Research paper

Protection against mycobacterial infection: A case-control study of mycobacterial immune responses in pairs of Gambian children with discordant infection status despite matched TB exposure

Robindra Basu Roy¹,²,³, Basil Sambou², Muhamed Sissoko², Beth Holder⁴,⁵, Marie P Gomez², Uzochukwu Egere⁶,⁷, Abdou K Sillah², Artemis Koukounari⁸, Beate Kampmann²,⁶,⁷,⁸,*

¹ Department of Academic Paediatrics, Section of Paediatric Infectious Disease, Imperial College London, St. Mary’s Hospital, Praed Street, London W2 1NY, United Kingdom
² Vaccines and Immunity Theme, MRC Unit The Gambia at the London School of Hygiene and Tropical Medicine, Atlantic Road, Fajara, The Gambia
³ Clinical Research Department, Faculty of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom
⁴ Institute of Reproductive and Developmental Biology, Department of Metabolism, Digestion & Reproduction, Imperial College London, Du Cane Road, W12 0HS, United Kingdom
⁵ Department of Reproductive and Developmental Biology, Department of Metabolism, Digestion & Reproduction, Imperial College London, Du Cane Road, W12 0HS, United Kingdom
⁶ Department of Infectious Disease Epidemiology, Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom
⁷ The Vaccine Centre, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom

ARTICLE INFO

Article History:
Received 17 April 2020
Revised 29 June 2020
Accepted 29 June 2020
Available online 13 July 2020

Keywords:
Paediatric
Tuberculosis
Latent tuberculosis infection
Correlates of protection
Mycobacterial growth inhibition assay

ABSTRACT

Background: Children are particularly susceptible to tuberculosis. However, most children exposed to Mycobacterium tuberculosis are able to control the pathogen without evidence of infection. Correlates of human protective immunity against tuberculosis infection are lacking, and their identification would aid vaccine design.

Methods: We recruited pairs of asymptomatic children with discordant tuberculin skin test status but the same sleeping proximity to the same adult with sputum smear-positive tuberculosis in a matched case-control study in The Gambia. Participants were classified as either Highly TB-Exposed Uninfected or Highly TB-Exposed Infected children. Serial luminescence measurements using an in vitro functional auto-luminescent Bacillus Calmette–Guérin (BCG) whole blood assay quantified the dynamics of host control of mycobacterial growth. Assay supernatants were analysed with a multiplex cytokine assay to measure associated inflammatory responses.

Findings: 29 pairs of matched Highly TB-Exposed Uninfected and Highly TB-Exposed Infected children aged 5 to 15 years old were enrolled. Samples from Highly TB-Exposed Uninfected children had higher levels of mycobacterial luminescence at 96 hours than Highly TB-Exposed Infected children. Highly TB-Exposed Uninfected children also produced less BCG-specific interferon-γ than Highly TB-Exposed Infected children at 24 hours and at 96 hours.

Interpretation: Highly TB-Exposed Uninfected children showed less control of mycobacterial growth compared to Highly TB-Exposed Infected children in a functional assay, whilst cytokine responses mirrored infection status.

Funding: Clinical Research Training Fellowship funded under UK Medical Research Council/Department for International Development Concordat agreement and part of EDCTP2 programme supported by European Union (MR/K023446/1). Also MRC Program Grants (MR/K007602/1, MR/K011944/1, MC_UP_A900/1122).

© 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license. (http://creativecommons.org/licenses/by/4.0/)

1. Introduction

The WHO have set the ambitious goal of ending TB by 2035 and achieving zero childhood TB deaths [1,2]. Key to this is the development of effective vaccines, which requires identification of correlates.
Research in context

Evidence before this study

Over one million children develop tuberculosis each year, often through household exposure to an adult with pulmonary tuberculosis. However, most children with such exposure develop neither tuberculosis disease, nor latent tuberculosis infection (LTBI) and these individuals are important to study to understand protective immunity.

Studies using functional whole blood mycobacterial growth assays have shown superior growth control by adults with LTBI compared to healthy controls, whilst another study including children found no difference. Production of the cytokine interferon-γ (IFN-γ) is the basis of blood tests for LTBI, whilst Tumour-Necrosis Factor-α (TNFα) production has also been linked to responses in the tuberculin skin test for LTBI. However, differences in TNFα responses to mycobacterial stimulation have not been consistently found in household TB contact studies.

Search strategy: PubMed was searched using the terms “immun” AND (“latent tuberculosis” OR “tuberculosis infection”) with a filter of “Child: birth-18 years” and no date or language limitations on 10/6/20.

Added value of this study

To our knowledge, this is the first study of children with high exposure to TB but who have not developed disease or LTBI that has used an exposure-matched case-control design. We recruited pairs of asymptomatic children with discordant tuberculosis infection status but the same sleeping proximity to the same adult with sputum smear-positive tuberculosis in The Gambia. Using a whole blood autoluminescent mycobacterial growth assay we found that High TB-Exposed Uninfected children had less control of mycobacterial growth at 96 h than matched High TB-Exposed Infected children. Multiplex cytokine analysis of supernatants from this assay showed interferon-γ responses reflected LTBI status but no significant differences in TNFα and other measured cytokines.

Implications of all the available evidence

Children with effective immunity against tuberculosis infection are an important study population to try to identify correlates of protection that can guide vaccine design and evaluation. In vitro experiments using clinical samples that focus on gene expression, inflammatory and innate immune responses, and antibody effector function may be helpful in future studies.
2. Materials and methods

Recruitment: All household compounds that were originally enrolled in the childhood TB household contact platform at MRC Unit The Gambia between 1st January 2015 and 31st December 2015 were evaluated for inclusion in this study [34,36–38]. Briefly, adult index cases (>15 years old) with newly identified smear-positive pulmonary tuberculosis were approached for consent to visit their household compounds. A compound was defined as a cluster of homes or buildings often owned by members of the same family, with typically 7–8 child contacts per index case [34]. Families within this compound were then consented for their children <15 years old to be screened for symptoms of tuberculosis and a TST. Those with a TST ≥10 mm or symptoms were referred to clinic for evaluation. All participants were screened for symptoms of tuberculosis 3-monthly for one year. Neonatal BCG immunisation coverage in The Gambia is estimated at 98% [41].

Sample size calculation: This was based upon unpublished pilot data from children with tuberculosis exposure using the BCG-lux assay (Supplementary Fig. 1). Assuming a paired test to allow for matching, data were bootstrapped with age matching. Taking 10,000 bootstrapped samples of size 30 in each group gave a power of 80% for demonstrating a significant difference for a two-sided non-parametric Wilcoxon signed-rank test at the 5% level.

Case definitions: Case definitions and inclusion and exclusion criteria that were used to identify Highly TB-Exposed Infected and Highly TB-Exposed Uninfected children for this study are summarised in Supplementary Tables 1 and 2 [34]. Matched pairs of children had the same sleeping proximity in the same building to the same adult with smear-positive pulmonary tuberculosis. Children aged less than 5 years old were excluded as they were prescribedisoniazid preventive therapy in line with WHO recommendations [38]. Participants in this study were not treated for LTBI in keeping with WHO guidelines at the time in view of their age and the absence of HIV infection [42]. All participants were asymptomatic at the time of initial household symptom screening and were recruited to this study at least 3 months after the initial screening for a repeat TST to confirm that the children identified as Highly TB-Exposed Uninfected had clear evidence of having remained uninfected (TST ≤5 mm). Highly TB-Exposed Infected children had an initial TST ≥10 mm in keeping with WHO thresholds for a positive TST, a negative HIV test, a normal chest radiograph, and a normal physical examination by a paediatrician [42]. An in-house IGRA was taken from Highly TB-Exposed Infected children at this initial clinic visit. Where more than one child in the compound met the criteria for being Highly TB-Exposed Uninfected or Highly TB-Exposed Infected, a single child from those eligible was selected at random. Demographic and exposure data were collected by the study fieldworker. The consent process included permission to access the HIV status of the tuberculosis index case that had been tested as part of routine clinical care in this low HIV prevalence setting [34].

Sample procedures: Venous blood from matched Highly TB-Exposed Uninfected and Highly TB-Exposed Infected children was collected at the same visit. Samples were collected using lithium heparin tubes for the whole blood assay and EDTA tubes for full blood count (both BD, Oxford, UK). Full blood counts were analysed by the Clinical Services Diagnostic Haematology Laboratory at MRC Unit The Gambia at LSHTM using the Cell Dyn 3700 Haematology Analyser (Abbott Diagnostics, Illinois). If this was not available, full blood count samples were run using Medonics M-series (Boule Medical AB, Sanga, Sweden) in the Immunology Research Laboratory. Samples from both members of a pair were always analysed with the same method. Monocyte: lymphocyte ratios were also calculated [43]. In-house interferon gamma release assays on Highly TB-Exposed Infected children were conducted as has previously been described [36,44,45].

Autoluminescent BCG growth monitoring in whole blood: The method has been fully reported [39]. In brief, BCG Danish was transformed to express the Luciferase Full Operon of Photobradus luminescens (RRID:Addgene_49999 with RRID: Addgene_50000) and green fluorescent protein (RRID:Addgene_30173) with a ratio of 0.05 Relative Light Units (RLU)/ml/s: 1 Colony Forming Unit (CFU). Aliquots were grown to logarithmic growth phase in liquid culture with antibiotic selection markers (kanamycin 20 μg/ml [Glyco/Life Technologies, Carlsbad, CA, USA] and hygromycin 50 μg/ml [Sigma-Aldrich, Gillingham, UK]) and diluted to 3.3 × 10^{5} RLU/ml/s immediately prior to venepuncture. Samples from both members of a pair were always analysed in the same experiment. Heparinised whole blood was diluted 1:1 with RPMI 1640 culture medium (Sigma-Aldrich, Gillingham, UK) containing 2.5% 1 M HEPES buffer and 1% -glutamine (both Sigma-Aldrich). BCG-GFP-LuxFO was added at a ratio of 1 part BCG-GFP-LuxFO: 9 parts whole blood in culture medium (equivalent to 1.3 × 10^{5} CFU per 100 μl undiluted whole blood). For the control samples, bacterial medium alone was added in the same ratio of 1:9. 500 μl was aliquoted in triplicates for each experimental condition at each timepoint into sterile lidelled 75 × 12 mm polystyrene tubes (Corning B.V Life Sciences, Amsterdam, The Netherlands) and transferred to a rocking incubator at 37 °C. Luminescence readings of each tube were measured immediately after removal from the incubator using a Sirius Tube Luminometer (Berthold Detection Systems GmbH, Pforzheim, Germany) 1, 4, 24, 48, 72 and 96 hours after the bacteria were added, and corrected for background luminescence. The baseline luminescence reading was taken one hour after inoculation of samples to ensure equilibration to a temperature of 37 °C in the incubator [39]. In addition, at baseline (0 h), 24, and 96 hours, further sets of triplicate samples were centrifuged at 2000 x g for ten minutes and supernatants stored at −70 °C and shipped on dry ice to Imperial College London for cytokine analysis. Experimenters were not allocated to groups of assignment for the experimental design.

