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Research paper

Clinical implications of serum neurofilament in newly diagnosed MS patients: A longitudinal multicentre cohort study

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ABSTRACT

Background: We aim to evaluate serum neurofilament light chain (sNfL), indicating neuroaxonal damage, as a biomarker at diagnosis in a large cohort of early multiple sclerosis (MS) patients.
Methods: In a multicentre prospective longitudinal observational cohort, patients with newly diagnosed relapsing-remitting MS (RRMS) or clinically isolated syndrome (CIS) were recruited between August 2010 and November 2015 in 22 centers. Clinical parameters, MRI, and sNfL levels (measured by single molecule array) were assessed at baseline and up to four-year follow-up.
Findings: Of 814 patients, 54.7% (445) were diagnosed with RRMS and 45.3% (369) with CIS when applying 2010 McDonald criteria (RRMS[2010] and CIS[2010]). After reclassification of CIS[2010] patients with existing CSF analysis, according to 2017 criteria, sNfL levels were lower in CIS[2017] than RRMS[2017] patients (9.1 pg/ml, IQR 6.2–13.7 pg/ml, $n = 45$; 10.8 pg/ml, IQR 7.4–20.1 pg/ml, $n = 213$; $p = 0.036$). sNfL levels corre-

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lated with number of T2 and Gd+ lesions at baseline and future clinical relapses. Patients receiving disease-modifying therapy (DMT) during the first four years had higher baseline sNfL levels than DMT-naïve patients (11.8 pg/ml, IQR 7.5-20.7 pg/ml, *n* = 726; 9.7 pg/ml, IQR 6.4–15.3 pg/ml, *n* = 88). Therapy escalation decisions within this period were reflected by longitudinal changes in sNfL levels.

Interpretation: Assessment of sNfL increases diagnostic accuracy, is associated with disease course prognosis and may, particularly when measured longitudinally, facilitate therapeutic decisions.

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1. Introduction

Neuroaxonal damage is the major underlying pathologic substrate of disability accumulation over time in patients with relapsing-remitting multiple sclerosis (RRMS). Patients with existing signs of neuroaxonal loss are at a high risk of developing secondary progressive and disabling disease courses [1]. Various studies suggest that tissue loss quantified by optical coherence tomography (OCT) [2,3] or MRI assessment [4] could be used to predict subsequent disability. However, both methods require either standardised longitudinal measurements or entail technical challenges and have therefore not yet been implemented in routine management. Serum neurofilament light chain (sNfL) has recently been proposed as a possible candidate for a reliable, easy-to-use biomarker of neuroaxonal damage [5]. Neurofilament proteins are part of the neuronal cytoskeleton and are elevated in various neurological diseases associated with neuronal damage including neurodegenerative diseases [6] and stroke [7], as well as MS [8]. Neurofilament light chain proteins are released after axonal injury into the cerebrospinal fluid (CSF) and to a lesser extent into the peripheral blood, where they can be measured by highly sensitive single molecule assays (SiMoA) [5]. sNfL has been shown to correlate with brain and spinal cord atrophy, as well as clinical relapses, T2 lesion load, and gadolinium-enhancing (Gd+) lesions in patients with RRMS [8–11]. However, to take the next step towards translation of sNfL into routine clinical use, there is a need for the investigation of large-scale real-world cohorts and longitudinal intra-individual samples.

Recent findings by us and others have indicated that sNfL might serve as a biomarker from very early stages of MS, namely in patients with clinically isolated syndrome [8] (CIS), pediatric MS patients [12], or even in presymptomatic stages of the disease [13]. This raises the possibility that sNfL could be included in diagnostic algorithms. The diagnosis of MS is based on McDonald criteria, which were first presented in 2001 and which underwent regular revisions in 2005, 2010 and 2017 as our understanding of this autoimmune CNS disease improved. The basic pillars of the McDonald criteria are identification of CNS lesions, using surrogate markers, and their dissemination in time (DIT) and space (DIS).

The criteria enable the diagnosis of RRMS in patients already at the first clinical presentation and to differentiate it from CIS, in which the chronicity of established MS is not yet proven, as is reflected by a lack of either DIT or DIS [14]. The definition of DIT has changed markedly in the current 2017 McDonald revision by additionally including a) symptomatic Gd+ lesions [15] and b) the presence of oligoclonal bands (OCB) in the CSF as a substitute for clinical or imaging evidence of DIT. The intention was to define chronicity earlier in the disease course allowing an earlier initiation of disease-modifying therapy (DMT), which should improve long-term prognosis. Retrospective analysis of the 2017 McDonald criteria in different cohorts [16–18] has demonstrated that the current revision allows an earlier diagnosis of RRMS compared to the 2010 criteria, in particular through the use of OCB to fulfil the DIT criterion. Additional markers are warranted to select patients at high risk of developing future disability (beyond making the diagnosis of MS *per se*) in order to initiate early and effective treatment of RRMS patients.

We here address, in a multicentre approach in 814 patients from the German National MS cohort with newly diagnosed CIS/RRMS, the potential clinical implications of assessing sNfL in a real world setting for diagnosis, prognosis, and therapeutic decisions.

Research in context

Evidence before this study

Serum neurofilament light chain protein (sNfL) levels have been shown to correlate with neuroaxonal damage in multiple sclerosis (MS) and various other neurological disorders. We used the terms “neurofilament light chain”, “NfL”, “multiple sclerosis”, “MS”, “prediction”, and “McDonald criteria” in PubMed to find publications from any date up to February 14, 2020. Several publications support an earlier diagnosis of relapsing-remitting MS (RRMS) with the 2017 McDonald criteria compared to the 2010 criteria based on magnetic resonance imaging (MRI) or clinical experience. There is strong evidence that sNfL levels correlate with specific clinical and MRI parameters that are commonly used in practice to monitor disease progression and treatment response. However, we found no report that uses sNfL to investigate the value of the 2017 McDonald criteria in order to identify patients at risk of disease progression and its impact on treatment decision and only a limited number of small pilot studies about the value of sNfL in very early MS patients.

Added value of this study

We classified patients as clinically isolated syndrome (CIS) or early RRMS according to 2010 and 2017 McDonald criteria and linked this with sNfL, a peripheral blood biomarker capable of detecting neuroaxonal damage. Thereby, we showed in a prospective multicentre study that the revised McDonald criteria 2017 are superior to detect patients at risk of neuroaxonal damage. Furthermore, we investigate in detail the relation of sNfL and disease course, as well as treatment decisions within the first four years after diagnosis. The observations are of additional value since they have been made in a young and initially untreated cohort at disease onset. Until now, this prognostically relevant group has been underrepresented in robust publications with high numbers of participants.

Implications of all the available evidence

In a prospective, multicentre cohort of 814 patients with CIS and early RRMS at disease onset, assessment of sNfL increased diagnostic accuracy and facilitated prognosis of the disease course over the next four years. Especially when it was measured longitudinally, sNfL reflected therapeutic decisions and may facilitate early therapeutic stratification of patients. Longitudinal measurement of sNfL rather than absolute cut-off values are recommended for clinical decision-making.

2. Materials and methods

2.1. Mainz cohort

To assess the impact of 2010 versus 2017 McDonald criteria on patients with newly diagnosed CIS and different levels of neuroaxonal damage, we first performed a retrospective cross-sectional single centre pilot study. Blood specimens of MS patients were collected and processed at the University Medical Center Mainz as described below. Datasets were available for 61 patients who had sNfL measurements, MRI measurements, and CSF analysis at baseline. These patients were initially classified according to 2010 McDonald criteria, and patients with an initial diagnosis of CIS were reclassified according to 2017 McDonald criteria.

2.2. German National MS cohort

The German National MS (NationMS) cohort is a multicentre prospective longitudinal observational study comprising (a) detailed assessment of patients with first diagnosis of MS or CIS according to 2005 McDonald criteria and (b) yearly assessment with a standardised protocol across 22 centres in Germany. All centres belong to the German Competence Network Multiple Sclerosis (KKNMS). The study was approved by the ethics committee of Ruhr-University Bochum (Registration no. 3714-10), and subsequently, by all local ethics committees of the participating centres. All patients provided written informed consent. Inclusion and exclusion criteria as well as assessment plans have been described previously [19]. All patients (n = 1,124) were included at least 30 days after relapse, but prior to DMT initiation. Thereafter, DMT was initiated in a "real world" setting by each centre. Complete datasets were available for 814 patients who had sNfL measurements and MRI at baseline and were followed up at least two years. For an additional 598 patients, clinical parameters were available after four years of follow-up. To exclude a selection bias, we analyzed baseline and clinical characteristics of both cohorts (814 vs 1124 patients in the whole NationMS cohort) for age, sex, disease duration, first clinical manifestation, and extended disability status scale (EDSS) and found no major differences for all parameters arguing against a selection bias. In our cohort the median age at inclusion was 33 years (IQR 26-41 years) compared to 32.4 years (26.6–41.0 years) in the total cohort. We reported a baseline EDSS median of 1·5 (IQR 1.0-2.0) which is in line with the median reported in the entire cohort ([19] and Table 1). For our study, patients were classified according to 2010 McDonald criteria, and patients with an initial diagnosis of CIS were reclassified according to 2017 McDonald criteria.

2.3. sNfL measurements

To ensure a high degree of standardisation, blood withdrawal was performed at the same day of MRI investigation but before the application of contrast medium using a standard protocol in all centres. Blood was collected in 10 ml Serum-Vacutainer®-tubes (Becton Dickinson, USA); samples were spun at 1300 g at room temperature for 15 min within 2 h after sampling. Directly after centrifugation, the serum was evenly transferred (300–600 μl/tube) in 1·1 ml polypropylene tubes and locally stored at −80 °C. Serum samples from all centres were then sent on dry ice to the KKNMS biobank and centrally stored at −80 °C.

