

# CTRIUMPh Pediatric Biomarker Substudy

IMPAACT TB Scientific Committee Meeting  
30<sup>TH</sup> May 2017

Jeffrey Tornheim, MD MPH  
Johns Hopkins University

Mandar Paradkar, DCH MPH  
BJMC Clinical Research Site



JOHNS HOPKINS  
CENTER FOR CLINICAL  
GLOBAL HEALTH EDUCATION



- **Background – CTRIUMPh Study**
- **Cohort for TB Research by Indo-US Medical Partnership**
- Study Type: Prospective Observational Study
- Study Location: BJGMC in Pune and NIRT in Chennai
- Study Population: 3 Prospective Cohorts
  - Active TB Cohort (800 PTB, 200 EPTB, **200 Pediatric Cases**)
  - Household (HH) Contact Cohort (**1200 Adults and Children**)
  - Control Cohort (100 Adults and 50 Children)
- Part of “The **R**egional **P**rospective **O**bservational **R**esearch in **T**uberculosis” (**RePORT**) **India Consortium** which aims to establish a biorepository in India with an associated database of well-characterized specimens for future tuberculosis (TB) research.
  - Funded in collaboration between the governments of USA (NIH) and India (DBT)



# Study Objectives

- Aim 1: To measure the **host and microbial factors associated with TB treatment outcomes** in Indian adults and children using the Active TB Cohort
- Aim 2: To investigate the **host and microbial factors associated with the progression from infection to active TB disease** in Indian adults and children using the Household Contacts of participants from the Active TB Cohort
- Aim 3: To explore the **host and microbial factors associated with TB transmission** using the Household Contacts Cohort

# Schedule of Evaluations – Cohort A

| Samples for Biorepository                | Entry | 2W                | 4W | 8W | 5M | 6M | 12M | 18M | 24M | End of Tx | TB Recurrence |
|--|-------|-------------------|----|----|----|----|-----|-----|-----|-----------|---------------|
| Stored Plasma sample for PK <sup>7</sup> |       |                   | X  |    | X  |    |     |     |     |           |               |
| Stored Plasma                            | X     |                   | X  | X  |    | X  | X   |     | X   | X         | X             |
| Whole Blood for DNA                      |       | (X) <sup>12</sup> |    |    |    |    |     |     |     |           |               |
| Stored Sputum                            | X     | X                 | X  | X  |    | X  |     |     |     | X         | X             |
| Stored Urine                             | X     |                   | X  | X  |    | X  | X   |     | X   | X         | X             |
| Stored Hair                              | X     |                   | X  |    | X  | X  |     |     |     | X         | X             |
| Stored Mtb isolate                       | X     |                   |    | X  | X  | X  |     |     |     |           | X             |
| Whole Blood for Host mRNA (PAXgene)      | X     |                   | X  | X  |    | X  | X   |     | X   | X         | X             |
| Whole Blood for PBMC                     | X     |                   | X  | X  |    | X  | X   |     | X   | X         | X             |
| Whole blood for Quantiferon supernatant  | X     |                   | X  | X  |    | X  | X   |     | X   | X         | X             |

**Total Pediatric samples- 406 plasma, 404 PBMCs, 367 paxgene, 153 plasma for PK, 148 QGIT supernatants, 77 DNA, 513 sputum/gastric aspirates, 401 urine, 312 hair and 18 MTB isolates.**

# Schedule of Evaluations – Cohort B

| Time                       | Entry | 4M             | 6M | 12M            | 18M | 24M            | Active TB <sup>1</sup> |
|----------------------------|-------|----------------|----|----------------|-----|----------------|------------------------|
| TT Skin Test               | H     | H <sup>5</sup> |    | H <sup>5</sup> |     | H <sup>5</sup> |                        |
| Quantiferon Gold in Tube   | H     | H <sup>5</sup> |    | H <sup>5</sup> |     | H <sup>5</sup> | F                      |
| Samples for Biorepository  |       |                |    |                |     |                |                        |
| Stored Plasma              | H     | H              |    | H              |     |                | F                      |
| Stored DNA                 | H     |                |    |                |     |                |                        |
| Stored Sputum              | H     | H              |    |                |     |                | F                      |
| Stored Urine               | H     | H              |    | H              |     |                | F                      |
| Stored Hair                | H     | H              |    | H              |     |                | F                      |
| Stored MTB isolate         | H     |                |    |                |     |                | F                      |
| Stored QGIT supernatant    | H     | H              |    | H              |     | H              | F                      |
| Stored Host mRNA (Paxgene) | H     | H <sup>7</sup> |    | H <sup>7</sup> |     |                | F                      |
| Stored PBMC                | H     | H <sup>7</sup> |    | H <sup>7</sup> |     |                | F                      |

We now have a repository of Pediatric samples- 436 plasma, 436 PBMCs, 379 paxgene, 344 QGIT supernatants, 204 DNA, 318 sputum/gastric aspirates, 395 urine, 236 hair and 8 MTB isolates.

