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Research paper

Host transcriptomic signature as alternative test-of-cure in visceral leishmaniasis patients co-infected with HIV

Wim Adriaensen[a,*], Bart Cuypers[b,c], Carlota F. Cordero[a], Bewketu Mengasha[d], Séverine Blesson[e], Lieselotte Cnops[a], Paul M. Kaye[f], Fabiana Alves[e], Ermias Diro[d], Johan van Griensven[a]

[a] Unit of Neglected Tropical Diseases, Department of Clinical Sciences, Institute of Tropical Medicine, Nationalestraat 122, 2000 Antwerp, Belgium
[b] Unit of Molecular Parasitology, Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 122, 2000 Antwerp, Belgium
[c] Adrem Data Lab, Department of Mathematics and Computer Science, University of Antwerp, Middelheim 1, 2020 Antwerp, Belgium
[d] Leishmaniasis Research and Treatment Centre, University of Gondar, Gondar, Ethiopia
[e] Drugs for Neglected Diseases initiative, 15 Chemin Louis-Dunant, 1202 Geneva, Switzerland
[f] York Biomedical Research Institute, University of York, United Kingdom

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ABSTRACT

Background: Visceral leishmaniasis (VL) treatment in HIV patients very often fails and is followed by high relapse and case-fatality rates. Hence, treatment efficacy assessment is imperative but based on invasive organ aspiration for parasite detection. In the search of a less-invasive alternative and because the host immune response is pivotal for treatment outcome in immunocompromised VL patients, we studied changes in the whole blood transcriptional profile of VL-HIV patients during treatment.

Methods: Embedded in a clinical trial in Northwest Ethiopia, RNA-Seq was performed on whole blood samples of 28 VL-HIV patients before and after completion of a 29-day treatment regimen of AmBisome or AmBisome/miltefosine. Pathway analyses were combined with a machine learning approach to establish a clinically-useful 4-gene set.

Findings: Distinct signatures of differentially expressed genes between D0 and D29 were identified for patients who failed treatment and were successfully treated. Pathway analyses in the latter highlighted a downregulation of genes associated with host cellular activity and immunity, and upregulation of antimicrobial peptide activity in phagolysosomes. No signs of disease remission nor pathway enrichment were observed in treatment failure patients. Next, we identified a 4-gene pre-post signature (*PRSS33, IL10, SLFN14, HRH4*) that could accurately discriminate treatment outcome at end of treatment (D29), displaying an average area-under-the-ROC-curve of 0.95 (CI: 0.75–1.00).

Interpretation: A simple blood-based signature thus holds significant promise to facilitate treatment efficacy monitoring and provide an alternative test-of-cure to guide patient management in VL-HIV patients.

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1. Introduction

Visceral leishmaniasis (VL) is a potentially fatal, yet neglected, vector-borne disseminated infection caused by protozoans of the *Leishmania donovani* spp. complex [1]. Typical symptoms include fever spikes, substantial weight loss, splenomegaly and alterations of haematopoiesis. With a global estimate of 90,000 cases annually, Ethiopia together with Brazil, India, Kenya, Somalia, South Sudan and Sudan host more than 90% of all VL cases [2].

Compared to varying cure rates of around 90–95% in VL patients, treatment of patients with a concurrent Human Immunodeficiency Virus-1 (HIV) infection (referred to herein as 'VL-HIV patients') in endemic regions of East-Africa, Brazil and India frequently fails. Treatment failure results in extended treatments and case-fatality rates up to 25% [3,4]. This is particularly true for East Africa where antileishmanial drugs show lower efficacy rates and HIV prevalence rates of 10–20% are reported amongst VL patients [3]. Even if

* Corresponding author.
E-mail address: wadriaensen@itg.be (W. Adriaensen).

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Research in context

Evidence before this study

Human immunodeficiency virus (HIV) infection has been identified as a significant challenge facing visceral leishmaniasis (VL) control. VL treatment in HIV patients very often fails and results in extended treatment time followed by high relapse and case-fatality rates. In addition to limited and toxic treatment options, the treatment outcome assessment thus becomes imperative in VL-HIV patients to guide decisions on treatment extension, treatment adjustment or secondary prophylaxis initiation. However, with no alternatives to assess treatment efficacy, repeated invasive and painful aspiration from infected organs for microscopical detection of the parasite remains the only approach. Chronic patients thus undergo repeated tissue aspirates or empirical optimization of treatment regimens. Hence, the development of a less-invasive alternative to assess treatment efficacy represents an urgent and important unmet clinical need to achieve VL control.

Added value of this study

Our study is the first to investigate whole transcriptome changes in the severely neglected group of visceral leishmaniasis patients with a concurrent HIV infection, where the host immune response is largely uncharted but believed to be pivotal for treatment outcome. In a translational effort, the findings were biologically interpreted and systemically reduced to a 4-gene biomarker that could accurately discriminate treatment outcome.

Implications of the all the available evidence

This signature holds significant promise to facilitate treatment efficacy monitoring in R&D and could provide an alternative test-of-cure to guide patient management in VL-HIV patients.

apparent parasitological clearance at end of treatment and viral suppression with ART is achieved, up to 60% of VL-HIV patients will develop recurrent relapse, typically within 3–6 months after initial cure (compared to 1–5% in immunocompetent VL patients) [3,5]. Hence, treatment outcome assessment is imperative in VL-HIV patients to guide decisions on treatment extension, treatment adjustment or secondary prophylaxis initiation.

To date, a repeated invasive and painful aspiration for microscopical detection of the parasite from infected organs (spleen, bone marrow or lymph nodes) remains the only approach for test-of-cure. Although spleen aspiration shows the highest sensitivity [6], it has a life-threatening risk of splenic haemorrhage that renders it unsuitable in patients with severe thrombocytopenia. In addition, these techniques require a great level of expertise, training of personnel and appropriate facilities where blood transfusion and management of intraabdominal bleeding is possible. Due to these reasons, chronic patients often undergo repeated tissue aspirates or empirical optimization of treatment regimens. Hence, the development of a less-invasive alternative to assess treatment efficacy represents an urgent and important unmet clinical need.

Molecular techniques for parasite detection seem promising, but could be less suitable as the parasitic load in blood decreases steeply after two days of treatment and gives no information on the host's immunological recovery [7]. In immunocompromised individuals in particular, host immune response restoration has been shown to be pivotal in the efficacy of VL treatment [8]. Therefore, transcriptomic signatures in peripheral blood may reflect immunological responses underpinning clearance or persistence of parasites. In recent years, gene signatures derived from blood transcriptomic profiling have shown great promise in treatment monitoring for a number of infectious diseases [9–11]. With a 5-gene signature, robust prediction of treatment failure in tuberculosis patients could be achieved after 1 or 4 weeks of therapy [11]. Similarly, Liu et al. identified and validated a 10-gene signature that predicted Ebola treatment outcome with an accuracy of 85% to 92% [9]. Yet, previous studies were often confined to single timepoint measurements and purely statistical approaches that could be complicated by patient-to-patient variation and little biological relevance of selected genes, all jeopardizing their generalizability.

To date, two small-scale studies in VL patients described distinct expression profiles in respectively the blood and the lymph nodes before and after treatment with amphotericin B [12] or sodium stibogluconate [13]. Likewise, Gardinassi et al. also defined distinct immunological signatures in the blood for active and cured L. *infantum* infected patients [14]. Although performed in immunocompetent VL patients, these findings support the pursuit of a blood-based test-of-cure. None of the previous studies, however, sought to translate such knowledge into a clinically useful signature to guide the challenging clinical management of VL-HIV patients and facilitate the evaluation in clinical trials of urgently needed novel therapeutics.

Here, to minimize the impact of heterogeneity in gene expression levels amongst individual VL-HIV patients we studied relative changes in the gene expression profile during VL treatment. By combining biological insight with a stringent machine learning approach, we have identified a relevant and robust 4-gene blood signature that accurately determined treatment outcome in VL-HIV patients.

2. Materials and methods

2.1. Study population and design

In 2014–16, a total of 30 patients recruited at the Leishmaniasis Research and Treatment Centre (Gondar, Ethiopia) in a randomized clinical trial (RCT) on combination treatment (NCT02011958, sponsored by the Drugs for Neglected Diseases Initiative) were included in this study for additional whole blood sampling [15]. In short, AmBisome monotherapy and a combination treatment of AmBisome and miltefosine were administrated. AmBisome monotherapy dosage was 40 mg/kg total dose, IV infusion of 5 mg/kg on day 1–5, 10, 17 and 24. In the combination treatment AmBisome dosage was 30 mg/kg total dose, IV infusion of 5 mg/kg on day 1,3,5,7,9 and 11 and miltefosine every day for 28 days (50 mg if patient weight $<$ 25 kg, 100 mg if patient weight is $>$25 kg). Treatment success was defined as absence of parasites in spleen aspirate at D29. Treatment failure at D29 was defined as presence of parasites at the D29 assessment, or no clinical response to treatment requiring rescue medication on or before D29. Treatment was extended or rescue medication was provided after D29 based on parasitological examination and clinical improvement. Once the patients had a negative parasitology result, they started a follow-up period of one year (up to D390) to assess long-term relapse-free survival and safety. Rescue therapy was also given to all patients who had a confirmed VL relapse during follow-up (for more details, see [16]). After exclusion of 2 patients due to a technical error in the RNA isolation process, 28 VL-HIV patients were included in the herein described analyses (Fig. 1).