In a previous study, several days of processing did not significantly affect NfL levels in plasma, indicating stability of the protein and a robust assay procedure [20]. For this project, serum samples of our included patients were sent on dry ice from the central biobank in Munich to Mainz. Here, measurements from the multicentre cohort were performed in one single centre at one machine with a standardised protocol and a single batch. sNfL was measured in several rounds by SiMoA HD-1 (Quanterix, USA) using the NF-Light Advantage Kits (Quanterix) from the same batch according to manufacturer's instructions. Resorufin-β-D-galactopyranoside (RGP) was incubated at 33 °C for 60 min prior to running the assay.

Samples were measured in duplicates. The coefficient of variation (CV, as a percentage) of each sample was obtained by dividing the

Table 1
Clinical and demographic data of CIS/early RRMS patients according to 2010 McDonald criteria included in this study at baseline.

Variable		CIS	RRMS	p-value
n		369	445	
		Median (IQR)		
sNfL (pg/mL)		10.1 (6.9–17.1)	12.5 (8.0–22.8)	<0.0005[a]
Age (years)		33 (26–41)	31 (26–40)	0.272[a]
EDSS		1.5 (1–2)	1.5 (1–2)	0.010[a]
		Mean ± SD: 1.3 ± 1.0	1.5 ± 1.0	
Disease duration (months)		2 (1–2)	2 (1–3)	0.141[a]
		n (%)		p-value
Sex	male	113 (30.6%)	146 (32.8%)	0.505[b]
	female	256 (69.4%)	299 (67.2%)	
OCB	neg.	23 (6.2%)	23 (8.9%)	0.593[b]
	pos.	188 (50.9%)	222 (86.0%)	
	unknown	158 (42.8%)	13 (5.0%)	
T2-lesion count	1–8	110 (30.1%)	132 (29.8%)	0.936[b]
	> 8	256 (69.9%)	311 (70.2%)	
	unknown	3 (0.8%)	2 (0.4%)	
GD-enhancement	no	269 (75.6%)	218 (50.3%)	<0.0005[b]
	yes	85 (23.9%)	213 (49.2%)	
	unknown	2 (0.6%)	2 (0.5%)	
Ring-enhancement	no	321 (93.9%)	364 (89.0%)	0.019[b]
	yes	21 (6.1%)	45 (11.0%)	
	unknown	27 (7.3%)	36 (8.1%)	
Treatment	no treatment	369 (100%)	445 (100%)	

IQR: interquartile range; CIS: clinically isolated syndrome; RRMS: relapsing-remitting multiple sclerosis; sNfL: serum neurofilament light chain; EDSS: Expanded Disability Status Scale; OCB: oligoclonal bands; SD: standard deviation.
[a]Mann–Whitney-U tests were conducted to compare group differences.
[b]Distributions were compared using chi-square tests of homogeneity.

standard deviation of both replicates by the mean of both replicates multiplied by 100. Since the range of sNfL concentrations in serum is smaller than in CSF, some samples with a sample CV above 20% (or missing replicate result) were measured twice, as in previous publications [8,9]. Finally, the mean intra-assay CV of 6.2% was obtained by averaging all individual sample CVs. The two same low and high controls, consisting of recombinant human NfL antigen, were run in duplicates with each sample run to monitor plate-to-plate variation. The mean concentration over all runs was 4.4 pg/ml for the low control and 141.5 pg/ml for the high control. We obtained inter-assay CVs of 6.0% and 13.2% for the low and high control, respectively. sNfL measurements were performed in a blinded fashion without information about clinical data.

2.4. Multiple Sclerosis Functional Composite (MSFC)

The MSFC is a score composed of three objective quantitative neurological tests for assessing arm, leg, and cognitive function and was developed to improve the measurement of clinical outcome in extension to the EDSS [21]. Subtests of the MSFC in the NationMS cohort have recently been published to investigate changes in cognitive impairment from baseline up to 12 months [22]. We here used our baseline cohort as a reference population for the z-standardisation instead of normative control cohorts in the previous publication, which leads to different z-scores without affecting the ratio of the amount of change to the standard deviation of the change. A detailed description of the administration and calculation of the MSFC were already published [23]. Z-scores were obtained by standardising all subtest scores to the baseline results of all patients included in this study. Finally, the MSFC score was calculated using the following formula: MSFC score = (Z-score $_{TW}$ + Z-score $_{9\text{-HPT}}$ + Z-score $_{PASAT}$)/3. (TW = Timed 25-Foot Walk; 9-HPT = Nine-Hole Peg Test; PASAT = Paced Auditory Serial Addition Test).

2.5. Statistics

Statistical analyses were performed with SPSS version 23 (IBM Corp., USA), MATLAB R2018a (MathWorks, USA) and RStudio version 1.1.456 (RStudio Inc., USA). The normal distribution of data was tested using the Kolmogorov–Smirnov and Shapiro–Wilk tests. We used a Mann–Whitney test or Kruskal–Wallis-Test with adjusted p values by Bonferroni correction, as appropriate. Effect size after applying a Mann–Whitney-U test was estimated using the formula ($r^2 = \eta^2 = \frac{Z}{\sqrt{n}}$) where Z is the standardised value for the U-value, r the correlation coefficient, and r^2 or η^2 indicate the percentage of variance in the dependent variable that can be explained by the independent variable when multiplied by 100%. Non-parametric correlation was determined by Spearman's rank correlation coefficient and partial non-parametric correlation when considering age as a covariate. Age as cofounding factor needs to be taken into account in older patients as sNfL seems to considerably increase in particular above the age of 60 years with rather stable values in younger patients [24]. In agreement, in our cohort of mainly young patients (median age 32 years, percentage of patients > 60 years old: 0.7%), we found no significant correlation between age and sNfL values (r = −0.044, p = 0.21, Supplementary Fig. 1A+B) and hence no age correction was necessary. However, all analyses were additionally performed with age as a covariate using one-way or two-way mixed ANCOVA where appropriate and can be found in Supplementary Table 1. Within-subject factors over time were analyzed by mixed linear models or two-way mixed ANOVA after log-transformation of sNfL values. This was followed by one-way ANOVA with Tukey post-hoc test for multiple comparison to calculate simple main effects. Delta sNfL values (two-year follow-up minus baseline) were reflected and set to a minimum of 1 followed by non-negative transformation of the resulting values. A Chi-square test of homogeneity was applied to investigate differences in proportions. When performed, post hoc analysis involved pairwise comparison using multiple z-test of two proportion with a Bonferroni correction. Boxplots are shown with the median represented by a horizontal line. Boxes extend from the 25th to 75th percentile. The upper whiskers expand from the 75th percentile to the highest value that is smaller than or equal to the interquartile range (IQR) multiplied by 1.5 and added to the 75th percentile. The lower whiskers extend from the 25th percentile to the smallest value that is higher or equal to the IQR multiplied by 1.5 and subtracted from the 25th percentile. For better visualisation, scatterplots were graphically modified using the syntax command *dodge* (Fig. 1A) or *jitter* (all other figures) in SPSS. All statistical analyses were performed using the original data without modifications. P values < 0.05 were considered statistically significant.

2.6. Bayesian analyses

To deal with imbalanced sample sizes for data with a non-Gaussian distribution (Fig. 2C and D), we used the Bayesian posterior distribution analyses as additional validation of significant group differences already determined by Mann–Whitney-U tests. This analysis provides complete distribution of credible values for group means and their differences [25]. Specifically, we tested for sNfL markers based on the two groups with and without taking age as a covariate for the capability of credible separation.

