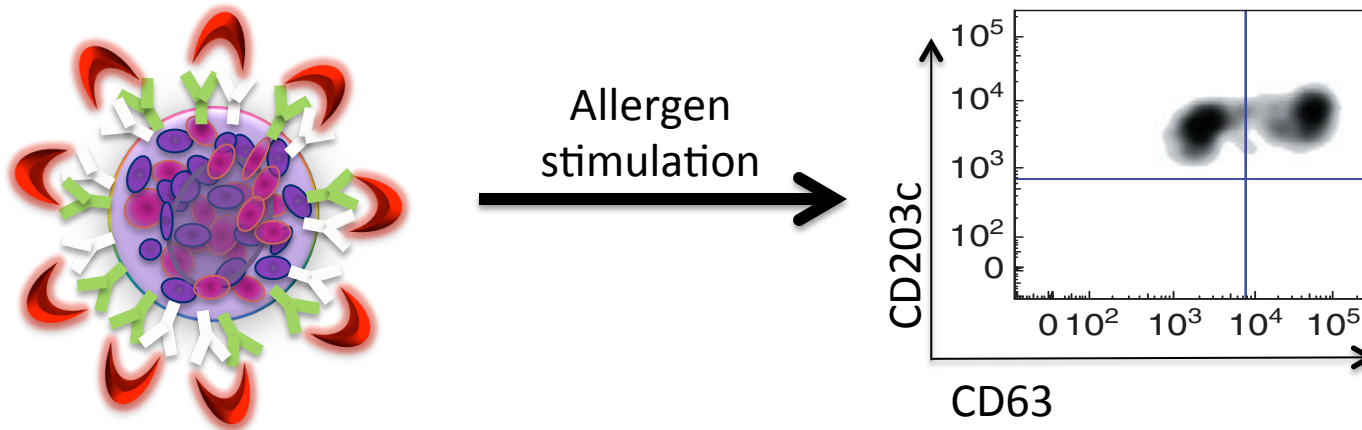# The Application of Data-Driven Approaches to Flow Cytometric Analysis of Basophil Activation Testing
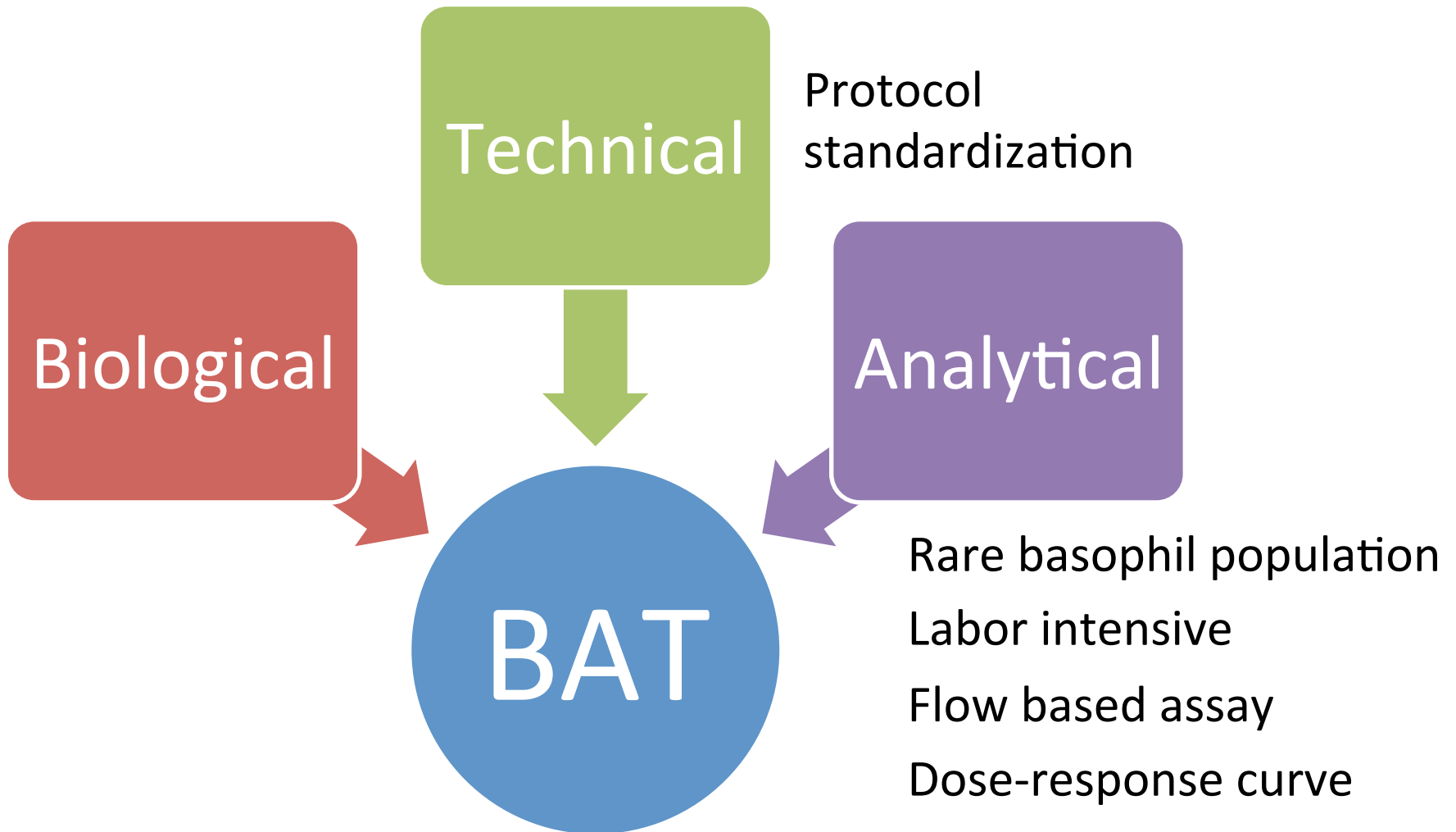
Sarita Patil, MD
Instructor, Allergy and Immunology
Massachusetts General Hospital
Harvard Medical School

# The basophil activation test (BAT)

Allergen stimulation

CD203c

CD63

- Basophil activation testing
  - Ex vivo allergen stimulation
  - Upregulation of activation markers
  - Data acquisition by flow cytometry
- IgE-mediated allergies
  - Allergens: Environmental, Venom, Drug, Food
  - Clinical applications: Diagnosis, Immunotherapy

Hoffman, et.al. 2016. Allergy.

# Variability in BAT

**Biological**

**Technical** — Protocol standardization

**Analytical**

**BAT**

Rare basophil population

Labor intensive

Flow based assay

Dose-response curve

# Programmatic flow cytometry data analysis

| Manual Gating | Autogating |
|:---:|:---:|
| Gold standard | Reproducible |
| Subjective | Transparent |
| Permissive | Quality control |
| Labor intensive | High-throughput |

