# Can trials in West Africa study both off-target effects and determinants of severe morbidity/mortality?

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# Non-specific effects: the need for biological insights

- The epidemiology of NSE's have been well-documented
- The current challenge: we lack biological insight behind NSE's

  *Why* do immunized infants survive?

  *What* are unimmunized infants dying of?

- The necessary trials must: **measure the right variables**
- Be conducted using SOPs that can be successfully carried out in **low-resource environments**

# Which variables should we select to measure NSE's?

**The Challenge:** How do you select assays and variables to measure?

- Previous targeted approaches failed to identify correlates of even specific protection by BCG or infant responses to infection that lead to death or survival
- Small blood volumes obtained from infants, low-resource settings, and field work limit feasible downstream *in vitro* assays

# Unbiased approaches: omics

- Allows characterization of immune responses to different classes of pathogens:

  *host-centered vs. pathogen-centered diagnoses*

- Successfully used to identify signatures of both response to vaccines and infection


ARTICLE

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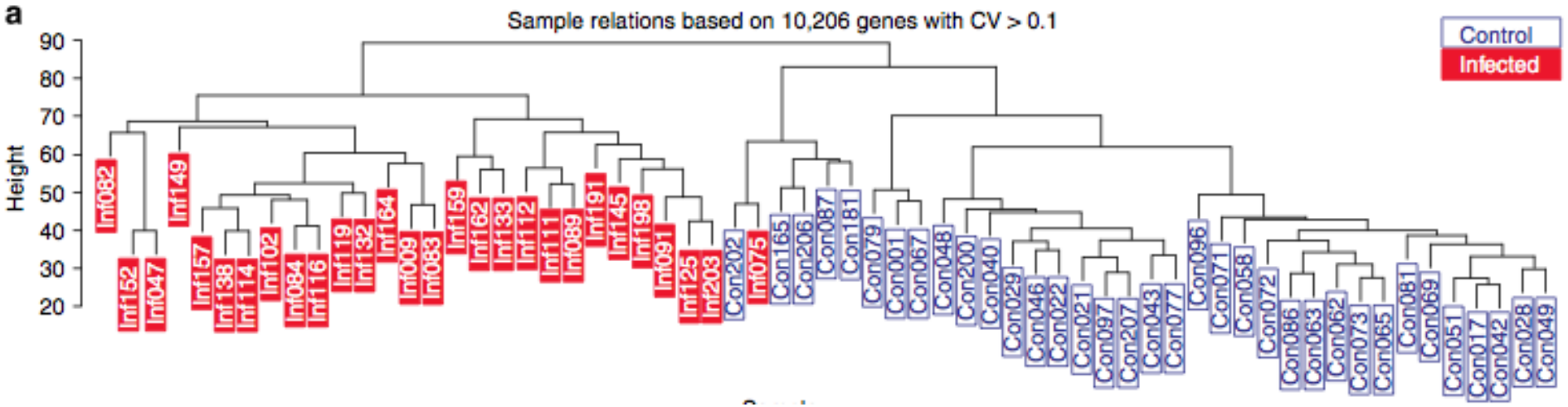
## Identification of a human neonatal immune-metabolic network associated with bacterial infection