# C-TRIUMPh Substudy – Pediatric Biomarkers

- **Problem**: Lack of biomarkers of treatment response & correlates of progression
  - 2-month smear conversion unreliable for outcome (24% sensitive 85% specific)
  - 2 month culture conversion unreliable for outcome (40% sensitive and 85% specific)
  - Most pediatric patients are probable/possible, not confirmed
  - Recent transcriptomic studies have had few Indian or confirmed pediatric cases:



# C-TRIUMPh Substudy – Pediatric Biomarkers

- **Problem**: Lack of biomarkers of treatment response & correlates of progression
  - 2-month smear conversion unreliable for outcome (24% sensitive 85% specific)
  - 2 month culture conversion unreliable for outcome (40% sensitive and 85% specific)
  - Most pediatric patients are probable/possible, not confirmed
  - Recent transcriptomic studies have had few Indian or confirmed pediatric cases:

| Author        | Timeframe                   | # of Genes        | Confirmed Indian Cases            | Confirmed Pediatric Cases (Derivation) | Confirmed Pediatric Cases (Validation) |
|---------------|-----------------------------|-------------------|-----------------------------------|--|--|
| Anderson      | Cross-sectional             | 51                | 0                                 | 114                                    | 35                                     |
| Berry         | Longitudinal Post-Treatment | 86                | 0                                 | 0                                      | 0                                      |
| Bloom         | Longitudinal Post-Treatment | 664 (Dx) 320 (Tx) | 0                                 | 0                                      | 0                                      |
| Jenum         | Cross-sectional             | 12                | 40                                | 40 (single set)                        |  |
| Kaforou       | Cross-sectional             | 44                | 0                                 | 0                                      | 0                                      |
| Laux da Costa | Cross-sectional             | 3                 | 0                                 | 0                                      | 0                                      |
| Maertzdorf    | Cross-sectional             | 4                 | 120                               | 0                                      | 0                                      |
| Sweeney       | Longitudinal Post-Treatment | 3                 | 113 (same patients as Maertzdorf) | 37                                     | 111 (same patients as Anderson)        |
| Zak           | Longitudinal Pre-Treatment  | 16                | 0                                 | 37 (adolescents)                       | 9 (adolescents)                        |



# Aims and Hypotheses

- AIM 1 – Confirm the presence of previously published gene signatures and evaluate their relative accuracy among a cohort of confirmed positive Indian pediatric TB patients
- AIM 2 – Evaluate whether positive gene signatures return to normal after 6 months of treatment in a cohort of Indian confirmed pediatric TB patients
- AIM 3 – Evaluate whether negative gene signatures remain consistently negative in exposed household contacts over time





# Methods

- All confirmed TB cases <15 years old (N=16) were matched by age and sex to household contacts with negative TST/IGRA at enrollment in a 2:1 ratio (N=32)
  - Whole blood was collected at t= 0,1, and 6 months for cases
  - Whole blood was collected at t= 0,4, and 12 months for exposed controls. TST and IGRA were repeated until either test converted or 1 year passed
- mRNA was extracted using Qiagen PAXgene Blood RNA Kits and sequenced on Illumina HiSeq 2500 to generate 100bp paired-end sequences. Reads were aligned to the human genome (GRCH38.10) using the STAR aligner and annotated using GENCODE.
- Count data were exported to R for analysis. Data were filtered to include only protein coding genes. Differential expression analysis was conducted using DESeq2 with a BH-false discovery rate of <0.05. Genes with absolute  $\log_2$  intensity ratio of at least 1 were considered differentially expressed.

# Clinical Characteristics of Study Participants

| Characteristic                     | CTRIUMPh<br>Cases (N=101) | Biomarker Study<br>Cases (N=16) | Exposed Household Controls |                      |
|------------------------------------|---------------------------|---------------------------------|----------------------------|----------------------|
|                                    |                           |                                 | Converters (N=11)          | Nonconverters (N=21) |
|                                    | N (%)                     | N (%)                           | N (%)                      | N (%)                |
| <b>Median Age in Years (range)</b> | 8 (1-14)                  | 9.5 (3-14)                      | 9 (6-14)                   | 10 (2-14)            |
| <b>Male</b>                        | 51 (50.5)                 | 8 (50.0)                        | 6 (54.5)                   | 9 (42.9)             |
| <b>BCG Scar</b>                    | 78 (77%)                  | 8 (50.0)                        | 5 (45.5)                   | 12 (57.1)            |
| <b>Pulmonary TB</b>                | 74 (73.3)                 | 8 (50.0)                        | NA                         | NA                   |
| <b>EPTB<sup>1</sup> Lymph Node</b> | 11 (36.7) <sup>1</sup>    | 6 (75.0)                        | NA                         | NA                   |
| <b>EPTB<sup>1</sup> Abdominal</b>  | 14 (46.7) <sup>1</sup>    | 0                               | NA                         | NA                   |

<sup>1</sup>EPTB = Extrapulmonary TB. Percentage reflects N = 30 for CTRIUMPh, N=8 for biomarker substudy.

# Diagnostic Characteristics of Participants With TB

| Characteristic                                   | CTRIUMPh Cases<br>N (% of Disease Type) | Biomarker Study Cases<br>N (% of Disease Type) |
|--|---|--|
| <b>History of TB Contact</b>                     | 66 (65.3)                               | 9 (56.3)                                       |
| <b>Median Illness Duration (range)</b>           | 30 Days (0-120)                         | 52.5 Days (25-60) ←                            |
| <b>Smear Positive PTB<sup>1</sup></b>            | 9 (12.2)                                | 3 (37.5)                                       |
| <b>Xpert MTB/RIF Positive PTB<sup>1</sup></b>    | 14 (18.9)                               | 6 (75.0)                                       |
| <b>Culture Positive PTB<sup>1</sup></b>          | 13 (16.7)                               | 5 (62.5)                                       |
| Culture Negative by 2 Weeks                      | 8 (61.5)                                | 4 (80.0) ←                                     |
| Culture Negative by 6 Months                     | 13 (100.0)                              | 5 (100.0)                                      |
| <b>Pathologically Confirmed EPTB<sup>2</sup></b> | 11 (36.7)                               | 8 (100.0)                                      |
| <b>Chest X-ray with Cavitory Disease</b>         | 20 (27.4)                               | 2 (25.0)                                       |
| <b>Chest X-ray Score (Range 0 – 40)</b>          | 25 (0 – 110)                            | 22 (0 – 130)                                   |

<sup>1</sup>PTB = Pulmonary TB. Percentage reflects N=74 for CTRIUMPh, N=8 for biomarker substudy.