# Clinical Dataset:
# Peanut oral immunotherapy

**Blood Draw**    *Modified Rush*    *Build-up (44 Weeks)*    *Maintenance (12 Weeks)*    **Oral Food Challenge**

| (Week #) | 0 | 1 | 1-44 | 44-56 | 56 |

12mg → 4000mg → Challenge

Peanut

Time on PNOIT

©Golden Peanut Company, LLC
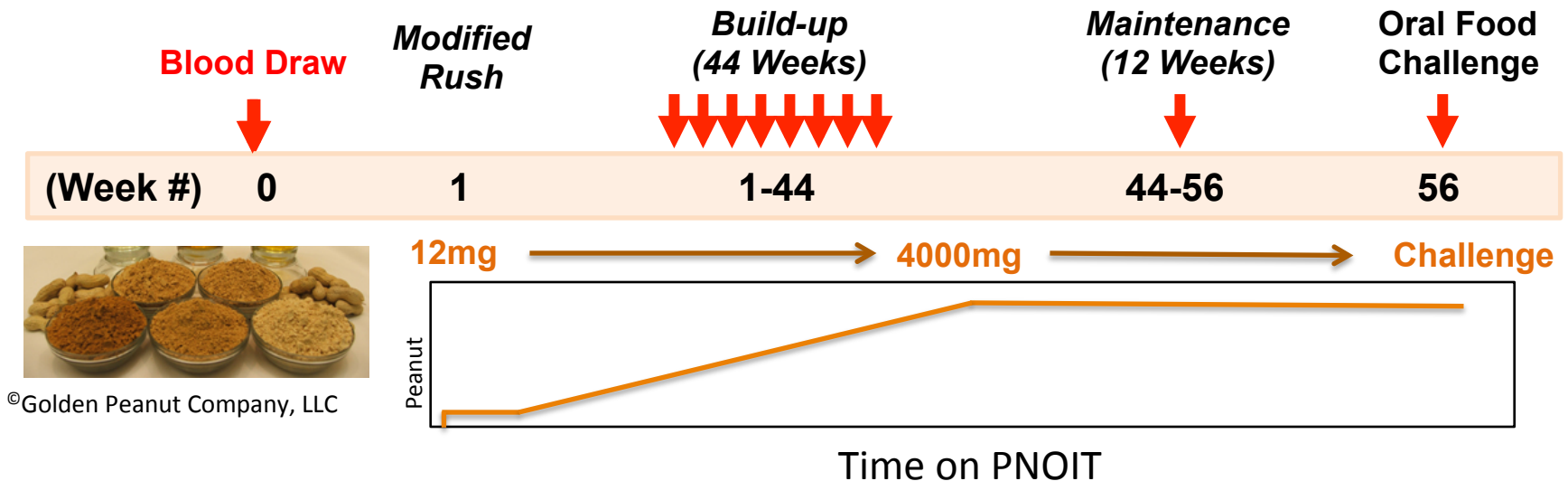
- Single center trial (N=30)
  - Pediatric (7-21 years old)
  - Peanut allergic (+SPT, +sIgE)
  - Increasing doses of peanut flour

# Basophil activation testing (BAT) analysis



**Antigen stimulation**
(Ara h 1, Ara h 2, Ara h 6, whole peanut extracts)

15 minute stimulation at 37°C

**Flow Cytometry**
FCS files

**Analysis**
Compensation
Transformation
Removal of boundary events

**Quality Control**
Expert Analysis
Outlier Identification

**Statistical Analysis**
Dose-response curves
Area under the curve
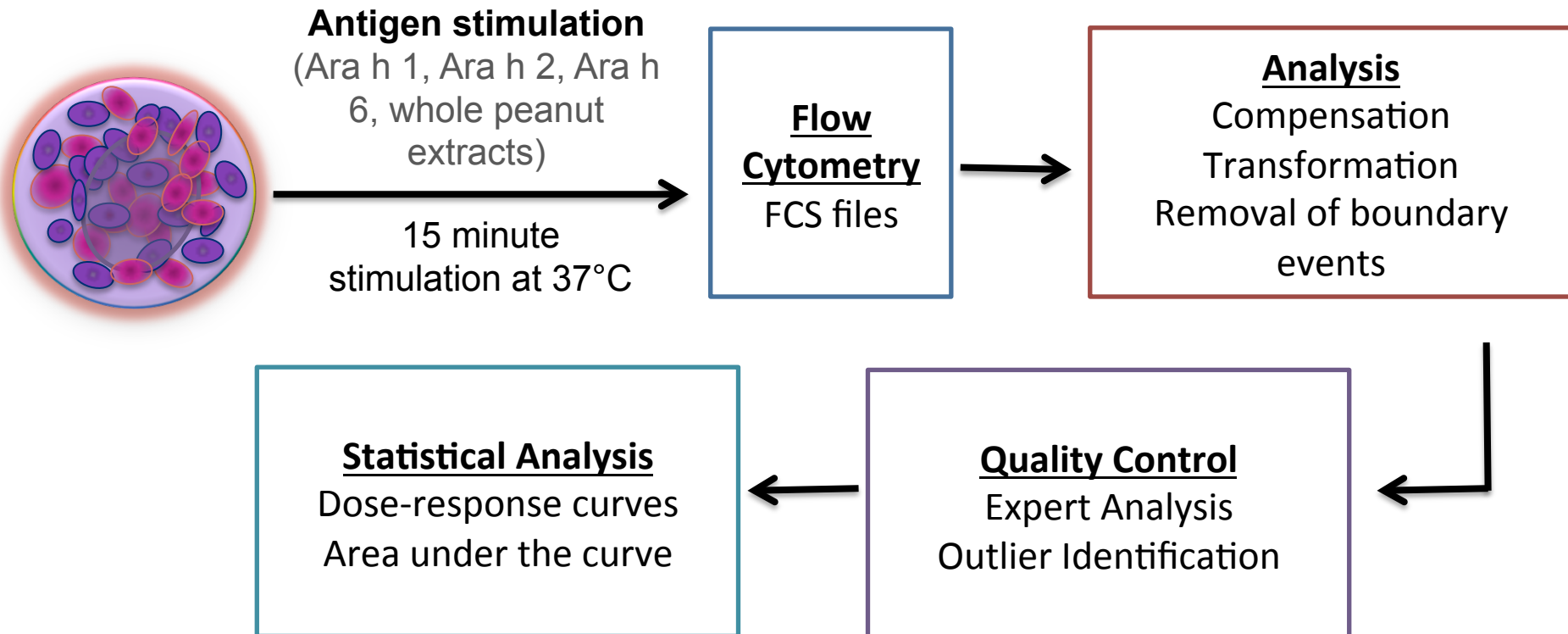
# DEVELOPMENT OF THE PROGRAMMATIC APPROACH