Cytokine analysis: Tripleticate supernatant aliquots were thawed, pooled, and sterile filtered by spinning for 10 min at 5000 RPM with 0.22 μm centrifuge Spin-X tube filters (Corning B.V Life Sciences, Amsterdam, The Netherlands), transferred to 96 well plates and stored at −70 °C prior to analysis. A standardised custom commercial multiplex magnetic bead-based immunoassay, Bio-Plex Pro (Bio-Rad, Hercules, CA, USA), was used to quantify levels of IL-1α and IL-1β, IL-10, IFN-γ, TNF-α following the manufacturer’s instructions. All samples from matched Highly TB-Exposed Infected / Highly TB-Exposed Uninfected pairs were run on the same plate and read using a Luminex 200 plate reader (LuminexCorp, Austin, TX, USA). Quality control samples made up of pooled supernatants from non-study assays were run in duplicate on each plate and a normalisation factor applied to ensure comparability between plates. Samples falling below the quantification level of the assay were allocated values 1/4 of the lower limit of detection.

Statistical analysis: We used normal quantile plots and Shapiro-Wilk tests to assess data distribution. To conduct unadjusted bivariate tests for association, the matched paired t-tests was used for normally distributed data, Wilcoxon’s matched pairs signed rank test for non-normally distributed data, and McNemar’s χ² test for binary variables. To examine multivariable associations of luminescence data multi-level linear regression (or mixed effects linear modelling) was used [46]. Luminescence data was log transformed due to the skewness of the data. The matched pairs and the individual participants were considered as random effects, whilst Highly TB-Exposed Uninfected / Highly TB-Exposed Infected status, age (as a categorical variable in tertiles), being a sibling of the adult index case and experimental time point were considered as fixed effects, the combination of all of which were used to predict log luminescence (dependant variable). Estimated marginal means and 95% confidence intervals for luminescence by group and time were predicted from the model treating all fixed effects including age and whether the
child was a sibling of the adult index case as balanced. Statistical interpretation of differences between groups at each time point was carried out through pairwise comparisons [46]. STATA Statistical Software: Release 12.1. (StataCorp LP, College Station, TX) and Prism 7 for MacOS X (Graphpad Software Inc) were used.

**Ethical approvals:** The study was approved by The Gambia Government/MRC Joint Ethics Committee (SCC1405 and SCC1273) and the Imperial College Healthcare Tissue Bank (R13071).

### 3. Results

#### 3.1. Participant characteristics

A total of 58 children were recruited to the study from 29 different residential compounds. The study recruitment profile is shown in Fig. 1. Demographic features of the study participants together with descriptive statistics are detailed in Table 1. Highly TB-exposed infected children were significantly older than Highly TB-exposed uninfected children (Highly TB-exposed infected: 10.37, interquartile range (IQR) 9.43–12.36; Highly TB-exposed uninfected: 7.89, IQR: 7.01–10.86, p = 0.027). 54 (93%) of the children were sleeping in the same house as the adult index case, and 4 (7%) were sleeping in the same room as the adult index case. The proportion of study participants where the adult index case was their parent did not significantly differ between groups (Highly TB-exposed infected children: 7/29, Highly TB-exposed uninfected children: 4/29; p = 0.45). Highly TB-exposed infected children were significantly more likely to be a sibling of the adult (>15 years old) index case (10/29) than Highly TB-exposed uninfected children (3/29, p = 0.02). Highly TB-exposed infected children had a median TST of 18 mm with a range of 13 mm to 25 mm (interquartile range 16 to 20 mm). All 29 Highly TB-exposed uninfected children had a TST of 0 mm both at baseline and between 3 and 12 months later. None of the Highly TB-exposed infected children who were seen in clinic had HIV infection and 28/29 of the adult index cases had negative HIV tests. The index case who did not consent for HIV testing was not a parent of the Highly TB-exposed infected or Highly TB-exposed uninfected children. 25 of the 29 Highly TB-exposed infected children had a positive in-house IGRA at the initial clinic visit concordant with their positive TST result. 3 Highly TB-exposed infected children had a negative IGRA and 1 had an indeterminate IGRA at their initial clinic visit (Fig. 1). There were no significant differences in haematological parameters, including monocyte: lymphocyte ratio, between Highly TB-exposed infected and Highly TB-exposed uninfected groups (Table 1). No participant developed tuberculosis during 12 months of follow-up.

**In vitro control of BCG bacterial growth:** Bacterial growth in whole blood was measured by luminescence at 6 timepoints, resulting in longitudinal bacterial growth profiles in blood from all 58 participants. Infected blood from Highly TB-exposed infected children had 8.5% lower luminescence at baseline (median 402.4, IQR: 327.6–416.7 RLU/s) compared to Highly TB-exposed uninfected children (median 439.7, IQR: 356.4–554 RLU/s; p = 0.0069). This difference was not statistically significant at intermediate timepoints, but at 96 hours, there was 38% lower bacterial luminescence in samples from Highly TB-exposed infected (median 11,511, IQR: 8210–15,272 RLU/s) compared to Highly TB-exposed uninfected children (median 15,895, IQR 9655–19,585 RLU/s; p = 0.0455) (Fig. 2, Supplementary Fig. 2). Mixed effects modelling, treating age, whether the child was a sibling of the index case, and other variables as balanced between groups, modelled Highly TB-exposed infected children to have lower luminescence than Highly TB-exposed uninfected children at baseline, although this was not statistically significant (Highly TB-exposed infected: Modelled estimated marginal mean (MEMM): 400.8, 95% CI: 332.7–482.8 RLU/s; Highly TB-exposed uninfected: MEMM: 456.6, 95%CI 374.8–556.2 RLU/s; p value=0.09, Table 2 and Supplementary Table 3). Highly TB-exposed infected children had significantly lower modelled luminescence than Highly TB-exposed uninfected children at 96 hours (Highly TB-exposed infected: MEMM: 11,262 RLU/s, 95%
CL: 9347–13,568; Highly TB-Exposed Uninfected MEMM: 13,304 RU/Ls, 95% CI: 10,921–16,206, \( p = 0.031 \). Similar patterns were seen in the sub-analysis where the Highly TB-Exposed Infected children with negative or indeterminate baseline IGRA responses were similar between

| Characteristic                     | Highly TB-exposed uninfected children | Highly TB-exposed infected children | \( p \) |
|-----------------------------------|--------------------------------------|-------------------------------------|-------|
| \( n \)                            | 29                                   | 29                                  | –     |
| Sleeping proximity                 | Same room: 2                         | Same room: 2                        | –     |
|                                   | Same house: 27                       | Same house: 27                      | –     |
|                                   | Different house: 0                   | Different house: 0                  | –     |
| Median age in years (IQR)          | 7.89 (7.01–10.86)                    | 10.37 (9.43–12.36)                  | 0.027a|
| % Males                           | 14 (48)                              | 16 (55)                             | 0.62a |
| HIV infection in child (n = 29)    |                                     |                                     |       |
| Relationship of child to adult index case (%) |                                |                                     |       |
| Child                             | 4 (13.8)                             | 7 (24.1)                            | 0.45b |
| Cousin                            | 6 (20.7)                             | 2 (6.9)                             | 0.13b |
| Grandchild                        | 1 (3.4)                              | 2 (6.9)                             | 1.00b |
| Nephew or Niece                   | 9 (31.0)                             | 6 (20.7)                            | 0.45b |
| Sibling                           | 3 (10.3)                             | 10 (34.5)                           | 0.02  |
| Distinct relation                 | 1 (3.4)                              | 1 (3.4)                             | 1.00b |
| Unrelated                         | 5 (17.2)                             | 1 (3.4)                             | 0.13b |
| Median initial TST result in mm (IQR) | 0 (0)                             | 18 (16–20)                          | 0.000c|
| Median repeat TST result in mm (IQR) | 0 (0)                             |                                     |       |
| Initial Interferon Gamma Release Assay | Positive                        | 25 (86.2%)                          |       |
|                                   | Negative                             | 3 (10.3%)                           |       |
|                                   | Indeterminate                        | 1 (0.3%)                            |       |
| Median absolute White blood cell count k/µL (IQR) | 6.38 (5.38–7.18)                  | 6.02 (5.6–6.75)                     | 0.563a|
| Mean absolute monocyte count k/µL (95% CI) | 0.467 (0.381–0.552)            | 0.443 (0.352–0.534)                 | 0.657 |
| Mean absolute neutrophil count k/µL (IQR) | 2.44 (1.88–2.83)                 | 2.61 (2.17–3.19)                    | 0.492a|
| Mean absolute lymphocyte count k/µL (95% CI) | 2.90 (0.99–4.80)                | 2.67 (0.91–4.44)                    | 0.391 |
| Mean Monocyte/Lymphocyte ratio (95% CI) | 0.174 (0.141–0.206)                | 0.171 (0.138–0.204)                 | 0.905a|

\( a \) denotes Wilcoxon matched pairs signed rank test.

\( b \) McNemar’s \( \chi^2 \) test.

\( c \) denotes paired \( t \)-test. Haematological statistics based upon results available from CellDyn machine (Highly TB-Exposed Infected \( n = 25 \), Highly TB-Exposed Uninfected \( n = 25 \)).

### 4. Discussion

Given the importance of understanding human protective immunity to LTBI to guide vaccine design and evaluation, we applied a novel functional mycobacterial assay in a tuberculosis-endemic country to children with a persistently negative TST despite defined household MTB exposure. We conducted a carefully matched study of children with discordant infection status despite the same sleeping proximity to the same adult with smear-positive pulmonary tuberculosis. We utilised a whole blood autoluminescent BCG growth assay to identify differences in functional control of mycobacteria and cytokine responses in these individuals that could correlate with protection against infection. The potential to identify functional differences in the dynamics of host-mycobacterial interactions using small volumes of blood is a major advantage of the autoluminescent properties harnessed in this method, and is not possible with other whole blood mycobacterial assays. [25,26,29,39,47,48]

We hypothesised that Highly TB-Exposed Uninfected children would exhibit superior mycobacterial control as measured by lower experimental luminescence at early experimental timepoints than Highly TB-Exposed Infected children. Non-parametric bivariate tests of luminescence one hour after infection instead showed a statistically significant opposite effect. The observation was not significant in mixed effects modelling incorporating the longitudinal nature of the data, age, and inter-individual variability, so further investigation is needed. The possible differences within only one hour after infection of samples suggest further research focusing on the trained innate immune response may be of value [18,49,50]. A difference in inoculum is unlikely to account for the differences in baseline and 96 h luminescence between the groups as all samples from a pair were handled in the same way and inoculated with the same volume from the same stock of BCG at the same time. Baseline luminescence was measured one hour after inoculation to allow samples to equilibrate to 37 °C in the incubator, and each triplicate tube was individually removed, measured, and returned, so there was a maximum ten-
minute interval between measuring samples from the two children in a pair [39]. We did not see differences in cytokine response at baseline. Both non-parametric statistics and mixed effects modelling showed significantly greater mycobacterial control by Highly TB-Exposed Infected children at 96 h, mirroring results from two adult studies, which used different functional mycobacterial growth assays and suggest that MGIA are likely measuring adaptive responses at this time-point [25,29,51]. However, a comparison of unmatched groups of 20 IGRA-positive and 28 IGRA-negative 8 year-old South African children did not demonstrate a difference in mycobacterial growth inhibition [48]. Our luminescence data did not support superior mycobacterial control by Highly TB-Exposed Uninfected children and we therefore rejected our hypothesis and found that luminescence was not a correlate of protection in the assay’s current form.

