Precision Medicine. METHODS/STUDY POPULATION: Transurethral resection of bladder tumors were selected for testing based on availability and tissue composition. A wash step was used to generate daughter aliquots composed of dislodged cells and a solution with prior contact to the parent tissue. This wash step served two purposes: 1) reduce the amount of contaminating material from spreading to other cases, a problem known to be associated with this type of specimen; and 2) create aliquots from which additional informative data could be generated. These daughter aliquots were then examined to determine their value as a source for exosome profiling, metabolomic studies, molecular characterization and organoid development. The parent tissue was not compromised, was able to undergo conventional processing and yielded results equivalent to unwashed specimens. RESULTS/ANTICIPATED RESULTS: Exosomes secreted by the tumor cells were identified to be present in the daughter aliquots by a combination of their isolation using CD31 and detection of miR-21 expression. These exosomes were confirmed to be not related to fragmented cells from testing for beta-tubulin. A global/discovery-based approach using mass spectrometry provided insights into early characterization of metabolomic profiles present in these tumor cells. Ample amounts of high quality DNA (226 ng/ul concentrations; 11.3 ug total) were recovered from the dislodged, excess cells in the wash for molecular studies. Finally, from viable cells recovered in one of the daughter wash aliquots, the ability to grow organoids was proven to be possible and reproducible. DISCUSSION/SIGNIFICANCE: Based on these results, the value of the clinical specimen can be markedly expanded for utilization in research and possible clinical use without detracting from the parent tissue. This non-destructive, easy to adopt wash procedure can potentially lead to an influx of data that may ultimately prove useful in improving patient care.

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WDR5 represents a therapeutically exploitable target for cancer stem cells in glioblastoma

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OBJECTIVES/GOALS: Glioblastomas (GBMs) are heterogeneous, treatment-resistant tumors that are driven by populations of cancer stem cells (CSCs). In this study, we perform an epigenetic-focused functional genomics screen in GBM organoids and identify WDR5 as an essential epigenetic regulator in the SOX2-enriched, therapy resistant cancer stem cell niche. METHODS/STUDY POPULATION: Despite their importance for tumor growth, few molecular mechanisms critical for CSC population maintenance have been exploited for therapeutic development. We developed a spatially resolved loss-of-function screen in GBM patient-derived organoids to identify essential epigenetic regulators in the SOX2-enriched, therapy resistant niche. Our niche-specific screens identified WDR5, an H3K4 histone methyltransferase responsible for activating specific gene expression, as indispensable for GBM CSC growth and survival. RESULTS/ANTICIPATED RESULTS: In GBM CSC models, WDR5 inhibitors blocked WRAD complex assembly and reduced H3K4 trimethylation and expression of genes involved in CSC-relevant oncogenic pathways. H3K4me3 peaks lost with WDR5 inhibitor treatment occurred disproportionally on POU transcription factor motifs, required for stem cell maintenance and including the POU5F1(OCT4)::SOX2 motif. We incorporated a SOX2/OCT4 motif driven GFP reporter system into our CSC cell models and found that WDR5 inhibitor treatment resulted in dose-dependent silencing of stem cell reporter activity. Further, WDR5 inhibitor treatment altered the stem cell state, disrupting CSC in vitro growth and self-renewal as well as in vivo tumor growth. DISCUSSION/SIGNIFICANCE: Our results unveiled the role of WDR5 in maintaining the CSC state in GBM and provide a rationale for therapeutic development of WDR5 inhibitors for GBM and other advanced cancers. This conceptual and experimental framework can be applied to many cancers, and can unmask unique microenvironmental biology and rationally designed combination therapies.

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A Novel Animal Model of Radiation-Induced Heart Disease Using Photon Radiation

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OBJECTIVES/GOALS: The purpose of this study is to develop a clinically relevant mouse model of Radiation-Induced Heart Disease (RIHD) and characterize the resulting phenotype to find biomarkers and therapeutic targets as well as to understand the changes in cellular and molecular mechanisms of bioenergetics. METHODS/STUDY POPULATION: We used a two-beam method in the axillary region targeting the heart to irradiate male BALB/c mice at an isodose of 22, 16 and 8 Gray (Gy). We examined cardiac damage (i.e., vacuolization), inflammation, and DNA damage at 10 days post irradiation using histology and immunohistochemistry of heart tissue and cardiac function at day 35 by echocardiography. Additionally, cardiac tissue of mice irradiated at 22 Gy was collected at day 10 and day 35 post irradiation and sent for RNA sequencing. Data from RNA sequencing was analyzed using gProfiler, GSEA, and Cytoscape to enrich and visualize differentially expressed genes. RT-qPCR was performed to validate findings of significantly differentially expressed genes. RESULTS/ANTICIPATED RESULTS: Significantly increased phosphorylation of H2A.X indicated that irradiated mice were undergoing DNA double strand break repair indicating cardiac damage. Additionally, we found that regulators of mitochondrial function were decreased in the heart at day 10 for all doses. We found

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that mice that received 22 Gy developed cardiomyopathy at day 35 based on increased global longitudinal strain (GLS). Radiation decreased T cells, macrophages, and mast cells in the heart of irradiated mice by RT-qPCR at day 10 indicating damage to immune cells by radiation at all doses. Thus, we successfully created a clinically relevant model of RIHD in male BALB/c mice. DISCUSSION/SIGNIFICANCE: Patients undergoing radiation therapy for thoracic malignancies can develop cardiomyopathy (DCM) due to radiation damage. Previously published animal models utilized mouse strains resistant to developing DCM (female mice, C57BL/6 strain) and used high doses of radiation. Establishing a translational model is crucial for prevention of RIHD.

