responses are affected by FOLFOX, we utilized a model antigen expressing murine colon cancer cell line syngeneic to C57BL/6 (MC38-CEA). Treatment was initiated when tumor size reached 50 mm^2. Mice were treated with either vehicle (PBS), 5-Fluorouracil (5-FU), Oxaliplatin, or combination (FOLFOX). Antigen-specific cytotoxic T cell (tet + Tc) were detected using Db-CEA-tetramer obtained from the NIH-tetramer core facility. Flow cytometry was performed for phenotypic analysis and tetramer positivity. Tumor growth was measured using standard caliper measurements. Statistical analysis was performed using *t*-test for continuous variables and ANOVA was used when comparing multiple groups. Statistical analysis was performed using SPSS. All arms were completed with n = 3–7. RESULTS/ANTICIPATED RESULTS: To determine how systemic treatment with chemotherapy affects cytotoxic T cell development (Tc), we established that we could detect antigen-specific Tc (tet + Tc) in the spleen, tumor, and draining lymph nodes of tumor-bearing mice. After establishing that the system worked appropriately, tumor-bearing mice were treated with different chemotherapy regimens and tumor growth was monitored. As expected, the combination of FOLFOX was significantly better than either drug individually (2-way ANOVA, $p < 0.01$). FOLFOX therapy also showed a significant ($p < 0.05$) increase in the number of tumor-associated tet + Tc, and tet + Tc expressing phenotypic markers of effector (Te) and resident memory (Trm) subsets. Tumor-associated tet + Tc highly expressed PD-1 (>50%); however, this was not significantly different between treatment or vehicle arms. Since 5-FU, one component of FOLFOX has previously shown a selective reduction of myeloid-derived suppressor cells, we also investigated the myeloid compartment. There were no significant differences in conventional or plasmacytoid dendritic cells, myeloid-derived suppressor cells, or tumor-associated macrophages. DISCUSSION/SIGNIFICANCE OF IMPACT: The future of cancer care involves multi-modality care tailored to patients. To more effectively combine therapy it is critical that we understand how currently utilized therapy works. In this study, we show that the primary chemotherapy regimen utilized in colorectal cancer increases tumor-associated antigen-specific cytotoxic T cells and the majority of these cells are PD-1 positive. This suggests that FOLFOX may work in concert with immune-based therapy when selected appropriately. Further study is warranted to determine optimal combination therapy and ways to maximize anti-tumor immunity in order to improve the treatment of patients with this deadly disease.

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Investigation of patient-reported outcomes following ACL reconstruction using Rasch analysis

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OBJECTIVES/SPECIFIC AIMS: The knee injury osteoarthritis and outcomes survey (KOOS) is a commonly used instrument to measure patient-reported quality of life (QOL) post-ACLR. The purpose is to evaluate the psychometric properties of the QOL subscale of the KOOS. METHODS/STUDY POPULATION: Rasch analysis of KOOS QOL subscale from 39 individuals 1–2 years post ACLR was conducted. Measurement properties and model fit of the rating scale, items, and persons were evaluated. Relationship of item difficulties and person measures was evaluated using probability curves and item maps. Reliability indicators were also examined. RESULTS/ANTICIPATED RESULTS: All items demonstrated infit and outfit mean squares and standard *z*-scores. The majority of persons (n = 38, 97.4%) demonstrated fit to the Rasch model. However, ceiling effects were noted (n = 4, 10.26%), indicating some participants report higher QOL than is measurable. The mean person measure was 1.73 logits higher than the mean item measure: this sample is skewed toward higher QOL. Person reliability was adequate (0.67) and person separation was 1.42. Calculation of person strata revealed that the KOOS QOL separated participants into 2 strata. DISCUSSION/SIGNIFICANCE OF IMPACT: Although all items of the KOOS QOL fit the model, not all categories of the rating scale were used. Overall, this sample reported high QOL, which is to be expected given the time since ACLR. If participants with a broader range of time since ACLR were included, that the KOOS QOL could identify additional person strata.

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L1 expression analysis in adipose-derived stem cells