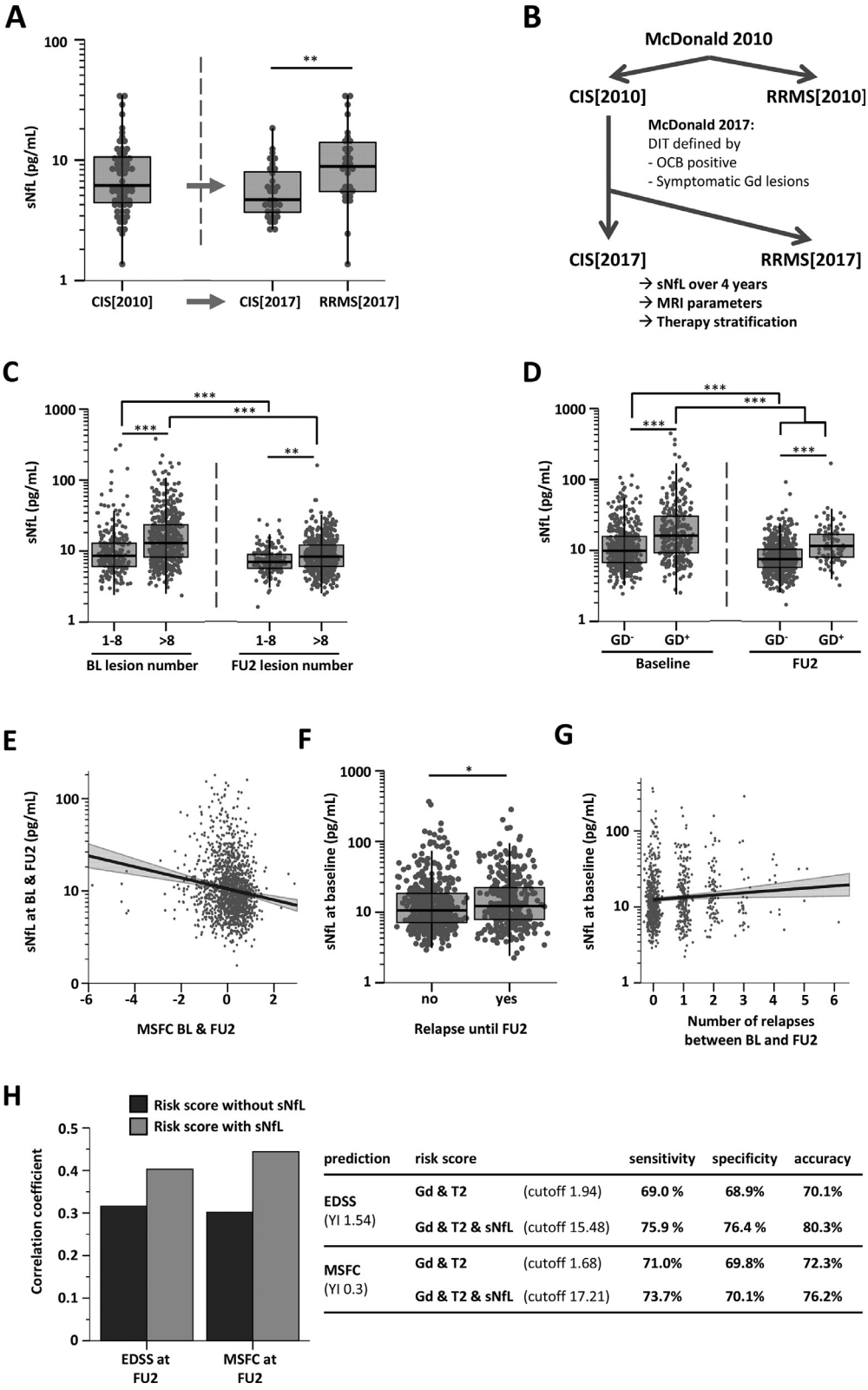
2.7. Composite score analyses

Composite scores were calculated in a two-step procedure. First, we performed a cluster analysis by grouping all possible combinations (always a pair) for the variables that had an area under the curve (AUC) > 0.5. Second, from the significant ($p < 0.05$) clusters corrected for multiple comparisons using Bonferroni corrections, we estimated a composite score using the partial least squares method (PLS) to assign the weights for each combination [26].

See also Supplementary Methods for more detailed methods.

3. Results

The overall aim of the study was to assess potential clinical implications of measuring sNfL for diagnostic accuracy, prognosis, and therapeutic decisions in early MS patients. To evaluate the impact of 2010 versus 2017 McDonald criteria on patients with newly diagnosed CIS and different levels of neuroaxonal damage, we first performed a pilot study. Patients diagnosed with CIS according to 2010 McDonald criteria (CIS[2010]) were reassessed and classified either as CIS or RRMS based on the 2017 criteria (CIS[2017], RRMS[2017]). Interestingly, in this single-center cohort, sNfL levels were higher in RRMS (8.9 pg/ml, IQR 5.5–14.3 pg/ml, n = 30) than in CIS patients according to the new criteria (4.7 pg/ml, IQR 3.6–8.5, p = 0·001, Fig. 1A). Based on this promising data, we designed a multicentre approach (Fig. 1B) where 814 patients were included at baseline and up to four-year follow-up (for baseline characteristics see Table 1). At baseline and year two, patients with ≥9 cranial T2 lesions according to Barkhof criteria [27] had significantly higher sNfL levels (baseline: 13.0 pg/ml, IQR 8.2–23.5 pg/ml, n = 567; two year follow-up: 8.4 pg/ml, 6.1–12.2 pg/ml, n = 573) than those with 1-8 T2 lesions (baseline: 8.6 pg/ml, IQR 6.1–12.9 pg/ml, n = 242; $p < 0.0005$; two-year follow-up: 7.0 pg/ml, 5.7–9.0 pg/ml, n = 161, p = 0.001, Fig. 1C). Comparable findings were obtained for sNfL and Gd+ lesions as Gd+ lesions correlated with high sNfL levels in all patients (Fig. 1D). Furthermore, we found a weak correlation between sNfL and EDSS values (r = 0.13, $p <$ 0.0005), between sNfL levels and ring enhancing lesions (Supplementary Fig. 2A+B), and an inverse correlation between sNfL levels and MSFC score at baseline and two-year follow-up (r = -0.170, $p <$ 0.0005, Fig. 1E). Patients who suffered from at least one relapse in the following two years had significantly higher levels at baseline



prediction	risk score		sensitivity	specificity	accuracy
EDSS (YI 1.54)	Gd & T2	(cutoff 1.94)	69.0 %	68.9%	70.1%
	Gd & T2 & sNfL	(cutoff 15.48)	75.9 %	76.4 %	80.3%
MSFC (YI 0.3)	Gd & T2	(cutoff 1.68)	71.0%	69.8%	72.3%
	Gd & T2 & sNfL	(cutoff 17.21)	73.7%	70.1%	76.2%

Fig. 1. sNfL at time point of diagnosis correlates with baseline MRI parameters in a multicentre cohort and predicts clinical activity within the next two years. **A)** In a single-centre pilot study, patients with CIS according to 2010 McDonald criteria (n = 61; CIS[2010]) were reclassified based on the 2017 version of the McDonald criteria. sNfL levels were significantly higher in patients switching to RRMS (8.9 pg/ml, IQR 5.5-14.3 pg/ml, n = 30; RRMS[2017]) compared to patients remaining CIS (4.7 pg/ml, IQR 3.6-8.5 pg/ml, n = 31; CIS [2017]; p = 0.001 determined by Mann−Whitney-U test). **B)** Study design of a multicentre approach to assess reclassification of CIS[2010] patients according to 2017 McDonald criteria. **C)** 1543 MRIs from 809 patients were assessed for sNfL levels comparing MRIs with > 8 T2 lesions (baseline: n = 567; two-year follow-up: n = 573) and 1-8 T2 lesions (baseline: n = 242; two-year follow-up: n = 161). sNfL was higher in patients with > 8 than 1-8 T2 lesions at baseline (13.0 pg/ml, IQR 8.2-23.5 pg/ml; 8.6 pg/ml, IQR 6.1-12.9 pg/ml, $p < 0.0005$) and two-year follow-up (8.4 pg/ml, IQR 6.1-12.2 pg/ml; 7.0 pg/ml, IQR 5.7-9.0 pg/ml, p = 0.001). Both lesion groups showed a significant decrease of sNfL concentration after two years. **D)** Patients with Gd+ lesions at the time of serum sampling (n = 298; two-year follow-up: n = 94) had significantly higher sNfL levels than patients without Gd+ lesions (n = 487; two-year follow-up: n = 606). **E)** Baseline and two-year follow-up sNfL levels were negatively correlated with corresponding MSFC values (r = -0.170, $p < 0.0005$). **F)** sNfL

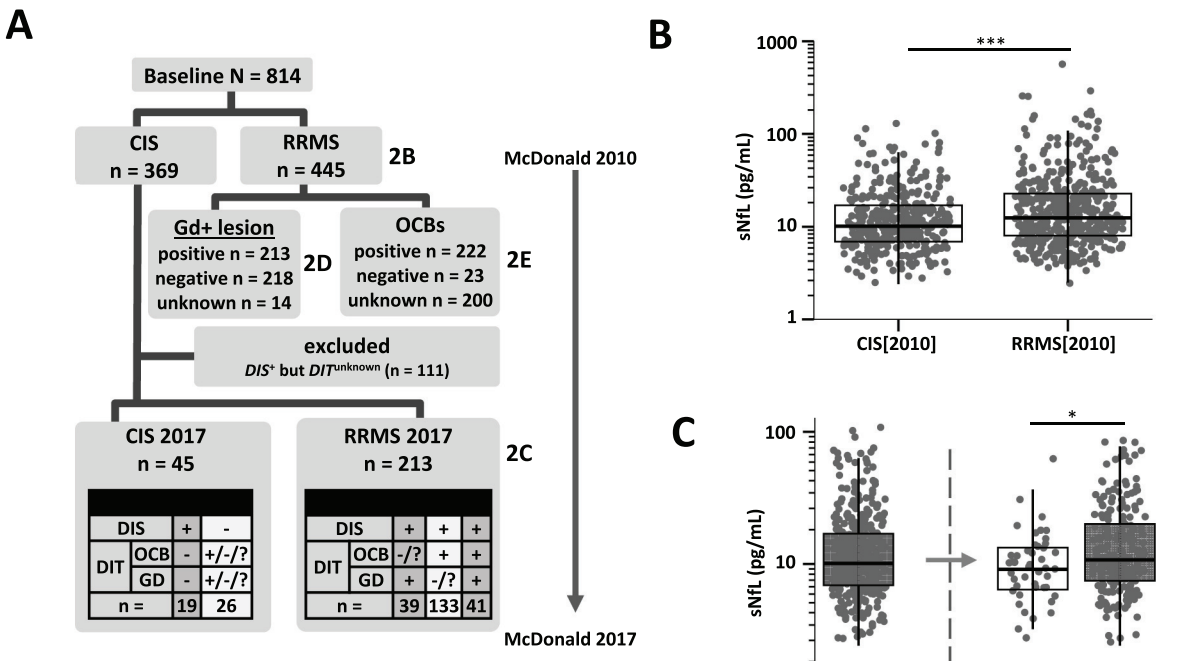
compared to patients experiencing no further relapses (12.2 pg/ml, IQR: 7.9–22.2 pg/ml, n = 337; no relapse: 10.6 pg/ml, IQR: 7.9–22.2 pg/ml, n = 477; p = 0.015, Fig. 1F). In agreement with this, we found a correlation between baseline sNfL levels and the absolute number of relapses in this two-year period (r = 0.092, p = 0.008, Fig. 1G). In order to further evaluate the predictive value of baseline sNfL values after diagnosis for future risk stratification, we developed a risk score using support vector machine (SVM) algorithms (see Supplementary Material and Methods). Using the two MRI parameters i) presence or absence of Gd+ lesions and ii) 1-8 T2 lesions or more, a composite risk score was assigned to each individual patient at baseline. The predictive capacity of the risk score for the two outcome parameters EDSS and MSFC in a two-year follow-up was calculated by SVM analysis. Correlation coefficients drastically increased by including the sNfL baseline values in the composite risk score (EDSS: 0.32 to 0.40, MSFC: 0.30 to 0.44, Fig. 1H), underlining that evaluation of both MRI parameters and sNfL had an added-value compared to only using one single approach.