**52**

- Development

**294**

- Application

# AutoBAT
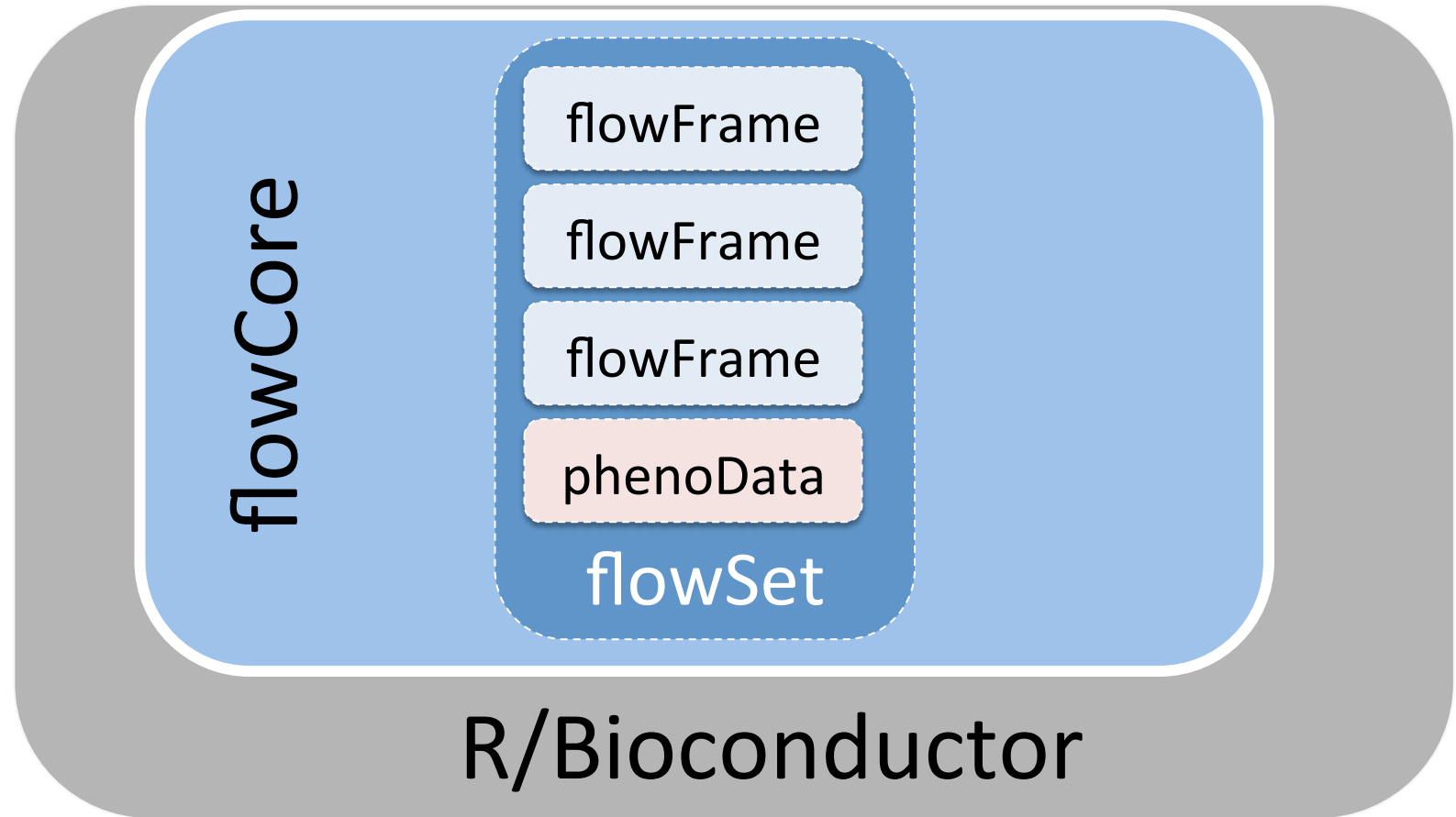


flowCore

flowFrame

flowFrame

flowFrame

phenoData

flowSet

R/Bioconductor

https://github.com/saritaupatil/AutoBAT

# Programmatic application: BATgate

- ## BATgate()
  - Function designed for basophil gating and identification of activated basophils (%CD63hi)
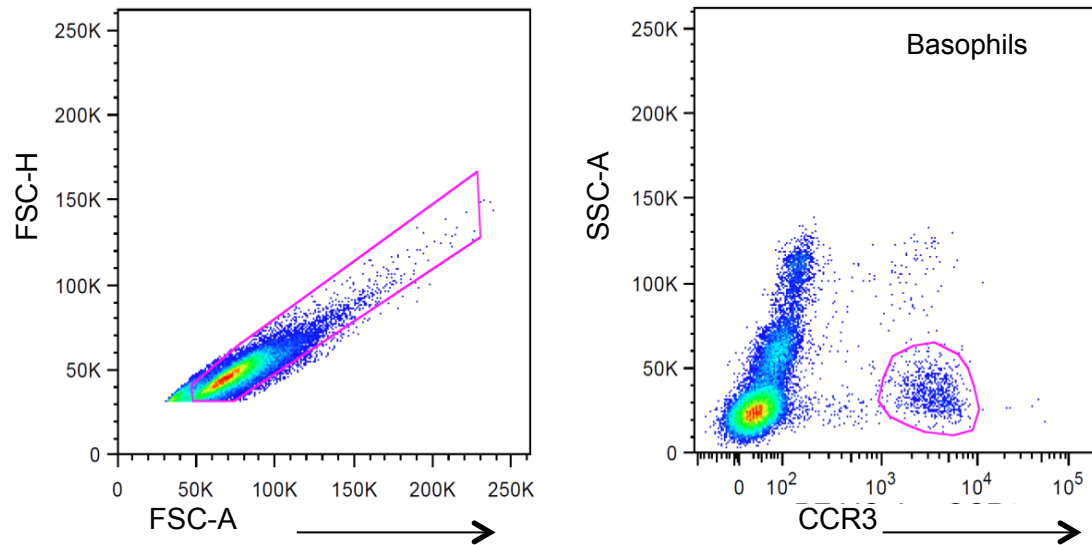  - Step-wise gating strategy
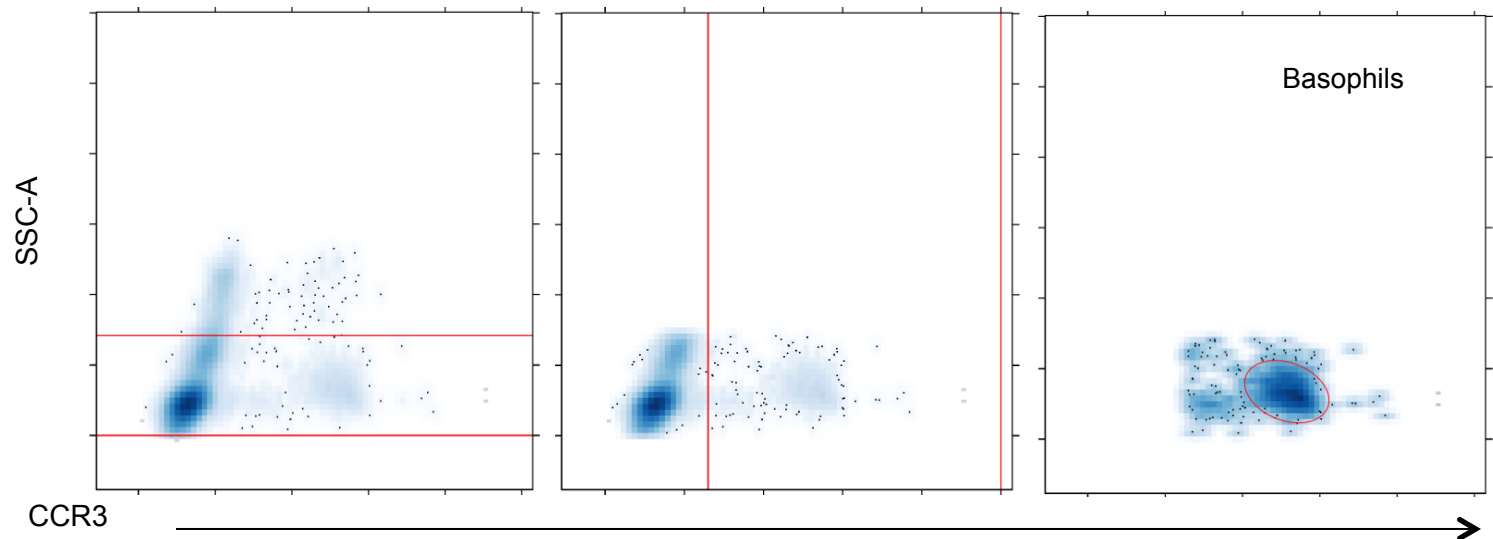
# Step-wise gating approach
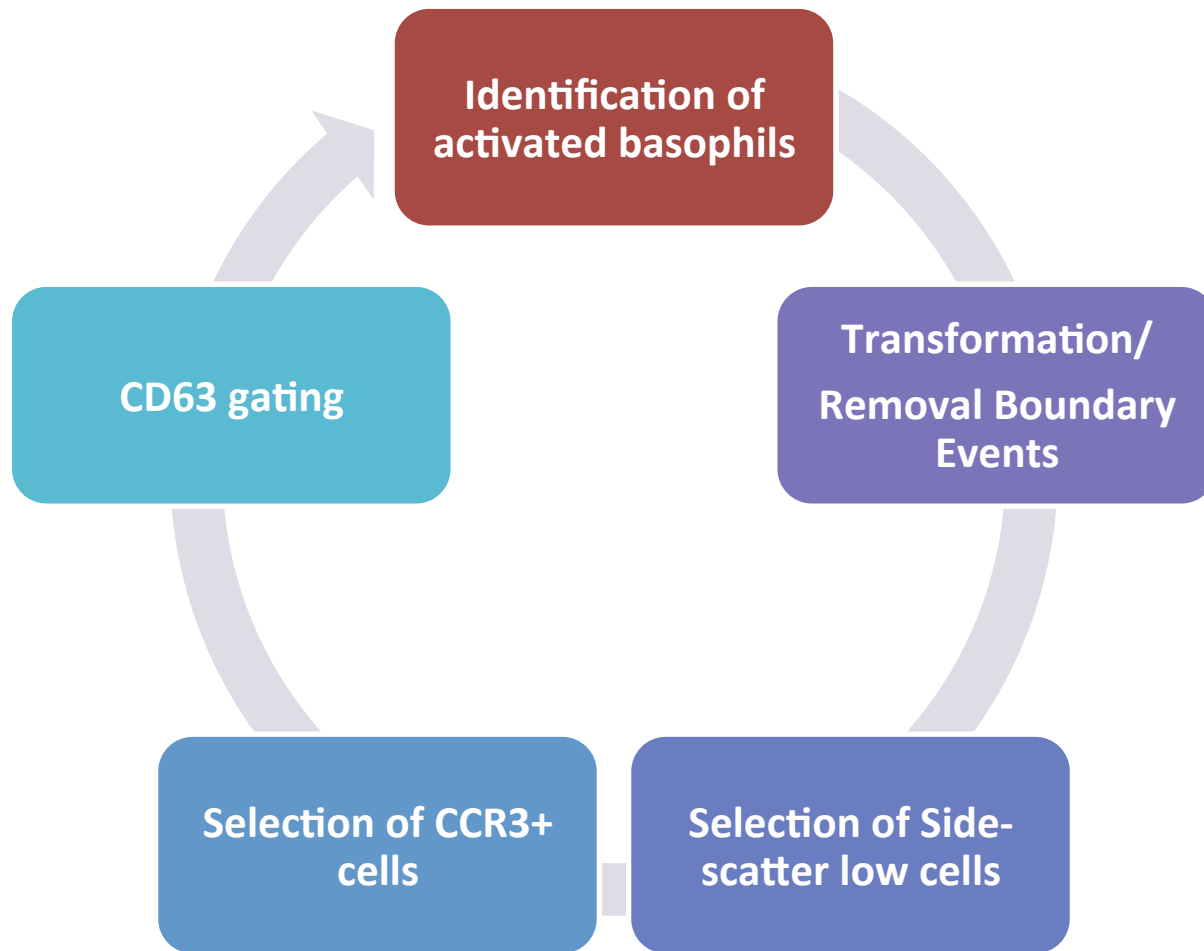