2.2. Ethics approval and consent to participate

The study protocol was approved by the University of Gondar Institutional Review Board, the Ethiopian National Research Ethics Review Committee, the Médecins Sans Frontiers Ethics Review Board, the London School of Hygiene and Tropical Medicine Research Ethics Committee, the Antwerp University Hospital Ethics Committee, the Prince Leopold Institute of Tropical Medicine Institutional Review Board and for this specific study by the University of York Biology

Ethics Committee. All patients provided informed consent and the study was carried out in accordance with international guidelines (Helsinki declaration, Good Clinical Practices and local regulations).

2.3. RNA isolation and sequencing

Whole blood was collected and stabilized in 2.5 mL PAXgene Blood RNA tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland) and stored at −80 °C. Isolation and purification of total RNA was performed using the PAXgene blood RNA kit (PreAnalytiX GmbH, Hombrechtikon, Switzerland) according to the manufacturer's instructions. Next, messenger RNA was enriched by depleting ribosomal and globulin RNA (Globin-zero gold removal kit, Illumina Inc, San Diego, USA). RNA concentrations were measured with NanoDrop 1000 Spectophotometer (NanoDrop Technologies, USA) and RNA integrity using an Agilent 2100 Bioanalyzer (Nano kit, Agilent, CA, US). Subsequently, cDNA amplification, adaptor ligation and indexing were carried out on 1 μg of total RNA by using TruSeq stranded mRNA library preparation (Illumina Inc, San Diego, USA). Libraries were sequenced on an Illumina NextSeq500 instrument (single-end, 75 bp) using 1.2 pM and 1.89% PhiX with a total of 4 runs and an average coverage of 19.3 million reads per sample. 98% of the trimmed passed-filter reads mapped against the human genome.

2.4. Mechanistic approach

Longitudinal and inclusive DGE analyses (FDR corrected p-value $\leq$ 0.05 and $a \geq$ 1.5 absolute fold difference) between the timepoints D0 and D29 were performed for both the treatment success and failure group, based on a paired generalized linear model with CLC Genomic Workbench software V12 (Qiagen Bioinformatics). Next, the pre-ranked feature within Gene Set Enrichment Analysis (GSEA) software v.3 (BROAD institute, California, USA) was used to determine enriched gene sets in the treatment groups. The well-defined hallmark, curated canonical and gene ontology datasets from the Molecular Signature Database (MsigDB) were used for the enrichment analyses. In addition, previously described whole blood transcription modules (BTMs) were evaluated [17,18]. Gene sets were considered significant if their false discovery rate (FDR) was less than 25%. Default parameters were used with 1000 permutations and the conservative classic enrichment statistics was used for calculation of the enrichment score (ES). Gene sets that did have not a minimum of 10 genes shared with the pre-ranked gene list were excluded from the analyses, and normalized enrichment scores were depicted. Leading edge analyses were run on the GSEA results to identify core group genes.

Non-redundant biological terms were visualized in a functionally grouped network by means of the ClueGo plug-in v2.5.4 for Cytoscape v.3.7.1 [19,20]. Enrichment/depletion analyses were carried

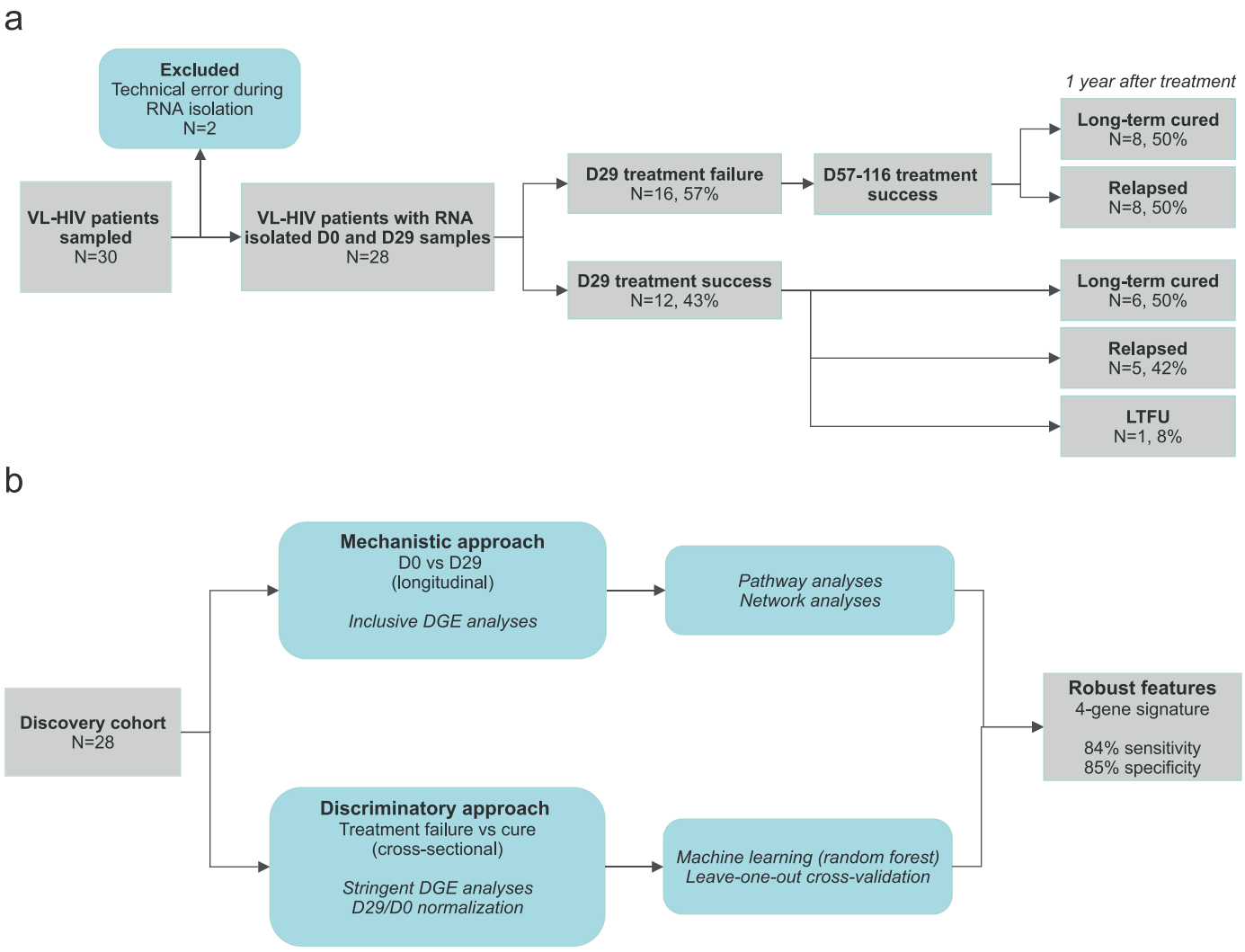

Fig. 1. Flow charts of study participants and study design. (a) Flow chart of inclusion and stratification by treatment outcomes of study participants (b) Lay-out of the study design and generation of 4-gene signature. *LTFU: lost to follow-up, D: Day of treatment, VL-HIV: visceral leishmaniasis-HIV co-infection, DGE: differential gene expression.*

out with a two-sided hypergeometric test for datasets from the EBI-UniProt GO, KEGG, REACTOME and WikiPathways databases. All genes were recognised and 45 (11.3%) were not functionally annotated. The applied kappa statistics for connectivity was kept at the default value of 0.4. GO term fusion was selected to reduce redundancy. In addition, only terms with a *p*-value of <0.100 were shown, as well as a GO tree interval level of 3 to 8 (medium to detailed network specificity). The autoannotate plug-in was used for cluster delineation and naming.

2.5. Discriminatory approach

A cross-sectional and stringent machine learning approach was designed. The count table was normalized for the 'per sample' sequencing depth using the DESEQ2 package in R. Genes that had a read count of zero in 80% of the samples were removed. The D29/D0 expression values were used as input for the machine learning approach to correct for patient specific expression patterns. We adopted a random forest (RF) classification with leave-one-out cross-validation. Within each cross-validation loop, a feature selection was first applied on the remaining samples, only selecting the differentially expressed genes between treatment cure and failure based on a more stringent DGE analyses (FDR corrected p-value ≤ 0.01 and $a \geq 2$ absolute fold difference) to target a limited gene set of highly discriminatory genes. These DEGs were then used to construct the RF classifier. The number of trees, with a maximum depth of 4 and equal minimal number of samples in each leaf, was limited to 100. The relative importancies of the genes in the forest decision making were stored if the left-out sample was correctly predicted. After summing the classifier importancies for each gene from every successful loop, the top 20 genes were depicted. The final 4-gene RF classifier was constructed similarly, but to reduce overfitting on the smaller training sets during bootstrapping (see 'Statiscal and visual analyses'), we applied a maximum tree depth of 3 and a minimal number of 3 samples in each leaf. All these analyses were carried out using the sklearn package in Python v3.7.0 [21].