The TST phenotype was dramatically different between the Highly TB-Exposed Infected and Highly TB-Exposed Uninfected groups, with Highly TB-Exposed Infected children strongly positive, and all Highly TB-Exposed Uninfected children consistently having 0 mm results, tested twice with a minimum of three months intervals. We found that Highly TB-Exposed Infected children were older than Highly TB-Exposed Uninfected children.

| Experimental timepoint | Highly TB-exposed Infected (95% CI) | Highly TB-exposed Uninfected (95% CI) | p |
|------------------------|-------------------------------------|---------------------------------------|---|
| Baseline               | 400.78 (332.66 – 482.84)            | 456.59 (374.81 – 556.21)              | 0.092 |
| 4 h                    | 848.10 (703.95 – 1,021.76)          | 889.31 (730.02 – 1,083.34)            | 0.54 |
| 24 h                   | 872.47 (724.18 – 1,051.12)          | 970.13 (796.37 – 1,181.80)            | 0.171 |
| 48 h                   | 2,164.54 (1,780.05 – 2,583.66)      | 2,307.49 (1,894.20 – 2,810.95)        | 0.345 |
| 72 h                   | 5,282.15 (4,384.38 – 6,363.76)      | 6,005.02 (4,929.47 – 7,315.24)        | 0.098 |
| 96 h                   | 11,261.95 (9,347.84 – 13,568.01)    | 13,303.89 (10,921.06 – 16,206.62)     | 0.031 |

![Fig. 2. Luminescence kinetics in the whole blood BCG assay for all Highly TB-Exposed Uninfected and Highly TB-Exposed Infected children.](image-url)

Table 2

Modelled estimated marginal means for luminescence of Highly TB-Exposed Infected and Highly TB-Exposed Uninfected pairs of children. Estimated marginal means and 95% confidence intervals by group and time were predicted from the model treating all fixed effects including age as balanced. P-values derived from pairwise comparisons of adjusted predictions.
Fig. 3. BCG-specific cytokine levels for the 29 Highly TB-Exposed Infected and 29 Highly TB-Exposed Uninfected children.

a) IFNγ, b) TNFα, b) IL1α, d) IL1β, and e) IL10. Median and interquartile ranges. P values from Wilcoxon matched pairs signed rank test. Scale on y axis for each plot selected to optimise data display.
| Time (h) | Highly TB-exposed infected | Highly TB-exposed uninfected | p |
|---------|-----------------------------|-----------------------------|---|
| 0 h     | 0 (0)                       | 0 (0)                       |   |
| 24 h    | 0.042                       | 0.023                       | 0.098 |
| 96 h    | 0.023                       | 0.023                       | 0.335 |

To our knowledge, our study is the first to apply a matched study design to the evaluation of whole blood cytokine responses to mycobacteria in children without LTBI despite exposure. Highly TB-Exposed Infected children produced significantly more IFN-γ in response to BCG at 24 and 96 hours. IFN-γ is recognised as a necessary but not sufficient mediator of host response to mycobacteria and is of course the basis of IGRAs [5]. In keeping with our findings, in two distinct cohorts in Uganda and Pakistan, IFN-γ levels in response to mycobacterial stimulation were also found to be lower in persistently TST negative individuals compared to those with LTBI or those who convert to a positive TST [53–55].

We did not find statistically significant differences in TNF-α levels as has also been previously observed [53,54,56]. The absence of significantly different cytokine results between groups is also in concordance with other published studies. Although there was not a matched design and the number of paediatric participants is not clear, persistently IGRA negative Indonesian hospital-based TB contacts in comparison to those who converted to a positive IGRA showed no difference in levels of TNF-α, IL1β, IL-1RA, IL-10, IL-6 and IL-8 in response to in vitro BCG infection or stimulation with an MTB lysate [18]. There was also no difference in IL-2, IFN-γ, TNF-α, and/or IL-17-expressing subsets of CD4, CD8 and γδ T-cells between IGRA+ and IGRA- South African adults following 12 h of BCG stimulation, nor correlations with MTB growth in a cross-sectional study [48]. Cytokine responses to MTB antigens of samples from babies born to mothers with or without LTBI also do not differ [57].

Our study had notable strengths in its matched design, clear differences in the TST phenotype, and confirmation of persistent TST negativity for at least 3 months in the Highly TB-Exposed Uninfected children. The application of the novel autoluminescent BCG whole blood assay enabled the possibility to examine the kinetics of host-mycobacterial interactions in children using minimal blood volumes. However, our study also had limitations. The sample size was not large, although the number of children was greater or comparable to other studies in the field [18,48]. Due to the limited sample capacity of laboratory equipment and the importance of simultaneous conduct of the functional mycobacterial assay on samples from matched children in the same experiment, it was not possible to recruit every Highly TB-Exposed Infected and Uninfected child. The study was conducted in a single centre. IGRA status was only available at baseline for the Highly TB-Exposed Infected children and was not available for the Highly TB-Exposed Uninfected children. Nevertheless, excluding the four IGRA/TST discordant Highly TB-Exposed Infected children did not affect the results. Also, all of the Highly TB-Exposed Infected children maintained their TST results of 0 mm at baseline and did not affect the results. Also, all of the Highly TB-Exposed Uninfected children in the study had no detectable BCG-specific IL1-β at baseline and therefore an accurate p-value for the Wilcoxon matched pairs signed rank test could not be calculated.
adults with pulmonary tuberculosis was not routine within the Gambian National Leprosy and Tuberculosis Control Program so data on MTB lineage and strains in the index cases are not available. Our study used BCG as the mycobacterial stimulus rather than pathogenic MTB. However, inoculum strain has been shown not to be a major determinant in mycobacterial growth inhibition assays in a high TB-prevalence setting, where BCG, MTB H37Rv, a W/Beijing MTB strain HN878, and Euro-American MTB strain, CDC1551 were all used to compare individuals with and without LTBI, including children and young adults [48]. The experimental stimulus being BCG in a population with near universal coverage of neonatal BCG vaccine, may have contributed to the similarity of cytokine responses between groups, except for IFN-γ levels that matched infection status. Nevertheless, the significant difference in luminescence at 96 h between groups suggests that even against this background of BCG vaccination and environmental mycobacterial exposure, the assay is able to distinguish children by their LTBI status. It may be that we were not able to detect differences in luminescence at early timepoints due to the need for a relatively high multiplicity of infection, as a consequence of the relatively low autoluminescence of the bacteria, in order for baseline measurements to be within the dynamic range of the luminescence counter [39].

We have demonstrated the importance of careful exposure-matched study design when comparing experimental data on samples from patients with LTBI to those who remain uninfected despite TB contact. We would encourage such study designs to be adopted more widely. We have also employed for the first time an autoluminescent mycobacterial growth assay specifically designed for paediatric studies where serial non-destructive luminescence measurements and supernatant analysis were possible with less than 5mls total blood. In conclusion, we found no evidence for a superior biological insights that can be applied to guide vaccine design and improvement. Identifying correlates of protection against LTBI remains challenging. We suggest a continued research focus on persistently TST negative individuals despite exposure, with application of novel and improved functional assays, broader investigation of trained innate immunity, antibody effector function, gene expression and genetic diversity, and metabolic differences needed to yield further biological insights that can be applied to guide vaccine design and evaluation [3, 4, 18, 20, 50, 58].

Declaration of Competing Interest

BK reports grants from MRC UK, during the conduct of the study; In addition, BK has a patent for a paediatric biomarker signature issued (United Kingdom Patent Application Number 1602305). The authors do not have any other commercial or other association that might pose a conflict of interest. The data presented here form part of RB’s PhD thesis “Protection from Mycobacterium tuberculosis infection: Learning from exposed but uninfected children” awarded March 2018 by Imperial College London.

Acknowledgements

We are grateful to the study participants and their families. We are also grateful to the Childhood TB team and Clinical Services Diagnostic Haematology Laboratory at MRC Unit The Gambia at LSHTM, the Gambian National Leprosy and Tuberculosis Control Program, and to Imperial College Healthcare Tissue Bank. We are grateful to Dr David Jeffries, Senior Statistician at the MRC Unit The Gambia for his help with the sample size calculation.

Funding Sources

RB was the recipient of a Clinical Research Training Fellowship that was jointly funded by the UK Medical Research Council (MRC) and the UK Department for International Development (DFID) under the MRC/DFID Concordat agreement and was also part of the EDCTP2 programme supported by the European Union (MR/K023446/1). The project was enabled by BK’s MRC Program Grant (MR/K007602/1, MR/K011944/1, MC_UP_A900/1122). The funders did not have any role in study design, data collection, data analysis, interpretation, or writing of the report.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2020.102891.

References

[1] World Health Organization. The end TB strategy. Geneva, Switzerland: World Health Organization; 2015.
[2] The World Health Organization. The roadmap for childhood TB: toward zero deaths (WHO/HTM/TB/2013.12), 2013.
[3] Treatment Action Group, Child & Adolescent TB Working Group. Research priorities for paediatric tuberculosis. 2018. http://www.treatmentactiongroup.org/content/research-priorities-pediatric-tuberculosis (accessed April 2, 2020).
[4] Seddon JA, Whitaker E, Kampmann B, et al. The evolving research agenda for paediatric tuberculosis infection. Lancet Infect Dis 2019;19:e322–9.
[5] Basu Roy R, Whitaker E, Seddon JA, Kampmann B. Tuberculosis susceptibility and protection in children. Lancet Infect Dis 2019;19:e96–8.
[6] Seddon JA, Chang SS, Esmail H, Coussens AK. The wonder years: what can primary school children teach us about immunity to mycobacterium tuberculosis? Front Immunol 2018;9:2946.
[7] Martinez L, Cords O, Horsburgh CR, et al. The risk of tuberculosis in children after close exposure: a systematic review and individual-participant meta-analysis. Lancet 2020;395:973–84.
[8] Campbell JR, Winters N, Menzies D. Absolute risk of tuberculosis among untreated populations with a positive tuberculin skin test or interferon-gamma release assay result: systematic review and meta-analysis. BMJ 2020;368. doi: 10.1136/bmj.m549.
[9] Roy A, Eisenhut M, Harris RJ, et al. Effect of BCG vaccination against Mycobacterium tuberculosis infection in children: systematic review and meta-analysis. BMJ 2014;349:g664.
[10] Basu Roy R, Sotgiu G, Alket–Gómez N, et al. Identifying predictors of interferon-γ release assay result in pediatric latent tuberculosis: a protective role of bacillus Calmette-Guérin. Am J Respir Crit Care Med 2012;186:378–84.
[11] Nennes E, Geldenhuyx H, Rozot V, et al. Prevention of M. tuberculosis infection with H37Rv vaccine or BCG revaccination. N Engl J Med 2018;379:138–49.
[12] Behr MA, Edelstein PH, Ramakrishnan L. Is Mycobacterium tuberculosis infection lifelong? BMJ 2019;367. doi: 10.1136/bmj.l5770.
[13] Cobat A, Barrera LF, Henao H, et al. Tuberculin skin test reactivity is dependent on host genetic background in Colombian tuberculosis household contacts. Clin Infect Dis 2012;54:968–71.
[14] Cobat A, Hoal EG, Gallant CJ, et al. Identification of a major locus, TNF1, that controls BCG-triggered tumour necrosis factor production by leukocytes in an area hyperendemic for tuberculosis. Clin Infect Dis 2013;57:963–70.
[15] Cobat A, Poirier C, Hoal E, et al. Tuberculin skin test negativity is under tight genetic control of chromosomal region 11p14-15 in settings with different tuberculosis endemcities. J Infect Dis 2015;211:317–21.
[16] Steen CM, Nohuti L, Chiunda AB, et al. Evidence for a major gene influence on tumor necrosis factor-alpha expression in tuberculosis: path segregation and analysis. Hum Hered 2005;60:109–18.
[17] Steen CM, Zalwango S, Chiunda AB, et al. Linkage and association analysis of candidate genes for TB and TNFα cytokine expression: evidence for association with IFNγR1, IL-10, and TNF receptor 1 genes. Hum Genet 2007;121:663–73.
[18] Verrall AJ, Schneider M, Aljisahbana B, et al. Early clearance of mycobacterium tuberculosis is associated with increased innate immune responses. J Infect Dis 2020;221:1342–50.
[19] Hawn TR, Day TA, Scriba TJ, et al. Tuberculosis vaccines and prevention of infection. Microbiol Mol Biol Rev 2014;78:850–71.
[20] Lu X, Smith MT, Yu XQ, et al. IFN-γ-independent immune markers of Mycobacterium tuberculosis exposure. Nat Med 2019;25:977–87.
[21] Seshadri C, Sedaghat N, Campo M, et al. Transcriptional networks are associated with resistance to Mycobacterium tuberculosis infection. PLoS One 2017;12:e0175844.
[22] Garand M, Goodier M, Owolabi O, Donkor S, Kampmann B, Sutherland JS. Functional and phenotypic changes of natural killer cells in whole blood during Mycobacterium tuberculosis infection and disease. Front Immunol 2018;9:257.
[23] Roy Chowdhury R, Vallania F, Yang G, et al. A multi-cohort study of the immune factors associated with M. tuberculosis infection outcomes. Nature. 2018; 560:644–8.

