cell carcinoma (OSCC) organoids will be encapsulated in an interpenetrating network hydrogel engineered with tunable stiffness and viscosity to mimic healthy, dysplastic, and cancerous tissue. Organoid growth, morphology, and expression of mechanosensitive markers will be analyzed by imaging and immunostaining. Single-cell RNA sequencing will identify mechanoresponsive markers across genetically diverse patient lines. Targeted inhibitors of EGFR, YAP, and p53 pathways will text whether blocking these signals can prevent mechanics-driven malignant transformation. RESULTS/ANTICIPATED RESULTS: We anticipate that OED organoids in stiffer matrices similar to OSCC tumor stroma will exhibit increased proliferation, altered morphology, and activation of tumorigenic markers similar to OSCC. scRNA-seq is expected to reveal conserved and patient-specific mechanosensitive pathways. Targeted inhibition of these pathways should mitigate mechanics-driven malignant transformation. These outcomes will define molecular mechanisms linking ECM biomechanics to tumor progression and reveal new therapeutic opportunities for early intervention. DISCUSSION/SIGNIFICANCE OF IMPACT: This hydrogel-organoid model links patient-specific genetics and ECM biomechanics to tumor progression, enabling controlled studies of how mechanical cues drive malignancy and therapeutic response in a clinically relevant, precision medicine context.

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Leveraging modular TriKE-PACC molecules capable of dual-antigen targeting to enhance NK-cell immunotherapy in AML.

Anders Matson, Caden Caligiuri, Katherine M. Koehler, Behiye Kodal, Emma M Eungard, Yvette Soignier, Samreen Fathima, Jeffrey S. Miller and Martin Felices
University of Minnesota

OBJECTIVES/GOALS: Acute myeloid leukemia (AML) is an aggressive blood cancer that hides from the immune system, which led to ~140,000 deaths worldwide in 2024. AML often recurs or resists therapy; therefore, our goal is to develop and test TriKE-PACCs, which combine dual tumor antigen targeting with NK cell activation, to overcome antigen escape and heterogeneity in AML. METHODS/STUDY POPULATION: TriKE-PACC molecules were engineered by combining a TriKE [anti-CD16 single-domain antibody (sdAb), an IL-15 moiety, and an antitumor antibody domain single-chain variable fragment (scFv) or an sdAb] with a PACC arm containing a second antigen-binding scFv/sdAb linked to IL-15Rα. TriKE-PACC constructs were produced in Expi293 cells. To identify clinically relevant AML tumor targets, we analyzed the expression of tumor antigens in AML bone marrow samples obtained from 20 patients using flow cytometry. We modeled antigen escape and heterogeneity using CRISPR/Cas9-generated knockout AML lines. TriKE-PACC-mediated NK cell activity was measured via degranulation, cytokine production, and Incucyte-based cytotoxicity assays against AML cell lines and CRISPR knockout models. RESULTS/ANTICIPATED RESULTS: Our examination of AML patient bone marrow samples revealed diverse antigen profiles, highlighting the need for modular TriKE-PACC designs. The dual-targeting TriKE-PACCs enhanced NK cell activation via CD107a expression and the production of IFNγ and TNFα in co-culture assays with AML cell lines. Notably, TriKE-PACCs retained activity against AML models lacking a targeted antigen, demonstrating the ability of the second tumor targeting arm to overcome antigen escape. Furthermore, NK cell-mediated killing was improved against antigen-deficient AML cells when treated with the TriKE-PACCs compared to standard TriKEs. DISCUSSION/SIGNIFICANCE OF IMPACT: TriKE-PACCs offer a modular and customizable NK-engaging immunotherapy to address AML heterogeneity and resistance. Our data, collected utilizing primary patient samples, support the use of this platform for the future development of personalized, dual-targeted therapies to enhance the efficacy of AML treatments.