Out of the 814 patients at disease onset, 45.3% (n = 369) had a diagnosis of CIS[2010] and 54.7% (n = 445) had a diagnosis of RRMS [2010] (Fig. 2A). sNfL levels were higher in RRMS[2010] (12.5 pg/ml, IQR 8.0–22.8 pg/ml, n = 445) than in CIS[2010] (10.1 pg/ml, IQR 6.9-17.1 pg/ml, n = 369, $p < 0.0005$, Fig. 2B) despite similar baseline characteristics such as disease duration (Table 1). When applying the 2017 McDonald criteria to the same patients, both the presence of OCB and the assessment of symptomatic Gd+ lesions could change the classification of CIS[2010] to RRMS[2017]. We excluded 111 patients due to missing information regarding DIT. To prevent a selection bias, subgroup analyses were performed showing no significant differences (see Supplementary Table 2). Upon reclassification of CIS[2010] patients with existing CSF analysis, according to 2017 criteria, only 17.4% (45/258) remained CIS[2017] whilst 82.6% (213/258) were reclassified as RRMS[2017]. Importantly, patients who were reclassified from CIS[2010] to RRMS[2017] had elevated sNfL levels (10.8 pg/ml, IQR 7.4–20.1 pg/ml, n = 213) compared to CIS[2010] patients remaining CIS[2017] (9.1 pg/ml, IQR 6.2-13.7 pg/ml, n = 45, p = 0.036, Fig. 2C). Taking into account the imbalanced sample sizes, this analysis was additionally confirmed by Bayesian analysis, which resulted in a discrimination accuracy of 93.0%. These findings demonstrate that application of 2017 McDonald criteria to CIS patients results in diagnosis of RRMS compared to a diagnosis of CIS when applying the previous McDonald criteria in patients with increased neuroaxonal damage. Additionally, we evaluated whether the presence or absence of OCB and Gd+ in RRMS[2010] patients separates patients with high and low sNfL levels. Both OCB-positive RRMS [2010] patients (13.1 pg/ml, IQR 8.0–24.3 pg/ml, n = 222) and RRMS [2010] patients with Gd+ lesions (16.4 pg/ml, IQR 9.3–31.3 pg/ml, n = 213) had significantly higher sNfL levels than OCB-negative (10.1 pg/ml, IQR 6.7–15.8 pg/ml, n = 23, p = 0.035, accuracy of discrimination = 96.4%) and Gd-negative (10.2 pg/ml, IQR 7.2–16.1 pg/ml, n = 218, $p < 0.0005$) patients (Fig. 2D+E).

To unravel whether inclusion of sNfL in the McDonald diagnostic criteria algorithm would increase the discrimination accuracy between patients with CIS and RRMS, receiver operating characteristic (ROC) analysis was performed in order to reclassify CIS[2010] as CIS[2017] or RRMS[2017]. Inclusions of the 90th percentile of sNfL (31.2 pg/ml) led to a significantly increased (p = 0.035) area under the curve (AUC = 0.84, CI 0.79– 0.89, $p < 0.0005$) compared to OCB and/or Gd+ (AUC = 0.76, CI 0.70–0.83, $p < 0.0005$, Fig. 2F). We confirmed these linear classification data by predictive analysis using a machine learning algorithm SVM which is a non-linear classifier. Importantly, the prediction accuracy of OCB and/or Gd+ (sensitivity: 72%, specificity: 76%, accuracy: 79%) were again further increased by including the 90th percentile of sNfL in addition to the above two variables (sensitivity: 73%; specificity: 79%, accuracy: 84%; Fig. 2G, for more data on 50th to 90th percentile see Supplementary Table 3). These findings point towards a potential value of especially high sNfL levels (>31 pg/ml) at time of first demyelinating event as indicators of ongoing chronic CNS neuroinflammation and may be considered for inclusion in a future refinement of the McDonald criteria.

The changes in the 2017 McDonald criteria are intended to allow earlier diagnosis of RRMS and thus to facilitate initiation of DMT as early as possible in these patients [28]. Therefore, we assessed whether sNfL levels can predict later initiation of DMT and whether treatment would influence sNfL levels after two years of follow-up. The percentage of patients under therapy at two-year follow-up was comparable between CIS[2010] (76% (279/369)) and RRMS[2010] (81% (359/445)) patients (Fig. 3A). Reclassification of CIS[2010] patients according to 2017 criteria had no impact on whether DMT were administered or not (Fig. 3A). At baseline, all patients were treatment naïve. Indeed, patients without DMT initiation during the first two years showed lower sNfL levels at baseline (9.5 pg/ml, IQR 6.4–14.1 pg/ml, n = 87) than patients with at least one (transient) DMT during the observation period (11.8 pg/ml, IQR 7.5–20.9 pg/ml, n = 727, p = 0.002, Fig. 3B). We next grouped patients into four classes based on the type of DMT they were receiving at two-year follow-up ("no DMT": n = 176; "basic": interferons and glatirameracetate, n = 392; "moderate": teriflunomide and dimethylfumarate, n = 134; and "high": natalizumab, rituximab, fingolimod, ocrelizumab, daclizumab, alemtuzumab, mitoxantrone, n = 107). Five patients were excluded from DMT analysis due to unknown treatment. Of note, sNfL baseline levels significantly correlated with the established treatment group at two-year follow-up (r=0.223, p<0.0005, Fig. 3C). While all treatment groups had comparable sNfL levels after two years (no DMT: 8.4 pg/ml, IQR 6.2–11.6 pg/ml; "basic": 7.7 pg/ml, IQR 5.8–11.4 pg/ml; "moderate": 7.5 pg/ml, IQR 5.6–11.0 pg/ml; "high": 8.2 pg/ml, IQR 6.4–11.6 pg/ml; all $p > 0.05$), patients on "high" therapies had significantly elevated baseline levels ("high": 21.0 pg/ml, IQR 12.0–45.3 pg/ml) compared to other groups (no DMT: 10.0 pg/ml, 6.6–18.3 pg/ml, $p < 0.0005$; "basic": 10.5 pg/ml, 7.1–17.1 pg/ml, $p < 0.0005$; "moderate": 12.0 pg/ml, 7.4–22.0 pg/ml, $p < 0.0005$; Fig. 3D). This indicates that high sNfL levels at disease manifestation correlate with real world therapy decisions since sNfL levels were not known to treating physicians at the time point of treatment initiation. After adjustment for the baseline sNfL concentrations, comparing the delta sNfL values (two-year follow-up minus baseline) of the different treatment groups between each other further showed a massive sNfL decline in the "high" treatment group (median −11.3 pg/ml, IQR −37.3 to −2.9 pg/ml) that was significantly higher than in the "basic" group (−2.2 pg/ml, −7.6 to 0.8 pg/ml, p = 0.001) and no DMT group (−0.8 pg/ml, −6.5 to 1.6 pg/ml, p = 0.001, Fig. 3E). This might be due to resolving initial inflammation (regression to the mean phenomenon), as all patients were included at least 30 days after relapse, but prior to DMT initiation, and acute inflammatory

levels at baseline were significantly higher in patients suffering at least one relapse up to FU2 (median: 12.2 pg/ml, IQR: 7.9-22.2 pg/ml, n = 337) than patients without relapse (median: 10.6 pg/ml, IQR: 7.9-22.2 pg/ml, n = 477, p = 0.015). **G)** Baseline sNfL levels correlated with the number of relapses between baseline and FU2 (r = 0.092, p = 0.008). **H)** A baseline risk score consisting of either presence of Gd+ lesions and T2 lesions (1-8 lesions or more than 8) alone (EDSS cutoff point = 1.94; MSFC cutoff point = 1.68) or in addition to sNfL (EDSS cutoff point = 15.48; MSFC cutoff point = 17.21) was determined for risk stratification at study initiation. The predictive power of the model for the two outcome parameters EDSS (YI = 1.54) and MSFC (YI = 0.3) and at FU2 was assessed by SVM algorithm. Correlation coefficients were increased (EDSS: 0.32 to 0.40, MSFC: 0.30 to 0.44) by the inclusion of baseline sNfL levels in the risk score computation. 10-fold cross validation was performed for the correlation and accuracy parameters. Detailed information on the model specifications are depicted in the table. Group differences were analyzed by mixed linear model procedure or Mann-Whitney-U test. Correlation analysis was performed by Spearman's rank correlation coefficient after exclusion of normally distributed data by Kolmogorov-Smirnov-Test and Shapiro-Wilk-Test. BL: baseline, CIS: clinically isolated syndrome FU2: two year follow-up, Gd: gadolinium enhancing lesions, OCB: oligoclonal bands, RRMS: relapsing remitting multiple sclerosis, T2: lesions in T2 weighted MRI scans, sNfL: serum neurofilament, MSFC: Multiple Sclerosis Functional Composite, YI: Youden's index, SVM: support vector machine. $^{**}p < 0.01$, $^{***}p < 0.001$.