kmeans 2 or 3

# Gating on basophils in BAT

**Manual**



**Autogating**

Patil, et.al. 2017. Flow Cyto B.

# Programmatic application: BATgate

- ## BATgate()
  - Function designed for basophil gating and identification of activated basophils (%CD63hi)
  - Step-wise gating strategy
  - Input
    - Workingdir
    - Fluorophores
    - SSno
    - Medcontroltube
  - Outputs
    - Csv file with data statistics for each stimulation condition
    - Pdf file of gating graphics for each condition for visualization

# Programmatic application: BATmeasures

- BATmeasures()
  - Function designed to derive dose-response measures of antigen stimulated basophils
  - Measures:
    - ED50
    - Area-under-the-curve by using log of the stimulation concentration
  - Input
    - data: the data.frame being used for measurement of BAT
      - Needs to have removal of any non-dose response data
    - groupvariables: the grouping variables to identify each dose-response series
      - Ie: Patient, visit, stimulation condition
    - output.file: name of the output file
  - Output
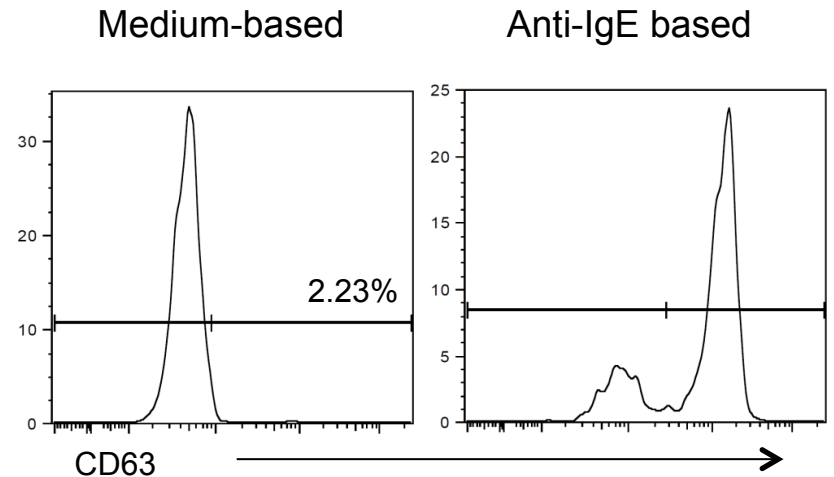    - An csv file with the name of the output.file, with ED50 and AUC

