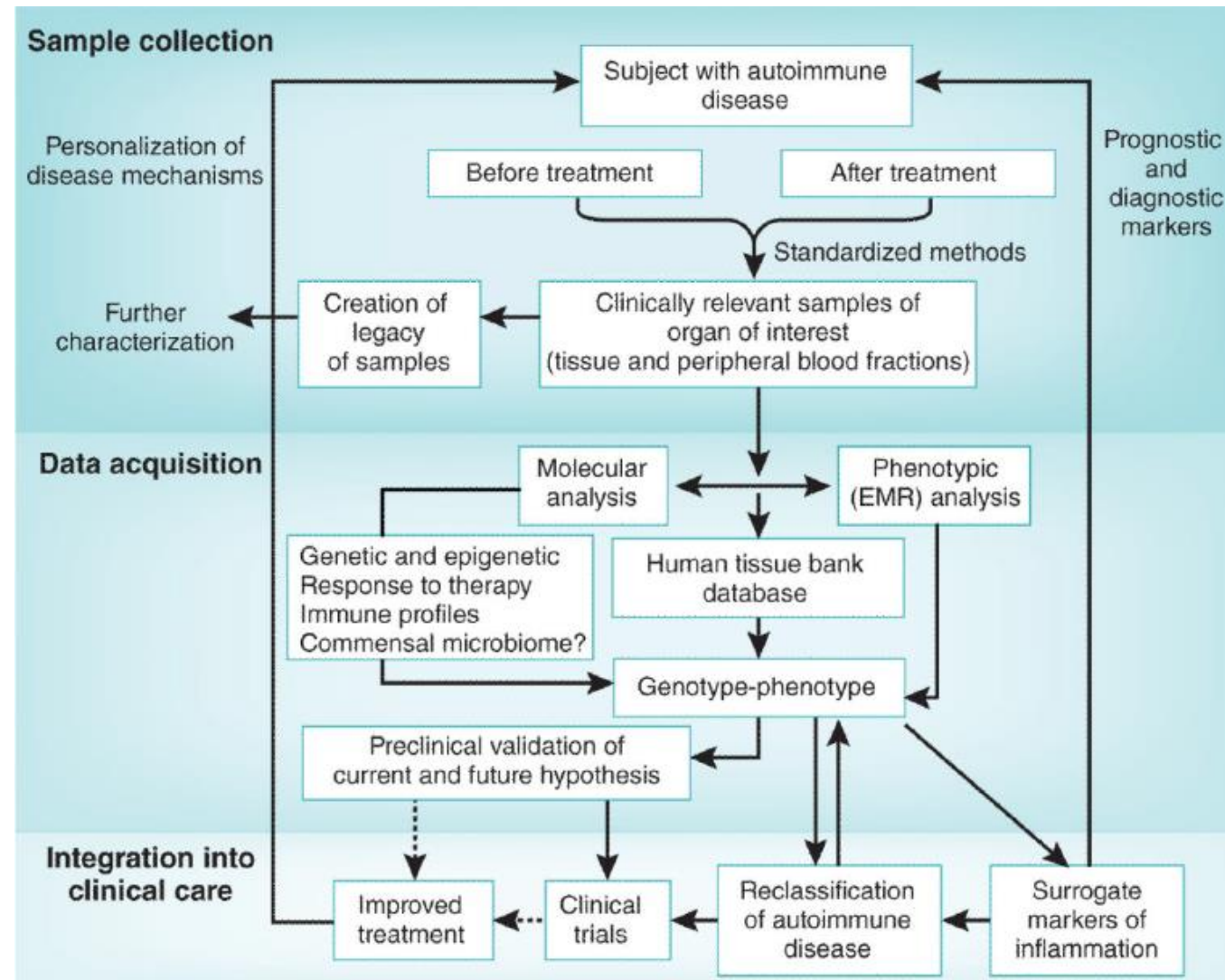
Single cell analysis

Cell populations-flow cytometry

Challenges

- Cost
- Storage
- Stability
- Validity
- Reproducibility

Samples in autoimmunity-related research



Biomarkers- Minimizing Bias and Error

Flow cytometry- unbiased gating and ongoing monitoring for batch effects

Double entry of data

Close collaboration with biostatistics and bioinformatics

Ongoing training and education that centers on scientific rigor and reproducibility