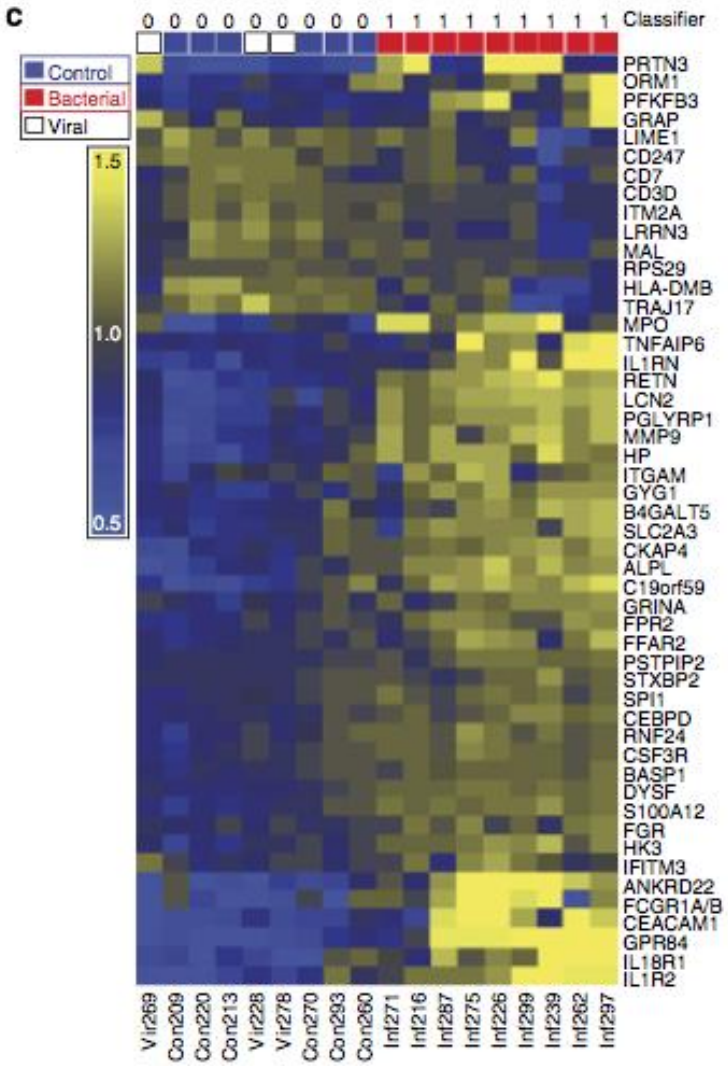
Claire L. Smith[1,2,*], Paul Dickinson[2,3,*], Thorsten Forster[2,3], Marie Craigon[2], Alan Ross[2], Mizanur R. Khondoker[2,†], Rebecca France[2], Alasdair Ivens[4,†], David J. Lynn[5,†], Judith Orme[1], Allan Jackson[1], Paul Lacaze[2], Katie L. Flanagan[6,†], Benjamin J. Stenson[1] & Peter Ghazal[2,3]

# Using transcriptomics to identify infection

- Whole blood from infants having blood draws for suspect infections vs. blood draws for unrelated reasons
- Microarray for whole-genome expression (48,000 transcripts)



# Validation across cohorts




A 50-gene classifier built from their original cohort was applied to an independent set of samples.

The algorithm was able to separate bacterial from control/viral infections.

# Applying the omic approach to NSE: BCG-Immediate-Sub-Study

**The BCG-Immediate (BCGIMED) study**

- Infants admitted to main NICU in Guinea-Bissau
- Randomized trial, providing **BCG/OPV immediately upon NICU admission**, as opposed to the normal routine of BCG at discharge
- Major predictors for NICU admission are birth weight <1500 g and Apgar score ≤3.
- Average stay in NICU is 5 days.

**Hypothesis:** *BCG immunization induces an enhanced state of innate immunity that leads to immediate protection from early-life infection*