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Association between self-monitoring and ketogenic diet adherence in a technology-assisted lifestyle intervention

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OBJECTIVES/GOALS: Self-monitoring (SM) improves adherence to low-fat low-calorie (LFLC) diet for weight management. Ketogenic diet (KD) is a promising alternative to LFLC, however, it is unclear whether SM improves KD adherence. We examined the association between SM and KD adherence during the first 12 weeks of a 6-month technology-assisted lifestyle intervention. METHODS/STUDY POPULATION: We included 30 (50.8 ± 12.4 years, 70% female) overweight/obese (body mass index: 37.1 ± 7.2 kg/m2) participants in the analysis. They received personalized KD goals with very low-carbohydrate (22–62 g/d), moderate protein (52 -87 g/d), and high-fat (115 - 219g/d) and calorie intake goals (1338–2554 kcal/d). Additionally, participants performed daily diet, exercise, and weight SM. Adherence to KD was measured by (1) self-monitored dietary intake, and (2) percent of days in ketosis state (blood ketone≥0.5 mmol/L) captured by a fingerstick blood ketone meter. SM frequency was defined as percent of days participant logged food intake, wore fitness tracker, and weighed body weight. Pearson correlation coefficients were computed to examine the correlation between SM in diet, exercise, and weight with KD adherence. RESULTS/ANTICIPATED RESULTS: Percentage of days participants SM for diet, exercise, and weight was 58.4 ± 32.2%, 66.4 ± 30.9%, and 59.0 ± 32.6%, respectively. Correlational analysis more frequent diet SM was positively correlated with more days in ketosis ($r = 0.58$, $p = 0.003$), higher fat intake ($r = 0.68$, $p = 0.0001$), and higher calorie intake ($r = 0.67$, $p = 0.002$) within the fat and calorie goals set; more frequent weight SM was positively correlated with more days in ketosis ($r = 0.48$, $p = 0.02$), higher fat intake ($r = 0.45$, $p = 0.023$), and higher calorie intake ($r = 0.44$, $p = 0.027$). DISCUSSION/SIGNIFICANCE: We found that diet and weight SM were positively associated with fat and calorie intake, as well as days in ketosis. Given the reported promising effect of KD on weight loss and the challenges of adhering to KD, our findings suggested that promoting SM on diet and weight might be a promising avenue for improving KD adherence leading to successful weight loss.

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Beneficial Actions of SGLT2 Inhibition and H2S Therapy in Heart Failure with Preserved Ejection Fraction

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OBJECTIVES/GOALS: SGLT2i therapy is currently a cornerstone in heart failure with preserved ejection fraction (HFpEF) therapy. Similarly, H2S has been shown to be beneficial in preclinical models of heart failure. With this in mind, we sought to investigate the effects of the SGLT2i and H2S donor therapy alone or in combination in a rodent model of cardiometabolic HFpEF. METHODS/STUDY POPULATION: Male C57BL/6N mice (9 weeks of age) were fed a high fat, Western diet (HFD) and received L-NG-Nitro arginine methyl ester (L-NAME) in the drinking water (0.5 g/L) to induce HFpEF. At 5 weeks, animals were randomized to either control, H2S donor (SG-1002, 90 mg/kg/d, P.O), Empagliflozin (155 mg/L, P.O), or the combination of SG-1002 and Empagliflozin for an additional 5 weeks while being maintained on HFD and L-NAME. Echocardiography, left ventricular invasive LV and systemic hemodynamics, and exercise capacity testing were performed to assess cardiovascular disease severity. Fasted glucose, circulating triglyceride and cholesterol content were similarly measured to quantify key clinical metabolic parameters. H2S and its metabolite, sulfane sulfur, were quantified to assure adequate H2S donation. RESULTS/ANTICIPATED RESULTS: Administration of SG-1002 restored H2S and sulfane sulfur to normal circulating levels. All treatment groups exhibited similar improvements in LV diastolic dysfunction as measured by E/E'and LVEDP. Combination therapy significantly improved exercise capacity whereas the monotherapy groups did not. Treatment with SG-1002 decreased fasting glucose and circulating cholesterol while all treatment groups displayed decreased circulating triglycerides and body weight compared to HFpEF control. DISCUSSION/SIGNIFICANCE: These data indicate that restoring H2S or treatment with an SGLT2i in this preclinical HFpEF model attenuates pathology. Combination of both drugs exhibited greater benefit than either monotherapy in important HFpEF parameters such as exercise capacity. Further studies are underway to characterize the benefits observed from combination therapy.

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