Integrated data capture

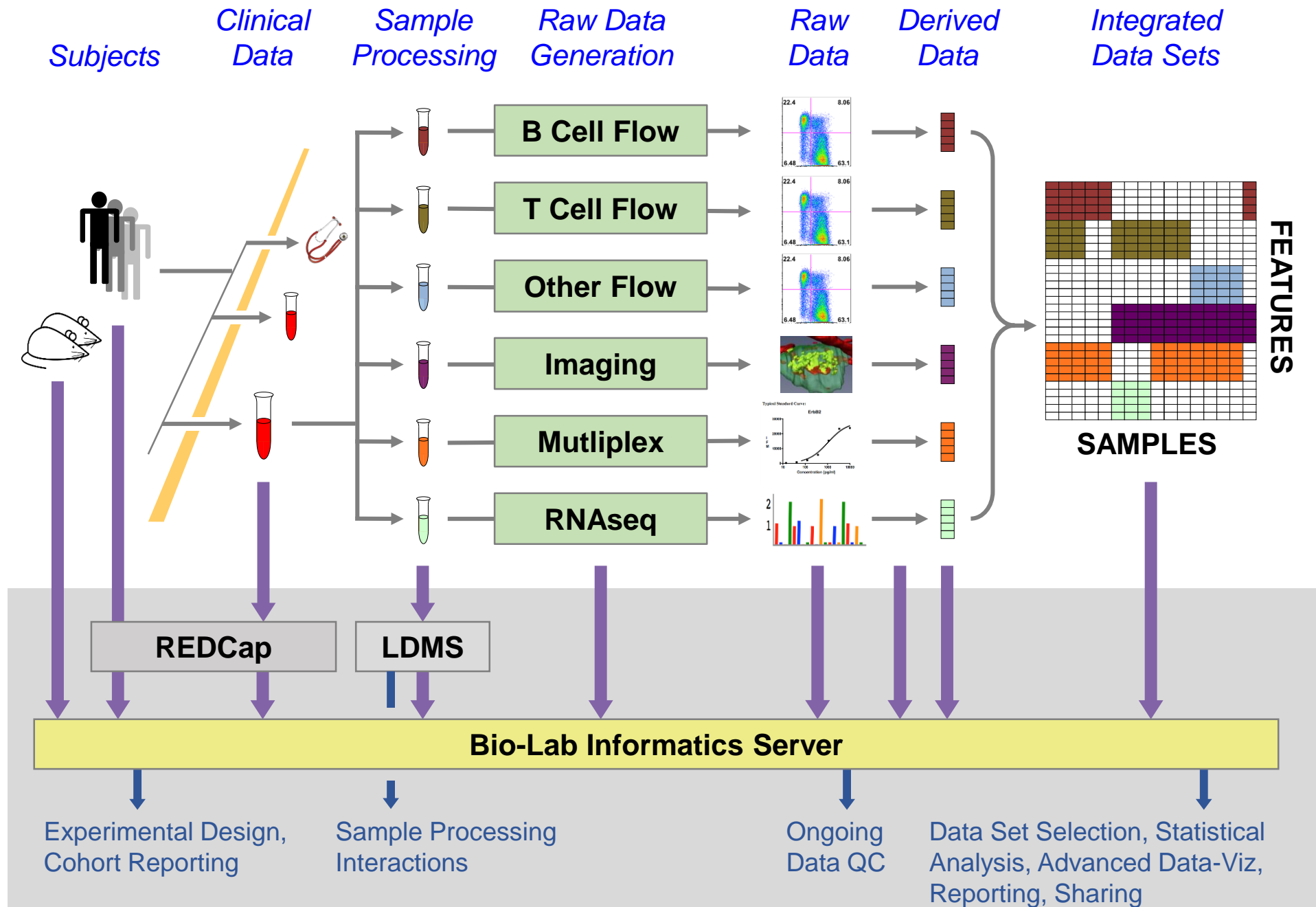


Data management is key to progress.