52 • Development

294 • Application

269 • Successful

**92%**

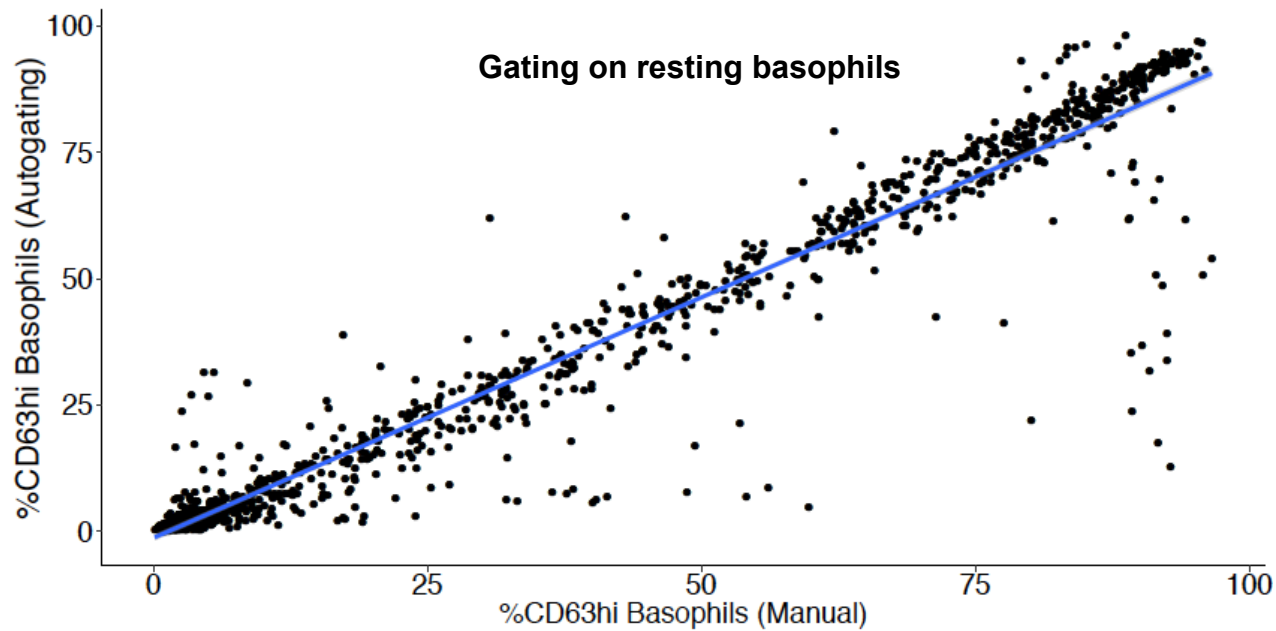**7,166 individual BAT**
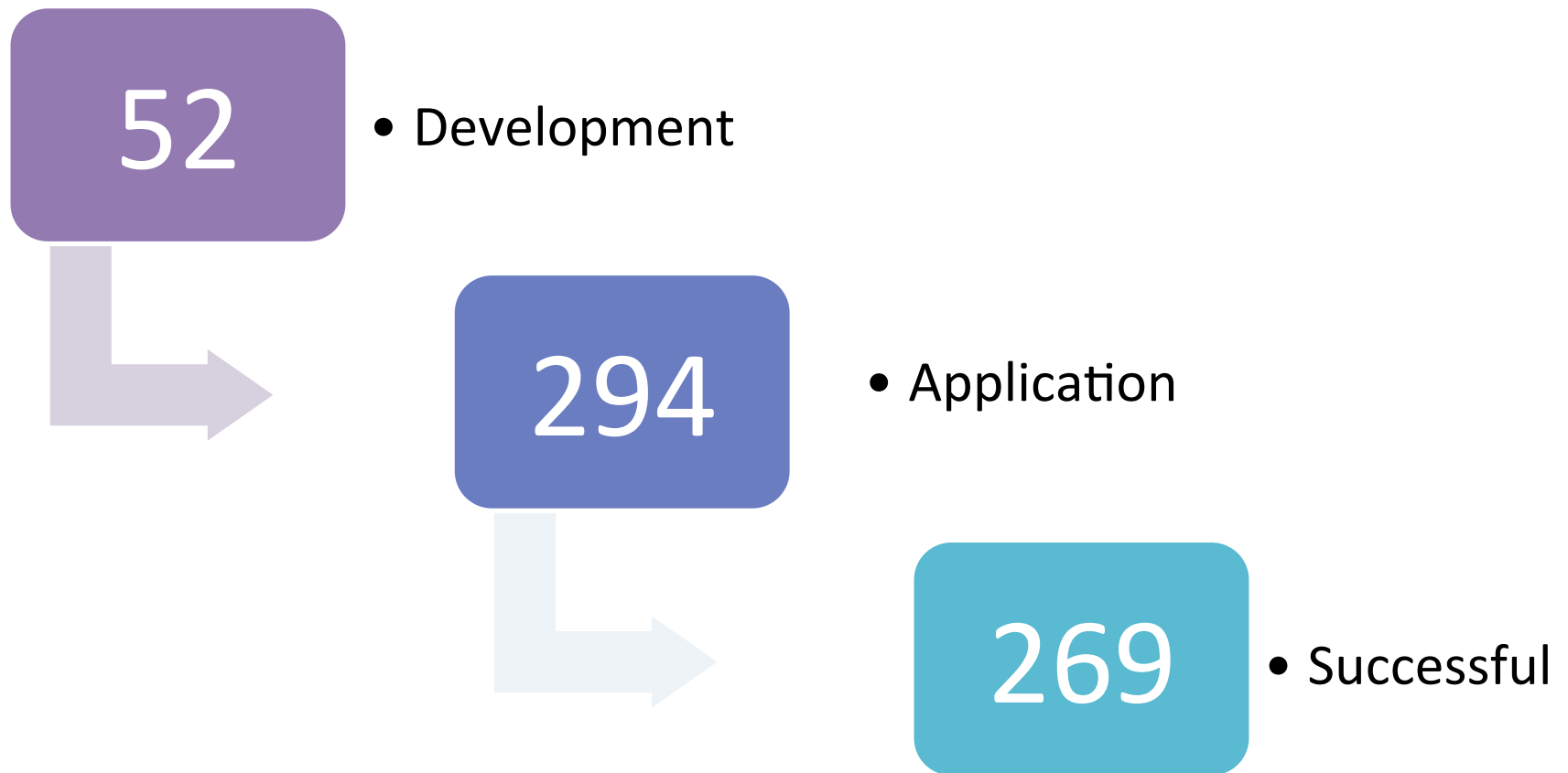
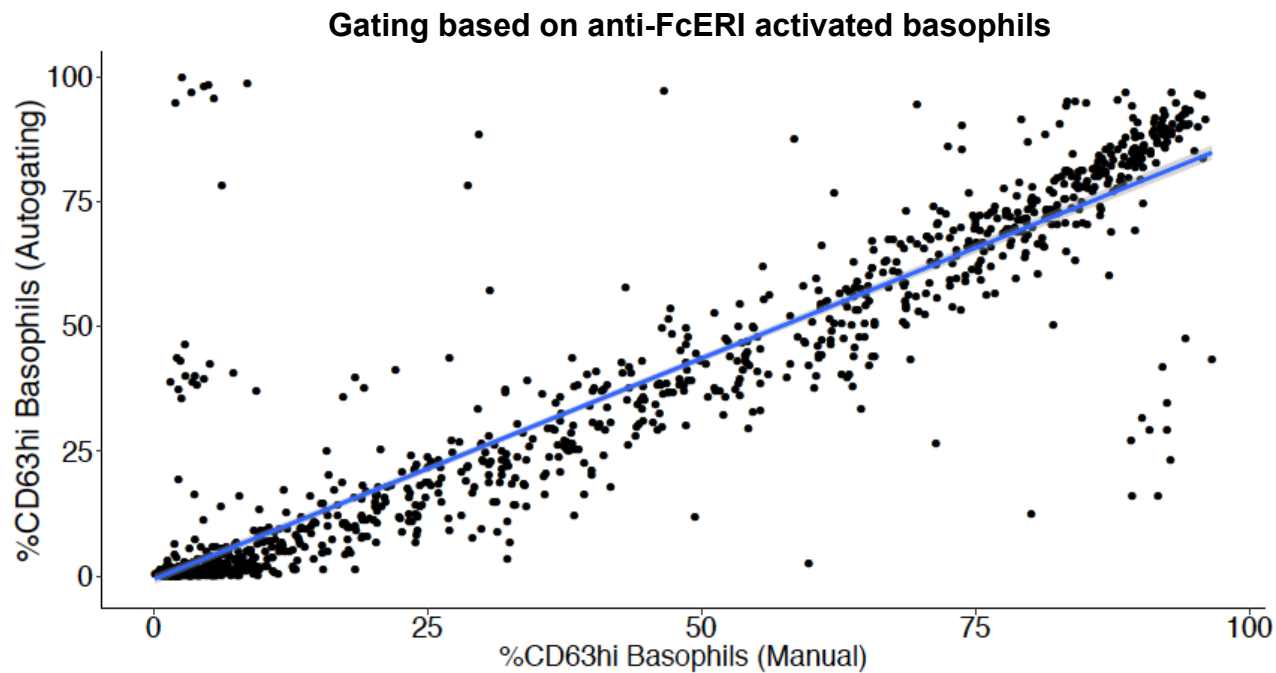Patil, et.al. 2017. Flow Cyto B.