2.6. Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Predesigned PrimeTime®qPCR assays (Integrated DNA Technologies (IDT), Iowa, USA) were used for human gene expression analysis of HRH4 (Hs.PT.58.4403324), IL10 (Hs.PT.58.2807216), PRSS33 (Hs.PT.58.3087623), SLFN14 (Hs.PT.58.993231) and the reference gene TBP (Hs.PT.58v.39858774) (*File S2*). The recommended assay with the highest algorithm score was selected for each gene. After read counts were normalized via DESEQ2, TBP was selected as reference gene based on lowest maximal deviation between samples, lowest coefficient of variation, lowest LOG2FC change, commercial availability and literature review. Previous studies have validated TBP as a reliable reference gene for qPCR [22,23] and it was detected in all study samples.

Samples with sufficient left-over volume to reach an equal amount of 0.5 μg in every RNA extract were included (Fig. S2*)*. Both, cDNA synthesis with iScript cDNA synthesis Kit (BioRad laboratories, California, USA) and qPCR were performed on the QuantStudio 5 real-time PCR system (Applied Biosystems, ThermoFisher, Waltham, MA USA). The PrimeTime gene expression master mix with low passive reference dye (IDT) was used. To reduce technical variation, all samples from 1 patient were run in duplicate for the four genes and reference gene on one plate simultaneously. As the efficiencies were almost all ranging between 90−110%, we used the comparative Ct-value method ($\Delta\Delta$Ct) for relative quantification between the timepoints. Individual LOG2FC between D29 and D0 were calculated based on CPM values or Ct-values for the RNA sequencing or RT-qPCR results, respectively.

2.7. Statistical and visual analyses

For patient characteristics (Table 1), continuous data was represented as medians with interquartile ranges and categorical data as numbers and frequency. Statistical significance between treatment success and failure patients was determined with the non-parametric Mann-Whitney U test for continuous variables and two-sided Fisher's exact test for categorical variables. *P*-values < 0.05 were considered significant. The ROC curve of the final 4-gene classifier was generated using the sklearn package in Python. The AUC, sensitivity and specificity were calculated over 1000 bootstrap iterations. For each iteration, the dataset was split in four parts (sklearn, StratifiedKFold), of which three parts were used for training and one part for testing. Principal component analysis (PCA) scatter plots, heatmaps (Euclidean distance, complete linkage), venn diagrams and volcano plots were created with the CLC Genomic Workbench software V.12 (Qiagen Bioinformatics). Violin plots were created with Graphpad Prism v8.0.1 (GraphPad Software, San Diego, USA).

3. Results

Nested in a clinical trial, 30 VL-HIV patients underwent additional whole blood sampling. After exclusion of two patients due to technical error, a total of 28 VL-HIV patients were included in the analyses (Fig. 1a). Twelve (43%) patients reached parasitological cure after 1 cycle of treatment and 16 required extended or rescue treatment. At admission, half of the cases had a history of VL and 82% were on antiretroviral therapy (ART) (Table 1). In both treatment outcome groups, a similar CD4 reconstitution of around 110 cells/μL was observed after 29 days of VL treatment compared to baseline. Except for a higher percentage of viral controllers in the treatment failure group (possibly linked to the higher number of people on ART), no significant differences in patient characteristics nor treatment regimens could be observed between the treatment success and failure group. These findings indicate that HIV-related markers were not associated with VL treatment outcome and that despite a successful suppression of the viral load or CD4 T cell recovery, VL treatment failure still occurred and that vice versa, non-controlled HIV patients were able to clear the infection.

3.1. Distinct gene expression changes in treatment success and failure patients

We first used principal component analysis (PCA) to reduce the dimensionality of the data and determine whether patients could be clustered. However, after pooling all the samples, the first principal component only explained 11.9% of the total variance in the dataset and this was associated with a lower read count in one of the samples (Fig. S1).

To account for this high variability in transcript abundance between individual VL-HIV patients, possibly due to diverse HIV or VL histories, genetic backgrounds, nutritional status and a high presence of comorbidities (>80%, Table 1), we performed longitudinal paired analyses of intra-individual changes in gene expression levels between Day 0 (D0) and D29 (using the mechanistic approach described in Methods, Fig. 1b). Differential gene expression (DGE) analyses between D0 and D29 resulted in distinct transcriptomic patterns by treatment outcome, with 397 and 194 unique DEGs for the treatment success and failure group, respectively (*Fig. 2a, File S1 for complete lists*). Only 142 were common DEGs between the groups, of which only one (*CTD-2545M3.6*) encoding an uncharacterised protein was regulated in the opposite direction in the groups. An almost equal distribution of up- (n = 218/397, 55%) and down- (n = 179/397, 45%) regulated genes was observed during treatment success, while in the treatment failure group less genes significantly changed in expression level and their expression was mainly downregulated

Table 1
Patient characteristics before and after treatment, stratified by treatment outcome.

	Total (n = 28)	Success (n = 12, 43%)	Failure (n = 16, 57%)	p-value
Demographic characteristics				
Age, median (1–99%)	34 (27–45)	33 (28–45)	34 (27–44)	0.815
Men, n (%)	28 (100)	12 (100)	16 (100)	1.000
Clinical characteristics at D0				
Treatment regime, n (%)				0.491
AmBisome	9 (32.1)	3 (25)	6 (37.5)	
AmBisome + Miltefosine	19 (67.9)	9 (75)	10 (62.5)	
Primary VL, n (%)	14 (50)	5 (41.7)	9 (56.3)	0.704
Site of parasite detection, n (%)				0.141
Spleen (+1 to +2)	1 (4)	1 (8)	0 (0)	
Spleen (+3 to +6)	26 (92)	11 (92)	15 (94)	
Bone marrow	1 (4)	0 (0)	1 (6)	
On ART, n (%)	23 (82.1)	8 (66.7)	15 (93.8)	0.133
HIV viral load, n (%)				0.049
Undetectable	8 (29)	1 (8)	7 (44)	
>20–10,000 copies/mL	7 (25)	3 (25)	4 (25)	
>10,000 copies/mL	13 (46)	8 (67)	5 (31)	
CD4 count, median (1–99%)	47 (11–159)	44.5 (11–159)	61.5 (15–152)	0.516
Concomitant diseases, n (%)	23 (82.1)	10 (83.3)	13 (81.3)	1.000
Platelet count (x$10^3/\mu$l), median (1–99%)	100 (47–195)	116 (47–181)	98 (55–195)	0.693
Clinical characteristics at D29				
Site of parasite detection, n (%)				NA
Spleen (+1 to +2)	6 (21)	NA	6 (37.5)	
Spleen (+3 to +6)	6 (21)	NA	6 (37,5)	
Bone marrow	4 (14)	NA	4 (25)	
CD4 count, median (1–99%)	155 (20–341)	153 (20–285)	171 (42–341)	0.807
Platelet count (x$10^3/\mu$l), median (1–99%)	221 (87–701)	206 (115–464)	235 (87–701)	0.710

VL: visceral leishmaniasis, ART; antiretroviral therapy, HIV: human immunodeficiency virus, D: day of treatment, NA: not applicable.

(n = 166/194, 86%) (Fig. 2a). This observation was also reflected in corresponding unsupervised hierarchical clustering of the expression data (Fig. 2b&c). In the treatment success group, all samples were distinctly clustered by timepoint and showed a reversed pattern at D29 (Fig. 2b). In the treatment failure group, no primary clustering by timepoint could be observed with a more scattered pattern (Fig. 2c), indicating no mutual delineated impact of VL treatment on host transcriptomic profile. Altogether, these results suggest that significant changes take place in the abundance of blood transcripts during successful VL treatment and that the patterns of gene expression depend on the treatment efficacy.

3.2. Functional annotation of unique gene transcripts underlying successful VL treatment

To better understand the ontology and function of the 397 unique genes that showed significant alterations in their expression levels during successful VL treatment, we assessed a total of 6161 published gene sets across 4 different databases for significant enrichment. In total, 77 biological GO terms, 12 canonical pathways, 8 hallmark gene sets and 5 B.M. were enriched (Fig. 3). Despite more than 200 upregulated genes in the treatment success cases, enriched gene sets across the four different databases showed a robust downregulation of pathways by D29, in particular those linked to cell cycle, apoptosis, proteolysis and adaptive immune response processes. The top 8 most influential genes that were part of the core enrichment group in more than 35 gene sets, included different alpha subunits of the proteasome alpha (*PSMA6, PSMA2, PSMA3, PSMA4*), one subunit of the proteasome 26 s (*PASMD14*), cyclin-dependant kinase 1 (*CDK1*), interferon-γ (*IFNG*) and interleukin 6 (*IL6*). In contrast, no significant enrichments could be observed within the 194 unique DEGs from the failure group, consolidating the observation of no clear delineated impact of VL treatment on host pathways amongst patients with treatment failure after one month of treatment.

To obtain insight in the correlations between enriched terms and define overarching pathways or processes, a functionally grouped network of enriched categories was generated that showed 2 distinct clusters (Fig. 4). The main cluster was downregulated and consisted of cell cycle features (green), including (stem) cell differentiation, cell division, antigen presentation, cell proliferation and related catabolic or proteolytic processes. The second cluster was upregulated and covered features of phagolysosomal activity to kill or stop the growth of fungal and microbial organisms (purple), including G-protein coupled receptor signalling and vacuolar activity with primary (azurophilic) granules loaded with defesins and serine proteases.