<sup>2</sup>EPTB = Extrapulmonary TB. Percentage reflects N = 30 for CTRIUMPh, N=8 for biomarker substudy.

# Results of Transcriptomic Analysis

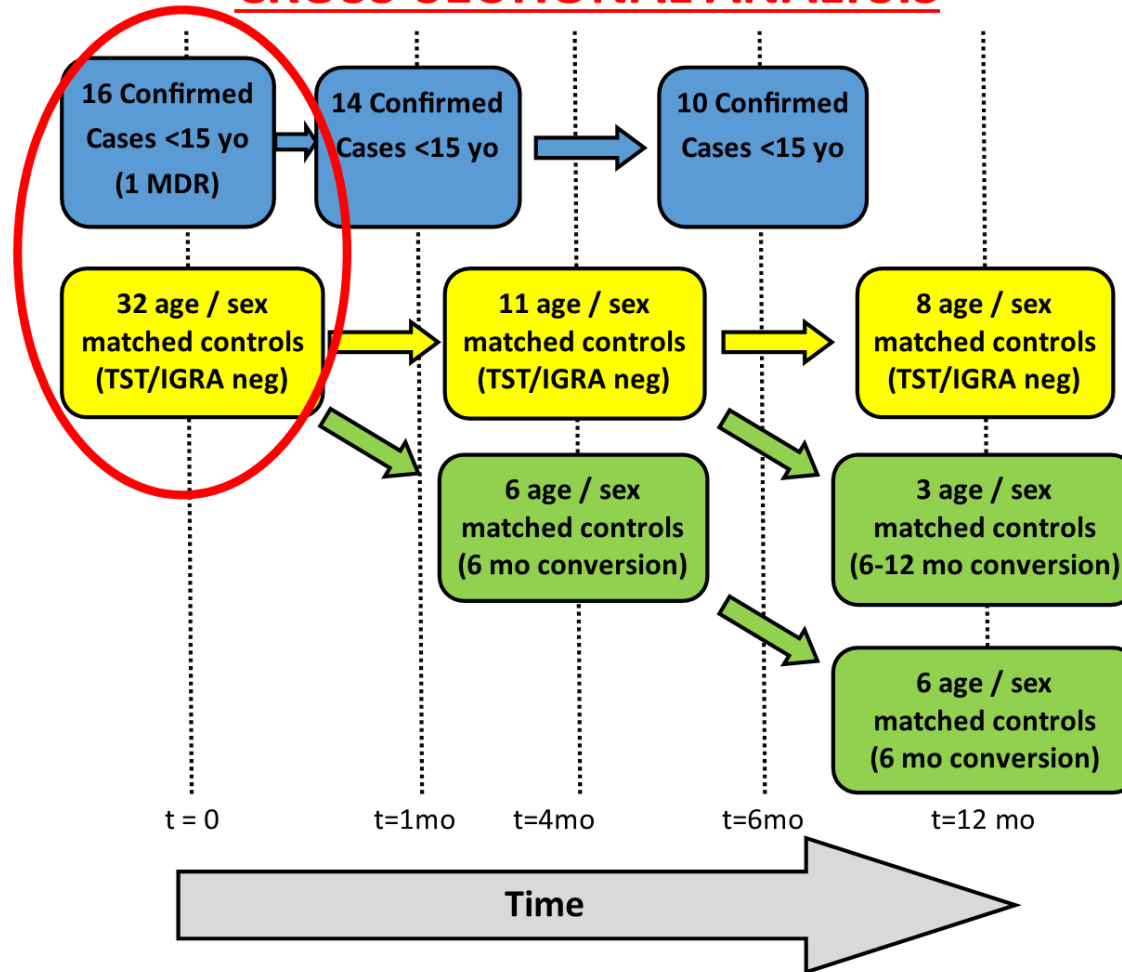


**RePORT**  
India Consortium



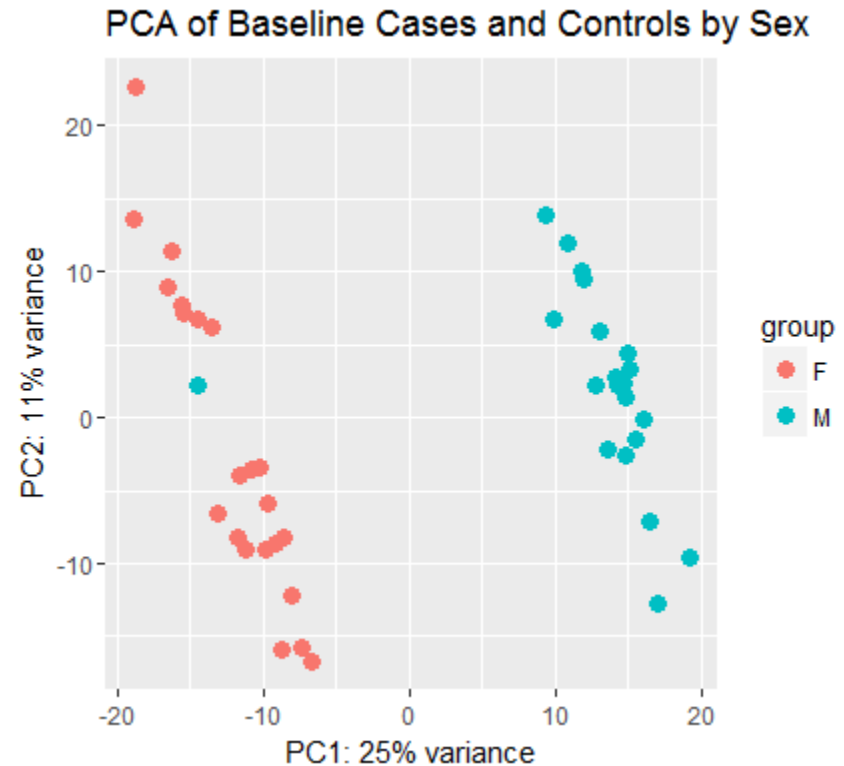
# Cross-Sectional Analysis of Cases vs. Controls