# GATING CD63 UPREGULATION

# CD63 gating

- Comparison of gating strategies for CD63 upregulation
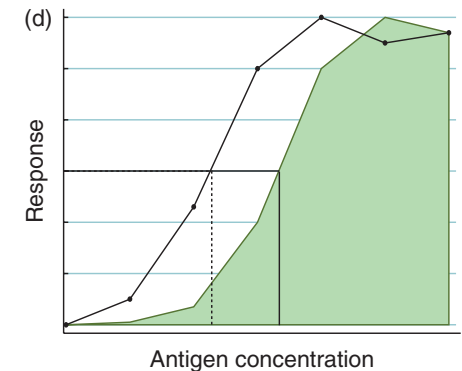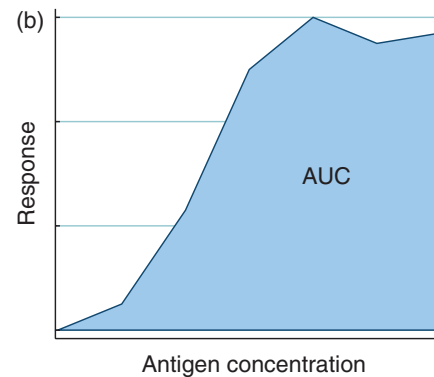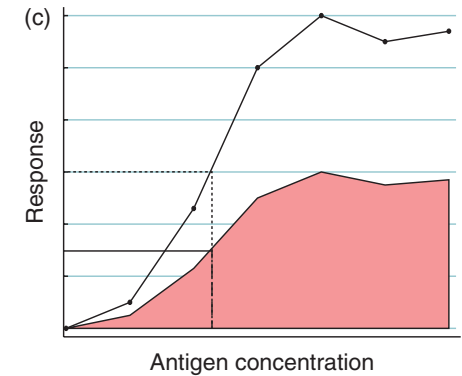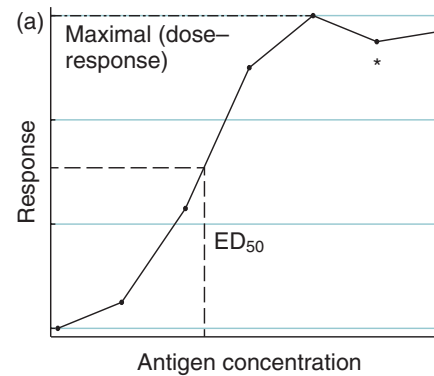
    – Medium-based
    – Anti-IgE based

Patil, et.al. 2017. Flow Cyto B.

**Gating on resting basophils**

%CD63hi Basophils (Autogating) vs. %CD63hi Basophils (Manual)

**Gating based on anti-FcERI activated basophils**

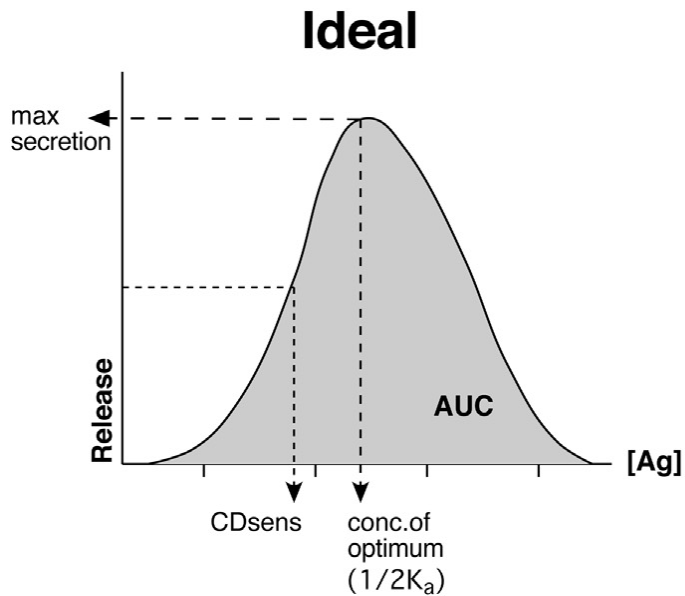%CD63hi Basophils (Autogating) vs. %CD63hi Basophils (Manual)
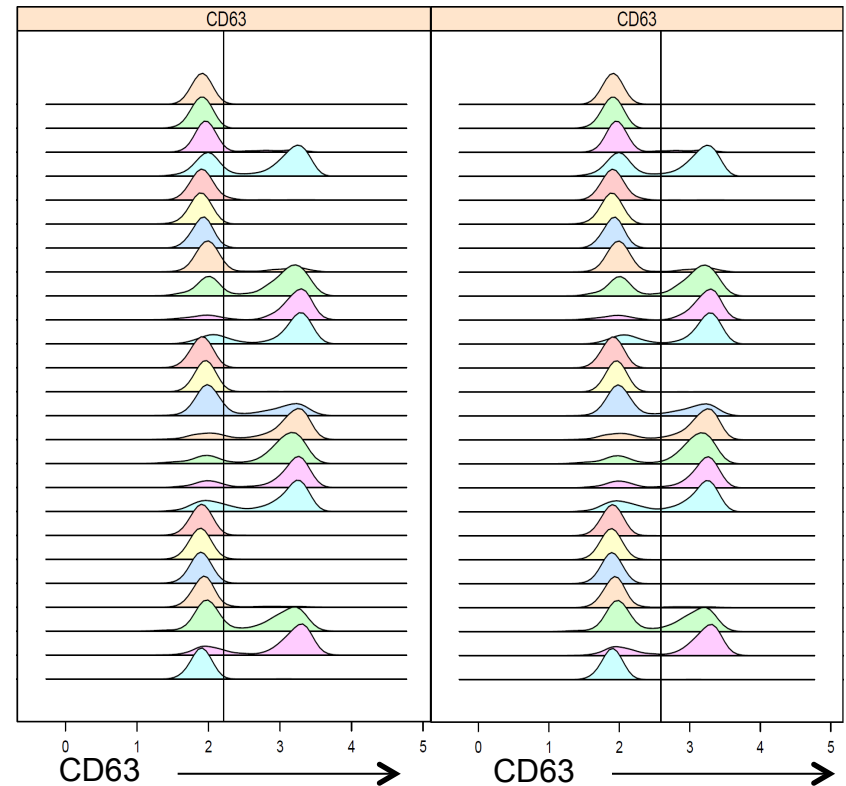
R=0.96 vs. R=0.84, $p < 0.001$
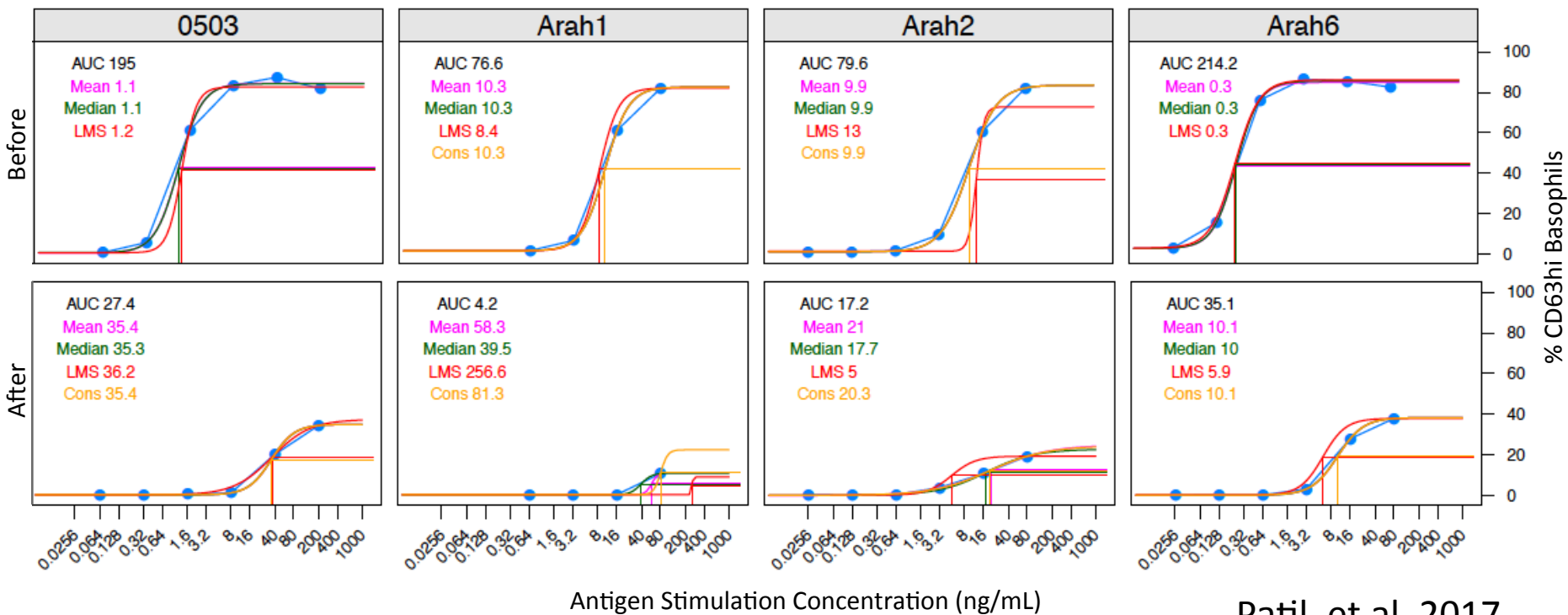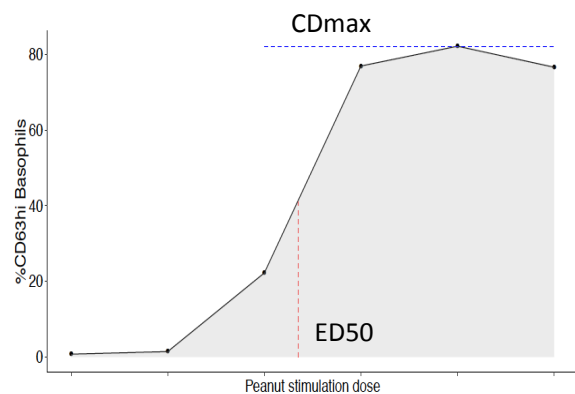
Patil, et.al. 2017. Flow Cyto B.