[24] Schragg LK, Vekemens J, Drager N, Lewinson DM, Olesen OF. The status of tuberculosis vaccine development. Lancet Infect Dis 2020;20:e28–37.

[25] Brennan MJ, Tanner R, Morris S, et al. The Cross-species Mycobacterial Growth Inhibition Assay (MGA) Project 2010–2014. Clin Vaccine Immunol 2017;24 e00142–17.

[26] Tannen R, O’Shea MK, Fletcher HA, McShane H. In vitro mycobacterial growth inhibition assays: a tool for the assessment of protective immunity and evaluation of tuberculosis vaccine efficacy. Vaccine 2016;34:4656–65.

[27] Tena GN, Young DB, Eley B, et al. Failure to control growth of mycobacteria in blood from children infected with human immunodeficiency virus and its relationship to T cell function. J Infect Dis 2003;187:1544–51.

[28] Kampmann B, Tena GN, Mzazi S, Eley B, Young DB, Levin M. Novel human anti-mycobacterial immune responses in HIV-infected children receiving HAART. AIDS 2006;20:1011–8.

[29] Kampmann B, Gaora PO, Snewin V, Gares MP, Young DB, Levin M. Evaluation of tuberculosis case-contact research in endemic tropical settings: design, conduct, and relevance to other infectious diseases. Lancet Infect Dis 2010;10:723–32.

[30] Lule SA, Mawa PA, Nkurunungi G, et al. Factors associated with tuberculosis infection, and with anti-mycobacterial immune responses, among five year olds BCG-immunised at birth in Entebbe, Uganda. Vaccine 2015;33:796–404.

[31] Lienhardt C, Sillah J, Fielding K, et al. Risk factors for tuberculosis infection in children in contact with infectious tuberculosis cases in the Gambia, West Africa. Pediatrics 2003;111:e608–14.

[32] Naranbhai V, Fletcher HA, Tanner R, et al. Distinct transcriptional and anti-mycobacterial responses using minimal blood volumes. Front Pediatr 2019;7:151.

[33] Schrager LK, Vekemens J, Drager N, Lewinsohn DM, Olesen OF. The status of tuberculosis vaccine development. Lancet Infect Dis 2016;16:1033.

[34] Brennan MJ, Tanner R, Morris S, et al. The Cross-species Mycobacterial Growth Inhibition Assay (MGA) Project 2010–2014. Clin Vaccine Immunol 2017;24 e00142–17.

[35] Zelner JL, Murray MB, Becerra MC, et al. Age-specific risks of tuberculosis infection from household and community exposures and opportunities for interventions in a high-burden setting. Am J Epidemiol 2019;180:853–61.

[36] O’Shea MK, Tanner R, Müller J, et al. Immunological correlates of mycobacterial growth inhibition describe a spectrum of tuberculosis infection. Sci Rep 2018;8:14480.

[37] Whalen CC, Chiunda A, Zalwango S, et al. Immune correlates of acute Mycobacterium tuberculosis infection in household contacts in Kampala, Uganda. Am J Trop Med Hyg 2006;75:55–61.

[38] Hussain R, Talat N, Shahid F, Dawood G. Biomarker changes associated with Mycobacterium tuberculosis vaccine efficacy trial of a whole blood mycobacterial growth inhibition assay to study immunity against Mycobacterium tuberculosis in a high tuberculosis burden population. PLoS One 2017;12:e0184563.

[39] Kleinnijenhuis J, Quinto J, Preijers F, et al. BCG-induced trained immunity in NK cells: role for non-specific protection to infection. Clin Immunol 2014;155:213–9.

[40] Khader SA, Divangahi M, Hanekom W, et al. Targeting innate immunity for tuberculosis vaccination. J Clin Invest 2019;129:3482–51.

[41] Kleinnijenhuis J, Quinto J, Preijers F, et al. BCG-induced trained immunity in NK cells: role for non-specific protection to infection. Clin Immunol 2014;155:213–9.

[42] Baguma R, Penn-Nicholson A, Smit E, et al. Application of a high-burden setting. Am J Epidemiol 2019;180:853–61.

[43] O’Shea MK, Tena GN, Mzazi S, et al. Identifying children with tuberculosis among household contacts in The Gambia. Int J Tuberc Lung Dis 2017;21:46–52.

[44] Mandalakas AM, Kirchner HL, Lombard C, et al. Well-quantified tuberculosis exposure is a reliable surrogate measure of tuberculosis infection. Int J Tuberc Lung Dis 2012;16:1033–9.

[45] Togun TO, Egere U, Gomez MP, et al. No added value of interferon-γ release to a prediction model for childhood tuberculosis. Eur Respir J 2016;47:223–32.

[46] Togun TO, Egere U, Sillah AK, et al. Contribution of Xpert MTB/RIF to the diagnosis of pulmonary tuberculosis among TB-exposed children in The Gambia. Int J Tuberc Lung Dis 2015;19:1091–7.

[47] Egere U, Sillah A, Togun T, et al. Isoniazid preventive treatment among child contacts of adults with smear-positive tuberculosis in The Gambia. Public Heal Action 2016;6:226–31.

[48] Basu Roy R, Sambou B, Uhiâ I, Roetynck S, Robertson BD, Kampmann B. An auto-luminescent fluorescent BCG whole blood assay to enable evaluation of paediatric mycobacterial responses using minimal blood volumes. Front Pediatr 2019;7:151.

[49] Mayer-Barber KD, Sher A. Cytokine and lipid mediator networks in tuberculosis. Immunol Rev 2015;264:264–75.

[50] World Health Organization, UNICEF. The Gambia: WHO and UNICEF estimates of immunization coverage: 2015 revision. 2016. https://data.unicef.org/wp-content/uploads/country_profiles/Gambia/Immunization_gmb.pdf (accessed June 18, 2017).

[51] The World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. 2nd ed. World Health Organizatio- n; 2014.

[52] Naranbhai V, Fletcher HA, Tanner R, et al. Distinct transcriptional and anti-mycobacterial profiles of peripheral blood monocytes dependent on the ratio of monocytes: lymphocytes. EBioMedicine 2015;2:1619–26.

[53] Sutherland JS, Lalor MK, Black GF, et al. Analysis of host responses to mycobacterium tuberculosis antigens in a multi-site study of subjects with different TB and HIV infection states in Sub-Saharan Africa. PLoS One 2013;8(3):1–14.

[54] Black GF, Thiel BA, Ota MO, et al. Immunogenicity of novel DoxR regulon-encoded candidate antigens of Mycobacterium tuberculosis in three high-burden populations in Africa. Clin Vaccine Immunol 2009;16:1203–12.

[55] Rabe-Hesketh S, Kronkal A. Multilevel and longitudinal modeling using stata: volume 1 — continuous responses. third ed. College Station, TX: Stata Press Public- ation; 2012. https://www.stata.com/bookstore/multilevel-longitudinal-model- ing-stata/(accessed April 2, 2020).

[56] Harris SA, Satti I, Matsushita M, et al. Process of assay selection and optimization for the study of case and control samples from a phase llb efficacy trial of a candidate tuberculosis vaccine, MVA8SA. Clin Vaccine Immunol 2014;21:1005–11.

[57] Buchwald UK, Adetifa IMO, Bottomley C, et al. Broad adaptive immune responses to M. tuberculosis antigens precede TST conversion in tuberculosis exposed household contacts. PLoS One 2014;9:e116268.

[58] Baguma R, Penn-Nicholson A, Smit E, et al. Application of a whole blood mycobacterial growth inhibition assay to study immunity against Mycobacterium tuberculosis in a high tuberculosis burden population. PLoS One 2017;12:e0184563.
Update

eBioMedicine

Volume 66, Issue , April 2021, Page

DOI: https://doi.org/10.1016/j.ebiom.2021.103295
Erratum regarding previously published research papers

The following Author Contribution statements were not included in the published versions of EBioMedicine. The appropriate Author Contribution statements are included below.

Cestrol-induced degradation of FANC D2 sensitises pediatric high-grade gliomas to the DNA-crosslinking agent carboplatin. (EBioMedicine 50: 81–92)

**Author contributions:** D.S.M. and E.H. conceived and designed the project. D.S.M., M.H.M., P.W. developed and validated the in vitro and in vivo models used in the study. D.S.M., B.B., M.H.M., and P.W. performed the functional in vitro experiments. D.S.M., P.W., and H.M. performed the functional in vivo experiments. J.K. provided bioinformatic expertise and support. B.B. and M.H.M. provided material and logistical support and advised on the project. G.J.K. and E.H. acquired funding and supervised the study. All authors contributed to writing the manuscript.

Epigenetically upregulated GEFT-derived invasion and metastasis of rhabdomyosarcoma via epithelial mesenchymal transition promoted by the Rac1/Cdc42–PAK signaling pathway. (EBioMedicine 50: 122–134)

**Author contributions:** CL and FL designed the whole study and wrote the manuscript. LZ, WC, YD, JZL, QI, HS, LM, WL, YW, YL, PW, YX, YW, LS, JH, and WZ contributed to experimental design and data collection. All authors have agreed with the manuscript and provide their consent for publication.

Combined identification of three miRNAs in serum as effective diagnostic biomarkers for HNSCC. (EBioMedicine 50: 135–143)

**Author contributions:** CL and QZ conceived the study. ZYY, SYH, and DSZ participated in the study design. QZ and YYJ conducted the experiments. Z.ZL collected the samples. X.YF analysed and interpreted the results, and wrote the manuscript. J.K. provided bioinformatics analysis. All authors critically reviewed, edited, and approved the manuscript and made the decision to submit for publication. All authors assume responsibility for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

Phosphorylated Rasa2 facilitates breast cancer progression. (EBioMedicine 50: 144–55)

**Author contributions:** X.W., Y.K. and Z.M.Q. conceived, organized and supervised the study; X.W., M.Y.L. and Y.L.Y. performed the experiments and data collection; Y.L.Y., X.W., C.Q, and K.Y. contributed to the analysis of data and double checking. X.W., C.Q., Y.K., and Z.M.Q. prepared, wrote and revised the manuscript.

Sprouty4 correlates with favorable prognosis in periheral cholangiocarcinoma by blocking the FGFR-ERK signaling pathway and arresting the cell cycle. (EBioMedicine 50: 166–177)

**Author contributions:** QB, C.TI, S.RQ, L.ZL, Z.XM, and L.ZP carried out experiments. Z.ZL collected the samples. X.YF analysed data. X.YF conceived experiments and wrote the paper. All authors had final approval of the submitted and published versions.