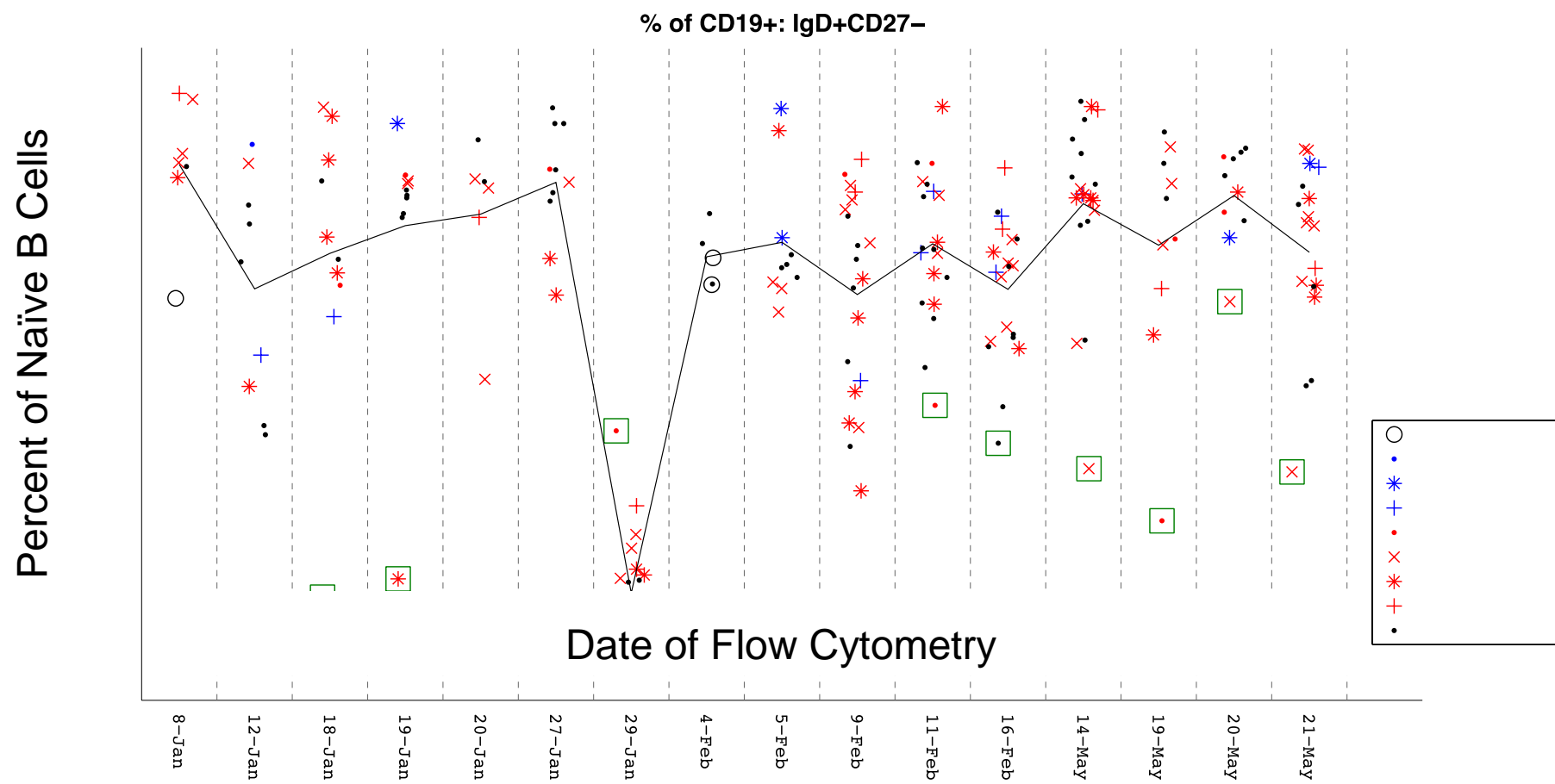
Extensive cellular and molecular profiling of human subjects generates vast amounts of disparate data. Effective data management and integration solutions are essential to the preservation of this information in an interpretable form.