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Designing nanoparticle therapies for age-related macular degeneration‡

Suleyman Bozal[1], Johnny Sheng Yue[2], Marcello DiStasio[1], Allison Rothrock[2], Xianzhi Zhang[2], Hande Eda Sutova[1], Sagar Bhatta[1], Emily Deschenes[2], Gyoung Ju[1], W. Mark Saltzman[2] and Brian P. Hafler[1]
[1]Yale School of Medicine, Yale University and [2]Yale University

OBJECTIVES/GOALS: Our objective in this study was to develop a nanoparticle-based RNA delivery system that reprograms Müller glia toward neuronal fates in human relevant models, enabling localized and transient regeneration without viral vectors. METHODS/STUDY POPULATION: - RESULTS/ANTICIPATED RESULTS: Fabricated nanoparticles encapsulating RNA cargoes were ~110–150 nm in size with polydispersity indices of ~0.2–0.3 and encapsulation efficiencies >95%. Nanoparticles successfully delivered GFP mRNA in both organoid and retinal explant models, showing significant expression of fluorescent reporters in both live cell imaging and in slides prepared using immunohistochemistry. We anticipate that our delivery system will lead to transcription factor expression after delivery of corresponding RNAs with evidence of proliferation and neuronal marker upregulation. We also hypothesize that this approach will enhance neurogenesis in retinal organoids and explants, potentially establishing a platform for non-viral retinal regeneration. DISCUSSION/SIGNIFICANCE OF IMPACT: This work demonstrates a clinically translatable, virus-free strategy for retinal repair through targeted RNA delivery. By enabling transient expression of neurogenic factors, nanoparticle-mediated reprogramming presents a scalable and adaptable approach for restoring vision in degenerative retinal diseases.

‡This has been updated since its original publication. A notice detailing this can be found here: https://doi.org/10.1017/cts.2026.10776

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A multi-institutional phase I study of acetazolamide with temozolomide in adults with newly diagnosed methylated malignant glioma (MGMT)

katayoun Hemmesi, Bakhtiar Yamini and Riley Driscoll
Department of Neurological Surgery, University of Chicago Medicine, Chicago, Illinois, USA

OBJECTIVES/GOALS: To assess the safety, tolerability, and adverse effects of adding acetazolamide to temozolomide in newly diagnosed

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MGMT-methylated malignant glioma, and to explore efficacy, survival outcomes, and Bcl-3 as a potential predictive biomarker. METHODS/STUDY POPULATION: Adults (>18 y) with newly diagnosed, MGMT-methylated, IDH-wildtype glioblastoma, and Karnofsky performance status > 60 receiving standard temozolomide (TMZ) + radiation (RT) were enrolled. Sixty patients (24 initial, 36 subsequent) received acetazolamide (ACZ) twice daily starting on the day of adjuvant TMZ initiation. ACZ began at 250 mg BID for 1 week, then escalated to 500 mg BID, given on days 1–21 of each 28-day cycle (TMZ days 1–5), for up to 6 cycles if not limited by tumor progression/toxicity. Clinical and MRI follow-up occurred every 2 months. RESULTS/ANTICIPATED RESULTS: Preliminary data (initial 24 patients) have shown that no patient had a dose-limiting toxicity. Adverse events were consistent with known sequelae of acetazolamide and TMZ. In the 23 WHO Grade 4 patients, the median overall survival (OS) was 30.1 months, and the median progression-free survival was 16.0 months. The 2-year OS was 60.9%. In total, 37% of the study population had high BCL-3 staining and trended toward shorter OS (17.2 months vs N.R., P=.06) (Riley et al., 2024) All of these factors will be evaluated in a prospective study including 60 patients DISCUSSION/SIGNIFICANCE OF IMPACT: Initial data in a smaller cohort showed that adding acetazolamide is safe and tolerable in GBM patients on standard temozolomide. Survival compares favorably to historical data in MGMT-methylated GBM. BCL-3 may be a prognostic biomarker. These findings will be assessed and discussed in a 60-patient prospective study.

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Assessing the impact of knee osteoarthritis phenotype on knee total joint moment in aging adults

Mariana Jacobs[1], Amara G. Sharp[1], Sarah Malek[2], Jeffrey Selby[1], Stephen T. Duncan[1], Christopher S Fry[1] and Michael A. Samaan[1]
[1]University of Kentucky and [2]Purdue University