## CROSS-SECTIONAL ANALYSIS



# Pediatric Gene Expression Confounding

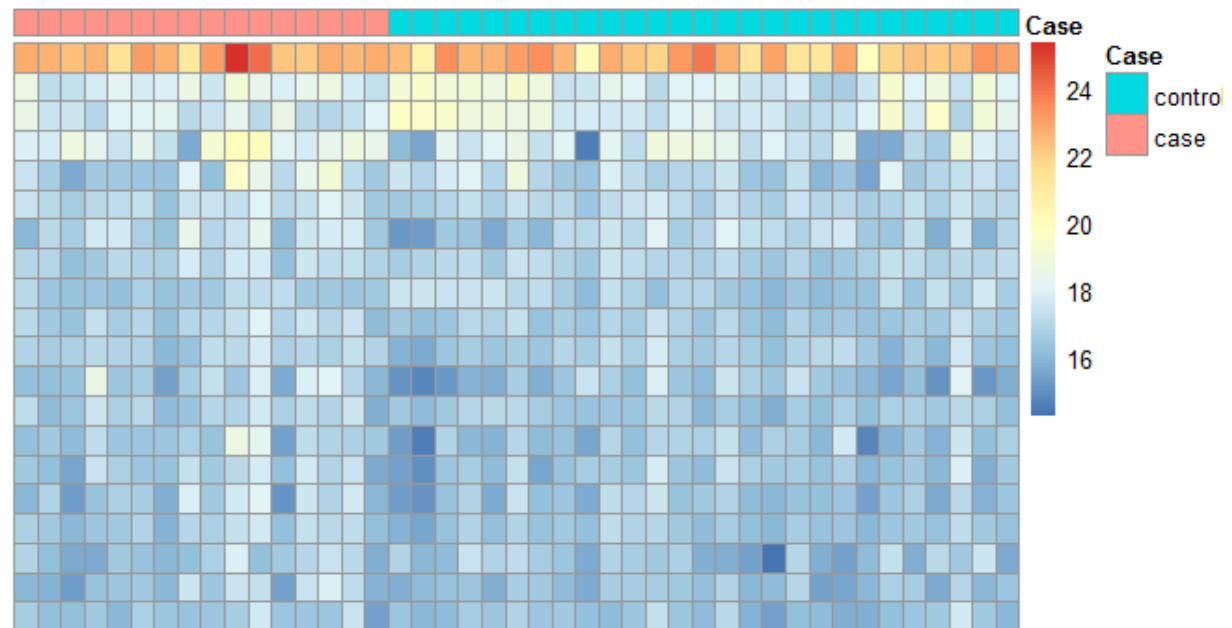
- Initial analysis demonstrated the importance of matching cases by age / sex
- Analyses from here forward are controlled for:
  - Age
  - Sex
  - Case vs. Control Status
  - Pulmonary vs. Extrapulmonary TB (Cases)
  - Baseline vs. Follow-Up Status
  - Time of Follow-Up (2<sup>nd</sup> vs. 3<sup>rd</sup> Visit)
  - Conversion vs. Nonconversion to LTBI



# Cross-Sectional Analysis of Cases vs. All Controls

- Out of 19384 protein-coding genes:
  - 131 genes were upregulated (0.68%)
  - 6 genes were downregulated (0.031)
  - 14 of those had  $\geq 1 \log_2$ -fold change