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	sensitivity	specificity	accuracy
OCBs + Gd⁺ lesions	71.9 %	75.9%	79.4 %
OCBs + Gd⁺ lesions + sNfL (90th percentile)	73.0 %	79.0 %	84.3 %

Fig. 2. Application of 2017 McDonald criteria selects patients with increased neuroaxonal damage. **A)** Flowchart of the study design. **B)** RRMS[2010] patients (n= 445) had significantly higher sNfL levels than CIS[2010] patients (12.5 pg/ml, IQR 8.0-22.8 pg/ml, n = 445; 10.1 pg/ml, IQR 6.9-17.1 pg/ml, n = 369, $p < 0.0005$). **C)** Application of 2017 McDonald criteria to CIS[2010] patients (n = 369) differentiated CIS[2017] (n = 45) from RRMS[2017] (n = 213) patients and resulted in significantly higher sNfL levels in patients reclassified to RRMS[2017] (10.8 pg/ml, IQR 7.4-20.1 pg/ml, n = 213) compared to patients remaining CIS[2017] (9.1 pg/ml, IQR 6.2-13.7 pg/ml, n = 45, p = 0.036 determined by Mann–Whitney-U test and 93.0% accuracy of discrimination computed by Bayesian analysis with caution of the imbalanced sample size). **D)** sNfL levels were significantly higher in RRMS[2010] patients with positive OCB (13.1 pg/ml, IQR 8.0-24.3 pg/ml, n = 222) compared to those without (10.1 pg/ml, IQR 6.7-15.8 pg/ml, n = 23, p = 0.035, discrimination accuracy = 96.4%). **E)** Significantly higher sNfL levels were found in RRMS[2010] patients with Gd+ lesions (16.4 pg/ml, IQR 9.3-31.3 pg/ml, n = 213) than in those without (10.2 pg/ml, IQR 7.2-16.1 pg/ml, n = 218, $p < 0.0005$). **F)** ROC curves generated for either positive OCB or Gd+ lesion alone (AUC = 0.76, CI 0.70 - 0.83) or in addition to the 90th percentile of sNfL (AUC = 0.84, CI 0.79 - 0.89). AUCs were compared using a Chi-square test (p = 0.035). **G)** Predictive analysis using SVM was performed yielding a prediction accuracy of 79% for OCBs

neuroaxonal injury also transiently increases NfL levels. A significantly higher proportion of patients without therapy (40%, 71/176) had higher sNfL values after two years compared to baseline in contrast to only 31% (120/392, $p < 0.05$) on "basic", 24% (32/134, $p < 0.05$) on "moderate" and 16% (17/107, $p < 0.05$) on "high" therapy (Fig. 3F). This also indicates that longitudinal sNfL changes rather than absolute sNfL values at a given time point might be indicative of disease activity and treatment stratification. Most interestingly, we found a remarkable impact of diagnostic classification on real-world treatment stratification. Compared to CIS [2010] patients, fewer RRMS[2010] patients were on "basic" treatment (RRMS[2010]: 53.7%, CIS[2010]: 72.4%), but received "moderate" (RRMS [2010]: 25.4%, CIS[2010]: 15.8%) or highly active treatment (RRMS [2010]: 20.9%, CIS[2010]: 11.8%) more frequently at year two (Fig. 3G). Importantly, evaluation of CIS[2010] patients showed that all patients but one on high therapy would have been classified as RRMS[2017] according to the new criteria (Fig. 3H). These data demonstrate that patients with initially high sNfL levels ended up on more efficient therapeutic agents two years later.

Where available, we analyzed current treatment decision at the four-year follow-up and the number of therapy changes made up to that point (no DMT: $n = 137$; "basic": $n = 209$; "moderate": $n = 132$; "high": $n = 120$, Fig. 4A). Intriguingly, without knowledge of sNfL levels, high efficacy DMT ("high" or "moderate") was initiated more often in patients with higher sNfL levels both at baseline (13.2 pg/ml, IQR 8.0–24.2 pg/ml, $n = 304$; 9.8 pg/ml, IQR 6.7–15.4 pg/ml, $n = 301$; $p < 0.0005$, Fig. 4B) and two-year follow-up (8.2 pg/ml, IQR 6.0–11.8 pg/ml, $n = 304$; 7.6 pg/ml, IQR 5.9–11.0 pg/ml, $n = 301$; $p = 0.007$). Being treated with at least one higher efficacy DMT was associated with a stronger relative decrease in sNfL levels compared to patients never treated with more than "basic" DMT ($p < 0.0005$). Furthermore, current sNfL levels at baseline or two-year follow up were significantly correlated to the number of therapy changes implemented in the subsequent two years of the observation period (sNfL at baseline $r = 0.179$, $p < 0.0005$, Fig. 4C upper panel; sNfL at two-year follow-up $r = 0.133$, $p = 0.001$, Fig. 4C lower panel).

Moreover, sNfL levels at two-year follow-up are increased in patients undergoing escalation therapy within the next two years compared to patients who stayed on the same therapy ("escalation": 9.1 pg/ml, IQR 6.4-13.7 pg/ml, $n = 107$; "no escalation": 7.7 pg/ml, IQR 5.9–11.2 pg/ml, $n = 358$, $p = 0.001$, Fig. 4D). On closer analysis, the sNfL levels changed significantly differently over time, depending on whether DMT was initiated/escalated, de-escalated or maintained in the same class between two and four years of follow-up ($p = 0.029$). The decision for "no escalation" was retrospectively confirmed by lower sNfL levels at two-year follow-up compared to patients with subsequent DMT initiation/escalation (7.1 pg/ml, IQR 5.8–10.4 pg/ml, $n = 50$; 9.1 pg/ml, IQR 6.4–13.7 pg/ml, $n = 107$, $p = 0.026$, Fig. 4D). Since different treatment strategies, namely early induction or later escalation to higher efficacy DMT, are part of the current debate we next separated all patients ending up in DMT groups "moderate" or "high" at four-year follow-up on whether they were already in these DMT classes at two-year follow up ("induction": $n = 153$) or not ("escalation", $n = 82$, Fig. 4E). Supporting the potential of neurofilament as a treatment biomarker we found a significant interaction between treatment strategy and time on sNfL levels ($p = 0.002$). Although patients in the "induction" group initially started with higher baseline sNfL levels (14.4 pg/ml, IQR 8.2–28.9 pg/ml; 10.9 pg/ml, IQR 7.5–19.1 pg/ml, $p = 0.035$), it declined much more sharply in the first two years and even crossed the line of the "escalation" group (7.8 pg/ml, IQR 6.0–12.3 pg/ml; 9.3 pg/ml, IQR 6.3–14.3 pg/ml, $p = 0.025$). Nonetheless, the escalated therapeutic regimen between year two and four was reflected by a stronger sNfL level decrease leading to similar four-year follow-up sNfL levels in both groups ("induction": 6.2 pg/ml, IQR 4.8–8.7 pg/ml; "escalation": 5.7 pg/ml, IQR 4.4–7.9 pg/ml, $p = 0.885$). These data underline that longitudinal assessment of sNfL levels might be a suitable approach for a real-world comparison of different treatment stratification algorithms.

4. Discussion

We here evaluated sNfL in a large cohort of early MS patients and provide supporting evidence for a role of starting longitudinal sNfL assessments directly at the time point of first diagnosis. Specifically, sNfL measurements have implications for i) diagnostic accuracy, ii) prognosis, and iii) treatment decision making in the four years after diagnosis.

In their 2017 revision of the McDonald diagnostic criteria, an international panel of experts reached a consensus that makes it easier to establish a diagnosis of MS earlier than was possible with previous criteria. All CIS[2010] patients but one under high efficacy therapies ($n = 22$) at follow-up would have already been diagnosed as "RRMS" according to the 2017 criteria, providing robust data for the superior diagnostic value of the 2017 diagnostic criteria. Previously, it was reported that increased CSF NfL in patients with radiologically isolated syndrome is an independent risk factor of developing CIS [29] and for further development of clinically definite MS in CIS patients [30]. Our findings even show that sNfL might be useful in differentiating CIS from RRMS and may thus be considered as a parameter for future revisions of diagnostic criteria if sNfL methology can be robustly improved to allow for clinical routine care settings. In fact, highest sNfL levels (in our patient cohort: cutoff 90th percentile) increased sensitivity, specificity, and accuracy over OCB or symptomatic Gd+ lesions to discriminate between CIS and MS. Further studies should specifically focus on an additional diagnostic value in patients with first disease symptoms and high initial sNfL levels and thus a high likelihood of axonal damage and an established CNS autoimmune inflammation. This notion is further supported by our data showing that the risk predicting EDSS/MSFC after two years based on baseline T2 lesions and Gd+ lesions was markedly elevated by additionally including sNfL values.