These findings were indicative of a clear remission of active disease in successfully treated patients. The data indicated a distinct and profound downregulation of the host cellular activity and immunity and suggested clearance of the parasite by antimicrobial peptides in phagolysosomes.

3.3. Gene set to accurately distinguish treatment outcome at D29

After having established that the whole blood transcriptome markedly changed during the course of treatment and reflected relevant biological processes, we investigated whether this transcriptomic change could be used to accurately determine the treatment outcome at D29. To answer this question, a machine learning approach was adopted that compared D0/D29 transcript abundance between treatment success and failure patients (using the discriminatory approach described in the Methods, Fig. 1b). To avoid overfitting, the random forest (RF) classifiers were evaluated by leave-one-out cross-validation. In total, 70% of the constructed classifiers could correctly predict the outcome of the left-out patient. The top 20 genes with the highest summed feature importance in the tree decision making over all correctly classifying classifiers were kept and included several pseudogenes and novel transcripts (Table 2, first column). To increase the biological relevance and robustness of selected genes, these 20 genes were subsequently compared with the list of DEGs from the mechanistic approach (D0 vs D29) for overlap (Table 2). Four genes were found to be shared with the treatment success group, but none with the failure group.

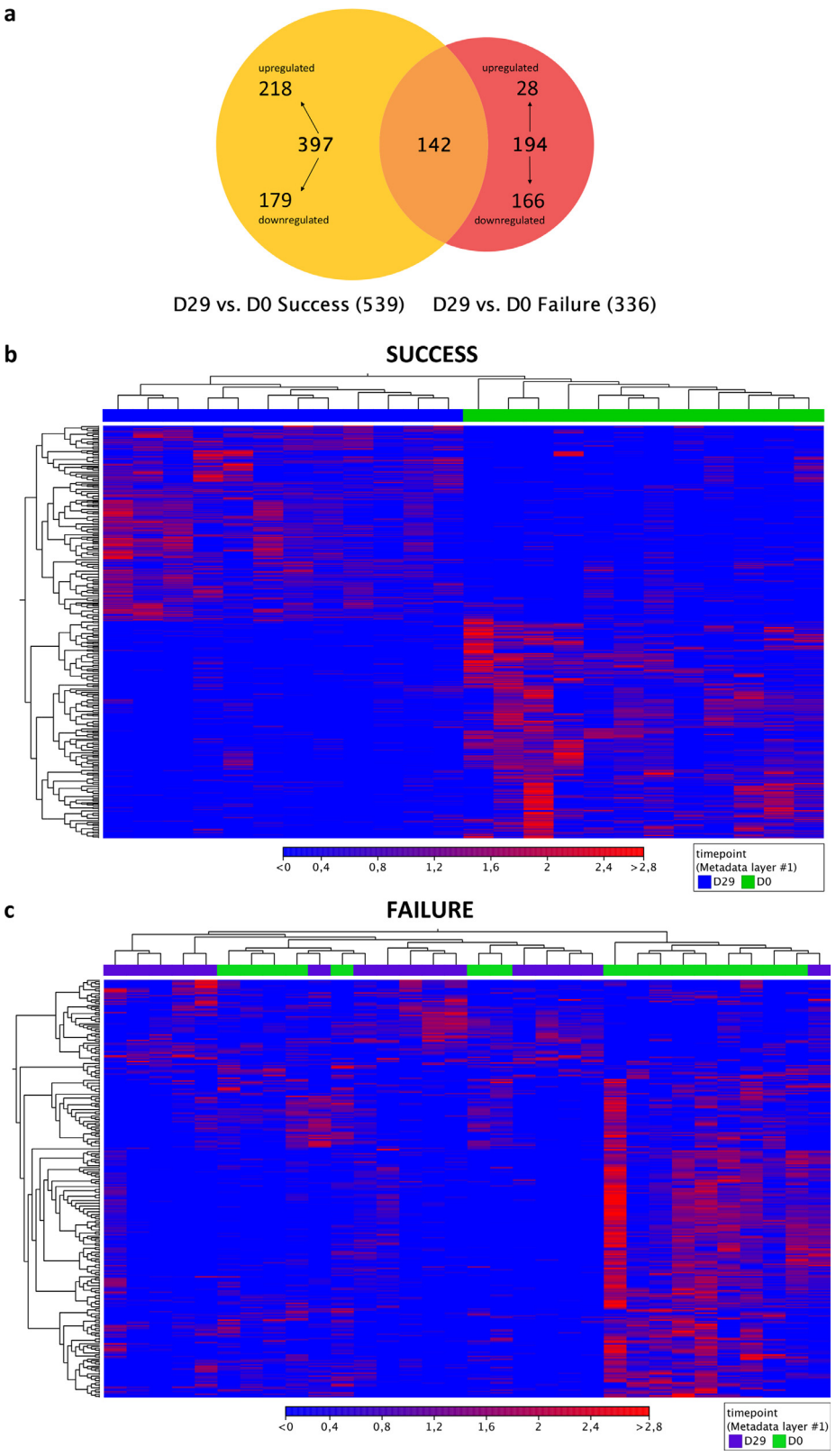

Fig. 2. Distinct patterns of differentially expressed genes (DEGs) in treatment success and failure patients. (a) Overlap of differentially expressed genes (DEGs) between D0 and D29 for treatment success and failure patients. Venn diagram showing the number, uniqueness and directionality of the DEGs (>1.5 absolute fold change with FDR *p*-value <0.05 – see mechanistic approach in materials and methods) between D0 and D29 for treatment success (orange, *n* = 12) and failure cases (red, *n* = 16) (b) Unsupervised clustering of differentially expressed genes (DEGs) in treatment success and (c) failure patients. Heat maps showing the z-scores (bottom scale) or extent of up (red) or downregulation (blue) of the DEGs (Y-axis) at D0 (green samples) and D29 (purple samples) for all individual treatment success patients (*n* = 12) and all individual treatment failure patients (*n* = 16). Unsupervised hierarchal clustering of the samples was applied and based on Euclidean distance and complete linkage. *D: Day of treatment.*

GO terms: biological process	NES	Size
CELL CYCLE	-3,79	44
CELL CYCLE PROCESS	-3,72	39
MITOTIC CELL CYCLE	-3,57	33
ORGANELLE FISSION	-2,89	22
MITOTIC NUCLEAR DIVISION	-2,86	19
NUCLEAR CHROMOSOME SEGREGATION	-2,73	10
SISTER CHROMATID SEGREGATION	-2,71	10
CHROMOSOME SEGREGATION	-2,71	13
REGULATION OF CELL CYCLE	-2,63	31
CHROMOSOME ORGANIZATION	-2,60	24
CELL DIVISION	-2,41	20
DNA CONFORMATION CHANGE	-2,37	12
REGULATION OF DNA METABOLIC PROCESS	-2,34	11
POSITIVE REGULATION OF CELL DEATH	-2,34	16
NEGATIVE REGULATION OF TRANSPORT	-2,24	11
REGULATION OF CELL CYCLE PROCESS	-2,23	19
REGULATION OF PROTEIN MODIFICATION BY SMALL PROTEIN ...	-2,20	13
POSITIVE REGULATION OF PROTEOLYSIS	-2,18	15
POSITIVE REGULATION OF CELLULAR PROTEIN CATABOLIC ...	-2,18	11
REGULATION OF PROTEOLYSIS	-2,16	26
POSITIVE REGULATION OF CATABOLIC PROCESS	-2,16	15
RESPONSE TO ALCOHOL	-2,15	12
NEGATIVE REGULATION OF CELL CYCLE	-2,11	13
REGULATION OF CELL CYCLE PHASE TRANSITION	-2,11	12
POSITIVE REGULATION OF PROTEIN CATABOLIC PROCESS	-2,08	14
NEGATIVE REGULATION OF PROTEIN MODIFICATION PROCESS	-2,08	20
NEGATIVE REGULATION OF PROTEIN METABOLIC PROCESS	-2,07	30
REGULATION OF CELLULAR PROTEIN CATABOLIC PROCESS	-2,04	14
REGULATION OF MITOTIC CELL CYCLE	-2,04	17
CELL CYCLE PHASE TRANSITION	-2,03	12
REGULATION OF CATABOLIC PROCESS	-2,02	24
REGULATION OF PROTEIN LOCALIZATION	-1,99	22
REGULATION OF PROTEIN CATABOLIC PROCESS	-1,98	19
REGULATION OF INNATE IMMUNE RESPONSE	-1,97	14
REGULATION OF CELL DEATH	-1,96	40
ESTABLISHMENT OF PROTEIN LOCALIZATION	-1,91	15
REGULATION OF CELLULAR PROTEIN LOCALIZATION	-1,89	16
NEGATIVE REGULATION OF TRANSFERASE ACTIVITY	-1,88	16
REGULATION OF PROTEIN SECRETION	-1,88	11
ACTIVATION OF IMMUNE RESPONSE	-1,86	18
ANTIGEN PROCESSING AND PRESENTATION OF PEPTIDE ANTIGEN	-1,86	10
INNATE IMMUNE RESPONSE	-1,84	23
REGULATION OF CELLULAR LOCALIZATION	-1,80	31
RESPONSE TO CYTOKINE	-1,80	34
POSITIVE REGULATION OF DEFENSE RESPONSE	-1,77	15
ESTABLISHMENT OF LOCALIZATION IN CELL	-1,77	34
PROTEIN LOCALIZATION	-1,77	23
NEGATIVE REGULATION OF CELL COMMUNICATION	-1,76	26
REGULATION OF TRANSFERASE ACTIVITY	-1,76	40
PROTEIN CATABOLIC PROCESS	-1,75	14
ANTIGEN PROCESSING AND PRESENTATION	-1,73	12
POSITIVE REGULATION OF IMMUNE RESPONSE	-1,72	24
CELLULAR MACROMOLECULE LOCALIZATION	-1,69	22
CELLULAR MACROMOLECULAR COMPLEX ASSEMBLY	-1,66	12
PROTEIN LOCALIZATION TO ORGANELLE	-1,65	10
IMMUNE RESPONSE	-1,63	53
REGULATION OF PROTEIN MODIFICATION PROCESS	-1,63	57
NEGATIVE REGULATION OF NITROGEN COMPOUND METABOLIC...	-1,63	29
REGULATION OF GROWTH	-1,62	22
MUSCLE ORGAN DEVELOPMENT	-1,61	10
REGULATION OF T CELL PROLIFERATION	-1,61	10
REGULATION OF ORGANELLE ORGANIZATION	-1,61	21
PROTEOLYSIS	-1,60	28
REGULATION OF CHROMOSOME ORGANIZATION	-1,59	11
NEGATIVE REGULATION OF CYTOKINE PRODUCTION	-1,58	12
DNA METABOLIC PROCESS	-1,58	16
RESPONSE TO DRUG	-1,54	17
MACROMOLECULE CATABOLIC PROCESS	-1,54	22
CELLULAR RESPONSE TO STRESS	-1,50	30
CELLULAR RESPONSE TO CYTOKINE STIMULUS	-1,50	31
POSITIVE REGULATION OF BIOSYNTHETIC PROCESS	-1,49	47