Analysis of gene expression signatures identifies prognostic and functionally distinct ovarian clear cell carcinoma subtypes. (EBioMedicine 50: 203–210)

**Author contributions:** RYH, TZT, and DSPT, designed and conceptualised the study. D.L. processed and reviewed OCCC samples. JY performed sample collection and experiments. NYLN curated and reviewed the clinical data of NUH cohort. TZT performed bioinformatics analyses. RYH, TZT, CVY, NYLN and DSPT analysed the data, interpreted the results, and wrote the manuscript.

Pro-inflammatory monocyte profile in patients with Major Depressive Disorder and suicide behavior and how ketamine induces anti-inflammatory M2 macrophages by NMDAR and mTOR. (EBioMedicine 50: 290–305)
Author contributions: W.N. designed and performed in vitro experiments, analysed and discussed results, and critically revised the manuscript; L.N.G. and D.E.R. recruited and followed up patients with MDD and performed sample collection; I.G.E. and N.E. performed in vitro experiments; A.R.A. processed samples of patients with MDD; M.P.A. and L.M.S. designed and performed in vivo murine experiments, analysed and discussed results, and critically revised the manuscript; F.M.D. conceived and designed the study, recruited and followed up patients with MDD, discussed results, and wrote the manuscript; E.A.C.S. and A.E.E. conceived and designed the study, designed and performed experiments, analysed and discussed results, and wrote the manuscript.

Radiomics analysis of placenta on T2WI facilitates prediction of postpartum hemorrhage: A multicentre study. (EBioMedicine 50: 355–365)

Author contributions: Conception and design: Xiaoan Zhang, Jie Tian, Meijun Wang. Collection and assembly of data: Qingxia Wu, Kuan Yao, Zhennu Liu, Longfei Li, Xin Zhao, Shuo Wang, Honglei Shang, Yousong Lin, Zewen Wen. Development of methodology: Kuan Yao, Zhennu Liu, Longfei Li, Shuo Wang, Yousong Lin, Jie Tian. Data analysis and interpretation: All authors. Manuscript writing: All authors. Final approval of manuscript: All authors.

TP63 Isoform Expression is Linked with Distinct Clinical Outcomes in Cancer. (EBioMedicine 51: 102,561)

Author contributions: A.B. designed experiments, analyzed data and wrote the manuscript; T.M. performed PCR and RT-PCR experiments; Y.W. performed western blot validation experiments; P.B. contributed to statistical design and analysis of data; P.P. supervised experimental design, analyzed data and prepared the manuscript. All authors read and approved of final manuscript.

Serum IGFBP-1 as a potential biomarker for diagnosis of early-stage upper gastrointestinal tumor. (EBioMedicine 51: 102,566)

Author contributions: Y-WX designed the study, searched the literature, performed the experiments, analysed and interpreted the data, did the statistical analysis, and wrote the manuscript. HC designed the study, collected patient samples, performed the experiments, analysed, and interpreted the data. C-QH designed the study, collected patient samples, searched the literature, did the statistical analysis, analysed, and interpreted the data. L-YC collected patient samples, performed the experiments, analysed and interpreted the data. S-HY analysed and interpreted the data. L-SH, and HG collected patient samples and clinical data. L-YC, C-CL, X-YH and L-HL and S-LC collected patient samples and clinical data. Z-YW, Y-HP, L-XY and E-ML conceptualized and designed the study, supervised the project, and revised the paper. All authors vouch for the respective data and analysis, and agreed to publish the manuscript.

Diagnostic accuracy and easy applicability of intestinal auto-antibodies in the wide clinical spectrum of coeliac disease. (EBioMedicine 51: 102,567)

Author contributions: Study concept and design: Luigina De Leo, Tarcisio Not. Acquisition of data: Luigina De Leo, Stefano Martelossi, Grazie Di Leo, Matteo Bramuzzo. Analysis and interpretation of data: Luigina De Leo, Tarcisio Not, Stefano Martelossi, Grazia Di Leo, Matteo Bramuzzo, Vincenzo Villanacci, Chiara Zanchi. Drafting of the manuscript: Tarcisio Not, Luigina De Leo. Critical revision of the manuscript: Alessandro Ventura, Vincenzo Villanacci, Matteo Bramuzzo, Chiara Zanchi. Clinical decisions: Stefano Martelossi, Grazia Di Leo, Matteo Bramuzzo. Histological evaluation of biopsy samples: Vincenzo Villanacci. Intestinal antibodies immunoassays: Luigina De Leo, Michela Pandullo, Petra Riznik.

Phage display antibody libraries: Fabiana Ziberna. Statistical analysis: Fabiola Giudici. All authors read and approved the final version of the manuscript.

MEF2C Repressor Variant Deregulation Leads To Cell Cycle Re-Entry and Development of Heart Failure. (EBioMedicine 51: 102,571)

Author contributions: AHMP, ACC designed and performed experiments, analyzed data, and wrote the manuscript; SRC, RRO, AS an MLBV designed and performed experiments, JRMS performed the echocardiography in animals. MFC analyzed data. AG, JLF, GCAV and MML provided human samples. JDM discussed the manuscript. KGF designed experiments, analyzed data, and wrote the manuscript. All authors reviewed and commented on the manuscript.

Developments in Zebrafish Avatars as radiotherapy sensitivity reporters – towards personalized medicine. (EBioMedicine 51: 102,578)

Author contributions: R.F. and M.G.F. conceptualized the research; R.F. and B.C. supervised the research; S.F., B.C., V.P. and R.F. performed research, acquisition, analysis and interpretation of data; P.F., RR-T, N.F. provided primary tumor samples; M.J.C., S.V., J.S., performed calculations and set-up the accelerator, O.P., J.S. for fruitful discussions; R.F. and B.C. wrote the manuscript. S.F., C.G., O.P. and M. G.F. did critical reading and editing of the manuscript.

Multi-cancer V-ATPase molecular signatures: A distinctive balance of subunit C isoforms in esophageal carcinoma. (EBioMedicine 51: 102,581)

Author contributions: JCVCS performed most of the experiments and analysis. PNN participated in the analysis and acquisition of data. EPC performed the in silico structural models. ARF and LFRP coordinated the project. JCVCS and ARF wrote the manuscript. JCVCS, PNN, TAS, ARF and LFRP performed study design. TAS and PNN participated in the collection of samples. ALOF and FFF provided specialized scientific and technical support. All authors discussed the results and manuscript text. All authors read and approved the final manuscript.

Heterogeneous nuclear ribonucleoprotein A2/B1 is a negative regulator of human breast cancer metastasis by maintaining the balance of multiple genes and pathways. (EBioMedicine 51: 102,583)

Author Contributions: The authors’ work in this study is listed as follows: In vitro and in vivo assays (YL, HL, FL, LBC, RH, CC and XD); RNA immunoprecipitation (YL); dual-luciferase reporter assay (YL and SL); signal pathways analysis (HL); proteomic analysis (YL); EMT markers test (HL, LBC and RH); real-time PCR (YL, SL, KL, LY, HMT, BBC and XL); and tissue microarray analysis (YL, DHX and XLD). SLS designed and supervised the study. YL and SLS analysed data and wrote manuscripts.

Genetic Risk for Dengue Hemorrhagic Fever and Dengue Fever in Multiple Ancestries. (EBioMedicine 51: 102,584)

Author contributions: GP, ML, KH, IL contributed to the design; ML, SE, LG, GK, AB, IL, LP, CP, CF, RS, ED, FB, YR, PB, JN, LW, DS, SP, GP, AW, CR, LP acquisition of data; GP, ML, AB, LG, GK Interpretation of data; GP, ML, PS, IL drafted the manuscript; IF, LW, DS, SP, GP, AW, AB, ED, LG, GK, MI, RS, KH revised it for critical intellectual content; ML, SE, LG, GK, AB, IL, LP, CP, CF, RS, ED, FB, YR, PB, JN, LW, DS, SP, GP, AW, PS, GK, KH approved the final manuscript; PG, ML, PS, SE, IF, LW, DS, SP, GP, AW, JN, AB, ED, LG, GK, RS, KH agree to be accountable for all aspects of the work.

Cortical haemodynamic response measured by functional near infrared spectroscopy during a verbal fluency task in patients with major depression and borderline personality disorder. (EBioMedicine 51: 102,586)

Author contributions: Syeda F. Husain: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing-review & editing. Tong-Boong Tang: Supervision, Writing – review & editing. Rongjun Yu: Supervision, Writing – review & editing. Wilson W. Tam: Supervision, Methodology, Writing – review & editing. Bach Tran: Supervision, Writing – review & editing. Travis T. Quek: Participant recruitment, Writing – review & editing. Shi-Hui Hwang: Participant recruitment, Writing – review & editing. Cheryl W. Chang: Participant recruitment, Writing – review & editing. Cyrus S. Ho: Supervision, Writing – review & editing. Roger C. Ho: Conceptualisation, Participant Recruitment, Methodology, Writing – review & editing.
Impact of sitagliptin on endometrial mesenchymal stem-like progenitor cells: A randomised, double-blind placebo-controlled feasibility trial. (EBioMedicine 51: 102,597)

**Author contributions:** Study concept, design, and overall supervision: J.J.B, S.Q. Prepared manuscript: J.J.B, S.T.e., E.S.L., S.Q. Edited manuscript: L.L, L.J.E, M.J.M.D.C., K.J.F., J.M., P.J.B., A.P., P.K.K., R.F. Obtained funding: S.Q., J.J.B, S.T.a. Regulatory approvals: S.Q., S.T.e. Patient enrolment, consenting, ultrasound and clinical assessments: S.Q., S.T.e., A.P., L.J.E, L.L. Cfu assays and analysis: E.S.L., P.J.B. Exploratory investigations: E.S.L., R.F., P.J.B, J.M, K.J.F., M.J.M.D.C., J.J.B. Data analysis: P.K.K., E.S.L., S.T.e., J.J.B, S.Q.

The CD24+ Cell Subset Promotes Invasion and Metastasis in Human Osteosarcoma. (EBioMedicine 51: 102,598)

**Author contributions:** Zhenhua Zhou wrote the manuscript. Zhenhua Zhou, Yan Li and Mu Yu Kuang performed cell culture, real-time PCR, flow cytometry and animal experiments. Xudong Wang carried out cell migration, invasion, proliferation assays, Western blot and protein mass spectrometry. Jingjing Hu and Jiashi Cao carried out the histological analysis and scores evaluation. Q.J. and Sujia Wu carried out progeny statistical analysis of clinical cases. Zhiwei Wang and Jianru Xiao conceived of the study and participated in its designation and helped to draft the manuscript. All authors read and approved the final manuscript.

The Transferability and Evolution of NDM-1 and KPC-2 co-producing Klebsiella pneumoniae from Clinical Settings. (EBioMedicine 51: 102,599)

**Author contributions:** HW conceived the project and designed the experiments. QW collected samples and performed microbial identification. YL collected the medical records. RW, YL and LJ performed the microbiological experiments. HG performed the computational analyses. YL, HG, RW and HW wrote the manuscript. All authors read and commented on successive drafts and all approved the final version of the manuscript. All authors contributed to critical revision of the final manuscript. RW and PG contributed to the study design. YZ and RH contributed to study design and data collection. RW performed statistical interpretation, analysis and drafted the manuscript. All authors contributed to critical revision of the final manuscript. RW approved the final version of the manuscript.