# Measures of BAT



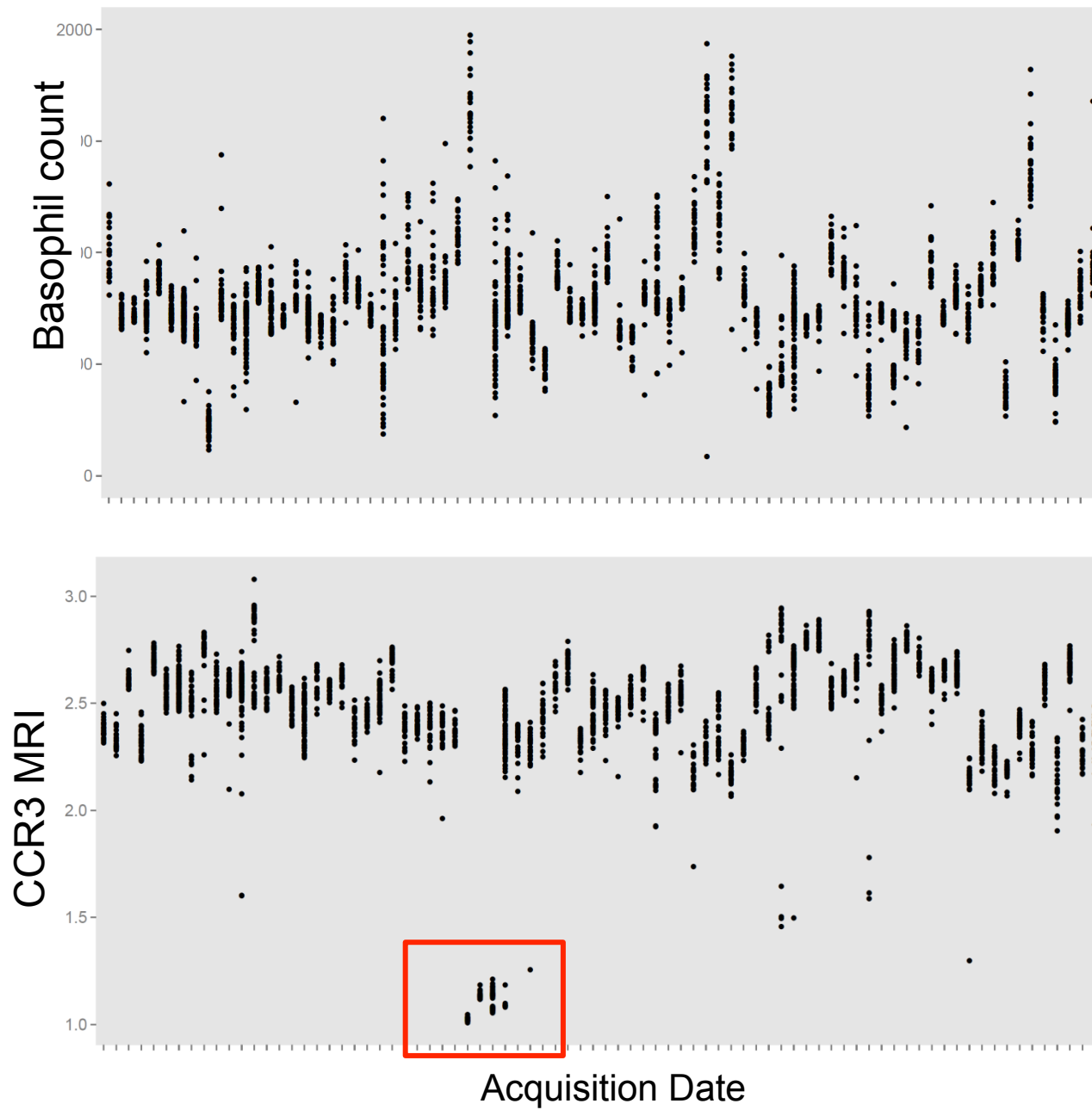MacGlashan, D. (2013) JACI.                    Patil, et.al. (2012) Clin Exp Immuno.
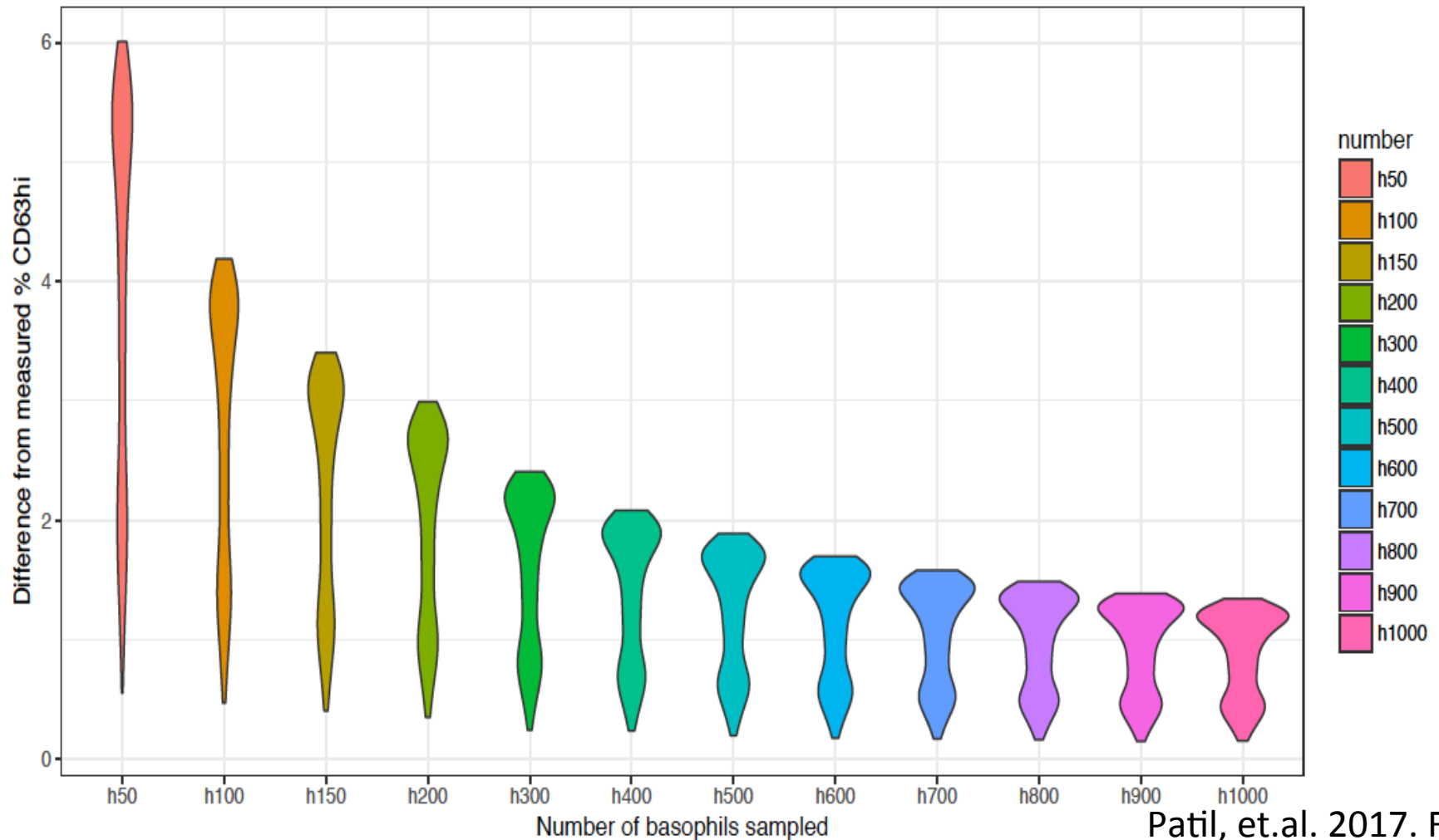
Patil, et.al. 2017.
Flow Cyto B.