Hallmarks	NES	Size
E2F TARGETS	-2,92	17
G2M CHECKPOINT	-2,76	17
MYC TARGETS V1	-2,70	17
INTERFERON GAMMA RESPONSE	-2,51	12
TNFA SIGNALING VIA NFKB	-1,74	11
MITOTIC SPINDLE	-1,69	10
MTORC1 SIGNALING	-1,63	12
HYPOXIA	0,78	10
Canonical pathways	**NES**	**size**
CELL CYCLE MITOTIC	-2,60	18
CELL CYCLE	-2,52	20
DNA REPLICATION	-2,45	12
MITOTIC M M G1 PHASES	-2,37	11
G1 S TRANSITION	-2,14	10
MITOTIC G1 G1 S PHASES	-2,13	10
IMMUNE SYSTEM	-2,02	38
CYTOKINE SIGNALING IN IMMUNE SYSTEM	-1,94	14
METABOLISM OF AMINO ACIDS AND DERIVATIVES	-1,57	11
CLASS I MHC MEDIATED ANTIGEN PROCESSING...	-1,47	10
ADAPTIVE IMMUNE SYSTEM	-1,40	17
GPCR LIGAND BINDING	1,36	10
Blood transcriptional modules	**NES**	**size**
M4.0 - CELL CYCLE AND TRANSCRIPTION	-3,53	37
M4.1 - CELL CYCLE (I)	-3,51	25
M4.5 - MITOTIC CELL CYCLE IN STIMULATED CD4 T ...	-2,07	10
M11 - ENRICHED IN MONOCYTES (II)	-1,23	10
M37 - IMMUNE ACTIVATION - GENERIC CLUSTER	1,43	18

Fig. 3. Enriched gene sets in the treatment success group (n = 12). Normalized enrichment scores for hallmarks, GO terms and canonical pathways from the MsigDB database and previously described blood transcriptional modules are depicted in blue (downregulated) or red (upregulated) scale. The size column represents the number of genes from the respective gene set found in the expression dataset. *NES: normalized enrichment score.*

Next, a new RF classifier was built to assess whether the combination of these 4 genes alone was able to accurately determine the treatment outcome. Bootstrapping this classifer a 1000 times (each iteration one quarter of dataset was used for testing) showed a mean area-under-the-ROC-curve (AUC) of 0.95 (95% CI: 0.75−1.00) - with a mean sensitivity of 84% (95% CI: 83−85) and specificity of 85% (95% CI: 83−86) - to correctly distinguish treatment failure from success cases (Fig. 5). We validated the findings by reverse transcription

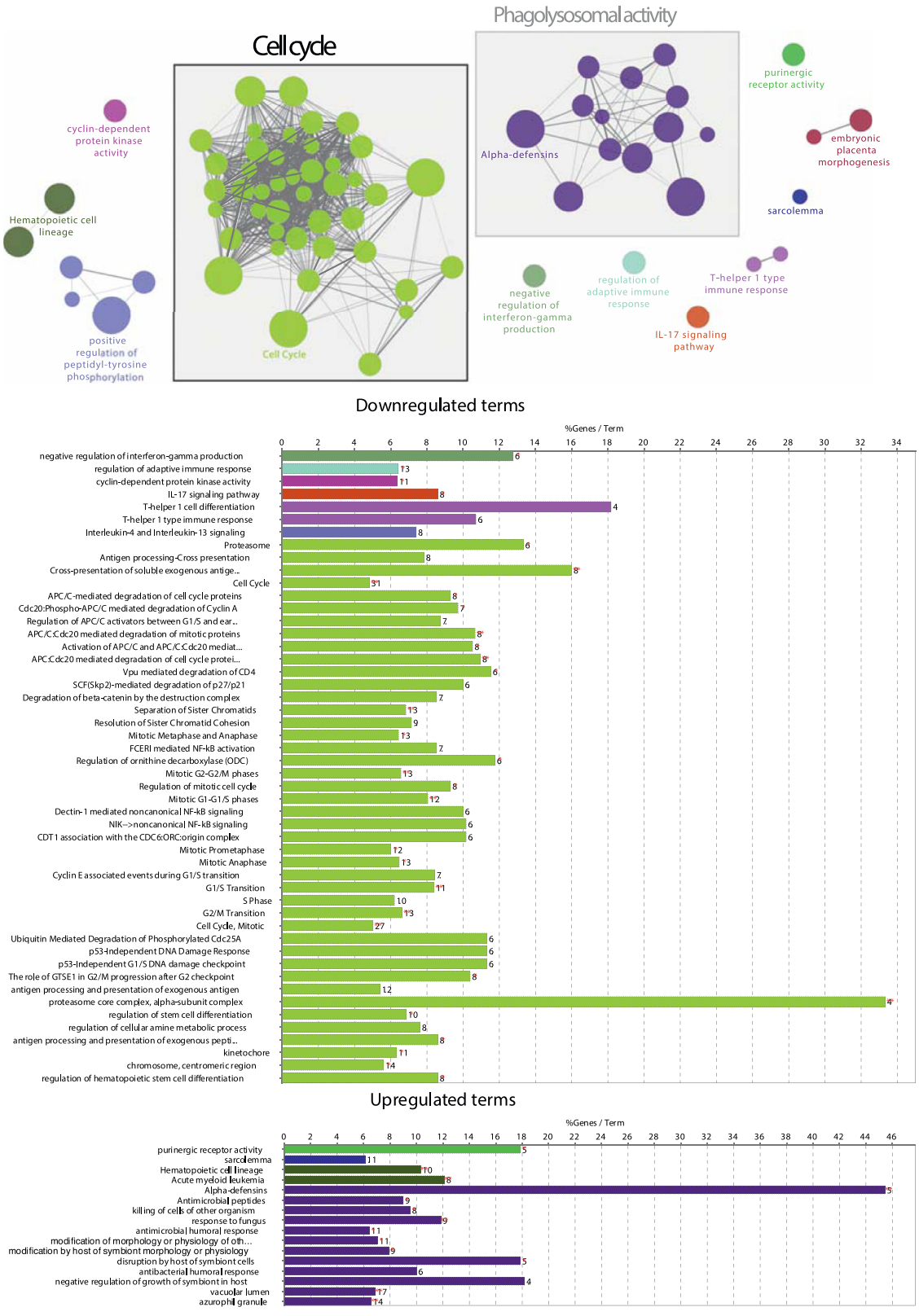

Fig. 4. Functionally grouped network analyses of enriched gene sets in the treatment success group (n = 12). Functionally related clusters of enriched gene sets are represented by different colours and the label of the most significant term per cluster is shown. The node size represents the term enrichment significance and the connectivity by grey lines (kappa statistics 0.4). Similar terms were fused to reduce redundancy. Bottom tables showed the directionality of significant terms and the frequency of genes from the respective terms detected in the expression dataset. The exact number of genes detected is indicated behind each bar.

quantitative polymerase chain reaction (RT-qPCR) and showed similar distribution patterns in $\log_2$(foldchange) (LOG2FC) changes for all 4 genes (Fig. S2 and File S2). Due to the lack of follow-up samples in the 16 treatment failure patients, we could not investigate whether these patients also expressed the 4-gene signature when reaching parasitological cure after extended treatment (Fig. 1a). We did,

Table 2
Comparison of differential gene expression approaches and identification of the 4-gene set.