Mucosal microbial load in Crohn’s disease: a potential predictor of response to fecal microbiota transplantation. (EBioMedicine 51: 102,602)

**Author contributions:** RL, WZ and HHZ contributed to the study design. YZ and RH contributed to data collection. RL performed statistical interpretation, analysis and drafted the manuscript. All authors contributed to critical revision of the final manuscript. RL approved the final version of the manuscript.

Author contributions: C.M. and G.S. conceived and supervised the study. G.S., E.V., D.C., A.S., J.W. performed the experiments and data analysis. M.P. and C.M. performed the 16s rRNA data analysis and interpretation. S.L., M.M. and E.P. provided the explant tissues and reviewed the manuscript. C.E. provided the patients’ clinical data. K.M. and S.V. provided the mucosal biopsies from CD patients and reviewed the manuscript. G.S. and C.M. wrote and reviewed the manuscript. A.C. revised the manuscript. All authors read and approved the final version of the manuscript.

Mesenchymal stem cells ameliorate β cell dysfunction of human type 2 diabetic islets by reversing β cell dedifferentiation. (EBioMedicine 51: 102,615)

**Author contributions:** Conceptualization, Z.S., S.W.; Funding acquisition, Z.S., S.W.; Study design, L.W., T.L., R.L.; Investigation, L.W., T.L., R.L.; Data analysis, L.W., T.L., R.L.; Methodology, L.W., T.L., G.W., R.L., N.L., B.Z., V.J.L., X.D., X.C., Y.L.; Data interpretation, S.W., Z.S., Z.W., X.X.; Supervision, S.W., Z.S., C.R.; Writing – original draft, R.L., L.W; Writing – review & editing, Z.S., S.W., X.X., C.R.

A practical model for the identification of congenital cataracts using machine learning. (EBioMedicine 51: 102,621)

**Author Contributions:** HL, DL, WC, and YL contributed to the concept of the study and critically reviewed the manuscript. HL, DL, JC, ZL, YX, and XL designed the study and performed the literature search. HL, DL, JC, ZL, XL, XW, ZL, and WC collected the data. KZ, JH, LZ, and CG contributed to the design of the statistical analysis plan. DL, KZ, and JHL performed the data analysis and data interpretation. DL and HL drafted the manuscript. HL, DL, CC, YX, LW, and YZ critically revised the manuscript. HL, DL, WC, and YL provided research funding, coordinated the research and oversaw the project. All authors reviewed the manuscript for important intellectual content and approved the final manuscript.

MiR-765 functions as a tumor suppressor and eliminates lipids in clear cell renal cell carcinoma by downregulating PLP2. (EBioMedicine 51: 102,622)

**Author contributions:** WX, CW and XPZ designed and performed the experiments. WX, JCX and CW wrote the manuscript. WX, KC and TW analyzed and performed the experiments. XGW and XPZ directed the experiments and analyzed and assembled the data. All authors read and approved the submitted manuscript.

Breast cancer induces systemic immune changes on cytokine signaling in peripheral blood monocytes and lymphocytes. (EBioMedicine 51: 102,631)

**Author contributions:** LW and PPL designed experiments; LW, DLS, YTY and CA conducted experiments; LW and XL analyzed experimental data; AYC, FMD, JY, JW identified and recruited patients into this study; LW and PPL wrote manuscript. All authors read and approved the manuscript.

Near Infrared Photoimmunotherapy Targeting DLL3 For Small Cell Lung Cancer. (EBioMedicine 51: 102,632)

**Author contributions:** The all authors checked and approved the final version of the manuscript. Y.L. and K.S. mainly conducted all the experiments, performed analysis and wrote the manuscript; K.T., S.T., H.Y., Y.N., R.E., M.S., C.K., N.K., H.Y., Y.B., and Y.H. conducted analysis; S.N., T.F, K.K. and T.F.C.Y. conducted surgical operation to gather the specimens; K.S. supervised and conducted the project.

Cutting microtubule composition during infancy and subsequent behavioural outcomes. (EBioMedicine 51: 102,640)

**Author contributions:** AL and PV proposed the analysis. AL, MOH, ALP, and FC contributed to the statistical analysis. AL, PV, APL, MOH, CS, FC, and MT contributed to data interpretation. FC contributed to biobanking. AL, PV, and MOH drafted the manuscript. All authors provided feedback and edits to the manuscript. Relevant grant funding applications were prepared by and awarded to: PV, APL, JC, CS, FC, MT, SR, KA, RS, LH, PS, and the BIS Investigator Group.

Intracavernous injection of size-specific stem cell spheroids for neurogenic erectile dysfunction: efficacy and risk versus single cells. (EBioMedicine 52: 102,656)

**Author contributions:** ZQL and YT designed the whole experiments and guided the entire experiments, and are responsible for the integrity of the data and the accuracy of the data analysis; YDX and ZQL contributed to perform the animal experiments, data analysis and manuscript drafting. LZ, CH, XMQ, YCZ, and RLG contributed to the experiments and data analysis. ZCX and ZQL analyzed and interpreted the data, and critically revised the manuscript for important intellectual content. All authors approved the final version of the manuscript.

Identification and external validation of IgA nephropathy patients benefiting from immunosuppresion therapy. (EBioMedicine 52: 102,657)

**Authors contributions:** Research idea and study design: Z-HL, G-TX, C-HZ, T-TC, E-YX, T-GC; data analysis: Z-HL, C-HZ, T-TC, E-YX, T-GC, XL; data analysis/intepretation: Z-HL, C-HZ, T-TC, E-YX, T-GC, XL, YZ; statistical analysis: T-TC, E-YXT-GC; YZ; supervision or mentorship: Z-HL, C-HZ, YQ, S-SL, FX, Q-DL. All authors read and approved the final version of the manuscript.

Classification of Primary Liver Cancer with Immunosuppression Mechanisms and Correlation with Genomic Alterations. (EBioMedicine 52: 102,659)
Author Contributions: H.N. conceived the study. M.F., R.Y., T.H., S.H., and K.K. performed the analysis. K.M., K.N., A.F., M.U., S.H., H.A., H.Y., K.C., and S.I. contributed materials and data. K.A. performed immunohistochemical analysis. S.S. and S.T. performed cell line experiments and expression analysis. H.T. and S.M. contributed to the supercomputer environment. M.F. and H.N. wrote the manuscript.

Silencing of circular RNA H19/2k in neural stem cells enhances functional recovery following ischaemic stroke. (EBioMedicine 52: 102,660)

Author contributions: H.Y. conceived and supervised this project. H.Y and G.W. designed the experiments. G.W., B.H., L.S., S.W., L.Y., J.L., F.W., M.L., S.L., F.Z., Y.Z., Y.B., Y.M. and B.C. conducted experiments acquired, and analysed the interpreted data. H.Y. and G.W. wrote the manuscript. All authors read and approved the final version of the manuscript.

Genome-wide identification of FHL1 as a powerful prognostic candidate and potential therapeutic target in acute myeloid leukaemia. (EBioMedicine 52: 102,664)

Author contributions: CC and YF designed the study. YF, MX, ZC performed the experiments. YF, ZY, ZZ, XY and XH analyzed the data. CC, MZ and XW obtained the funding. YF, MX, MZ and XW prepared the figures. YF, MX, ZC and CC wrote the manuscript. CC supervised the study. All authors read and approved the final manuscript.

Longitudinal Serum Autoantibody Repertoire Profiling Identifies Surgery-Associated Biomarkers in Lung Adenocarcinoma. (EBioMedicine 52: 102,674)

Author contributions: S.C.-T. and H.-C. L. developed the conceptual ideas and designed the study. Y. L, S-J. G., H.-W. J. performed the experiments. C.-Q. L. and W. G. collected the sera samples. S.-C.T., H.-C. L., Y. L. and C.-Q. L. wrote the manuscript with suggestions from the other authors.

A comprehensive analysis of candidate genes in familial pancreatic cancer families reveals a high frequency of potentially pathogenic germline variants. (EBioMedicine 53: 102,675)

Author contributions: Study design: JE, NM and AC. Data collection: JE, MEC, VP, RF, MRG, TRA, LRD, ICG, MR, EMC and MM. Experimental work: JE, CC, JE2, EB, SGM, DG, GM. Data Analysis: JE, JE2, EB, DG, GM and JR. Interpretation of the data: JE, VP, RF, TRA, LRD, ICG, MR, EMC, NM and AC. Preparation of the manuscript: all authors

CircRNA-CIDN mitigated compression loading-induced damage in human nucleus pulposus cells via miR-34a-5p/SIRT1 axis. (EBioMedicine 53: 102,679)

Author contributions: Q.X. and L.K. designed the study protocol and wrote the manuscript; Q.X., L.K. and J.W. conducted the experiments; Z.L and Y.S. established the ex vivo IVD cultured model; K.Z. and K.W. collected and analysed data; C.Y. collected the NP tissues and supervised the study; Y.Z. supported and supervised the study.

FGFR1 and FGFR4 oncogenicity depends on N-Cadherin and their co-expression may predict FGFR-targeted therapy efficacy. (EBioMedicine 53: 102,683)

Author contributions: Conceptualization: A.Q., I.F., S.M.P., A.C., and L.P.A.; Methodology: A.Q., A.C., S.V.C, I.F., and S.M.P.; Investigation: A.Q., A.C., I.F., S.V.C., L.P.A. and S.M.P.; Validation: A.Q., A.M., L.O., E.G., S.V.C, S.M.G, L.M., S.G. and F.L.R.; Formal Analysis: A.Q., I.F., J.Z., S.M.P; Writing – Original Draft: A.Q., I.F., A.C., S.M.P and L.P.A.; Writing – Review & Editing: A.Q., I.F., S.V.C, A.C., S.M.P. and L.P.A.; Supervision: A.C., I.F., S.M.P., and L.P.A.; Funding Acquisition: S.M.P., I.F. and L.P.A. All authors read and approved the final version of the manuscript.

BAP18 is involved in upregulation of CCND1/2 transcription to promote cell growth in oral squamous cell carcinoma. (EBioMedicine 53: 102,685)

Author contributions: Xue Wang, Chunyu Wang, and Guangqi Yang designed the study and wrote the manuscript, Xue Wang, Ge Sun, Yuanyuan Kang, Shengli Wang, Renlong Zou, Hongmiao Sun and Kai Zeng performed experiments and analyzed the data, Huijuan Song, Wei Liu, Ning Sun, and Wensu Liu conducted bioinformatic analyses and statistical analyses, Yue Zhao wrote and revised manuscript. All authors read the approved the final manuscript.

Systematic identification of CDC34 that functions to stabilize EGFR and promote lung carcinogenesis. (EBioMedicine 53: 102,689)

Author Contributions: The project was conceived and designed by G.B.Z. The experiments were conducted by X.C.Z, G.Z.W., Q.H., L.W.Q., S.H.G., J.L., L.M., Y.Z.F., C.Z., H.Y., D.L.Z., and M.W.. Biospecimens were harvested/provided by Z.S.W., Y.C.Z., Y.C.H., B.Z.L., and Z.L.. The EGFR transgenic mice were provided by L.C.. Data were analyzed by G.B.Z., Y.Z., Z.L., L.C., and X.C.Z.. The manuscript was written by G. B.Z.. The study sponsor had no role in the design of the study: the data collection, analysis, or interpretation; the writing of the article; or the decision to submit for publication.