# LESSONS FROM QUALITY CONTROL MEASURES
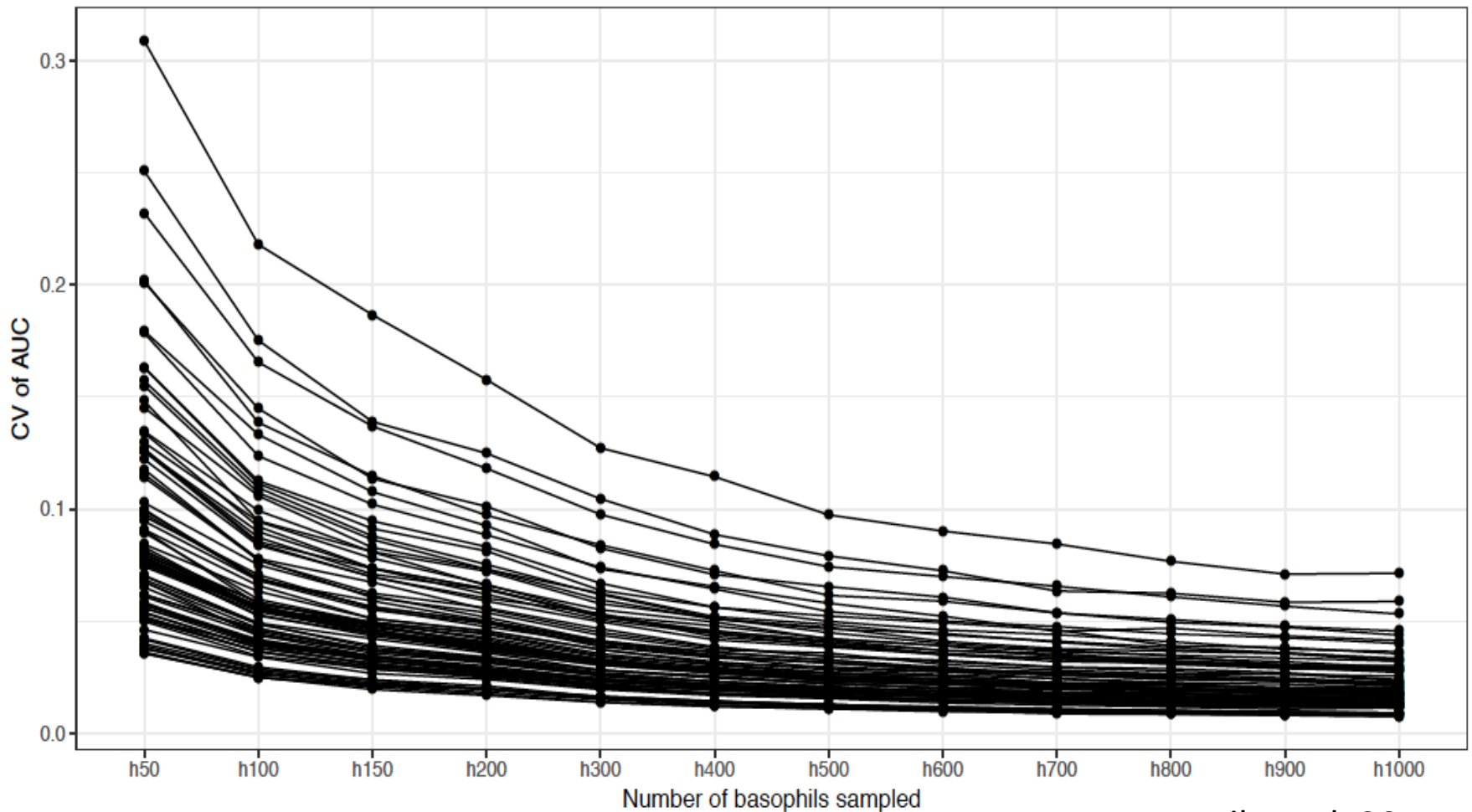
Patil, et.al. 2017. Flow Cyto B.

# Influence of basophil sampling on measurement of degranulated basophils



Patil, et.al. 2017. Flow Cyto B.

# Influence of basophil sampling on AUC measurement of dose-response curves



Patil, et.al. 2017. Flow Cyto B.

# The future of programmatic approaches

- Open-access to analytic method
- Cloud-based analytical platform
- Applications: Multicenter data
- Setting standards for flow cytometry gating
- Machine learning approaches
- Quality control
  - Longitudinal
  - Internal
- Broadening applications
  - Use with other allergens
  - Use with multi-marker basophil experiments

# Acknowledgements

- Agustin Calatroni (Rho, Inc)
- Bühlmann Laboratories
  - Michael Schneider

- Shreffler Laboratory (MGH)
  - Alex Ma
  - Elizabeth Fleming
  - Neal Smith
  - Johanna Steinbrecher
  - Cecilia Washburn
  - Huong Tang



  - Clinical Research Team
  - Food Allergy Research Team
  - Harvard Catalyst, MGH CRC
  - Patients and families