OBJECTIVES/GOALS: Differences in gait patterns related to post-traumatic (PTOA) and idiopathic (IDIO) knee osteoarthritis (OA) in aging adults is not well understood. Therefore, the purpose of this study was to assess the impact of knee OA phenotype (PTOA vs IDIO) on the knee total joint moment (TJM) during walking in aging adults with and without end-stage knee OA. METHODS/STUDY POPULATION: Six adults with PTOA and six adults with IDIO were tested prior to knee replacement. Data from 12 healthy, asymptomatic adults were used as control data. All subjects underwent 3D gait analysis at a self-selected speed. TJM was calculated as the square root of the sum of the squared planar internal sagittal, frontal, and transverse plane knee moments across the stance phase. The corresponding sagittal, frontal, and transverse plane moments at the peak TJM in the 1st (TJM1) and 2nd (TJM2) half of the stance phase were extracted. A repeated-measures ANOVA was used to assess group differences in biomechanical parameters of the dominant (control group) and surgical (OA groups) limb, while knee pain (VAS: 0 – 10) during the walking task for the PTOA and IDIO groups was compared using an independent t-test (alpha=0.05). RESULTS/ANTICIPATED RESULTS: Both the PTOA and IDIO groups walked at slower speeds compared to the control group ($p<0.01$). There was a main effect of knee OA phenotype on frontal plane TJM ($p=0.003$). The PTOA group exhibited a significantly lower frontal plane moment at TJM2 compared to the control ($p=0.047$) and IDIO ($p=0.019$) groups. There were no differences in sagittal, frontal, or transverse plane knee loading at TJM1 nor any differences in sagittal and transverse plane knee loading at TJM2 ($p > 0.05$). In addition, there were no differences in self-reported knee pain during walking between the PTOA and IDIO groups ($p=0.25$). DISCUSSION/SIGNIFICANCE OF IMPACT: People with PTOA exhibit more rapid knee joint degeneration than those with IDIO. Lower frontal plane loading during TJM2 in PTOA may be associated with a more rapid decline in knee muscle function. Further evaluation of knee muscle function in PTOA and IDIO will provide better insight into these differences in gait patterns between PTOA and IDIO.

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Urinary cell iron processing as a noninvasive indicator of iron toxicity and treatment response in CKD

Chantalle Campbell[1], Ana Munera[1], Tzeela Lifshitz[1], Avery Freund[1], Mary Choi[1], Francesca Vinchi[2] and Oleh Akchurin[1]
[1]Weill Cornell Medicine and [2]Emory University

OBJECTIVES/GOALS: Define how CKD and iron therapy influence tubular epithelial cell (TEC) iron handling through parallel kidney and urine studies in mice and patients, uncovering cellular signatures that predict injury or recovery and may guide individualized iron therapy in CKD. METHODS/STUDY POPULATION: We used two mouse models of CKD and fresh urine samples from pediatric CKD patients and healthy controls to assess tubular iron handling. Kidney tubular epithelial cells (TECs; $CD45^{-}AQP1^{+}$) were analyzed by flow cytometry for labile iron pool (LIP) using FerroOrange and sorted into LIP^hi and LIP^low subsets for RNA-seq. Expression of injury markers such as KIM1 and NGAL was used to evaluate tubular stress, linking iron accumulation to epithelial injury and adaptive responses. By combining mouse CKD models with patient urine analysis, we traced how kidney tubular epithelial cells (TECs) manage iron across species. RESULTS/ANTICIPATED RESULTS: In CKD mouse models, tubular epithelial cells (TECs) showed reduced labile iron pool (LIP) and increased ferroptosis, with a smaller population of iron-overloaded cells exhibiting elevated KIM-1 and EMT-related transcriptional changes. Iron chelation lowered LIP in tubular cells and iron therapy exasperated KIM-1 expression. Urinary TECs mirrored tissue findings, with LIP and KIM-1 levels reflecting systemic iron status and CKD severity. In human CKD, urinary LIPHI TECs expressed higher KIM-1 and injury-associated signatures, validating urinary TEC profiling as a noninvasive readout of tubular health. In both species, iron handling and toxicity proceeded differently depending on the nature of the damage (tubular or glomerular), identifying urinary TECs as a noninvasive window into kidney iron toxicity. DISCUSSION/SIGNIFICANCE OF IMPACT: Chronic kidney disease affects 10–15% of the world population, and anemia is a common complication. Iron therapy is toxic to kidney cells, contributing to tubular injury. Our studies identify urinary cells as a noninvasive test of renal iron status and link iron

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