

H3K4me3 (+ control), and HA (PTHrP) CUT&RUN on MCF7V and MCF7P cells, and submitted DNA for sequencing. This study will define where PTHrP binds the genome and identify pathways regulated by PTHrP. Previously, through ChIP-qPCR we showed that PTHrP binds the LIFR promoter to repress LIFR expression. Given this result, we expect that PTHrP binds to the promoters of dormancy-associated genes including LIFR in MCF7P cells compared to MCF7V cells. PTHrP may be involved in regulating other processes besides dormancy to induce expansion of breast cancer cells in the bone, so we will use GSEA to identify pathways that are altered in MCF7P cells when PTHrP is over-expressed compared to MCF7V cells. Together, this will define how PTHrP regulates gene expression of bone metastatic breast cancer cells. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This study will unveil mechanisms of metastatic breast cancer expansion in the bone by defining where PTHrP binds the genome to regulate gene expression. These findings will reveal therapeutic vulnerabilities that will be used to target bone-disseminated tumor cells to prevent lethal recurrence.

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A novel approach to developing and validating a predictive model of functional recovery for adults with stroke in post-acute rehabilitation

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OBJECTIVES/GOALS: To use patient-level Center for Medicare and Medicaid Services (CMS) mandated quality metrics for inpatient rehabilitation facilities (IRFs) to develop and validate predictive models of functional recovery and interactions of baseline characteristics with therapy time. **METHODS/STUDY POPULATION:** Retrospective cohort study of a national US sample of ~40,000 adults with a primary diagnosis of stroke admitted to IRFs in 2023. Records will be randomly allocated to equal training and validation samples. We will use a random forest approach to generate predictive models for self-care and mobility functional outcomes using patient baseline and demographic data from a CMS-mandated assessment for IRFs (Section GG). We will also examine how predictive variables modulate the effects of occupational, physical, and speech-language therapy minutes. The random forest is a machine-learning approach that trains multiple models and combines their predictions to improve their overall performance. **RESULTS/ANTICIPATED RESULTS:** Predictive models developed from the training sample will be applied to the validation sample to confirm their capacity to support new observations. Preliminary results will be reported, including the F1 score and area under the curve (AUC), with 95% confidence intervals. A unique feature of this study is the large sample, which contrasts with prior research in stroke rehabilitation using machine learning approaches. This study will produce powerful models that will inform the design of a clinical decision-support tool for application into clinical practice in a future study. **DISCUSSION/SIGNIFICANCE OF IMPACT:** By using CMS-mandated quality metrics that are collected as part of standard clinical practice in IRFs, results will support clinical interpretation and application of metrics and inform the development of a clinician-facing intervention to support personalized rehabilitation approaches.

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Comparison of profile and utility measures of health-related quality of life in pediatric Hodgkin lymphoma

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OBJECTIVES/GOALS: Our aim is to compare scores collected from a health utilities measure (Health Utility Index, HUI) to those collected from a profile measure (Child Health Ratings Inventories, CHRIs- Global) among youth with newly diagnosed, high-risk classic Hodgkin lymphoma. **METHODS/STUDY POPULATION:** We will analyze existing data collected during the Children's Oncology Group AHOD 1331 trial, which was a phase 3 clinical trial comparing the efficacy of adding brentuximab vedotin to standard-of-care treatment with multiagent chemotherapy in children and adolescents with high-risk Hodgkin lymphoma. The study also had a prespecified patient-reported outcomes (PRO) secondary aim, which involved recruiting a subset of the initial 309 patients aged 11 years or older enrolled in the trial for serial PRO measures taken over the trial period. Health-related quality of life (HRQoL) was assessed by CHRIs, HUI version 2, and HUI version 3 assessments at six planned points throughout treatment. **RESULTS/ANTICIPATED RESULTS:** The first step of our analysis will be to ascertain agreement in scoring for parent-child dyads for the HUI2, HUI3, and CHRIs scores by comparing mean scores via two-sample t-testing. Bland-Altman plots will be constructed to compare agreement between the scores for HUI2/3 and the CHRIs. Similarly, Spearman's correlation coefficients will be calculated for CHRIs with HUI2/3 for both parents and children. We hypothesize the CHRIs and HUI scores should roughly correlate with one another, but there may be divergence of correlation because the HUI has greater emphasis on functionality (e.g., sensation, mobility), and the CHRIs further emphasize social and emotional well-being in addition to physical health. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The composite score of the HUI 2/3 has allowed for direct comparison with other global HRQoL measures, providing greater clarity of its performance in different patient populations and clinical settings. The current study will improve understanding of the HUI 2/3 performance in a pediatric cancer population over time.

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Evaluating prediction models for conversion of clinically isolated syndrome to multiple sclerosis: A systematic review^{*†}

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OBJECTIVES/GOALS: Accurately stratifying patients with clinically isolated syndrome by risk of developing multiple sclerosis is of great clinical importance. Though numerous prediction models attempt to achieve this goal, no systematic review exists to independently evaluate these models. We aim to systematically identify and assess the risk of bias in all such models. **METHODS/STUDY POPULATION:** Studies developing or validating prediction models

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.

[†]The abstract has been updated since original publication. A notice detailing the change has been published at doi: [10.1017/cts.2025.10061](https://doi.org/10.1017/cts.2025.10061)

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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.