

to assess risk of developing MS in patients with CIS who are not receiving an MS-indicated disease-modifying therapeutic will be identified via a systematic literature search. Studies will be evaluated for overall risk of bias using PROBAST (Prediction model Risk Of Bias Assessment Tool). Briefly, data sources, predictor, and outcome definition and assessment, applicability, and analysis will be assessed for each model in each identified study, and an overall risk of biased judgment will be assigned. Identified studies, predictors incorporated, results, and risk of bias assessment with accompanying rationale will be summarized in the final report. RESULTS/ANTICIPATED RESULTS: Based on an initial exploratory search, we anticipate that most, if not all, identified prediction models will have high risk of bias. We anticipate that many studies will have limited applicability due to the use of outdated diagnostic criteria for definition of outcomes, or high risk of bias concerns originating from their analysis due to insufficient volume of included participants or poor model validation practices. We further anticipate that most, if not all, of the identified prediction models will have limited potential to be translated to use in a clinical setting. DISCUSSION/SIGNIFICANCE OF IMPACT: Understanding how to identify patients with high-risk CIS may inform and improve clinician treatment decisions, patient outcomes, and future research study design. This work may also reveal flaws in current prediction models for CIS, opening new avenues of research and prompting development of improved prognostic models for patients with CIS.

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### Functionalization of human dECM for incorporation into 3D pulmonary fibrosis models

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OBJECTIVES/GOALS: The goal of this project was to engineer 3D lung models by embedding human epithelial cells and fibroblasts within hybrid-hydrogels containing human decellularized extracellular matrix (dECM) from healthy and fibrotic lungs. This platform will enable us to study cell–matrix interactions involved lung fibrosis pathogenesis. METHODS/STUDY POPULATION: To incorporate dECM into hybrid-hydrogels it must be digested and functionalized. We determined the best conditions for pepsin digesting dECM from healthy and fibrotic human lung by collecting samples every 12 hours up to 96 hours and measuring total protein (BCA assay), total amine concentration (ninhydrin assay), and protein fragment size (SDS PAGE). Next, several molar excesses of Traut's reagent were tested and functionalization was verified by comparing amine content (ninhydrin assay) to thiol content (Ellman's assay). Hydrogel stiffness was measured initially and after stiffening using parallel-plate rheology. RESULTS/ANTICIPATED RESULTS: The dECM was successfully pepsin-digested, with the 48-hour time point yielding the highest free amine levels. A 75-molar excess of Traut's reagent was best for converting free amines to thiols. Dynamic stiffening allowed the creation of hybrid-hydrogels mimicking both healthy (1–5 kPa) and fibrotic (>10 kPa) lung microenvironments. We anticipate that this model will demonstrate differential fibroblast activation based on hybrid-hydrogel dECM source (healthy or

fibrotic), microenvironmental stiffness, and cell source (healthy or fibrotic). Validation of this 3D co-culture system could accelerate drug discovery by providing a more accurate in vitro platform for high-throughput screening. DISCUSSION/SIGNIFICANCE OF IMPACT: This work advances pulmonary fibrosis modeling by creating human dECM-based hydrogels that recapitulate the cellular and mechanical microenvironment of healthy and diseased lung, potentially enabling us to uncover novel therapeutic targets and improving drug efficacy testing in vitro.

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### Functional link between myelination integrity in the connectome of the cingulum bundle and information processing speed in RRMS\*

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OBJECTIVES/GOALS: This study tests how fiber microstructural integrity and myelination levels within the cingulum connectome are associated with information processing speed (IPS) in relapsing-remitting multiple sclerosis (RRMS). We investigate the functional impact of structural coherence, myelin content, and white matter hyperintensities (WMH) load on IPS. METHODS/STUDY POPULATION: Data from 63 RRMS and 25 healthy controls (HC) were used. We hypothesize that the structural integrity of the cingulum bundle and its structural network – or connectome – is distinctly associated with IPS function in people with RRMS (vs. HC) due to myelin-related plasticity across the wiring. Using diffusion spectrum imaging and high-resolution tract segmentation, we constructed individualized white matter connectomes. Diffusion quantitative anisotropy (QA) and myelin fractions (MWF) were used to quantify structural coherence and myelination. WMH load was measured with T2-FLAIR imaging. Bayesian–Pearson correlations, mixed-linear, and moderation models explored how fiber-specific QA, MWF, and WMH load relate to IPS function in RRMS, as measured by Symbol Digit Modalities Test (SDMT). RESULTS/ANTICIPATED RESULTS: We theorize that (1) QA in the cingulum connectome correlates with SDMT performance dimensionally, indicating that structural coherence in the white matter supports IPS function among both groups; (2) increased myelination will strengthen the positive association between QA and SDMT scores, suggesting that connectome-specific myelin content facilitates IPS; (3) conversely, WMH load within the cingulum connectome is expected to inversely correlate with SDMT scores, reflecting the detrimental impact of lesion burden on IPS function; (4) myelination in specialized tracts within the cingulum connectome play a compensatory role to support IPS function in the RRMS group. These investigations can offer a mechanistic clue to potential neuroplastic targets for cognitive interventions in MS. DISCUSSION/SIGNIFICANCE OF IMPACT: By linking white matter integrity to cognitive function at the connectome level, this study can support neuroregenerative strategies to mitigate cognitive burden in RRMS. Our findings may advance understanding of how structural coherence, tract myelination, and WMH affect IPS, shaping personalized prognostic and therapeutic interventions.