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OBJECTIVES/SPECIFIC AIMS: Long interspersed element-1s (L1s) are autonomous, mobile elements that are able to copy and insert themselves throughout the genome with their own reverse transcriptase and endonuclease.
These elements make up 17% of the human genome with over 500,000 copies, though the vast majority of L1s are defective with only a few dozen potentially responsible for L1 activity. Full-length L1s have the potential to contribute to mutagenesis through random insertion and increased genetic instability. Here we set out to study L1 expression at the specific loci level in bone marrow-derived stem cells (bmSCs) and adipose-derived stem cells (ASCs) and compare the levels of expression from ASCs from donor patients who are young and lean, obese, and old. Our hypothesis is that L1-related damage may contribute to mutation and inflammation that alters the function of these stem cells throughout the life of an individual. METHODS/STUDY POPULATION: ASCs and bmSCs were isolated from patient donors. The following samples were collected: ASCs from 3 young (under the age of 59) and lean (BMI < 30) patients, ASCs from 3 older patients (over the age of 59), ASCs from 3 patients with BMI > 30, and bmSCs from 4 young and lean patients. Cytoplasmic RNA from the cell populations was isolated and sequenced by RNA-Seq from the cell populations. Using our recently developed bioinformatics pipeline, we set out to quantify L1 expression and identify the few culprit L1s at specific loci that are actively transcribing to RNA in the ASC and bmSC samples. RESULTS/ANTICIPATED RESULTS: Here we provide proof of concept with the application of this novel method in characterizing full-length expressed L1s at the specific loci level in ASCs and bmSCs. We identified L1 loci that are commonly expressed in these cell types and observed an increase in L1 expression in the obese and old ASC cells compared with the young, lean ASCs and bmSCs. DISCUSSION/SIGNIFICANCE OF IMPACT: ASCs hold the promise of broad application in the biomedical field including regenerative treatment. There are reports that ASCs cultivated from older and obese donors are less effective in regenerative treatments. By demonstrating an increased expression of the mutagenic L1 element in ASCs from obese and old donors, this study provides further evidence suggesting the preferable use of ASCs from young and lean donors for regenerative therapies. These studies will also help us to understand the potential contribution of L1 expression to loss of stem cell function during the aging process.

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Lafora disease premature termination codons (PTCs) are likely candidates for suppression by aminoglycosides

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OBJECTIVES/SPECIFIC AIMS: A small molecule therapy is within reach to treat a molecular mechanism known to result in thousands of fatal diseases. For 10% of patients with a genetic disease, a nonsense/STOP mutation/premature termination codon (PTC) is the underlying cause of their malady. PTCs prematurely stop protein synthesis and yield truncated proteins. Truncated proteins typically provide little to no proper function or activity and are rapidly degraded; thus, disease is imminent. Recent work has demonstrated that small molecules including aminoglycosides can cause the ribosome to readthrough these PTCs. Thus, PTC readthrough with small molecules is a very attractive approach for treating diseases caused by PTCs. Small molecules that promote readthrough act on the ribosome and induce a ribosomal conformational change. In this conformation, the PTC is not recognized by the translational machinery and an amino acid is incorporated into the growing peptide chain, thus protein synthesis continues and does not stop. The use of a single small molecule to readthrough various PTC mutations has been repeatedly effective for in vitro studies and some of these have progressed to clinical trials. Although there has been success in defining these small molecules, the field has discovered that every PTC is unique and likely requires a different small molecule. Thus, developing a cell culture model to test read-through of Lafora PTCs and the functionality of the protein product is the first step to developing a readthrough therapy for a LD. METHODS/STUDY POPULATION: Method for in vitro quantification of

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readthrough: 24 hours before transfection, HEK293 cells were split in 6-well plates. On the following day, approximately 60% confluence, the cells were transiently transfected with the WT or PTC mutated constructs using Polyethylenimine HCl MAX. Cells were transfected with a total amount of 0.35 μg DNA/well and 2 μl Polyethylenimine HCl MAX/well. Four hours later, the transfection medium was removed and replaced with fresh medium, without streptomycin and penicillin. The fresh media contained gentamicin diluted to the indicated concentration per well. Fresh gentamicin-containing medium was replaced after 24 hours. After 48 hours, lysates were collected in 100 μL mRIPA supplemented with protease inhibitors for each construct. The lysates were run on a western blot and the *N*-terminal was probed with anti-FLAG. A malachite green phosphatase assay to measure inorganic phosphate release from phospho-glucans, that is glycogen or LBs. Glycogen is used in this laforin bioassay as the biologically relevant substrate in order to determine the specific activity of the readthrough products. All reactions are incubated for 40 minute the absorbance is measured at 620 nm and the pmoles of phosphate released/min/nmol protein was calculated using a standard curve. RESULTS/ANTICIPATED RESULTS: HEK293 cells were transfected with MeCP2 R241X, laforin R241X, or laforin WT NT-FLAG construct, treated with different concentrations of gentamicin for 48 hours, and laforin levels were assessed by Western analysis with anti-FLAG. HEK293 cells were transfected with WT laforin or a laforin PTC CT-FLAG construct, treated with different concentrations of gentamicin for 48 hours, and laforin levels were assessed by Western analysis with anti-FLAG. B. Quantification of read-through for PTC experiments. **p*-value ≤ 0.001. #*p*-value ≤ 0.001. Schematic of laforin bioassay. The assay has been performed with human and mouse tissue as well as cultured cells. B. Laforin bioassay results using laforin from PTC experiment. ***p*-value ≤ 0.001. **p*-value ≤ 0.01. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results suggest that gentamicin is not only responsible for inducing readthrough of the PTC mutations, but also for promoting translation of fully functional laforin. Therefore, our in vitro system for the analysis of PTC readthrough of laforin will be useful for determining which PTC mutations are suppressible with gentamicin or other small molecules, in what quantities laforin is recovered from PTC mutations, and if the protein products possess the appropriate enzymatic function.