In addition to a potential added value in the initial diagnosis of patients, sNFL might also serve as a marker of treatment response, as sNFL levels have been described to decrease after initiation of any DMT [8,10,20] and specifically after switch from injectable therapies to fingolimod [20] or after initiation of interferon-beta-treatment [31]. We here present findings from one of the so far largest and earliest cohorts of MS patients correlating treatment responses with sNfL levels. Importantly, data was acquired in a prospective, centralised and highly standardised manner. The number of patients without DMT after two years is comparable with other databases (e.g., MSBase ~30% of patients; [32] Swiss National Multiple Sclerosis Cohort ~50% of patients [10]). However, patients in our study were recruited at first demyelinating event and therefore earlier than in other studies. After a two-year follow-up, untreated patients had an elevated risk of higher sNfL levels than patients on DMT. It should be noted that mean sNfL levels were nevertheless reduced in all patient groups, and to a lesser extent in untreated patients, after two and four years compared to baseline. Previously, sNfL levels were found to be elevated 2 months before and 1 month after MRI scans showing Gd+ lesions [31]. Inclusion criteria for our cohort demanded a minimum interval of 30 days after relapse, but the exact duration of elevated sNfL due to clinical or MRI disease activity has not yet been established. This underlines the importance of both longitudinal

and/or Gd+ for discriminating CIS and RRMS according to 2017 McDonald criteria. Accuracy increased to 84% by including the 90th percentile of sNfL in addition to the above two variables. Group differences were analyzed by Mann–Whitney-U test and in cases of imbalanced sample sizes additionally validated by Bayesian analysis (C+D). sNfL levels are reported as median. IQR: interquartile range, CIS: clinically isolated syndrome, DIS: dissemination in space, DIT: dissemination in time, Gd: gadolinium, OCB: oligoclonal bands, sNfL: serum neurofilament, RRMS: relapsing-remitting multiple sclerosis, SVM: support vector machine algorithm, ROC: receiver operating characteristic, AUC: area under the curve. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns = not significant.

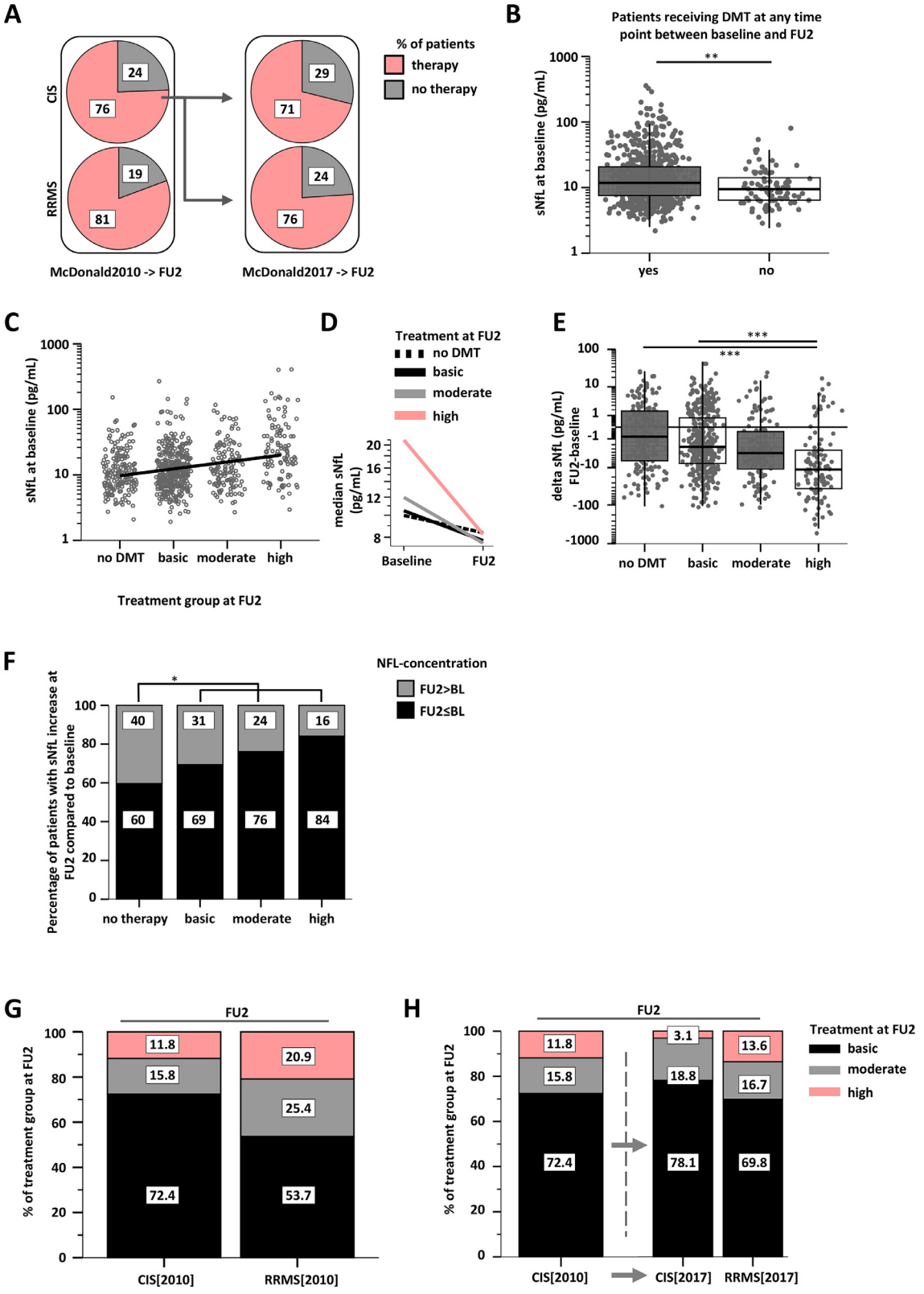

Fig. 3. Relationship between 2017 McDonald criteria, therapeutic decision and sNfL levels at two-year follow-up. **A)** Percentage of patients receiving DMT in CIS[2010], RRMS[2010], CIS[2010]→CIS[2017] and CIS[2010]→RRMS[2017] at two-year follow-up. **B)** sNfL levels at baseline were significantly higher in patients receiving DMT in the following two years (11.8 pg/ml, IQR 7.5-20.9 pg/ml, $n = 727$) than patients without specific MS medication until two-year follow-up (9.5 pg/ml, IQR 6.4-14.1 pg/ml, $n = 87$, $p = 0.002$). **C)** Patients were grouped into four classes based on the type of DMT they were receiving at year two follow-up. Median sNfL levels at baseline and two-year follow-up in four patient groups defined above (no DMT: $n = 176$, basic: $n = 392$, moderate: $n = 134$ and high: $n = 134$). Baseline sNfL levels were correlated with the patient's treatment group at two-year follow-up ($r = 0.223$, $p < 0.0005$). **D)** There was a statistically significant two-way interaction regarding the between- and within-subject factors (time*treatment groups) on sNfL concentration ($p < 0.0005$). At baseline the high treatment group showed elevated sNfL concentration compared to all other groups ($p < 0.0005$). We observed no significant difference between the treatment groups at two-year follow-up (ns) but a statistically significant effect of time on sNfL concentration for all four groups ($p < 0.0005$). **E)** Delta sNfL (two-year follow-up minus baseline) for patients without DMT and on basic, moderate and high DMT. Calculation was adjusted for baseline sNfL values. High treatment versus basic treatment

measurements and interpretation of sNfL results together with corresponding clinical information. Strikingly, patients who were on high efficacy therapies after two years, reflecting a severe early disease course, had the highest initial sNfL levels and strongest relative decrease. Furthermore, treatment decisions and escalation/de-escalation decisions within the first four years were reflected by changes in sNfL values. Future studies should further evaluate whether sNfL levels are suited for prospective treatment stratification. Furthermore, to pave the way into clinical practice, age-, demographic-, and comorbidity-associated normative values as well as optimal frequency of sampling and thresholds leading to distinct clinical decisions in patient management, need to be internationally agreed on and confirmed applying the exact same protocol prior to inter-laboratory comparisons and accreditation. It should, however, be mentioned that at present, there is in parallel still work to do with regards to missing mechanistic understanding about the underlying pathophysiologic processes best reflected by sNfL. How do inflammatory versus "diffuse" neurodegenerative processes impact sNfL both on a short- and long-term scale, and what would be the add-on value to use different surrogate parameters all considered to reflect neurodegenerative processes in MS (e.g., brain atrophy, OCT, sNfL).

Taken together, we report here findings in a large German multicentre cohort with early CIS/RRMS. In a clinical setting, determining sNfL levels at time point of diagnosis and thereafter longitudinally might not only increase the sensitivity of diagnostic criteria, but could also – at least according to our findings based on the NationMS cohort and German treatment practice in expert centres – provide the next step towards personalised and optimised MS therapy.

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Declaration of Competing Interest

