

bacteria growth over time to see how the donor microbiome would change during a 24 hour experiment in anaerobic conditions. Finally, we anticipate seeing increases in dATP with a Prevotella or Dialister supplemented donor microbiome compared to baseline donor microbiome. DISCUSSION/SIGNIFICANCE: The addition of vaginal dysbiosis to tissue model will increase accuracy of prediction of 100% protective TFVdp concentrations and is likely to provide a translational model that can be used to improve TFV-based PrEP in women and streamline development of future PrEP candidates, bringing more prevention options to women and ending the HIV epidemic.

300

### **Impact of the type of mechanical circulatory support (MCS) prior to transplant on development of post-orthotopic heart transplantation (OHT) infections**

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OBJECTIVES/GOALS: In 2018, the United Network for Organ Sharing began prioritizing patients on temporary MCS over those on durable MCS for OHT in an effort to prioritize sicker patients and decrease waitlist mortality. We explored the impact of this change by examining if the type of MCS prior to transplant affects the risk of post-transplant infection. METHODS/STUDY POPULATION: We will conduct a retrospective cohort study of approximately 350 patients that have undergone OHT at Tufts Medical Center between January 2014 and July 2021 who survived at least 72 hours post-transplant and have minimum post-transplant follow-up of one year or time to death if before one year. Chart review will determine the type of MCS in place prior to transplant and the occurrence of infections within one year of transplant. Data will also be collected on patient's age, sex, medical comorbidities, lab values, and open chest management practices. We will examine differences in the incidence rates of a composite outcome (blood stream infection, invasive fungal infection, skin and soft tissue infection of device sites, and mediastinitis) between patients that were on temporary versus durable MCS. RESULTS/ANTICIPATED RESULTS: We anticipate that this study will show a greater frequency of infections of all types in patients that received temporary as compared with durable mechanical circulatory support prior to transplantation. We will use Cox proportional hazards survival models to model multivariable relationships for predictors of infection. DISCUSSION/SIGNIFICANCE: This study will provide insights into the magnitude and type of infectious complications that patients experience after OHT and the impact that type of MCS and other factors have on their outcomes. The data obtained may have implications for choice of mechanical device prior to undergoing OHT surgery as well as antimicrobial prophylaxis.

301

### **Inhibition of lysine-specific histone demethylase 1A (KDM1A/LSD1) attenuates DNA double strand break repair and enhances efficacy of temozolomide in glioblastoma**

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OBJECTIVES/GOALS: Glioblastoma (GBM) patients face a poor prognosis. Glioma stem cells (GSCs), a chemo resistant GBM

subpopulation, possess enhanced DNA repair and elevated levels of epigenetic modifier KDM1A. This study aims to establish the significance of KDM1A in DNA repair and determine the potential of novel KDM1A inhibitor NCD38 to enhance TMZ efficacy in GSCs. METHODS/STUDY POPULATION: Patient derived GSCs were obtained via IRB-approved protocol from patient samples at UT Health San Antonio. KDM1A knockdown and knockout cells were generated by transduction of validated KDM1A-specific shRNA or gRNA, respectively. Brain bioavailability of KDM1A inhibitor NCD38 was established using LS-MS/MS. Effect of combination of KDM1A knockdown, knockout, or inhibition with TMZ was studied using cell viability, neurosphere, and self-renewal assays. Mechanistic studies were conducted using CUT&Tag-seq, RNA-seq, immunofluorescence, comet, Western blotting, RT-qPCR, homologous recombination (HR) or non-homologous end-joining (NHEJ) DNA repair reporter assays. In vivo efficacy of KDM1A knockdown or inhibitor alongside TMZ treatment was determined using orthotopic murine GBM models. RESULTS/ANTICIPATED RESULTS: KDM1A knockdown, knockout, or inhibition increased efficacy of TMZ in reducing cell viability and self-renewal of GSCs. Pharmacokinetic studies demonstrated KDM1A inhibitor NCD38 is readily brain penetrable. CUT&Tag-seq studies revealed KDM1A is enriched at DNA repair gene promoters. RNA-seq studies suggest KDM1A inhibition reduces DNA double strand break repair gene expression, with these findings validated using RT-qPCR and Western blotting. Knockdown, knockout, or inhibition of KDM1A attenuated HR and NHEJ-mediated DNA repair capacity. Immunofluorescence and comet assay support findings of increased DNA damage in NCD38/TMZ combination treated GSCs. Importantly, KDM1A knockdown or inhibition enhanced efficacy of TMZ and significantly improved survival of orthotopic GBM tumor-bearing mice. DISCUSSION/SIGNIFICANCE: Our results show compelling evidence that KDM1A is essential for DNA repair in GSCs and that KDM1A inhibition sensitizes GBM to TMZ via attenuation of DNA repair pathways. These findings suggest combination of KDM1A inhibitor NCD38 with TMZ could serve as a promising novel therapeutic strategy that can be translated to improve GBM patient outcomes.