Thus, data management efforts occurring 'behind the scenes' have an essential role to play in realizing the full potential of high throughput profiling approaches in human subjects.

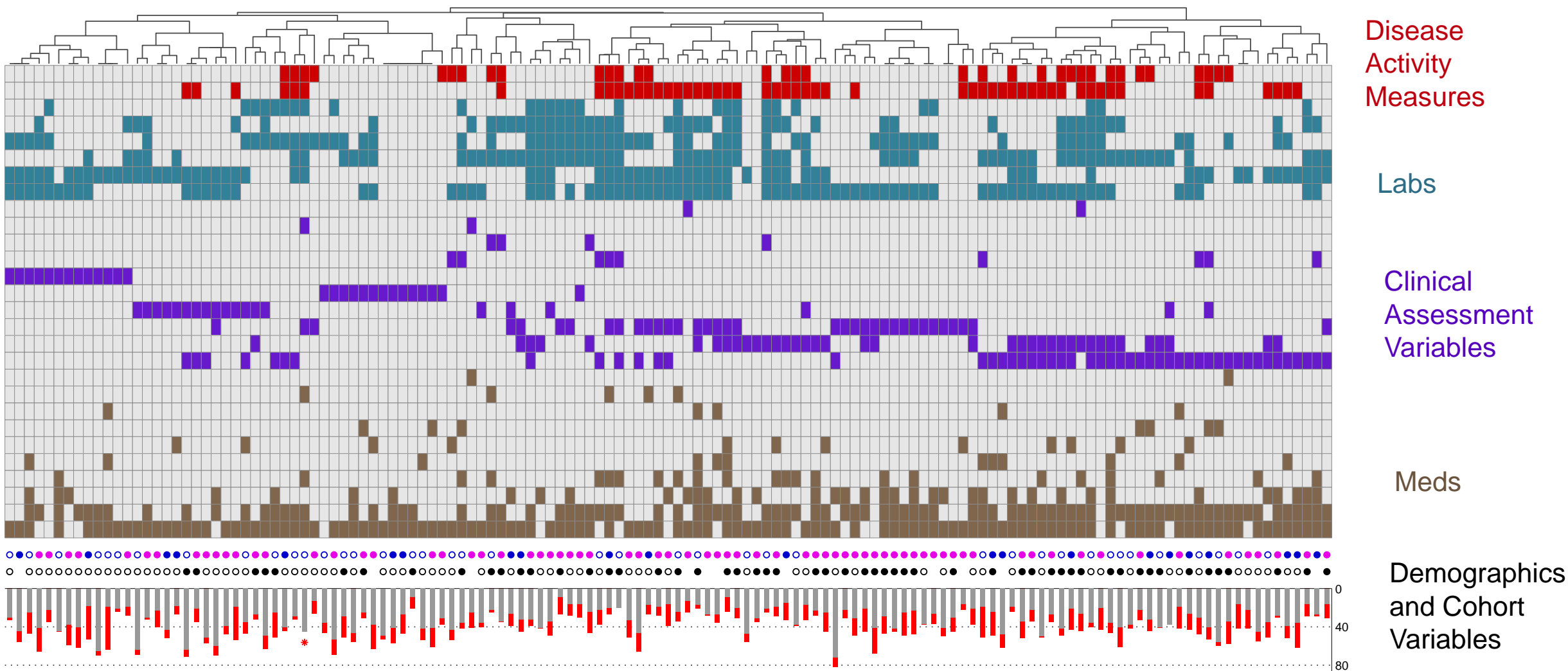
Chaussabel et al. (2010) Assessing the human immune system through blood transcriptomics. *BMC Biology* **8**:84 doi:10.1186/1741-7007-8-84