Falk Steffen, Vinzenz Fleischer, Muthuraman Muthuraman, Sergiu Groppa, and Mark Mühlau declare no competing interests. Stefan Bittner has received honoria and compensation for travel from Biogen Idec, Merck Serono, Novartis, Sanofi-Genzyme and Roche. Timo Uphaus received honoria from Merck Serono. Carsten Lukas received consulting and speaker's honoraria from BiogenIdec, Bayer Schering, Novartis, Sanofi, Genzyme, and TEVA; has received research scientific grant support from Bayer Schering, TEVA, and Merck Serono; holds an endowed professorship supported by the Novartis Foundation. Anke Salmen received speaker honoraria and/or travel compensation for activities with Almirall Hermal GmbH, Biogen, Merck, Novartis, Roche, and Sanofi Genzyme and research support by the Swiss MS Society, none related to this work. Felix Luessi received consultancy fees from Roche and support with travel cost from Teva Pharma. Achim Berthele reports personal fees from Bayer Healthcare, Biogen, Merck Serono, Mylan, Roche, and Sanofi Genzyme, and his institution received compensations for clinical trials from Alexion Pharmaceuticals, Biogen, Chugai, Novartis, Roche, Sanofi Genzyme, and Teva - all outside the submitted work. Luisa Klotz received honoraria for lecturing and serving on advisory boards, as well as travel expenses for attending meetings and financial research support from Immunic AG, Biogen, Janssen, Merck Serono, Novartis, Roche, Sanofi Genzyme, Teva, Grifols, Alexion, Santhera, Bayer Healthcare, the Deutsche Forschungsgemeinschaft (DFG; German Research Society), the German Ministry for Education and Research (BMBF), the Interdisciplinary Center for Clinical Studies (IZKF) Muenster and the program Innovative Medical Research (IMF) Muenster. Sven G. Meuth receives honoraria for lecturing, and travel expenses for attending meetings from Almirall, Amicus Therapeutics Germany, Bayer Health Care, Biogen, Celgene, Diamed, Genzyme, MedDay Pharmaceuticals, Merck Serono, Novartis, Novo Nordisk, ONO Pharma, Roche, Sanofi-Aventis, Chugai Pharma, QuintilesIMS and Teva. His research is funded by the German Ministry for Education and Research (BMBF), Deutsche Forschungsgemeinschaft (DFG), Else Kröner Fresenius Foundation, German Academic Exchange Service, Hertie Foundation, Interdisciplinary Center for Clinical Studies (IZKF) Muenster, German Foundation Neurology and Almirall, Amicus Therapeutics Germany, Biogen, Diamed, Fresenius Medical Care, Genzyme, Merck Serono, Novartis, ONO Pharma, Roche, and Teva. Antonios Bayas received personal compensation from Merck, Biogen, Bayer Vital, Novartis, TEVA, Roche and Sanofi-Aventis/Genzyme and grants for congress trips and participation from Biogen, TEVA, Novartis, Sanofi-Aventis/Genzyme, and Merck. Friedemann Paul receives honoraria for lecturing, and travel expenses for attending meetings from Guthy Jackson Foundation, Sanofi Genzyme, Novartis, Alexion, Viela Bio, Roche, UCB, Mitsubishi Tanabe and Celgene. His research is funded by the German Ministry for Education and Research (BMBF), Deutsche Forschungsgemeinschaft (DFG), Einstein Foundation, Guthy Jackson Charitable Foundation, EU FP7 Framework Program, Arthur Arnstein Foundation Berlin, Biogen, Genzyme, Merck Serono, Novartis, Bayer, Teva, Alexion, Roche, Parexel and Almirall. Hans-Peter Hartung has received fees for consulting, serving on steering committees and data monitoring committees from Bayer Healthcare, Biogen, GeNeuro, Medimmune, Merck, Novartis, Roche, Sanofi Genzyme, Teva and TG Therapeutics with approval by the Rector of Heinrich-Heine-University. Ralf Linker received Research Support and/or personal compensation for activities with Bayer Health Care, Biogen, Genzyme/Sanofi, Merck, Novartis Pharma, Roche, and TEVA Pharma; none related to this work. Christoph Heesen received research grants and speaker honoraria from Biogen, Genzyme, Roche, and Merck; none related to this work. Martin Stangel received honoraria for scientific lectures or consultancy from Alexion, Bayer Healthcare, Biogen, CSL Behring, Grifols, Janssen, Merck-Serono, Novartis, Roche, Sanofi-Aventis, Takeda, and Teva. His

($p = 0.001$) and no DMT ($p = 0.001$) were significantly different. **F**) sNfL levels were compared between baseline (BL) and two-year follow-up (FU2). Percentage of patients with sNfL increase (FU2>BL) and sNfL decrease (FU2<BL) are depicted for four treatment groups. The group without treatment showed significantly higher proportion of patients with increased sNfL levels as compared to the groups with either moderate or high treatment. **G)** Percentage of patients under basic, moderate and high efficacy DMT in CIS[2010] and RRMS[2010] after two-year follow-up. **H)** Percentage of patients under basic, moderate and high efficacy DMT in CIS[2010] patients and in CIS[2017] and RRMS[2017] patients after reassessment according to 2017 criteria. Group differences were analyzed by Mann–Whitney-U test (B) or a one-way ANCOVA with baseline sNfL values as a covariate (E). For two-way (treatment group*time) interaction a two-way mixed ANOVA with four additional separate one-way ANOVAs with Tukey post-hoc test for multiple comparison for simple main effects/between-subject factors for the different treatment groups was applied (D). Correlation analysis was performed by Spearman's rank correlation coefficient after exclusion of normally distributed data by Kolmogorov–Smirnov-Test and Shapiro–Wilk-Test. Chi-square test of homogeneity was applied to investigate differences in proportions. Post hoc analysis involved pairwise comparison using multiple z-test of two proportions and, where appropriate, a Bonferroni correction. sNfL levels are reported as median. FU2: two-year follow-up, CIS: clinically isolated syndrome, RRMS: relapsing remitting multiple sclerosis, DMT: disease-modifying therapy, basic: interferons and glatirameracetate; moderate: teriflunomide and dimethylfumarate; high: natalizumab, rituximab, fingolimod, ocrelizumab, daclizumab, alemtuzumab, mitoxantrone. ns: not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