Discriminatory Approach		Mechanistic Approach	Gene details				
DEG (success vs failure)		DEG (D0 vs D29)					
Gene symbol	Relative importance in RF classifiers	Shared gene (treatment group)	Protein	Function	Cell specificity	Associated enriched gene sets	Regulation at D29 (LOG2FC)
MAN1C1	0.158	No					
PRSS33	0.138	Yes (success)	Serine protease 33	Amidolytic activity	Predominantly expressed in macrophages and peripheral leukocytes	Proteolysis; protein kinase signalling; Ubiquitin/proteasome pathway	Upregulated (3.31)
AP001830.1	0.105	No					
FAM153B	0.088	No					
BCAS1	0.068	No					
NA	0.061	No					
AC007611.1	0.055	No					
HRH4	0.051	Yes (success)	Histamine receptor H4	Binds to histamine in peripheral tissue and mediates inflammation	Predominantly expressed on haematopoietic cells	Purinergic (G-protein coupled) receptor activity; inflammatory response	Upregulated (2.6)
NAV1	0.049	No					
VLDLR	0.042	No					
IL10	0.039	Yes (success)	Interleukin 10	Cytokine with pleiotropic effects on immunoregulation and inflammation. Downregulates Th1 cytokines and antigen presentation	Primarily produced by monocytes and lymphocytes	negative regulation of antigen presentation; negative regulation of IFN-γ production; Type 2 immune response; NF-κB signalling; defence response to protozoa/bacteria	Downregulated (−2.45)
KRT7	0.033	No					
AC135068.2	0.031	No					
AL390198.2	0.023	No					
H1F0	0.018	No					
LINC00205	0.017	No					
GEMIN7-AS1	0.011	No					
SLFN14	0.01	Yes (success)	Schlafen family member 14	Important role in platelet formation and function (endoribonuclease)	Abundant in immune cells, located in the nucleus	Regulation of DNA/protein metabolic and catabolic processes	Upregulated (1.74)
KLHL13	0.002	No					
AC093159.1	<0.001	No					

DEG: differential gene expression, RF: random forest, LOG2FC: $\log^2$(fold change), Th1: T-helper 1, IFN-γ: interferon-γ, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B-cells.

however, observe that three out of the four genes were also differentially expressed in the 13 VL-HIV patients with long-term cure (cf. no relapse) at 6 months after treatment end (Fig. S3a), of which eight were treatment failure cases at D29. In agreement, the four genes could not be detected in treatment failure patients at D29 with low parasite burden (1+,2+) in splenic aspirates, indicating a true cure signature that reflected disease remission after an apparent complete resolution of the parasite.

With regard to long-term cure prediction, distinct patterns at D29 could be observed in gene expression levels of successfully treated patients who relapsed in the following year (n = 5, 42%) and those who did not (Fig. 1). Yet, our 4-gene set was mostly found in the common DEGs between long-term cured and relapsed patients, except for *SLFN14*, indicating little value in relapse prediction (Fig. S3b). The relapsed patients overlapped for 61% with the failure group at D29, compared to only 30% with the long-term cured patients. These findings argue for the investigation of a predictive signature at D29 to predict relapse development in the next 6−12 months, but patient numbers were too small to perform robust analyses.

4. Discussion

In this study, we investigated the whole blood transcriptome of 28 well-characterized Ethiopian VL-HIV patients before and after 1 month of VL treatment. We observed a distinctive pattern of disease remission in successfully cured patients and the complete lack of it in treatment failure cases. Subsequently, we identified a 4-gene signature able to discriminate treatment success at D29 with a sensitivity of 84% and specificity of 85%. Application of this signature as a low-invasive tool to assess treatment efficacy in VL-HIV patients could have significant value in guiding patient management and future R&D.

Due to a possible homoeostatic mechanism to control persistent infection-induced inflammation in active VL, elevated levels of the regulatory cytokine IL-10 that significantly decreased following successful treatment, have been reported in numerous animal and human studies [24,25]. This marked downregulation of *IL10* transcription in successfully treated patients was strongly recognized in our 4-gene signature. This is, however, in contrast with the two previous transcriptomic studies in VL patients that did not find a significant regulation of *IL10* [12,13]. While we cannot rule out that our signature is HIV-specific, our longitudinal approach that accounts for natural variation in gene transcription between patients and the use of RNAseq instead of microarray, could have contributed to detecting these associations. Likewise, the other three genes (*HRH4, PRSS33* and *SLFN14*) have not been reported in the previous two studies.

In addition, we could not confirm the *IFNG* gene as the major regulator gene as reported in previous transcriptome studies of VL patients [12]. Although the *IFNG* gene and pathways were also strongly downregulated in treated VL cases compared to active cases, we rather observed a prominent upregulation of vacuolar activity with primary (azurophilic) granules loaded with defesins and serine proteases. These genes and terms suggested an enhanced phagocytosis, neutrophil involvement and complement activation; and may reflect the proteolytic degradation responsible for parasite clearance. This process was reflected in the 4-gene signature by the marked upregulation of the amidolytic activity of serine protease 33. Its predominant expression in peripheral macrophages and highest discriminatory importance in the classifier supports future studies into the role of *PRSS33* in phagolyosomal degradation of *Leishmania* parasites.

It has been postulated that the interactions of Leishmania spp. with eosinophils and mast cells influence the macrophage response to infection and the development of an adaptive immune response [26]. An important mediator in this process is the histamine receptor H4, also designated as the 'immune system histamine receptor' due to its

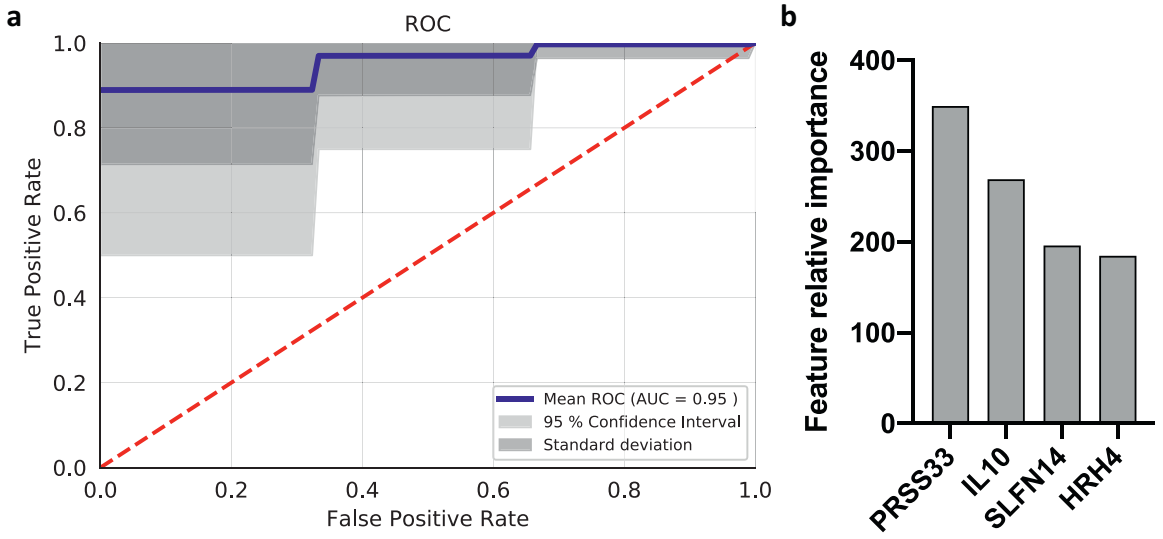

Fig. 5. Receiver operator curves (ROC) for the final random forest classifier based on the 4-gene set. (a) Showing the **average AUC value of the 4-gene random forest classifier, calculated over 1000 bootstrap replicates.** (b) Bar chart showing the sum of relative importancies in the classifier over 1000 bootstrap replicates.

chemotactic properties (eosinophil migration and mast call chemotaxis) and involvement in dendritic cell activation and T-cell differentiation. Because histamines have been described to have an assisting role in vitro clearance of *Leishmania* infection [26], the observed upregulation of *HRH4* in successfully treated patients could indicate clearance of parasites. Nevertheless, due to its importance in the 4-gene signature, the role of histamine, mast cells and its receptors on treatment efficacy in VL and VL-HIV patients merits further research. In particular because the H4 receptor is also being explored as a promising drug target in many chronic inflammatory disorders [27].

Defects in the *SLFN14* gene of the signature are associated with thrombocytopenia and excessive bleeding [28]. Likewise, the platelet count is known to be altered during VL infection due to splenic sequestration and often leads to bleeding tendency. Hence, the upregulation in *SLFN14* expression may reflect the described return of platelet counts to normal levels after successful cure. Yet, no clear correlation could be observed between *SLFN14* expression and recovery of platelet count after therapy in our study (Figure S4). It is of note that the gene was absent in the DEG list at D210 in long-term cured patients (Figure S4). Therefore, its importance should be validated as it could have been influenced by a small group of patients with severe thrombocytopenia. Our top 20 list from the discriminatory approach consisted of many pseudogenes and novel transcripts that also warrant further research, as they are not yet fully understood but are increasingly acknowledged as key contributors to immune responses [29].