# BCGIMED Substudy Design

8% NICU Mortality prior to discharge

600-1000

**BCG**
**OPV**

300

**Survive**

**Die**

**NILL**

300

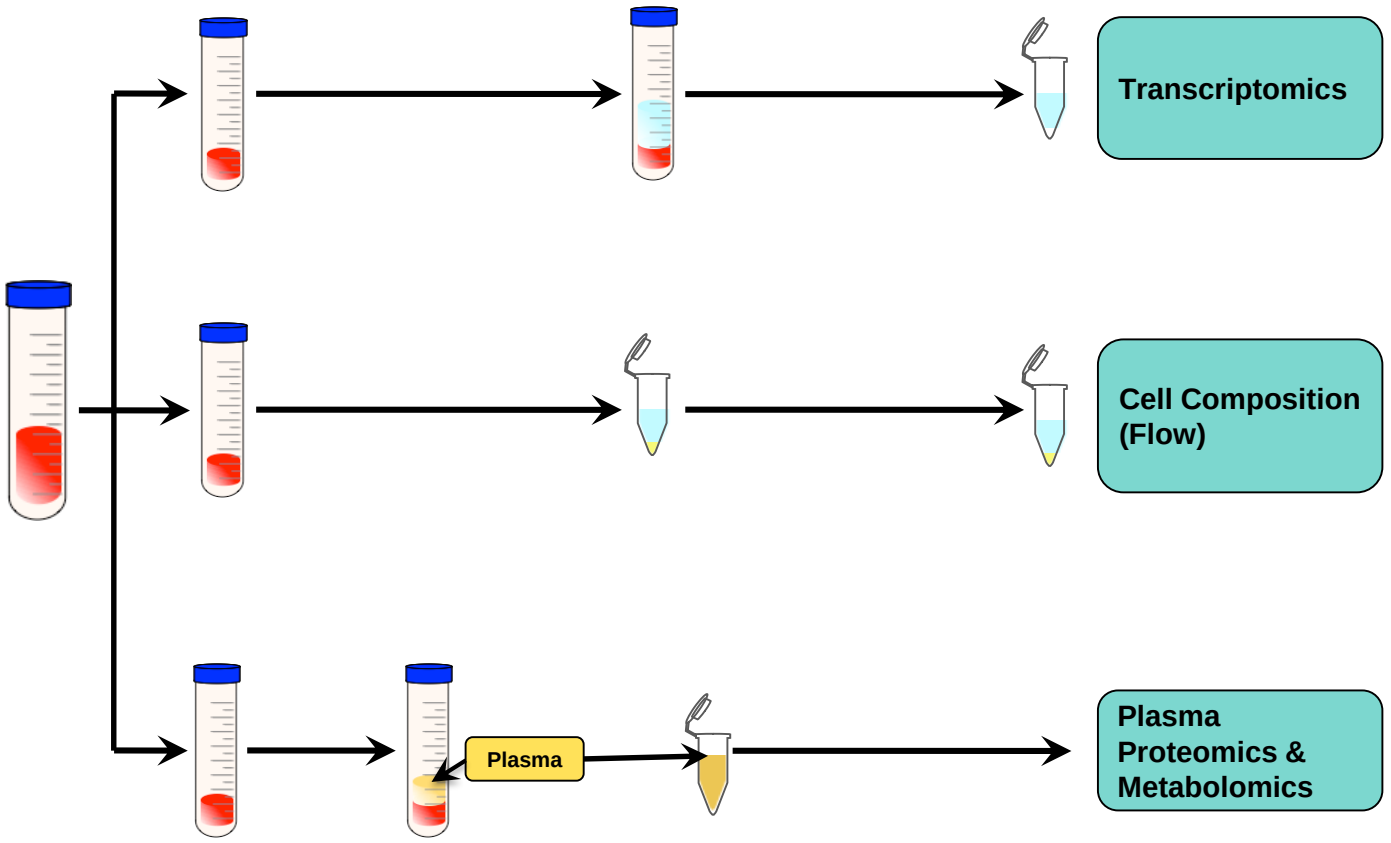
**Survive**

**Die**

24 hrs post randomization

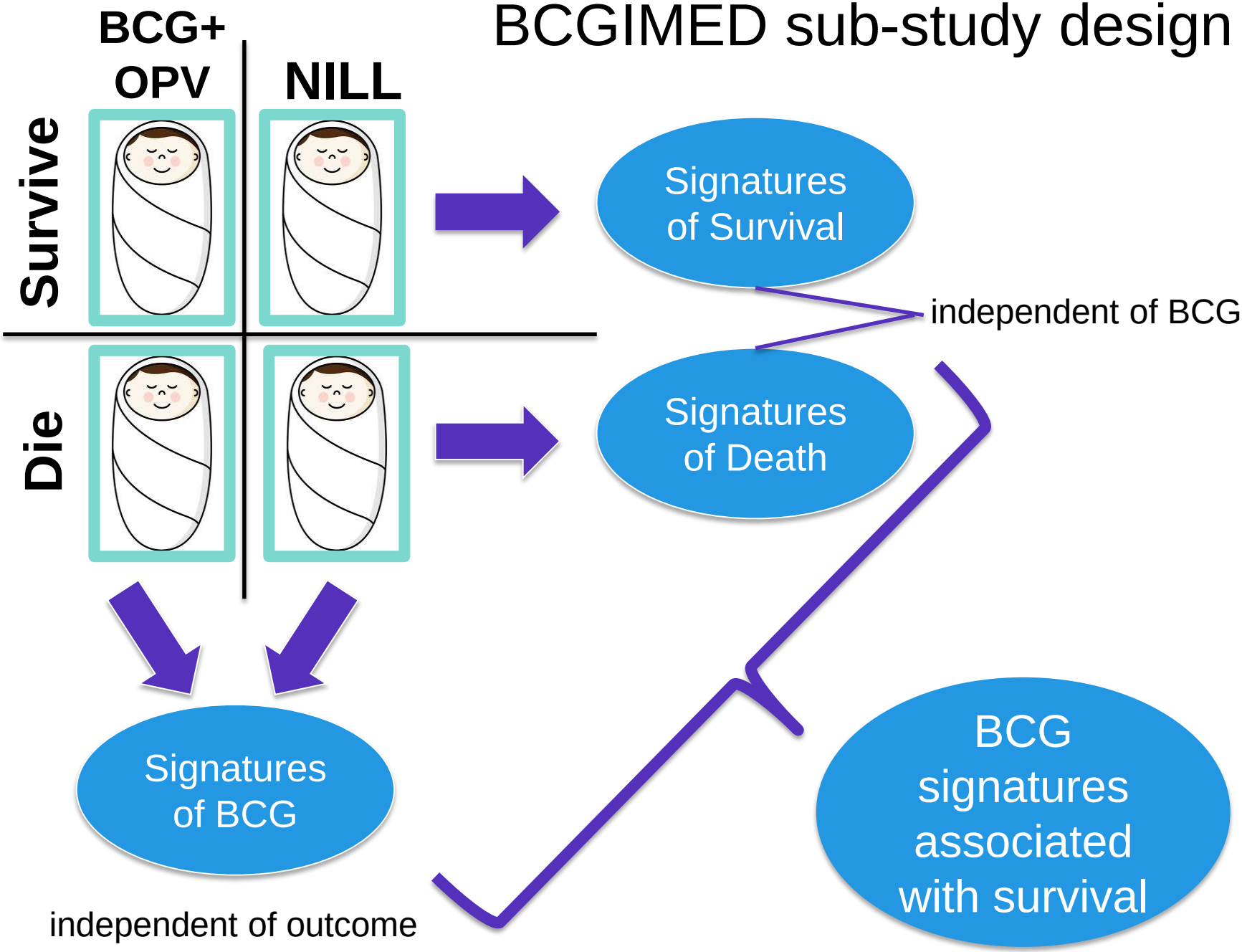
**Follow-up to 1 yr**
**Outcome**
**Demographics**

# BCGIMED sub-study design




Schematic by Dr. Simon Van Haren (Levy Lab; Boston Children's Hospital)

# BCGIMED sub-study design



# Easier said than done

Transcriptomics

mRNA transcripts quick to degrade after blood draw

Dvinge H. *et al.* *PNAS* 2014, **111**, 16802

Plasma Proteomics & Metabolomics

Protein content of plasma heavily influenced by:

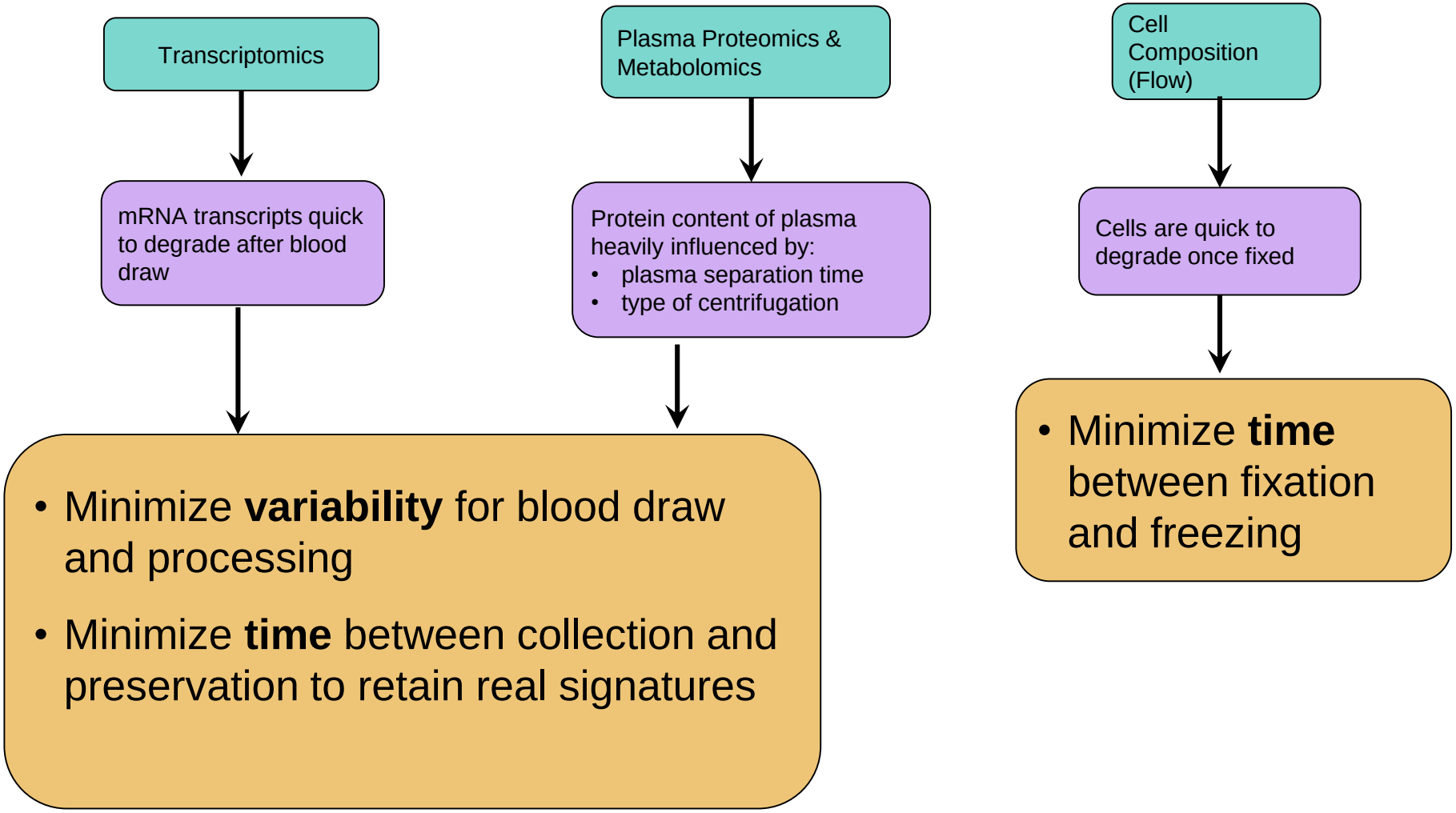
- plasma separation time
- Plasma freezing time

Pinto J. *et al.* *Analyst* 2014, **139,** 1168.
Lista S. *et al.* *Prog. Neurobiol.* 2013, **101,** 18.
Ostroff R. *et al.* *J. Proteomics* 2010, **73,** 649.

Cell Composition (Flow)

Cells are quick to degrade once fixed

# Knowing what's important



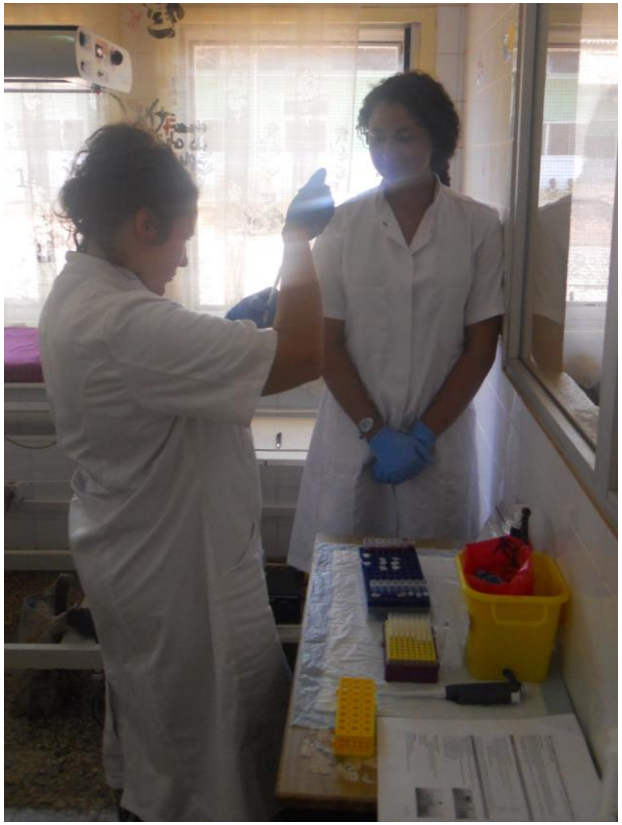
# Recruitment and sample collection: Maternity ward, Hospital Simao Mendes

- Minimize **variability** for blood draw and processing
- Minimize **time** between collection and preservation to retain real signatures