CBX4 transcriptionally suppresses KLF6 via interaction with HDAC1 to exert oncogenic activities in clear cell renal cell carcinoma. (EBioMedicine 53: 102,692)

Author contributions: Conception and design of the study: Jiang N, Zhang CH, Shen HM; Generation, collection, assembly, analysis of data: Jiang N, Niu G, Pan YH, Pan WW, Zhang MF; Drafting and revision of the manuscript: Jiang N, Zhang CH, Shen HM; Approval of the final version of the manuscript: all authors.

Enhanced O-linked GlcNAcylation in Crohn’s disease promotes intestinal inflammation. (EBioMedicine 53: 102,693)

Author contributions: Q.H.S. wrote the manuscript. Z.X.X. contributed to the conception and writing. W.Y.S., Y.L.L., and Z.X.X. designed research; Q.H.S., Y.P.J., M.D.L., D.Z., R.X.Z., J.C., and Y.L., performed research; C.S.Q., Y.S.W., G.L., H.L.Z., Q.D., J.L., Y.L.L., and Z.X.X. analyzed the data. Q.H.S., G.L., H.L.Z., Q.D., and Z.X.X. revised the manuscript. All authors read and approved the final manuscript.

Elevated myocardial SORB52 and the underlying implications in left ventricular noncompaction cardiomyopathy. (EBioMedicine 53: 102,695)

Author contributions: Yingjie Wei. supervised the work; Yingjie Wei, Chunyan Li. designed the experiments with help from Fan Liu, Shenghua Liu, Haizhou Pan, Haiwei Du, Jian Huang, Yuanyuan Xie, Yanfen Li and Ranxu Zhao. Yingjie Wei, Chunyan Li and Fan Liu analyzed the data; Chunyan Li and Yingjie Wei cowrote the manuscript. All authors discussed the results and commented on the manuscript.

Artificial intelligence-assisted prediction of preeclampsia: development and external validation of a nationwide health insurance dataset of the BPJS Kesehatan in Indonesia. (EBioMedicine 54: 102,710)

Author contributions: HS and ECVS developed the concept and design of this study. Dataset access was requested by HS. This author and YWW, and ECVS had full access to all data in the study. HS extracted and processed the data, performed training and validation of machine learning algorithms, conducted the literature search and wrote the draft of the manuscript. HS, YWW, and ECVS independently assessed the eligibility criteria of reviewed studies. YWW and ECVS critically revised the drafted manuscript. HS and ECVS take responsibility for data integrity and the accuracy of the analysis. All authors reviewed the final manuscript.

Planter temperatures in stance position: A comparative study with healthy volunteers and diabetes patients diagnosed with sensor neuropathy. (EBioMedicine 54: 102,712)

Author Contributions: UN, MS, JM, AM and PRM contributed equally to this study. PRM and SK conceived and designed the study. ED, JK, SK, JM, AM, and IW recruited participants and performed the experiments. UN, MS, JM, AM and PRM analyzed the data. UN, MS, JM, and PRM drafted the manuscript. TS and PRM were responsible for the design and performance of the sensor-equipped insoles and for data retrieval.

TRAF4 acts as a fate checkpoint to regulate the adipogenic differentiation of MSCs by activating PKM2. (EBioMedicine 54: 102,722)
Author contributions: SC, JL, ZC and YP designed the study and performed the experiments. ZS, ZL and GY performed the statistical analyses. GZ, ML, WL, WY and SW contributed study material and reagents. SC, ZX, PW and HS wrote the manuscript. ZX, PW and HS are the corresponding authors. All authors read and approved the final manuscript.

Identification, clinical manifestation and structural mechanisms of mutations in AMPK associated cardiac glycogen storage disease. (EBioMedicine 54: 102,723)

Author Contributions: Dan.H, and Dong.H designed the study. Dong.H, H.B.M, L.W.L., N.B.S., Y.L., B.W., F.Z., B.L.S., A.A., L.M., Y.X., S.W., C.A., M.H.G., P.M.E., Dan.H performed clinical and pathological phenotyping of study subjects. Dan.H, H.B.M, M.H.G., P.M.E., and Dong.H supervised and coordinated the genetic laboratory work. Y.L., Y.X., S.W., D.W., and D.B., performed history analysis. H.M., K.M., K.I., Dan.H, and D.B., performed computational modeling calculations and transfer entropy analysis. Dan.H, H.B.M, and Dong.H. organized and summarized the database. Dan.H, H.B.M, L.W.L., D.B, and Dong.H analyzed the data. Dan.H, D.B.C.A., M.H.G., P.M.E., and Dong.H developed the conceptual approaches to data analysis. Dan.H, Dong.H, D.B, and H.B.M, wrote the manuscript. All co-authors contributed to critical editing of manuscript.

Precise pulmonary scanning and reducing medical radiation exposure by developing a clinically applicable intelligent CT system: Toward improving patient care. (EBioMedicine 54: 102,724)

Author contributions: Conceptualization: Yang Wang and Bing Zhang; Experimental and data studies: Yang Wang, Xiaofan Lu, Yingwei Zhang, Xin Zhang, Kun Wang, Jian Li, and Xin Li; Technical Support: Renfang Hu, Xiaolin Meng, Shidun Dou, Huayin Yao, Xiaofeng Zhao, Wei Hu, Cheng Li, and Yaoyong Gao; Statistical analysis: Xiaofan Lu and Fangrong Yan; Construction of artificial intelligence network: Renfang Hu, Xian Li; Meng, Shidun Dou, Huayin Yao, Xiaofeng Zhao, Wei Hu, Cheng Li, and Yaoyong Gao; Manuscript editing: Yang Wang, Xiaofan Lu, Zhishun Wang, Guangming Lu, Fangrong Yan, and Bing Zhang; Funding acquisition: Fangrong Yan and Bing Zhang; Resources: Fangrong Yan and Bing Zhang; Supervision: Fangrong Yan and Bing Zhang. All authors read and approved the final version of the manuscript.

Clinical and genomic insights into circulating tumor DNA-based alterations across the spectrum of metastatic hormone-sensitive and castrate-resistant prostate cancer. (EBioMedicine 54: 102,728)

Author Contributions: Conception of idea, MK; Acquisition of data, MK, WT, LH, KM, HF, EK, AA, SY; Data generation, AM, CM, CW; Analysis and interpretation of data, TZ, JY, MK, AW, CW, PD, HF, EK, AA; Drafting of the manuscript, MK, AA, TZ, JY, WT; Critical revision of the manuscript for important intellectual data, WT, LH, SJ, KM, JY, TZ, SJ, HF, SY, EK, AA; Obtaining funding, MK, LH, AA, EK, KM.

Lifetime risk of autosomal recessive mitochondrial disorders calculated from genetic databases. (EBioMedicine 54: 102,730)

Author Contributions: MW and TK conceived the study. JT and MW defined a comprehensive list of mitochondrial disease genes and set up a list of pathogenic variants in these genes, supported by SLS, TMS, and SBW. JT and MW queried two databases (gnomAD and in house) to assess the allele frequencies of disease-causing variants in the general population and calculated the lifetime risks, supported by HP, TM, KO and TK. JT and MW drafted the manuscript which was then refined by all other authors and finalized by MW and TK.

Transcriptional and clonal characterization of B cell plasmablast diversity following primary and secondary natural DENV infection. (EBioMedicine 54: 102,733)

Author contributions: A.T.W conceived of the project, designed and executed experiments, analyzed data, and wrote the paper. G.G. and W.R. designed and executed experiments, analyzed data, and provided subject matter expertise. K.M.K. and B.G. analyzed data. T.L., H.S., K.V., C.K., A.G., M.E.F., and J.L. generated data. A.M, A.S., E.D., S.J. provided subject matter expertise and supervised data generation. B.J.D. secured funding, T.E., S.T., and A.L.R. secured funding and provided subject matter expertise. R.G.J. provided project oversight, secured funding, and provided subject matter expertise. D.E. provided project oversight and subject matter expertise. J.R.C and H.F. conceived of the project, designed and executed experiments and analyzed data.

Zika Virus Envelope Nanoparticle Antibodies Protect Mice without Risk of Disease Enhancement. (EBioMedicine 54: 102,738)

Author contributions: Literature search: SS; Figures: RS, RKS, SS, NK; Study design: SS, NK, JKL, FK; Data collection: RS, RKS, VR, UA, GB, JAA; Data analysis and interpretation: SS, NK, JKL, FK; Writing: SS and NK; Approval of final manuscript: all authors.

Bio responsive self-assembling of Au-miRNAs for targeted cancer theranostics. (EBioMedicine 54: 102,740)

Author contributions: The authors' responsibilities were as follows: WC, LY, YW and XW devised the experiments and wrote the manuscript. WC conducted the synthesis of materials, purification, and materials/biological characterizations etc. HF contributed to the mouse model experiment. All other authors contributed to materials synthesis, purification/characterization, and/or discussion of the results.

Large-scale network dysfunction in the acute state compared to the remitted state of bipolar disorder: A meta-analysis of resting-state functional connectivity. (EBioMedicine 54: 102,742)

Author Contributions: Yanlin Wang and Xiaoqi Huang designed the study, Yanlin Wang and Shi Tang collected data and performed analyses; Lu Lu, Lianqing Zhang, Xinyu Hu, Xuan Bu, Hailong Li, Xiaoxiao Hu, Xinyu Hu, Ping Jiang, and Zhiyun Jia provided helpful suggestions; Yanlin Wang, Yingxue Gao and Shi Tang drafted the main article; John A. Sweeney, Qiyouong Gong and Xiaoqi Huang critically reviewed the manuscript.

Dynamics of within-host Mycobacterium tuberculosis diversity and heteroresistance during treatment. (EBioMedicine 55: 102,747)

Author contributions: Study design: CN, JB, FB; Data collection: CN, KB, JM, AG, NP, MO; Data analysis: CN, FB; Data interpretation: CN, JM, MO, FB; Writing: CN, FB; Review and approval of manuscript: CN, KB, JM, AG, NP, MO, JB, FB; All authors have read and approved the final version of this manuscript.

Host transcriptomic signature as alternative test-of-cure in visceral leishmaniasis patients coinfected with HIV. (EBioMedicine 55: 102,748)

Author Contributions: All authors read and approved the final version of the manuscript. Wim Adriaensen: Conceptualization, data collection, formal analysis, visualization, writing & editing Bart Cuppers: Formal analysis, methodology, writing, review & editing Carlota F. Cordero: Formal analysis Bewuketu Mengasha: Data collection and curation Séverine Blesson: Data curation, project coordination Lieselotte Cnops: Formal analysis, writing, review & editing Paul M. Kaye: Methodology, supervision, review & editing Fabiana Alves: Data curation, funding acquisition, project administration, review & editing Ermias Diro: Data curation, project coordination, funding acquisition, review & editing Johan van Griensven: Conceptualization, methodology, funding acquisition, project administration, supervision, review & editing.

Motor transmission defects with sex differences in a new mouse model of mild spinal muscular atrophy. (EBioMedicine 55: 102,750)

Author Contributions: Marc-Olivier Deguise: Generated the mouse model, designed study, produced and analyzed data for all figures, and wrote the manuscript. Yves De Repentigny: Data acquisition, data analysis and method description. Alexandra Tierney: Data acquisition and data analysis.

Ariane Beauvais: Assistance with experiments. Jean Michaud: Assessment of histology of the skeletal muscle. Lucia Chehade: Data acquisition and data analysis. Mohamed Thabet: Assistance with electrophysiology. Britany Paul: Data acquisition and data analysis. Aoife
Reilly: Assistance with experiments. Sabrina Gagnon: Maintenance of mouse models and genotyping. Jean-Marc Renaud: Electrophysiology and data analysis. Rashmi Kothary: Designed study and wrote manuscript.