Unfortunately, we could not study differences in treatment regimens due to sample size restrictions. Therefore, it remains unknown whether the broad antiproliferative activities of miltefosine contributed to the strong predominance of the downregulated cell cycle cluster in the network analyses. This predominance is however believed to reflect a cessation of the massive proliferation and differentiation of T and B cell clonotypes during the acute adaptive immune response, as similar results were observed in studies of immunocompetent VL patients in India and Brazil treated with amphotericin B (inciting cell wall disruption) and pentavalent antimonials (broad macromolecule inhibitor), respectively [12,14]. This would indicate it is not HIV-specific nor affected by the administered drugs. Nevertheless, a true mechanistic evaluation of affected organs with single cell evaluations should be studied to assess the causal and time relationship with drug modulation and parasite reduction. It is also to be noted that we could not observe enriched gene sets in the treatment failure group but noticed a significant decline in expression of many IgG-related genes (almost 40% of all DEGs). Many of these genes were also shared at D29 with the seemingly cured patients who relapsed within the next year (35% of common DEGs). This finding could suggest the initial presence of hypergammaglobulinemia at diagnosis as a risk factor for treatment failure or relapse, and merits further research.

Despite the high inter-patient variation in gene levels, we were able to construct a good classifier. We believe our methodology applied some good general practices that could be incorporated in future studies. Previous studies targeting limited transcriptomic gene sets for diagnosis or treatment outcome prediction in VL or even infectious diseases in general were mostly confined to cross-sectional comparisons at a single timepoint [9,10,30]. Such studies can be complicated by patient-to-patient variation in transcript abundance and are highly dependant on the stability of the used housekeeping gene for quantification in future validation studies with RT-qPCR. Therefore, we targeted intra-individual and relative changes in transcript abundance that are likely to enhance its generalizability but require both pre and post sampling. Additionally, purely statistical approaches were often adopted to select the best discriminatory genes. Such approaches can have the inherent danger of identifying genes with little biological relevance or interpretation, jeopardizing their validation in other patient cohorts [31]. Correspondingly, our top 20 list from the discriminatory approach consisted of many pseudogenes and functionally uncharacterized transcripts. By overlaying the machine learning results with mechanistic insight analyses, we believe the 4 genes represent relevant but distinct pathways that reflect underlying biological changes during treatment. When monitoring of treatment outcome is envisioned, the specificity of these pathways or genes to *Leishmania* infection is also less critical, compared to signature development for diagnosis or disease development prediction.

A limitation of this study is that the 4-gene set identified here is derived from data collected from a single, rather limited cohort of 28 male VL-HIV patients in a single geographic area in East Africa. Although it will be necessary to further validate this biomarker in larger cohorts of VL-HIV patients, and to include patients from other regions/countries, this should not detract from the value of such a biomarker. Even if restricted to use in East Africa, it would still be of high interest as the burden of VL-HIV is significant in this area. It will be of future interest to evaluate performance of this biomarker as the chemotherapeutic landscape alters, though the combination of AmBisome/miltefosine is the most effective treatment of VL-HIV patients

in East Africa [15] and our data suggested that the identification of the 4-gene signature was not merely a reflection of drug choice.

In the near future, a composite endpoint consisting of clinical signs, antigen detection methods and a host 4-gene biomarker could be a powerful, less-invasive tool for research and drug development (R&D) in leishmaniasis, both for immunocompetent and VL-HIV patients. In particular, future studies should assess the predictive value of the signature at earlier timepoints and evaluate the methodology required to develop a more point-of-care detection system. This may guide early case management, treatment recommendation and could facilitate early futility analyses and dose-finding studies of novel compounds. It is also to be noted that its value in similar parasitic infections could be studied, as rather general disease remission signatures were detected. Our results also encourage future investigations in VL relapse prediction by blood-based transcriptomic signatures. Yet other processes are likely to be predictive in parasite recrudescence and to be reflected in such a signature. However, the specificity of the signature to *Leishmania* will also become more relevant. For example, an extensively validated blood-based 3-gene signature recently satisfied the WHO criteria for a non-sputum-based triage test for tuberculosis across heterogeneous culture-confirmed datasets [30].

In conclusion, we have identified an easily accessible 4-gene blood signature with high discriminatory value to assess treatment efficacy at the end of treatment in VL-HIV patients. Application of this signature as a low-invasive tool to assess treatment efficacy in VL-HIV patients could have significant value in guiding patient management and future R&D.

Declaration of Competing Interest

The authors declare that there are no competing interests.

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Author's contribution

All authors read and approved the final version of the manuscript. Wim Adriaensen: Conceptualization, data curation, formal analysis, investigation, visualization, writing & editing Bart Cuypers: Formal analysis, methodology, writing, review & editing Carlota F. Cordero: Formal analysis Bewketu 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.

Availability of data and materials

The raw dataset supporting the conclusions of this article is available in the Sequence Read Archive repository, [PRJNA595895, http://www.ncbi.nlm.nih.gov/bioproject/595895]. Additional metadata are also available upon request to jvangriensven@itg.be for researchers who meet the criteria for access to confidential data.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2020.102748.

References

[1] Burza S, Croft SL, Boelaert M. Leishmaniasis. Lancet 2018;392(10151):951–70.
[2] World Health Organization. Surveillance of leishmaniasis in the WHO European region. Wkly Epidomiological Rec 2018;93(40):521–40.
[3] Diro E, Lynen L, Ritmeijer K, Boelaert M, Hailu A, van Griensven J. Visceral leishmaniasis and HIV coinfection in East Africa. PLoS Negl Trop Dis 2014;8(6):e2869.
[4] Lindoso JAL, Cunha MA, Queiroz IT, Moreira CHV. Leishmaniasis-HIV coinfection: current challenges. HIV AIDS (Auckl) 2016;8:147–56.
[5] Alvar J, Aparicio P, Aseffa A, Den Boer M, Canavate C, Dedet JP, et al. The relationship between leishmaniasis and AIDS: the second 10 years. Clin Microbiol Rev 2008;21(2):334–59 2008/04/11.
[6] Srivastava P, Dayama A, Mehrotra S, Sundar S. Diagnosis of visceral leishmaniasis. Trans R Soc Trop Med Hyg 2011;105(1):1–6.
[7] Pourabbas B, Moghadam AG, Pouladfar G, Rezaee Z, Alborzi A. Quantification of leishmania infantum kinetoplast DNA for monitoring the response to meglumine antimoniate therapy in visceral leishmaniasis. Am J Trop Med Hyg 2013;88(5):868–71.
[8] Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. Clin Microbiol Rev 2006;19(1):111–26 2006/01/19.
[9] Liu X, Speranza E, Muñoz-Fontela C, Haldenby S, Rickett NY, Garcia-Dorival I, et al. Transcriptomic signatures differentiate survival from fatal outcomes in humans infected with Ebola virus. Genome Biol 2017;18(1):4.
[10] Robinson M, Sweeney TE, Barouch-Bentov R, Sahoo MK, Kalesinskas L, Vallania F, et al. A 20-Gene set predictive of progression to severe dengue. Cell Rep 26(5), 1104−11. e4. https://doi.org/10.1016/j.celrep.2019.01.033.
[11] Thompson EG, Du Y, Malherbe ST, Shankar S, Braun J, Valvo J, et al. Host blood RNA signatures predict the outcome of tuberculosis treatment. Tuberculosis 2017;107:48–58.
[12] Fakiola M, Singh OP, Syn G, Singh T, Singh B, Chakravarty J, et al. Transcriptional blood signatures for active and amphotericin B treated visceral leishmaniasis in India. PLoS Negl Trop Dis 2019;13(8):e0007673.
[13] Salih MAM, Fakiola M, Lyons PA, Younis BM, Musa AM, Elhassan AM, et al. Expression profiling of Sudanese visceral leishmaniasis patients pre- and post-treatment with sodium stibogluconate. Parasite Immunol 2017;39(6):e12431.
[14] Gardinassi LG, Garcia GR, Costa CHN, Costa Silva V, de Miranda Santos IKF. Blood transcriptional profiling reveals immunological signatures of distinct states of infection of humans with leishmania infantum. PLoS Negl Trop Dis 2016;10(11).
[15] Diro E, Blesson S, Edwards T, Ritmeijer K, Fikre H, Admassu H, et al. A randomized trial of ambisome monotherapy and ambisome and miltefosine combination to treat visceral leishmaniasis in HIV co-infected patients in Ethiopia. PLoS Negl Trop Dis 2019;13(1):e0006988.
[16] Diro E, Edwards T, Ritmeijer K, Fikre H, Abongomera C, Kibret A, et al. Long term outcomes and prognostics of visceral leishmaniasis in HIV infected patients with use of pentamidine as secondary prophylaxis based on CD4 level: a prospective cohort study in Ethiopia. PLoS Negl Trop Dis 2019;13(2):e0007132.
[17] Chaussabel D, Quinn C, Shen J, Patel P, Glaser C, Baldwin N, et al. A modular analysis framework for blood genomics studies: application to systemic lupus erythematosus. Immunity 2008;29(1):150–64.
[18] Li S, Rouphael N, Duraisingham S, Romero-Steiner S, Presnell S, Davis C, et al. Molecular signatures of antibody responses derived from a systems biology study of five human vaccines. Nat Immunol 2014;15(2):195–204.
[19] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13(11):2498–504.
[20] Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, et al. ClueGO: a cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics 2009;25(8):1091–3.
[21] Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: machine learning in python. J Mach Learn Res 2011;12:2825–30.