Heatmap of Differential Transcription  
Between Cases and Exposed Controls





# Cross-Sectional Analysis of Cases vs. All Controls

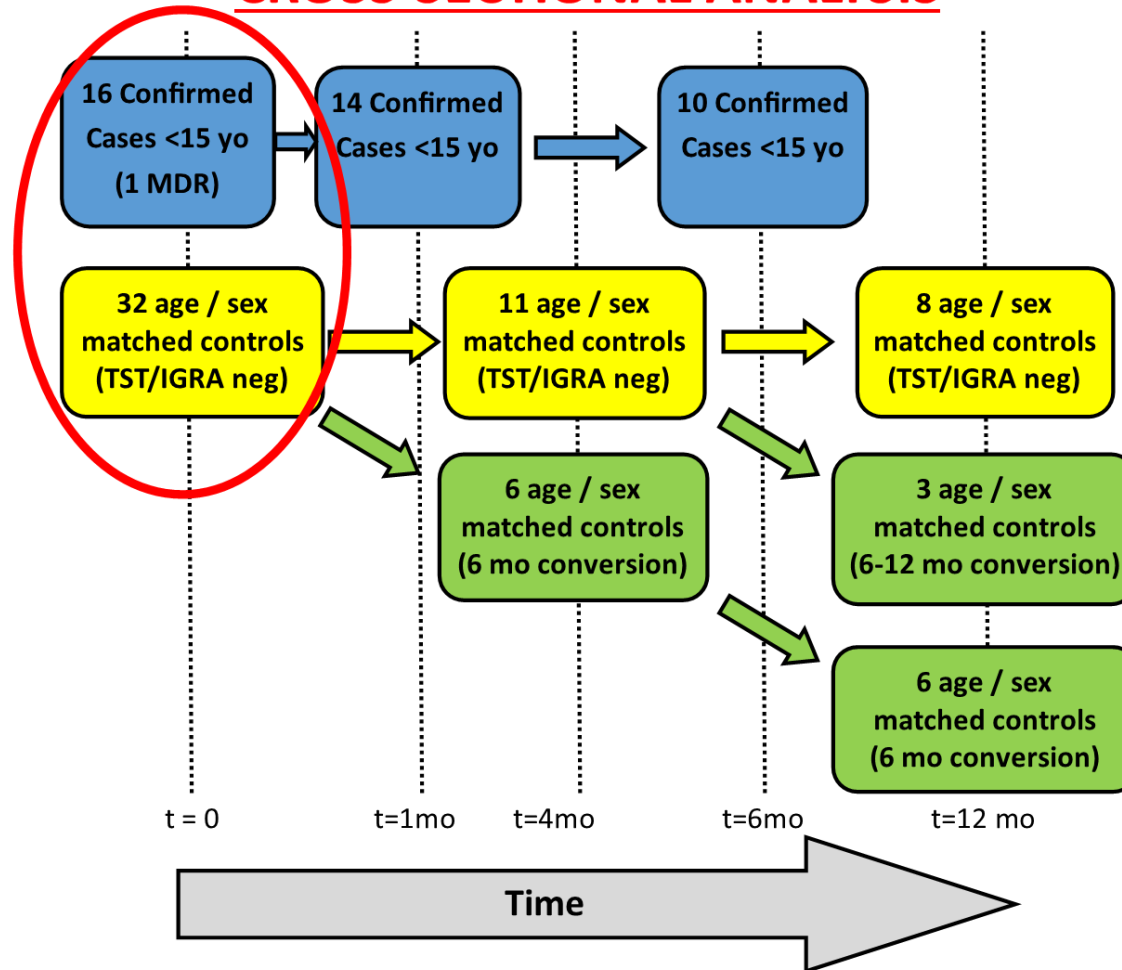
**Comparison of 14 Differential Expressed Genes Between Pediatrics TB Cases and Exposed Household Controls in CTRIUMPh with Other Published TB Signatures**

| CTRIUMPh | TB              |                  |                 | TB vs. OD         |                  | TB vs. LTBI       |                  | Interferon        |
|----------|-----------------|------------------|-----------------|-------------------|------------------|-------------------|------------------|-------------------|
|          | Berry 393 Genes | Kaforou 53 Genes | Sweeney 3 Genes | Anderson 51 Genes | Kaforou 44 Genes | Anderson 42 Genes | Kaforou 27 Genes | Obermose 79 Genes |
| APOL4    |                 |                  |                 |                   |                  |                   |                  |                   |
| AZU1     |                 |                  |                 |                   |                  |                   |                  |                   |
| C1QB     | X               |                  |                 | X                 |                  |                   | X                |                   |
| C1QC     |                 |                  |                 |                   |                  |                   | X                |                   |
| CLDN18   |                 |                  |                 |                   |                  |                   |                  |                   |
| CTSG     |                 |                  |                 |                   |                  |                   |                  |                   |
| DEFA3    |                 | X                |                 |                   |                  | X                 |                  |                   |
| ELANE    |                 |                  |                 |                   |                  |                   |                  |                   |
| GBP6     | X               | X                |                 | X                 | X                | X                 | X                | X                 |
| HBD      |                 |                  |                 |                   |                  |                   |                  |                   |
| METTL7B  |                 |                  |                 |                   |                  |                   |                  |                   |
| MPO      |                 |                  |                 |                   |                  |                   | X                |                   |
| PRTN3    |                 |                  |                 |                   |                  |                   |                  |                   |
| SEPT4    | X               | X                |                 |                   | X                |                   |                  | X                 |

➤ Differential expression demonstrated partial cross-over with other published signatures

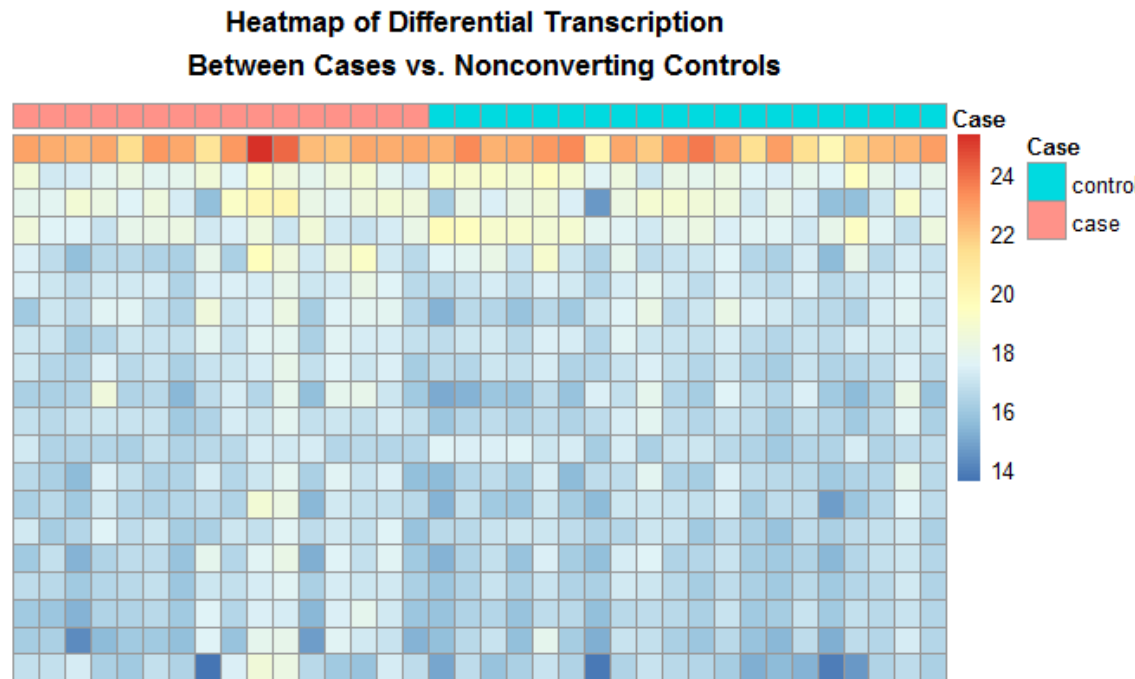
# Cross-Sectional Analysis of Cases vs. Controls