**Blood sample collection:**
HNSM ICU,
local Guinean nurses

**RNA processing:**
immediately after blood draw

**Plasma separation:**
immediately after RNA
*Separate person from sample collection*

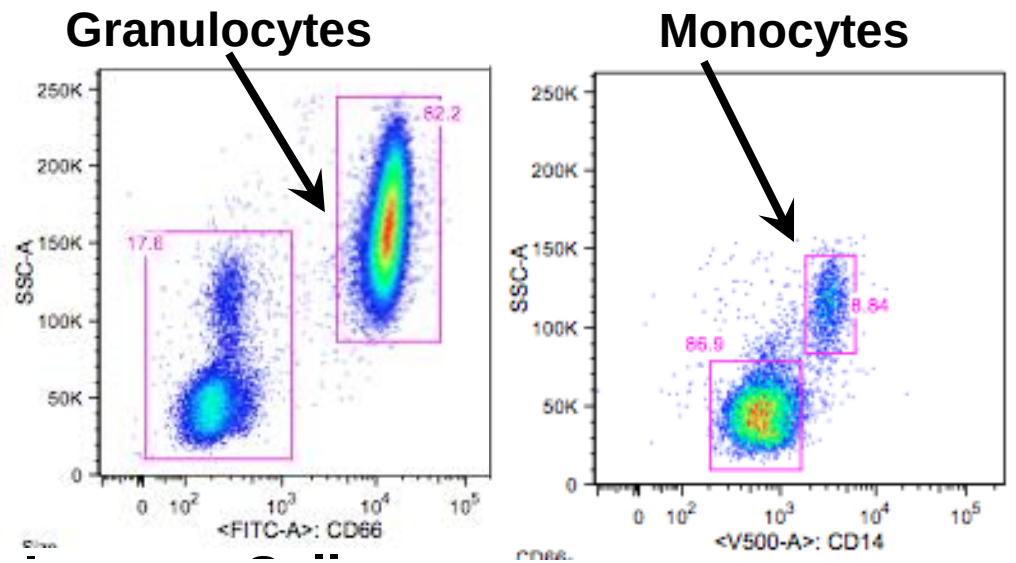


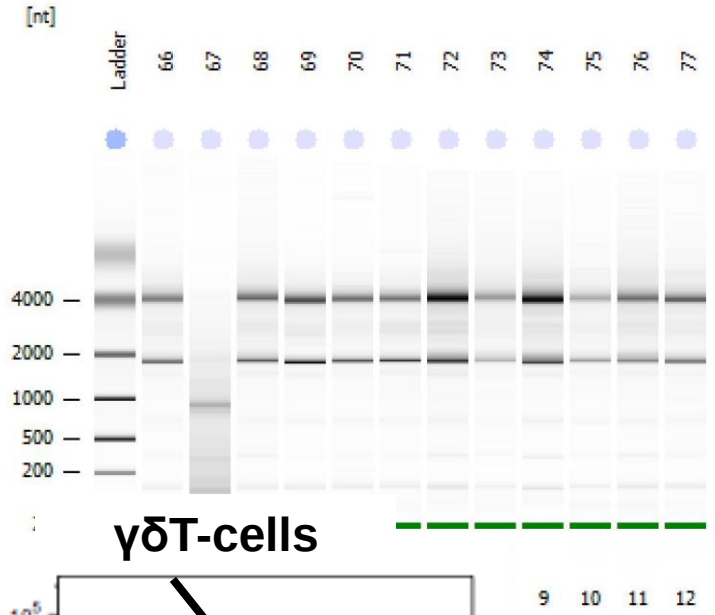
- Minimize **time** between fixation and freezing