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Lost and found: Detection of brain cardiolipins in plasma after cardiac arrest

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OBJECTIVES/SPECIFIC AIMS: Neurological injury remains as the main limiting factor for overall recovery after cardiac arrest (CA). Currently available indicators of neurological injury are inadequate for early prognostication after return of spontaneous circulation (ROSC). High diversification of brain mitochondrial cardiolipins (CL) makes them unique candidates to quantify brain injury and to predict prognosis early after ROSC. METHODS/STUDY POPULATION: CL content in plasma in 39 patients within 6 hours of ROSC and 10 healthy subjects as well as CL content in human heart and brain specimens were quantified using a high-resolution liquid chromatography mass spectrometry method. The quantities of brain-type CL species were correlated with clinical parameters of brain injury severity permitting derivation of a cerebral CL score (C-score) using linear regression. C-score and a single CL species (70:5) were evaluated in patients with varying neurological injury and outcome. Using a rat model of CA, CL was quantified in the plasma and brain of rats using similar methods and results compared with the controls. RESULTS/ANTICIPATED RESULTS: We found that brain and the heart fell on extreme ends of the CL diversity spectrum with 26 species of CL exclusively present in human brain not heart. Nine of these 26 species were present in plasma within 6 hours of ROSC with quantities correlating with greater brain injury. The C-score correlated with early neurologic injury and predicted discharge neurologic/functional outcome. CL (70:5) emerged as a potential point-of-care marker that alone was predictive of injury severity and outcome nearly as well as C-score. Using a rat CA model we showed a significant reduction in hippocampal CL content corresponding to CL released from the brain into systemic circulation. C-score was significantly increased in 10 minute Versus 5 minute no-flow CA and naïve controls. DISCUSSION/SIGNIFICANCE OF IMPACT: CA results in appearance and accumulation of CL in plasma, proportional to injury severity. Quantitation of brain-type CL species in plasma can be used to prognosticate neurological injury within 6 hours after ROSC.

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Longitudinal changes in EEG power envelope connectivity are proportional to motor recovery in chronic stroke patients

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OBJECTIVES/SPECIFIC AIMS: The objective of this study is to determine the degree to which the use of a contralesionally-controlled brain-computer interface for stroke rehabilitation drives change in interhemispheric motor cortical activity. METHODS/STUDY POPULATION: Ten chronic stroke patients were trained in the use of a brain-computer interface device for stroke recovery. Patients perform motor imagery to control the opening and closing of a motorized hand orthosis. This device was sent home with patients for 12 weeks, and patients were asked to use the device 1 hour per day, 5 days per week. The Action Research Arm Test (ARAT) was performed at 2-week intervals to assess motor function improvement. Before the active motor imagery task, patients were asked to quietly rest for 90 seconds before the task to calibrate recording equipment. EEG signals were acquired from 2 electrodes —one each centered over left and right primary motor cortex. Signals were preprocessed with a 60 Hz notch filter for environmental noise and referenced to the common average. Power envelopes for 1 Hz frequency bands (1–30 Hz) were calculated through Gabor wavelet convolution. Correlations between electrodes were then calculated for each frequency envelope on the first and last 5 runs, thus generating one correlation value per subject, per run. The chosen runs approximately correspond to the first and last week of device usage. These correlations were Fisher Z-transformed for comparison. The first and last 5 run correlations were averaged separately to estimate baseline and final correlation values. A difference was then calculated between these averages to determine correlation change for each frequency. The relationship between beta-band correlation changes (13–30 Hz) and the change in ARAT score was determined by calculating a Pearson correlation. RESULTS/ANTICIPATED RESULTS: Beta-band inter-electrode correlations tended to decrease more in patients achieving greater motor recovery (Pearson's $r = -0.68$, $p = 0.031$). A similar but less dramatic effect was observed with alpha-band (8–12 Hz) correlation changes (Pearson's $r = -0.42$, $p = 0.22$). DISCUSSION/SIGNIFICANCE OF IMPACT: The negative correlation between inter-electrode power envelope correlations in the beta frequency band and motor recovery indicates that activity in the motor cortex on each hemisphere may become more independent during recovery. The role of the unaffected hemisphere in stroke recovery is currently under debate; there is conflicting evidence regarding whether it supports or inhibits the lesioned hemisphere. These findings may support the notion of interhemispheric inhibition, as we observe less in common between activity in the 2 hemispheres in patients successfully achieving recovery. Future neuroimaging studies with greater spatial resolution than available with EEG will shed further light on changes in interhemispheric communication that occur during stroke rehabilitation.

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Mental illness public stigma, culture, and acculturation among Vietnamese Americans

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OBJECTIVES/SPECIFIC AIMS: Stigma has been recognized as a major impediment to accessing mental health care among Vietnamese and Asian

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