## CROSS-SECTIONAL ANALYSIS



# Cross-Sectional Analysis of Cases vs. Negative Controls

- A comparison of only controls who never converted TST or IGRA:
  - 135 genes were upregulated (0.7%)
  - 29 genes were downregulated (0.15%)
  - 8 of those had  $\geq 1 \log_2$ -fold change



# Cases vs. Age & Sex-Matched Exposed Controls

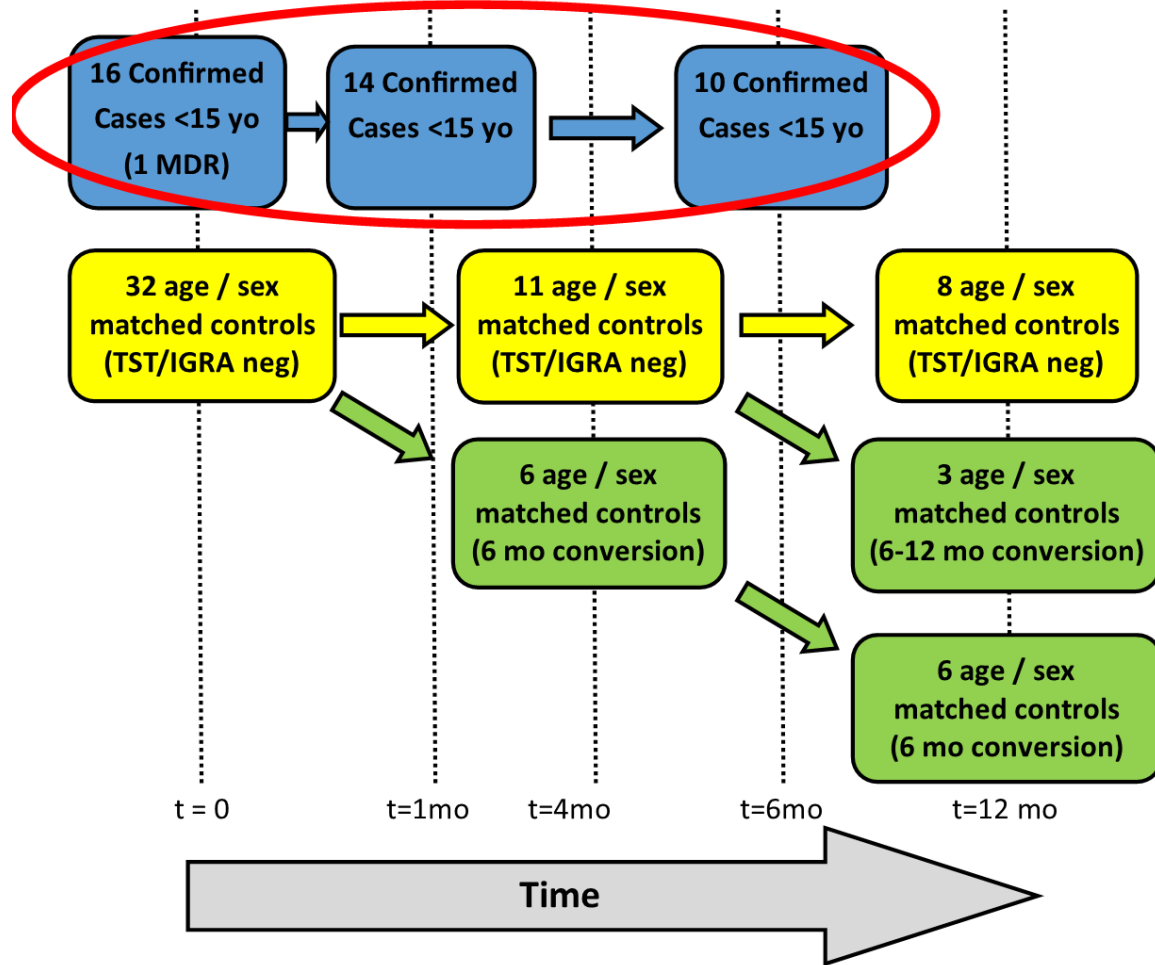
**Comparison of 8 Differential Expressed Genes Between Pediatrics TB Cases and Exposed Household Controls in CTRIUMPh with Other Published TB Signatures**

|          | TB              |                  |                 | TB vs. OD         |                  | TB vs. LTBI       |                  | Interferon        |
|----------|-----------------|------------------|-----------------|-------------------|------------------|-------------------|------------------|-------------------|
| CTRIUMPh | Berry 393 Genes | Kaforou 53 Genes | Sweeney 3 Genes | Anderson 51 Genes | Kaforou 44 Genes | Anderson 42 Genes | Kaforou 27 Genes | Obermose 79 Genes |
| APOL4    |                 |                  |                 |                   |                  |                   |                  |                   |
| AZU1     |                 |                  |                 |                   |                  |                   |                  |                   |
| CTSG     |                 |                  |                 |                   |                  |                   |                  |                   |
| DEFA3    |                 | X                |                 |                   |                  | X                 |                  |                   |
| GBP6     | X               | X                |                 | X                 | X                | X                 | X                | X                 |
| METTL7B  |                 |                  |                 |                   |                  |                   |                  |                   |
| MPO      |                 |                  |                 |                   |                  |                   | X                |                   |
| PRTN3    |                 |                  |                 |                   |                  |                   |                  |                   |