303

### **Investigation of the antibacterial and regenerative properties of a novel AHA dental coating for the treatment of deep caries\***

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OBJECTIVES/GOALS: Our objective is to investigate the antibacterial and regenerative properties of a novel AHA dental coating for the prevention and treatment of deep caries (cavities). Further, we aim to investigate and compare these properties through in vivo

murine models and assessment on human saliva samples and human pulpal cells collected from clinical samples. **METHODS/STUDY POPULATION:** In vitro antibacterial studies were performed by collecting and culturing human salivary bacteria with AHA substrates and quantifying survival of cariogenic Sm (*S. mutans*). In vivo, C57BL/6 mice were treated with AHA composite fillings, infected with Sm clinical isolates, and fed a high sucrose diet with cavity formation assessment after 6 weeks. To evaluate regeneration, mice were similarly given composite with AHA or MTA (standard of care) upon pulpal exposure with regeneration quantified by microCT and histological analysis of dentin bridge formation, ALP production, and odontoblast migration. In vitro, AHA substrates were cultured with MC3T3-E1 pre-osteoblast cells and dental pulp stem cells obtained from clinical samples over 21 days, with mineralization and ALP assessed indicating osteogenesis. **RESULTS/ANTICIPATED RESULTS:** In vivo studies have shown the reduction of cavity formation in mice treated with AHA as well as dentin regeneration upon pulpal exposure using microCT and histological image analysis. Coinciding with these findings, AHA substrates eradicated cariogenic Sm in human saliva samples and single species cultures in vitro. Further, preliminary results in vitro have shown increased mineralization of MC3T3-E1 cells when cultured with AHA substrates in comparison to uncoated substrates. We anticipate similar increased mineralization as well as increased ALP production of human pulpal stem cells from clinical samples when cultured with AHA substrates, suggesting osteogenesis. Further, we anticipate increased odontoblast migration and ALP production upon additional analysis of in vivo tissue samples. **DISCUSSION/SIGNIFICANCE:** This work will elucidate the antibacterial and regenerative properties of AHA dental coatings. These results further support the translation of AHA coatings into the clinic as a novel therapeutic for the prevention and treatment of dental decay, which may overcome the limitations associated with the current treatments.

304

### Let Kids Play: Using Virtual Reality as a Substitute for General Anesthesia for Minor Procedures in Children

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**OBJECTIVES/GOALS:** Minor procedures are anxiety-provoking and/or painful for children. Virtual reality (VR) is an emerging technology in medicine and largely has been used as an adjunct to analgesia and opioids. This study reviews the institutional use of VR in lieu of pharmacology and general anesthesia (GA) to perform minor procedures in a pediatric population. **METHODS/STUDY POPULATION:** A retrospective chart review was performed on all patients that presented to our institution from 2019 to 2022 for hormone implant placement, exchange, or removal with VR distraction. Demographic and procedure information was recorded. The primary outcome was successful procedure completion without requiring pharmacologic sedation or analgesia. **RESULTS/ANTICIPATED RESULTS:** A total of 111 patients underwent the following minor procedures with VR only. 14 patients underwent more than one procedure resulting in a total of 126 procedures. The mean age was  $11.3 \pm 3.6$  years. 43 patients were female, 23 were female to male, 6 were non-binary, 7 were male, and 32 were male to female. 58% had private insurance. The most common diagnosis was

precocious puberty (54%) followed by gender dysphoria (46%). The most common procedure was implant placement (72%). 69% of procedures were performed in the clinic and 31% in a procedural room. All procedures were completed without requiring sedation or GA. None of the patients required intravenous catheter placement for the procedure. No intra-procedural complications were recorded. **DISCUSSION/SIGNIFICANCE:** Despite the current trend toward minimizing GA and sedation in children, there is no widely accepted substitute. VR is a feasible option that can spare children from sedation or GA for minor procedures. This can enable procedures to be transitioned into more resource efficient settings and improve pediatric patient experience.

305

### Leveraging multi-timepoint blood samples to characterize cancer-associated mutations in the blood over time

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**OBJECTIVES/GOALS:** Clonal hematopoiesis of indeterminate potential (CHIP) is a common age-related condition that confers an increased risk of blood cancer, cardiovascular disease, and overall mortality. Larger proportions of blood cells with the CHIP mutation (clones) lead to worse outcomes. The goal of this study was to characterize CHIP clonal behavior over time. **METHODS/STUDY POPULATION:** While DNA biobanks have the ability to identify large cohorts of individuals with CHIP, they typically only contain blood from a single timepoint, limiting the ability to infer how CHIP clones change over time. In this preliminary study, we utilized multi-timepoint blood samples from 101 individuals with CHIP in Vanderbilt's biobank (BioVU) to characterize clonal behavior over time. Using a CHIP gene-specific sequencing pipeline, we were able to characterize each individual's CHIP mutation(s) and how the fraction of cells with the CHIP mutation expanded/reduced over time. By Spring 2023, we will also include ~300 additional individuals with CHIP in this study. **RESULTS/ANTICIPATED RESULTS:** CHIP mutations occurred 48% of the time in DNMT3A and 23% of the time in TET2, consistent with previous studies. 21% of individuals had more than one CHIP mutation. The mean difference in time between the two timepoints was 5.2 years (SD=2.9). Surprisingly, we observed both clonal expansion and clonal reduction across timepoints with 30% of DNMT3A, 0.6% of TET2, and 46% of JAK2 clones shrinking over time. The fastest average expansion was seen in TET2 clones (2% growth/year) and the slowest in DNMT3A clones (0.4% growth/year), but there was a significant amount of variation between individuals. In DNMT3A clones, there were no differences observed between loss of function mutations, missense mutations or DNMT3A R882 hotspot mutations. Clonal competition was observed in individuals with multiple driver mutations. **DISCUSSION/SIGNIFICANCE:** We used multi-timepoint blood samples to quantify the change in CHIP cell fraction over time on a per individual basis and observed novel clonal behavior and competition. Understanding the factors that influence the rate of CHIP progression can lead to personalized disease risk assessment for individuals with CHIP.