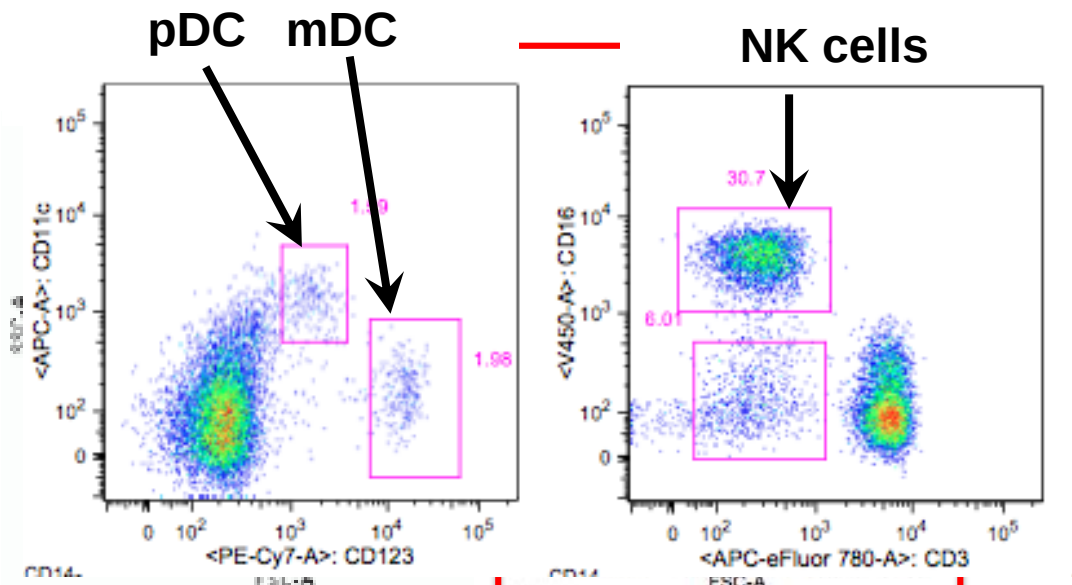
Establishing a laboratory wherever there needs to be one:

Fixed cells are frozen within 15 minutes of preservation

# Does it work? Pilot data

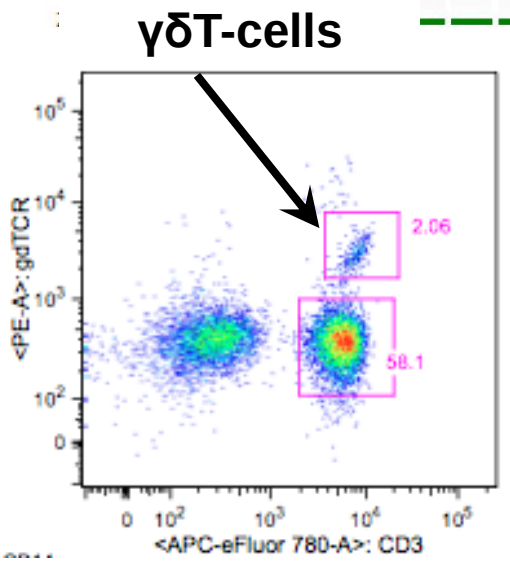




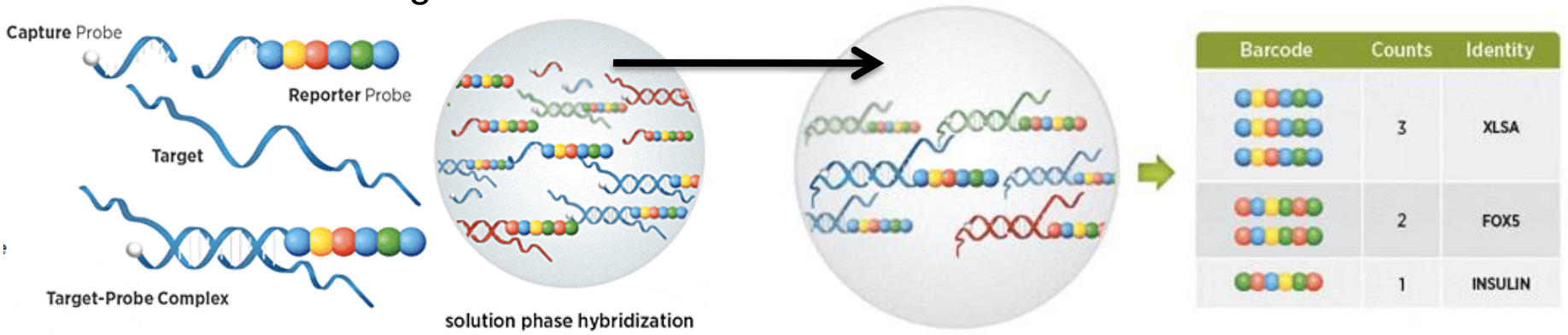




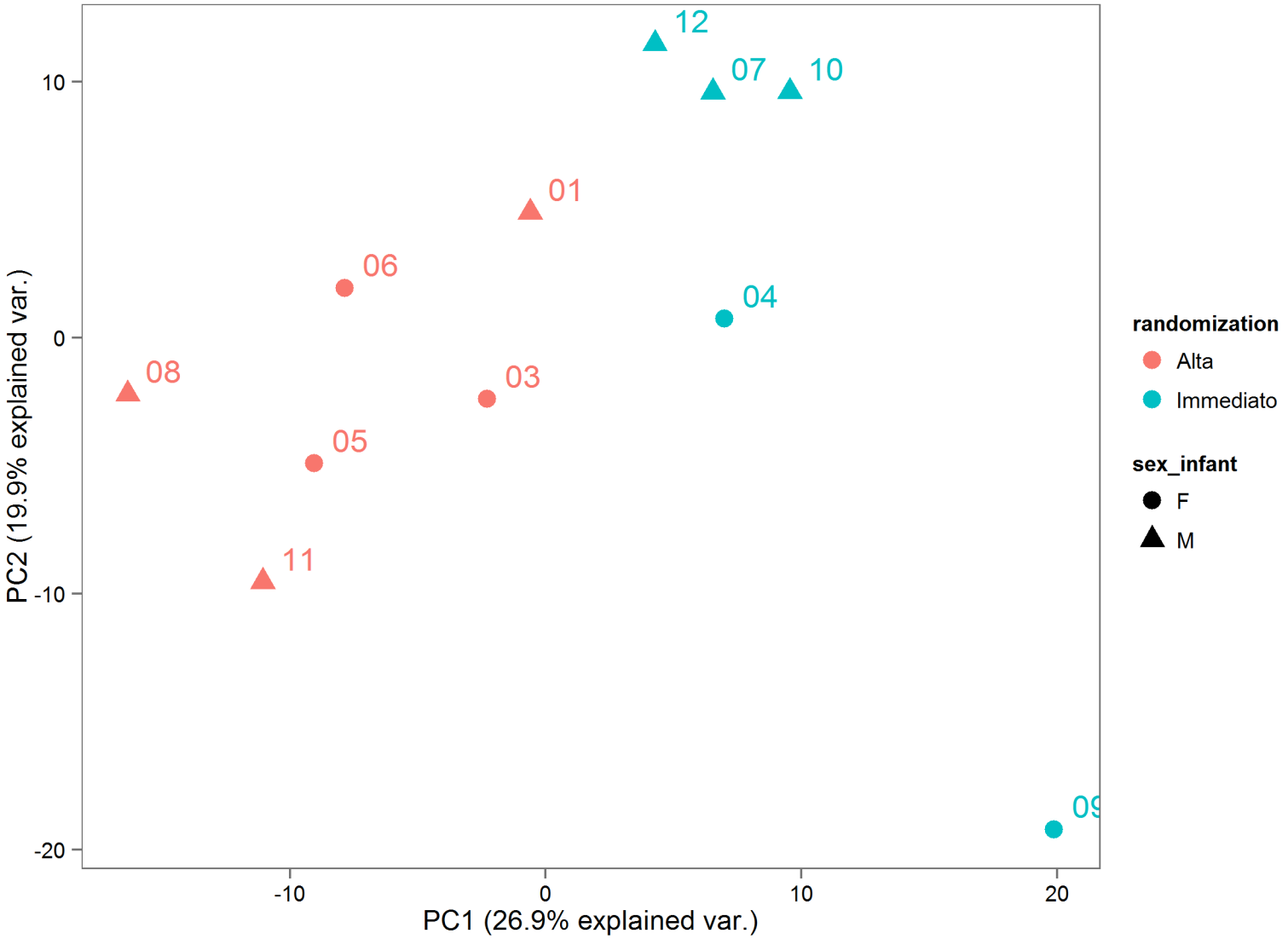
# Data quality: Nanostring RNA profiling

1. Probes bind to mRNA molecules overnight

2. The number of probes are counted by probe set scanner




# PCA – Unbiased clustering



# Can trials in West Africa study both off-target effects and determinants of severe morbidity/mortality?
# **YES!**

- Omic platforms provide tools to detect robust signatures of both **vaccine response & infection**
- These **highly controlled SOPs** can be carried out successfully in West Africa
- Preliminary data already demonstrate the feasibility, utility, and also necessity of these trials

# Acknowledgements

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