- C1QB, C1QC, CLDN18, ELANE, HBD, and SEPT4 (lower log<sub>2</sub>-fold changes (0.83–1)
- None of the 3 genes identified by Sweeney, et al. were significantly differentially expressed in our data (GBP5, DUSP3, and KLF2)
- No difference was noted between PTB and EPTB patients

# Longitudinal Analysis of Cases (0 vs. 1 and 6 Months)

## LONGITUDINAL ANALYSIS OF CASES



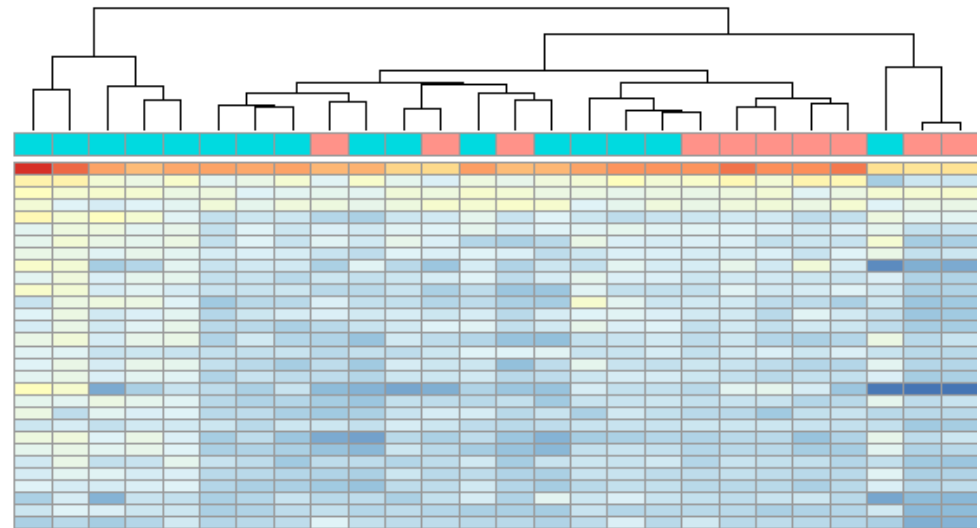
# Cases Over Time (0 vs. 6 Months of Treatment)

- 6 genes were upregulated (0.031%)
- 336 genes were downregulated (1.7%)
- 10 had  $\geq 1 \log_2$ -fold change at 1 month
- 2 had  $\geq 1 \log_2$ -fold change at 6 months

## 10 Differentially Expressed Genes Between Pediatrics TB Cases at Baseline and at 1 Month of Treatment

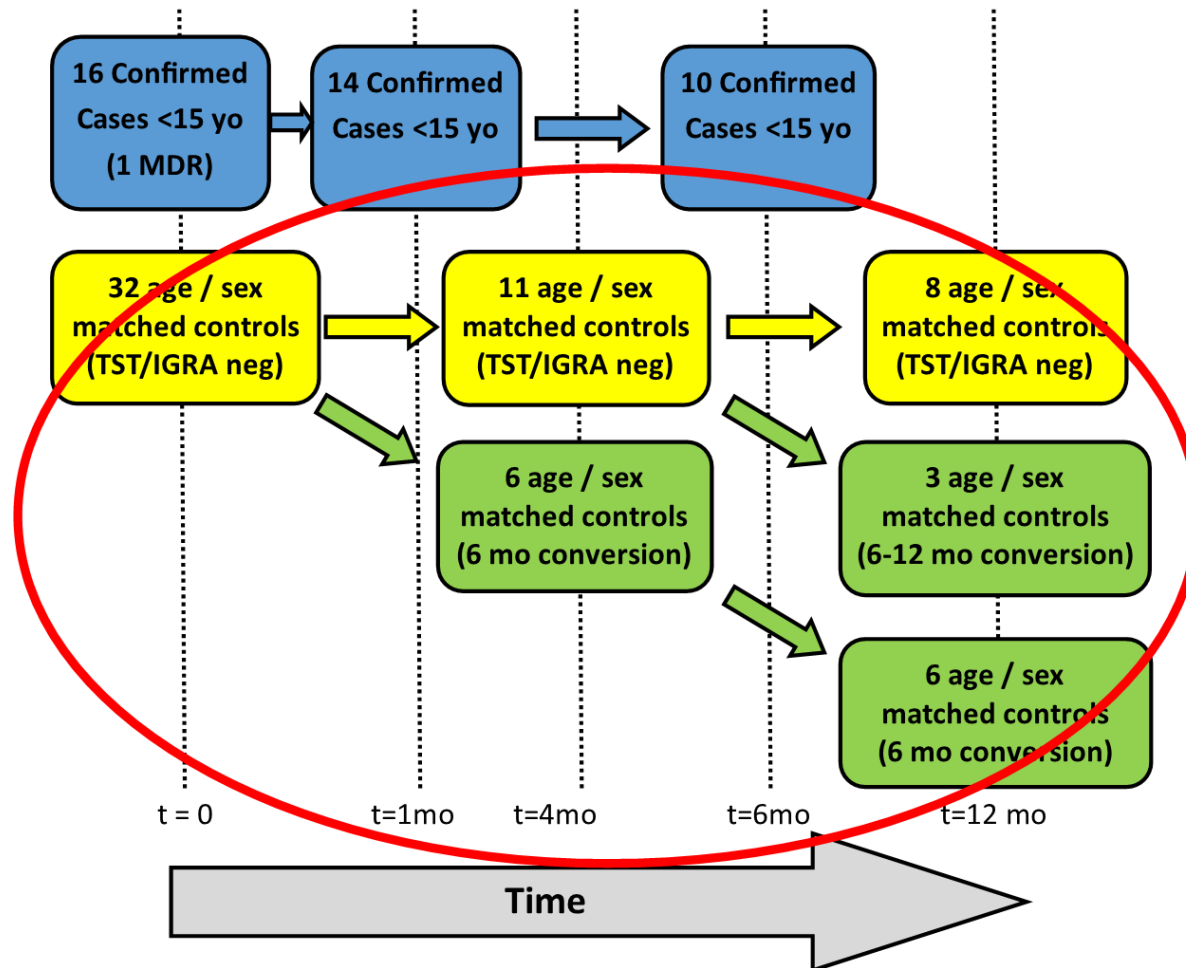
| CTRIUMPH | Kaforou 27 Genes<br>(TB vs. LTBI) |
|----------|-----------------------------------|
| C1QC     |                                   |
| CD177    |                                   |
| CLRN1    |                                   |
| ELANE    |                                   |
| GLDN     |                                   |
| GPR4     |                                   |
| MMP1     |                                   |
| MPO      | X                                 |
| PRTN3    |                                   |
| ZG16     |                                   |