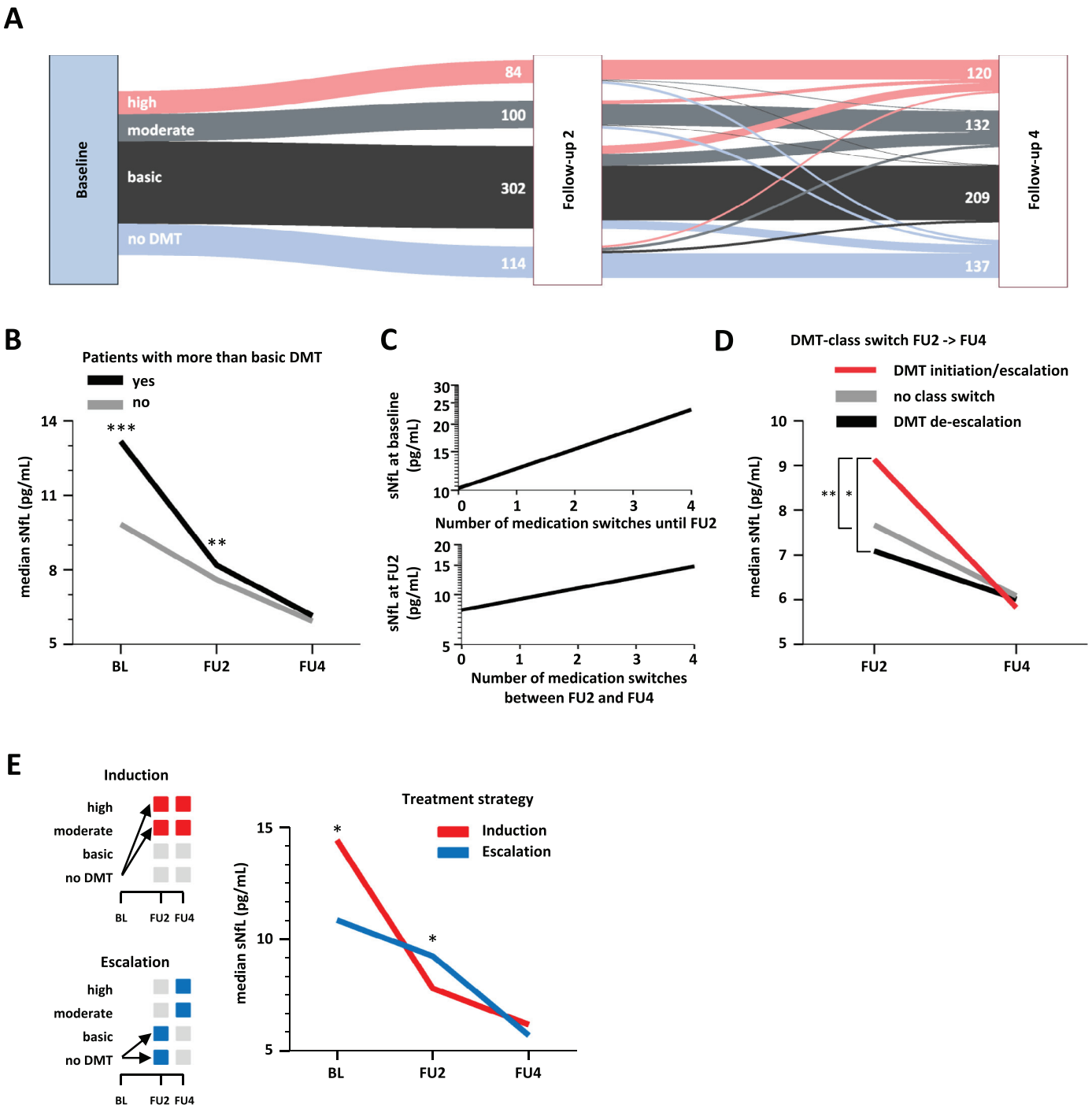

Fig. 4. sNfL levels reflect therapeutic decisions within four years after diagnosis. **A)** Sankey diagram illustrating treatment stratification at two-year follow-up and therapy changes between year two and four. Width of arrows reflects quantitative changes between groups. **B)** High efficacy DMTs ("high" or "moderate") were initiated more often in patients with higher sNfL levels both at baseline (13.2 pg/ml, IQR 8.0-24.2 pg/ml, n = 304; 9.8 pg/ml, IQR 6.7-15.4 pg/ml, n = 301; $p < 0.0005$) and two-year follow-up (8.2 pg/ml, IQR 6.0-11.8 pg/ml, n = 304; 7.6 pg/ml, IQR 5.9-11.0 pg/ml, n = 301; $p = 0.007$). There was a statistically significant two-way interaction on sNfL concentration ($p < 0.0005$). **C)** Top: baseline sNfL levels were correlated with the number of medication switches performed between baseline and two-year follow-up ($r = 0.179$, $p < 0.0005$). Below: FU2 sNfL levels were correlated with the number of medication switches performed between two-year and four-year follow-up ($r = 0.133$, p = 0.001). **D)** sNfL at two-year follow-up was significantly increased in patients with escalation of DMT treatment group between two-year and four-year follow-up compared to patients without escalation of DMT within this period (9.1 pg/ml, IQR 6.4-13.7 pg/ml, n = 107; 7.7 pg/ml, IQR 5.9-11.2 pg/ml, n = 358, $p = 0.001$). **E)** Patients with moderate or high DMT group at four-year follow-up were divided in two different groups depending on whether they have already been in these DMT classes at two-year follow up ("induction": n = 153) or not ("escalation": n = 82). A schematic illustration of this process is depicted in the left panel. Compared to the "escalation" group patients in the "induction" group showed higher sNfL levels at baseline (14.4 pg/ml, IQR 8.2-28.9 pg/ml; 10.9 pg/ml, IQR 7.5-19.1 pg/ml, $p = 0.035$) but lower sNfL concentrations at two-year follow-up (7.8 pg/ml, IQR 6.0-12.3 pg/ml; 9.3 pg/ml, IQR 6.3-14.3 pg/ml, $p = 0.025$). There was no significant difference at four-year follow-up ("induction": 6.2 pg/ml, IQR 4.8-8.7 pg/ml; "escalation": 5.7 pg/ml, IQR 4.4-7.9 pg/ml, $p = 0.885$). Correlation analysis was performed by Spearman's rank correlation coefficient after exclusion of normally distributed data by Kolmogorov-Smirnov-Test and Shapiro–Wilk-Test. Where appropriate, post hoc analysis was performed using a Bonferroni correction. sNfL levels are reported as median and interquartile range (IQR). FU2: two-year follow-up, FU4: four-year follow-up, DMT: disease-modifying therapy, basic: interferons and glatirameracetate; moderate: teriflunomide and dimethylfumarate; high: natalizumab, rituximab, fingolimod, ocrelizumab, daclizumab, alemtuzumab, mitoxantrone. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

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Bernhard Hemmer served on scientific advisory boards for Novartis; he has served as DMSC member for AllergyCare, Polpharma, and TG therapeutics; he or his institution have received speaker honoraria from Desitin; his institution has received research support from Regeneron; holds part of two patents; one for the detection of antibodies and T cells against KIR4.1 in a subpopulation of MS patients and one for genetic determinants of neutralising antibodies to interferon β during the last 3 years. Heinz Wiendl receives honoraria for acting as a member of Scientific Advisory Boards and as a consultant for Biogen, Evgen, MedDay Pharmaceuticals, Merck Serono, Novartis, Roche Pharma AG, Sanofi-Genzyme, as well as speaker honoraria and travel support from Alexion, Biogen, Cognomed, F. Hoffmann-La Roche Ltd., Gemeinnützige Hertie-Stiftung, Merck Serono, Novartis, Roche Pharma AG, Sanofi-Genzyme, TEVA, and WebMD Global. 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Ralf Gold serves on scientific advisory boards for Teva Pharmaceutical Industries Ltd., Biogen Idec, Bayer Schering Pharma, and Novartis; has received speaker honoraria from Biogen Idec, Teva Pharmaceutical Industries Ltd., Bayer Schering Pharma, and Novartis; serves as editor for Therapeutic Advances in Neurological Diseases and on the editorial boards of Experimental Neurology and the Journal of Neuroimmunology; and receives research support from Teva Pharmaceutical Industries Ltd., Biogen Idec, Bayer Schering Pharma, Genzyme, Merck Serono, and Novartis; none related to this work. Frauke Zipp has recently received research grants and/or consultation funds from the DFG, BMBF, PMSA, Novartis, Octapharma, Merck Serono, ONO Pharma, Biogen, Genzyme, Celgene and Roche.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2020.102807.

References

[1] Larochelle C, Uphaus T, Prat A, Zipp F. Secondary progression in multiple sclerosis: neuronal exhaustion or distinct pathology? Trends Neurosci 2016;39(5):325–39.
[2] Saidha S, Al-Louzi O, Ratchford JN, et al. Optical coherence tomography reflects brain atrophy in multiple sclerosis: a four-year study. Ann Neurol 2015;78(5):801–13.
[3] Zimmermann HG, Knier B, Oberwahrenbrock T, et al. Association of retinal ganglion cell layer thickness with future disease activity in patients with clinically isolated syndrome. JAMA Neurol 2018;75(9):1071–9.
[4] Steenwijk MD, Geurts JJ, Daams M, et al. Cortical atrophy patterns in multiple sclerosis are non-random and clinically relevant. Brain: J Neurol 2016;139(Pt 1):115–26.
[5] Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. Nat Rev Neurol 2018;14(10):577–89.
[6] Preische O, Schultz SA, Apel A, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. Nat Med 2019;25(2):277–83.
[7] Gattringer T, Pinter D, Enzinger C, et al. Serum neurofilament light is sensitive to active cerebral small vessel disease. Neurology 2017;89(20):2108–14.
[8] Siller N, Kuhle J, Muthuraman M, et al. Serum neurofilament light chain is a biomarker of acute and chronic neuronal damage in early multiple sclerosis. Mult Scler 2018;25(5):678–86.
[9] Barro C, Benkert P, Disanto G, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain: J Neurol 2018;141(8):2382–91.
[10] Disanto G, Barro C, Benkert P, et al. Serum Neurofilament light: a biomarker of neuronal damage in multiple sclerosis. Ann Neurol 2017;81(6):857–70.
[11] Canto E, Barro C, Zhao C, et al. Association between serum neurofilament light chain levels and long-term disease course among patients with multiple sclerosis followed up for 12 years. JAMA Neurol 2019 [Epub ahead of print]. doi: 10.1001/jamaneurol.2019.2137.
[12] Wong YYM, Bruijstens AL, Barro C, et al. Serum neurofilament light chain in pediatric MS and other acquired demyelinating syndromes. Neurology 2019;93(10):e968–e74.
[13] Bjornevik K, Munger KL, Cortese M, et al. Serum Neurofilament Light Chain Levels in Patients With Presymptomatic Multiple Sclerosis. JAMA Neurol 2019 [Epub ahead of print]. doi: 10.1001/jamaneurol.2019.3238.
[14] Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 2018;17(2):162–73.

[15] Filippi M, Rocca MA, Ciccarelli O, et al. MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. Lancet Neurol 2016;15(3):292–303.
[16] Beesley R, Anderson V, Harding KE, et al. Impact of the 2017 revisions to McDonald criteria on the diagnosis of multiple sclerosis. Mult Scler 2018;24(13):1786–7.
[17] McNicholas N, Lockhart A, Yap SM, et al. New versus old: Implications of evolving diagnostic criteria for relapsing-remitting multiple sclerosis. Mult Scler 2019;25(6):867–70.
[18] Schwenkenbecher P, Wurster U, Suhs KW, Stangel M, Skripuletz T. Applying the 2017 McDonald diagnostic criteria for multiple sclerosis. Lancet Neurol 2018;17(6):498.
[19] von Bismarck O, Dankowski T, Ambrosius B, et al. Treatment choices and neuropsychological symptoms of a large cohort of early MS. Neurol(R) Neuroimmunol Neuroinflamm 2018;5(3):e446.
[20] Piehl F, Kockum I, Khademi M, et al. Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. Mult Scler 2018;24(8):1046–54.
[21] Cutter GR, Baier ML, Rudick RA, et al. Development of a multiple sclerosis functional composite as a clinical trial outcome measure. Brain: J Neurol 1999;122(Pt 5):871–82.
[22] Johnen A, Burkner PC, Landmeyer NC, et al. Can we predict cognitive decline after initial diagnosis of multiple sclerosis? Results from the German National early MS cohort (KKNMS). J Neurol 2019;266(2):386–97.
[23] Fischer JS, Rudick RA, Cutter GR, Reingold SC. The multiple sclerosis functional composite measure (MSFC): an integrated approach to MS clinical outcome assessment. national MS society clinical outcomes assessment task force. Mult Scler 1999;5(4):244–50.
[24] Khalil M, Pirpamer L, Hofer E, et al. Normative values of serum neurofilament light levels. Mult Scler 2018;24(S(2)):530–737.
[25] Kruschke JK. Bayesian estimation supersedes the t test. J Exp Psychol Gen 2013;142(2):573–603.
[26] Schuberth F, Henseler J, Dijkstra TK. Partial least squares path modeling using ordinal categorical indicators. Qual Quant 2018;52(1):9–35.
[27] Barkhof F, Filippi M, Miller DH, et al. Comparison of MRI criteria at first presentation to predict conversion to clinically definite multiple sclerosis. Brain: J Neurol 1997;120(Pt 11):2059–69.
[28] Thompson AJ, Reingold SC, Cohen JA. International panel on diagnosis of multiple S. Applying the 2017 McDonald diagnostic criteria for multiple sclerosis – authors' reply. Lancet Neurol 2018;17(6):499–500.
[29] Matute-Blanch C, Villar LM, Alvarez-Cermeno JC, et al. Neurofilament light chain and oligoclonal bands are prognostic biomarkers in radiologically isolated syndrome. Brain: J Neurol 2018;141(4):1085–93.
[30] Teunissen CE, Iacobaeus E, Khademi M, et al. Combination of CSF N-acetylaspartate and neurofilaments in multiple sclerosis. Neurology 2009;72(15):1322–9.
[31] Varhaug KN, Barro C, Bjornevik K, et al. Neurofilament light chain predicts disease activity in relapsing-remitting MS. Neurol(R) Neuroimmunol Neuroinflamm 2018;5(1):e422.
[32] Brown JWL, Coles A, Horakova D, et al. Association of Initial disease-modifying therapy with later conversion to secondary progressive multiple sclerosis. JAMA 2019;321(2):175–87.

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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.