Ileo-colonic delivery of conjugated bile acids improves glucose homeostasis via colonic GLP-1–producing enteroequocrine cells in human obesity and diabetes. (EBioMedicine 55: 102,759)

**Author Contributions:** Conceptualization, AA, MC, Fmg, and AV; Methodology, AM, AA, JR, BG, MC, Fmg, and AV; Formal Analysis, GC, AM, JR, AA, Fmg, and AV; Investigation, GC, AM, AA, JR, JD, IZ, GF, DB, GR, BG, SN, AA; Resources, FR, BG, AV, NFl, Fmg, MC, AA. Writing – Original Draft: GC, AM, JR. Writing – Review & Editing, GC, AM, AA, JR, JD, GF, DB, GR, BG, AV, NFl, Fmg, MC, AA. Visualization, GC, AM, JR. Supervision FR, BG, AV, NFl, Fmg, MC, AA Funding Acquisition Fmg, MC, AA.

Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. (EBioMedicine 55: 102,763)

**Authors contributions:** Conceptualization: JL, SML, JL, YH, DLY, XZ. Acquisition of data: BYL, XBW, HW, WL, QXT, JHY, LZ, LJX, CGX, JT, JZL, JHY, RP, HS, CP, TL, QZ, JW, LX, SHL, BJW, ZHW, CRH, HBZ, RZ, HLZ, XC, PY, BZ, LW, WQQ, SYY, YWH, SHJ, PW, JAZ, YPL, WXX, LZ, LL, FQZ. Analysis and interpretation of data: JL, SML. Writing–original draft Preparation: JL. Writing-review and editing: UD, MJL, JL, DLY, XZ. All authors reviewed and approved the final version of the manuscript.

A dysregulated bile acid–gut microbiota axis contributes to obesity susceptibility. (EBioMedicine 55: 102,766)

**Author contributions:** Wei jia was principal investigator of this study. Zhaoxiang Bian provided valuable support for C. scindens gavage animal experiment. Wei jia, Aihua Zhao, Xiaoqiao Zheng, and Guoxiang Xie designed the study. Mellin Wei conducted key experiments of the study and perform the data analysis and drafted the manuscript. Fengjie Huang, Yunjing Zhang, Wei Yang, and Ling Zhao conducted the animal experiments. Kun Ge, Chun Qu, Mengci Li, Shouli Wang, and Xiaolong Han helped to perform the experiments and collected the data. Wei jia and Cynthia Rajani revised the manuscript.

Prognostic and predictive value of a five–molecule panel in resected pancreatic ductal adenocarcinoma: A multicentre study. (EBioMedicine 55: 102,767)

**Author contributions:** Concept and design: JGC, SL, TPZ. Provision of study material and patients: JCG, SL, TPZ, CGZ, BS, QL, MHD. Financial and administrative support: JGC, SL. Data analysis and interpretation: PZ, LZ, LY, QFL, ZYL, JL, JD, ADT, JS. Experimental support: PZ, LZ, LY, GXX. Manuscript writing: PZ, LZ, QFL. Final approval of the manuscript: All the authors.

CD24–targeted intraoperative fluorescence image–guided surgery leads to improved cytodestruction of ovarian cancer in a preclinical orthotopic surgical model. (EBioMedicine 56: 102,783)

**Author contributions:** Literature search: E. McCormack, L. Bjørge, K. Kleinmanns, V. Fosse; Study design: E. McCormack, L. Bjørge, K. Kleinmanns, V. Fosse; Development of methodology: E. McCormack, L. Bjørge, K. Kleinmanns, V. Fosse; Data collection (in vitro data, animal experiments, patient data): K. Kleinmanns, V. Fosse. B. Davidson, O. Tenstad, E. García de Jalón; Data analysis and interpretation of data: K. Kleinmanns, V. Fosse, B. Davidson, O. Tenstad, E. García de Jalón; Writing, review and/or revision of the manuscript: K. Kleinmanns, V. Fosse, E. McCormack, L. Bjørge; Study supervision: E. McCormack, L. Bjørge. All authors read and approved the final version of the manuscript.

Low oxygen saturation during sleep reduces CD1D and RAB20 expressions that are reversed by CPAP therapy. (EBioMedicine 56: 102,803)

**Author contributions:** TS, DJG, and SAG conceptualized the association study. TS, RL, RJ, HL, ACG, NK, BEC, JL, and SW performed statistical analysis and data harmonization. All authors critically reviewed the manuscript. YL, JR, and SR collected data and designed components of MESA and its gene expression study. DL collected data and designed components of FOS and the SABRe CVD initiative which collected genes expression data for FOS. RM, SRP, SFQ, SR, and DJG designed and executed the HeartBEAT study, and DJG and AS designed its gene expression study.

Clinical implications of serum neurofilament in newly diagnosed MS patients: A longitudinal multicentre cohort study. (EBioMedicine 56: 102,807)

**Author Contributions:** FS, VF, TU, M Muthuraman, SCG, SG: Analysis and interpretation of data and drafting the manuscript. AS, RG: Study protocol, design and ethics implementation of the KKNMS cohort study. CI, AS, FL, TK, M Mülhlau, LK, TR, A Bayas, A Berthele, FP, HPH, RL, CH, MS, BW, FTB, BT, TK, FW, UZ, UZ, HT, BH, HW, RG: Contributing data and revising the manuscript. SB, FZ: Design and conceptualisation of the study, analysis and interpretation of data, drafting the manuscript.

Molecular analysis of Chinese oesophageal squamous cell carcinoma identifies novel subtypes associated with distinct clinical outcomes. (EBioMedicine 57: 102,831)

**Author contributions:** Lin Feng and Xiyan Wang designed the study. Meng Liu performed the data collection and data analysis. Wei Sun and Yuan Zhang collected Chinese ESCC samples. Haiyan An and Meng Liu extracted and quantified RNA and DNA. Shujun Cheng provided constructive feedback. Lin Feng and Ruozheng Wang supervised research and provided data interpretation. Meng Liu wrote and reviewed the manuscript.

Using Recombination–Dependent Lethal Mutations to Stabilize Reporter Flaviviruses for Rapid Serodiagnosis and Drug Discovery. (EBioMedicine 57: 102,838)

**Author contributions:** C.B., XX, and A.M. performed experiments. K.F. provided critical reagents. C.B., XX, J.Z., and A.M. analyzed the data. C.B., XX, J.Z., K.F., and P-Y.S. interpreted results. C.B., XX, and P-Y.S. wrote the manuscript.

Broadly neutralizing antibodies potently inhibit cell-to-cell transmission of semen leukocyte-derived SHIV162P3. (EBioMedicine 57: 102,842)

**Author contributions:** Study conception and design: RLG and MC. Acquisition of data: KS, MT, and SH. Management of animals: DD, VL, HM and GS contributed with key reagents and expertise. Analysis and interpretation of the data: KS, NDB, and MC. Draft of the manuscript: KS and MC. Critical revisions: HM, GS, RLG, and MC. All authors read and approved the final version of the manuscript.

GSTM3 variant is a novel genetic modifier in Brugada syndrome, a disease with risk of sudden cardiac death. (EBioMedicine 57: 102,843)

**Author contributions:** MJM, TPL, and CA performed literature search, conceived and designed the study and the experiments. MJM, TPL, AB, IR, SJJ, CYJC, LCI, SFSY, EYC, and LPL conducted experiments and analysed the data. MJM, JH, WCC, YBI, LYL, CCC, LTH, and HCH enrolled patients, collected and interpreted data. MJM, AB, IR, TPL, and CA wrote the paper.

Tumor budding, poorly differentiated clusters, and T-cell response in colorectal cancer. (EBioMedicine 57: 102,860)

**Author contributions:** All authors contributed to review and revision. MG., J.A.N., and S.O.: developed the main concept and designed the study. A.T.C., C.S.F., M.G., and S.O.: wrote grant applications. K.F., J.P.V., J.B., D.J.P., J.A.M., A.T.C., C.S.F., J.K.L., J.A.N., and S.O.: were responsible for collection of tumor tissue, and acquisition of epidemiologic, clinical and tumor tissue data, including histopathological, immunohistochemical, and immunofluorescent characteristics. K.F., J.P.V., J.B., D.J.P., K.H., J.A.M., C.S.F., J.A.N., and S.O.: performed data analysis and interpretation. K.F., J.P.V., J.B., D.J.P., and S.O.:...
drafted the manuscript. K.A., K.H., J.K., N.A., T.U., M.C.L., S.G., S.S., M.Z., A.F.L.D.S., T.S.T, H.N., J.A.M., X.Z., K.W., M.G., J.A.N., and S.O.: contributed to editing and critical revision for important intellectual contents.

A surrogate of Roux-en-Y gastric bypass (the enterogastro anastomosis surgery) regulates multiple beta-cell pathways during resolution of diabetes in ob/ob mice. (EBioMedicine 58: 102,895)

Author contributions: F.A., C.A. and C.M. designed the experiments. C.A.; J.C.; C.G.; A.L.; F.M., C.R., J.D.; E.G.; S.M.L., O.T. conducted the experiments. C.A.; F.A.; C.M.; O.T.; C.G. G.R. and R.R. analyzed data. K.C. contributed to patient recruitment and coordinated clinical investigation, patient phenotyping, and sample collection. F.A. and C.A. wrote the manuscript and C.A.; F.A.; C.M.; O.T.; T.S.; C.G.; R.R.; S.L.; R.S.; H.L.S.; E.G. and G.R. contributed to data presentation and the manuscript. All authors reviewed the manuscript. F.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Protection Against Mycobacterial Infection: a case-control study of mycobacterial immune responses in pairs of Gambian children with discordant infection status despite matched TB exposure. (EBioMedicine 59: 102,891)

Author contributions: RB and BK conceived and designed the work. RB, MS, AS and UE conducted the clinical recruitment. RB and BS conducted and interpreted the BCG-GFP-LuxFO whole blood assays. BS and MG conducted the in-house interferon gamma release assays. BH conducted and interpreted the cytokine multiplex assays. RB and AK conducted the statistical analyses. RB and BK drafted the work. All authors revised the work for important intellectual content.

Brain Delivery of Supplemental Docosahexaenoic Acid (DHA): A Randomized Placebo-Controlled Clinical Trial. (EBioMedicine 59: 102,883)

Author contributions: IC, NC, BK, DB participated in recruitment and study visits. HNY and MGH did lumbar punctures. XH, NK, and WJM conducted data analysis. NH, NK and MNB did imaging analysis. LD, CM, and HCC planned cognitive testing. AM, AS, BZ assisted with biomarkers. IC, VS, HH, MH, HCC, WJM, MNB, LSS and HNY wrote the manuscript. HNY and LSS designed the study.

Obesity-related hypoxia via miR-128 decreases insulin-receptor expression in human and mouse adipose tissue promoting systemic insulin resistance. (EBioMedicine 59: 102,912)

Author contributions: B.A. and F.L.A. performed experiments in vitro and in vivo, in mouse systems; B.A. performed human tissue culture studies and analyzed data with the contribution of E.C., M.M., D.M.C., D.P.F and A.B; G.C. and G.N. provided tissues from surgery and clinical information; D.B., V.M. and UK contributed to the analysis of data from mouse experiments; B.A. and E.C. contributed to manuscript draft; F.S.B. helped collecting clinical data and drafted figures; I.D.G. edited the final version of the manuscript and contributed to data interpretation; A.B. conceived and supervised the study and wrote the manuscript.

All authors read and approved the final manuscript.