Clustered Heatmap of Differential Transcription  
Between Baseline and 6 Month of Treatment  
for Pediatric TB Cases



# Longitudinal Analysis of Controls (0 vs. 12 Months)

## LONGITUDINAL ANALYSIS OF CONTROLS





# Exposed Controls Over Time (0 vs. 12 Months)

## ➤ Infected Exposed Controls (N=9)

- 504 differentially expressed genes with  $\geq 1 \log_2$ -fold change at 12 months
- None of these were components of any of the published signatures reviewed

## ➤ Healthy Exposed Controls (N=8)

- Only 1 gene to be significantly differentially expressed at 12 months
- HES4 = transcriptional regulator

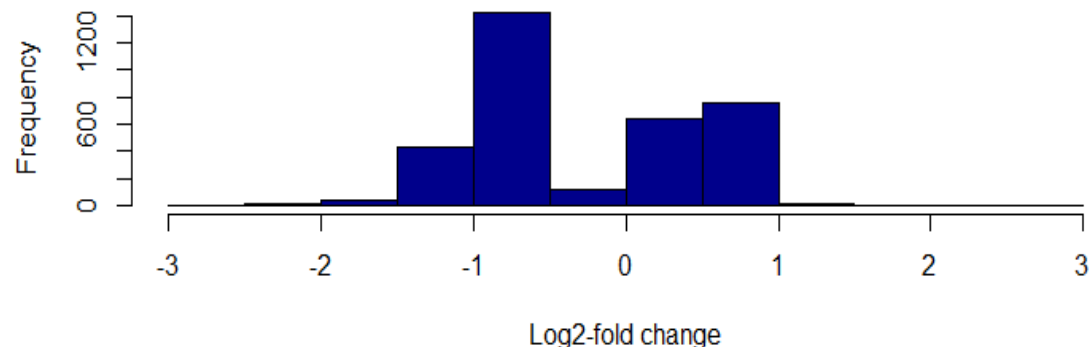
# Conclusions

- Transcriptomic evaluation of 16 confirmed pediatric TB patients and 32 age- and sex-matched exposed controls (TST and IGRA negative) found:
  - 14 differentially expressed genes that distinguished cases from controls
  - 8 of those distinguished cases from controls who never developed LTBI
  - 10 differentially expressed genes among cases between 0 and 1 month of treatment
  - 504 genes differentially expressed between 0 and 12 months for controls with LTBI
  - 1 gene differentially expressed between 0 and 12 months for nonconverting controls
- Comparison with published signatures found:
  - Partial crossover with TB signatures of Anderson, Berry, Kaforou, and Obermose
  - No cross-over with TB signature published by Sweeney, et al.
  - No cross-over with LTBI signature Zak, et al.

# Next Steps and Future Directions

- Additional longitudinal analyses
  - Address changes between 1 and 6 months for cases
  - Address changes during the months before conversion to LTBI
- Optimize gene lists based on sensitivity analysis
  - Need to optimize list in light of differing methods in published papers
  - Log-fold changes of 0.5, 1, 1.5, or 2
- Analysis of other biomarkers
  - miRNA analysis
  - Metabolite analysis

**Frequency Distribution of Differentially Expressed Genes  
Among Controls Who Converted to LTBI Over Time,  
(False Discovery Rate <0.05), by Log2-fold Changes**



# Acknowledgements

| BJMC-JHU Investigators |                   | Institute of Bioinformatics  | NIRT Investigators       |
|------------------------|-------------------|------------------------------|--------------------------|
| Sushant Meshram        | Amita Gupta       | Akhilesh Pandey              | Padmapriyadarsini C      |
| Dileep Kadam           | Vidya Mave        | Anil Madugundu               | Geetha Ramachandran      |
| Rahul Lokhande         | Akshay Gupte      | Sreelaskshmi Sreenivasmurthy | Beena Thomas             |
| Renu Bharadwaj         | Nikhil Gupte      |                              | Uma Devi                 |
| Anju Kagal             | Jonathan Golub    |                              | Gomathy N.S.             |
| Sandhya Khadse         | Mandar Paradkar   |                              | Luke Hannah              |
| Aarti Kinikar          | Rewa Kohli        |                              | Kavitha Danasekaran      |
| Vandana Kulkarni       | Nishi Suryavanshi |                              | Kannan Thiruvengadam     |
| Usha Balasubramanian   | Andrea DeLuca     |                              | Shri Vijay Bala Yogendra |
| Neeta Pradhan          | Petros Karakousis |                              |                          |
|                        | Noton Dutta       |                              |                          |