[22] Nakayama T, Okada N, Yoshikawa M, Asaka D, Kuboki A, Kojima H, et al. Assessment of suitable reference genes for RT-QPCR studies in chronic rhinosinusitis. Sci Rep 2018;8(1).
[23] Watson S, Mercier S, Bye C, Wilkinson J, Cunningham AL, Harman AN. Determination of suitable housekeeping genes for normalisation of quantitative real time PCR analysis of cells infected with human immunodeficiency virus and herpes viruses. Virol J 2007;4.
[24] Rodrigues V, Cordeiro-da-Silva A, Laforge M, Silvestre R, Estaquier J. Regulation of immunity during visceral leishmania infection. Parasit Vectors 2016; 9:118.
[25] Nylén S, Sacks D. Interleukin-10 and the pathogenesis of human visceral leishmaniasis. Trends Immunol 2007;28(9):378–84.
[26] Rodríguez NE, Wilson ME. Eosinophils and mast cells in leishmaniasis. Immunol Res 2014;59(1–3):129–41.
[27] Thangam EB, Jemima EA, Singh H, Baig MS, Khan M, Mathias CB, et al. The role of histamine and histamine receptors in mast cell-mediated allergy and inflammation: the hunt for new therapeutic targets. Front Immunol 2018;9:1873.
[28] Fletcher SJ, Johnson B, Lowe GC, Bem D, Drake S, Lordkipanidzé M, et al. SLFN14 mutations underlie thrombocytopenia with excessive bleeding and platelet secretion defects. J Clin Invest 2015;125(9):3600–5.
[29] Atianand MK, Caffrey DR, Fitzgerald KA. Immunobiology of long noncoding RNAs. Annu Rev Immunol 2017;35(1):177–98.
[30] Warsinske H, Vashisht R, Khatri P. Host-response-based gene signatures for tuberculosis diagnosis: a systematic comparison of 16 signatures. PLoS Med 2019;16(4).
[31] Burel JG, Babor M, Pomaznoy M, Lindestam Arlehamn CS, Khan N, Sette A, et al. Host transcriptomics as a tool to identify diagnostic and mechanistic immune signatures of tuberculosis. Front Immunol 2019;10:221.

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Erratum

Erratum regarding previously published research papers

The following Author Contribution statements were not included in the published versions of the Research Papers that appeared in previous volumes of *EBioMedicine*. The appropriate Author Contribution statements are included below.

Celastrol-induced degradation of FANCD2 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 bioinformatical 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, YP, JD, ZL, QL, 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 study, including acquisition, analysis, and interpretation of data. CL, ZZY, and ZWS drafted the manuscript. 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 Rasal2 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 perihilar cholangiocarcinoma by blocking the FGFR-ERK signaling pathway and arresting the cell cycle. (*EBioMedicine* **50:** 166–177)

Author contributions: Q.B, C. TL, 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. DL 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, interpret 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)

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https://doi.org/10.1016/j.ebiom.2021.103295

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, Meiyun Wang. Collection and assembly of data: Qingxia Wu, Kuan Yao, Zhenyu Liu, Longfei Li, Xin Zhao, Shuo Wang, Honglei Shang, Yusong Lin, Zejun Wen. Development of methodology: Kuan Yao, Zhenyu Liu, Longfei Li, Shuo Wang, Yusong 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-TL, X-YH L-HL and S-LC collected patient samples and clinical data. Z-YW, Y-HP, L-YX, 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, Grazie 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, GCAR 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., R.R-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, LBG, 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, LBG 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, IF, 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, ML, RS, KH revised it for critical intellectual content; ML, SE, LG, GK, AB, IL, LP, CP, IF, 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-Boon 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.Te., 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.Ta. Regulatory approvals: S.Q., S.Te. Patient enrolment, consenting, ultrasound and clinical assessments: S.Q., S.Te., 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.Te., 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 Muyu 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. Qi Jia and Sujia Wu carried out prognosis 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 content of the final version. tumor immune cell infiltration and survival after platinum-based chemotherapy in high-grade serous ovarian cancer subtypes: A gene expression-based computational study. (*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 analysis, interpretation and drafted the manuscript. All authors contributed to critical revision of the final manuscript. RL 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:** 102,611)

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.E. 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., Y.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 JLH 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, TYT 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.I. and K.S. mainly conducted all 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.

Gut microbiota 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, ALP, 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, ALP, 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 performance the animal experiments, data analysis and manuscript drafting. HZ, CH, XMZ, YCZ, and RLG contributed to the performance of the experiments. 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 immunosuppression therapy. (*EBioMedicine* **52:** 102,657)

Authors contributions: Research idea and study design: Z-HL, G-TX, C-HZ, T-YC, E-YX, T-GC; data acquisition: Z-HL, C-HZ, T-YC, E-YX, T-GC, XL; data analysis/interpretation: Z-HL, C-HZ, T-YC, E-YX, T-GC, XL, YZ; statistical analysis: T-YC, E-YX,T-GC,YZ; supervision or mentorship: Z-HL, C-HZ, YQ, S-SL, FX, D-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)

Authors 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 HIPK2 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 and acquired, analysed and interpreted the 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, CG, 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 Yan 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.F.Z., 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., C.L.W., 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 CZ, 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 CZ, 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 SORBS2 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 ECYS developed the concept and design of this study. Dataset access was requested by HS. This author and YWW, and ECYS 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 ECYS independently assessed the eligibility criteria of reviewed studies. YWW and ECYS critically revised the drafted manuscript. HS and ECYS take responsibility for data integrity and the accuracy of the analysis. All authors reviewed the final manuscript.

Plantar temperatures in stance position: A comparative study with healthy volunteers and diabetes patients diagnosed with sensoric 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, and Dong.H. coordinated the clinical evaluations. 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., Dan.H, and D.B., performed history analysis. H.M., K.M., K.L., 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, Jiani Liu, and Xin Li; Technical Support: Renfang Hu, Xiaolin Meng, Shidan Dou, Huayin Hao, Xiaofen Zhao, Wei Hu, Cheng Li, and Yaozong Gao; Statistical analysis: Xiaofan Lu and Fangrong Yan; Construction of artificial intelligence network: Renfang Hu, Xiaolin Meng, Shidan Dou, Huayin Hao, Xiaofen Zhao, Wei Hu, Cheng Li, and Yaozong Gao; Manuscript editing: Yang Wang, Xiaofan Lu, Zhishun Wang, Guangming Lu, Fangrong Yan, and Bing Zhang; Funding acquistion: 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, AW, CM, CW; Analysis and interpretation of data, TZ, JY, MK, AW, CM, 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. M.K.M. 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.F. 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-assembly 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, Qiyong 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 curation, formal analysis, investigation, visualization, writing & editing Bart Cuypers: Formal analysis, methodology, writing, review & editing Carlota F. Cordero: Formal analysis Bewketu 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. Brittany 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 enteroendocrine 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, FR, BG, AV, NFL, FMG, MC, AA. Visualization, GC, AM, JR, Supervision FR, BG, AV, NFL, FMG, MC, AA Funding Acquisition, FMG, 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, CXG, JT, JZL, JHY, RP, HS, CP, TL, QZ, JW, LX, SHL, BJW, ZHW, CRH, HBZ, RZ, HLZ, XC, PY, BZ, LW,WQZ SSH,YWH, SHJ, PW, JAZ, YPL, WXW, LZ, LL, FQZ. Analysis and interpretation of data: JL, SML, JW. 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, Aaihua Zhao, Xiaojiao Zheng, and Guoxiang Xie designed the study. Meilin 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: Conception and design: JCG, SL, TPZ. Provision of study material and patients: JCG, SL, TPZ, ZGZ, BS, QL, MHD. Financial and administrative support: JCG, SL. Data analysis and interpretation: PZ, LZ, LY, QFL, ZYL, JL, DY, ADT, JS. Experimental support: PZ, LZ, LY, GGX. Manuscript writing: PZ, LZ, QFL. Final approval of the manuscript: All the authors.

CD24-targeted intraoperative fluorescence image-guided surgery leads to improved cytoreduction 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 (statistical analysis): 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, SGM, SG: Analysis and interpretation of data and drafting the manuscript. AS, RG: Study protocol, design and ethics implementation of the KKNMS cohort study. CL, AS, FL, TK, M Mühlau, 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. Haiyin 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., X.X, and A.M. performed experiments. K.F. provided critical reagents. C.B., X.X., J.Z., and A.M. analyzed the data. C.B., X.X., J.Z., K.F., and P-Y.S. interpreted results. C.B., X.X., 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: JMJJ, TPL, and CA performed literature search, conceived and designed the study and the experiments. JMJJ, TPL, AB, IR, SJL, CYJC, LCL, SFSY, EYC, and LPL conducted experiments and analysed the data. JMJJ, JJH, WCC, YBL, LYL, CCY, LTH, and HCH enrolled patients, collected and interpreted data. JMJJ, 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. M.G., 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.