Detecting batch effects



Integrated data capture



IMMPROF / Lupus

Logged in as **arosenberg** from 10.162.12.79 [LOGOUT](#)

ACTIONS

- [All Subjects](#)
- [Flow Count Profiles](#)
- [Flow Frequency Profiles](#)
- [Analyte Collections](#)
- [Analysis Sets](#)
- [General Query](#)

DATABASE SUMMARY

208	Subjects
258	Draws
711	Flow Runs
144	LIPS Assays
202	IFNa Assays
711	Analyses
1728	LIPS Values
202	IFNa Values
54421	Counts
102255	Frequencies

Subject Quick Find: [find](#)

developed by [Alex Rosenberg](#) | © 2013, Division of Allergy, Immunology & Rheumatology, URM | [Contact Webmaster](#)

“Show me samples that have associated analyzed flow cytometry data using the ‘transitional B cell panel’ that are derived from lupus patients with:

- severe flare
- SLEDAI ≥ 8
- musculoskeletal or nephritis manifestation
- any positive autoantibody measurement
- has low complement”

IMMPROF / Lupus

Logged in as **arosenberg** from 10.162.12.79 [LOGOUT](#)

Generic Search Form

Retrieve: Draw with at least one Analysis [Run Query](#)

- Use checkboxes to filter results
- Wildcard for text fields is “%”
- Dates in format “YYYY-MM-DD”

Subject Attributes

- ☐ Subject Name
- ☐ Study
- ☐ Cohort
- ☐ Gender
- ☒ Has Lupus ☒
- ☐ Flow Control ☐
- ☐ Birth Year Range -
- ☐ Diagnosis Year Range -

Draw Attributes

- ☐ Draw Name
- ☐ Draw Date Range -
- ☒ Flare ☒
- ☒ Flare Level
- ☐ Draw Prior to Flare ☐
- ☒ SLEDAI Range -
- ☐ ACR Criteria Range -

Flow Attributes

- ☐ Flow Run ID
- ☐ Flow Date Range -
- ☒ Flow Panel
- ☐ Experiment Name
- ☐ FCS File
- ☐ Fresh Sample ☒

Clinical Manifestations (AND OR)

- ☒ CNS
- ☒ Musculoskeletal
- ☒ Nephritis
- ☒ Skin
- ☒ Serositis
- ☒ Hematologic
- ☒ SACQ
- ☒ Quiescent
- ☒ Gastrointestinal
- ☒ Vasculitis
- ☒ Pulmonary

Analysis Attributes

- ☐ Gating Depth
- ☐ CD19
- ☐ Painting Category
- ☐ Not Usable ☐

Medications (AND OR)

- ☒ Enbrel
- ☒ Methotrexate
- ☒ Medrol
- ☒ Rituxan
- ☒ Anti-BAFF
- ☒ Cytosan

Prednisone Dose -

WBC Range -

% Lymphocytes Range -

dsDNA Range -

Autoantibodies (AND OR)

- ☒ DNA
- ☒ RNP
- ☒ Sm
- ☒ Ro
- ☒ La

C3 Range -

C4 Range -

Has Low Complement ☒

developed by [Alex Rosenberg](#) | © 2013, Division of Allergy, Immunology & Rheumatology, URM | [Contact Webmaster](#)

Take Home Points

Develop and maintain good research practices

Formally train and empower young investigators

Embrace big science

Formalize and make SOPS available to all

Integrated system to capture and store patient, imaging, biomarker data

Recruit biostatisticians and bioinformatics specialists to your team

Resources

- NIH website
- NIH modules on scientific rigor and reproducibility
- ARRIVE guidelines
- *Resnik, DB. Reproducibility and research integrity. Account. Res.2017;24(2):122*