STUDY POPULATION: We differentiated a family trio (a heart-healthy daughter and EA/LVNC-affected mother and daughter) of induced pluripotent stem cells into cardiomyocytes (iPSC-CMs) in a blinded manner on three iPSC clones per subject. Using flow cytometry, immunofluorescence, and biomechanical, electrophysiological, and automated contraction methods, we investigated iPSC-CM differentiation efficiency between D10-20, contractility analysis and cell cycle regulation at D20, and sarcomere organization at D60. We further conducted differential analyses following label-free protein and RNA-Seq quantification at D20. Via CRISPR-Cas9 gene editing, we plan to characterize KLHL26 variant-specific iPSC-CM alterations and connect findings to discoveries from patient-specific studies. RESULTS/ANTICIPATED RESULTS: All iPSC lines differentiated into CMs with an increased percentage of cTnT+ cells in the affected daughter line. In comparison to the unaffected, affected iPSC-CMs had fewer contractions per minute and altered calcium transients, mainly a higher amount of total calcium release, faster rate of rise and faster rate of fall. The affected daughter line further had shorter shortening and relaxation times, higher proliferation, lower apoptosis, and a smaller cell surface area per cardiac nucleus. The affected mother line trended in a similar direction to the affected daughter line. There were no gross differences in sarcomere organization between the lines. We also discovered differential expression of candidate proteins such as kinase VRK1 and collagen COL5A1 from proteomic profiling. DISCUSSION/SIGNIFICANCE: These discoveries suggest that EA/LVNC characteristics or pathogenesis may result from decreased contractile ability, altered calcium transients, and cell cycle dysregulation. Through the KLHL26 variant correction and introduction in the daughter lines, we will build upon this understanding to inform exploration of critical clinical targets.

## *Regulatory Science*

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### Youth Nicotine Addiction: Strategic Defiance of Regulatory Oversight by the Disposable Electronic Nicotine Delivery System Industry

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OBJECTIVES/GOALS: To assess the impact of federal regulations and policies relating to disposable Electronic Nicotine Delivery Systems (ENDS) on youth consumption of these products by identifying factors enabling its growing consumption among youth users and its relation to adolescent addiction to nicotine. METHODS/STUDY POPULATION: Disposable ENDS are all-in-one devices with pre-filled nicotine liquid and a built-in battery. Recent data shows increased sales as users, including youth, are switching from pod-based to disposable ENDS. Thus, an understanding of the regulatory landscape for these products will provide insight on how to mitigate youth nicotine addiction. Data from the Centers for Disease Control and Prevention 2021 National Youth Tobacco Survey (NYTS) was analyzed for patterns of adolescent use. FDA statements and actions involving disposable ENDS companies were reviewed to evaluate the current FDA stance. Analyses of both data sets identified factors enabling the growth in sale of disposable ENDS. RESULTS/ANTICIPATED RESULTS: The NYTS reported 53.7% of youth ENDS users report using disposable ENDS and Puff Bar is the leading ENDS device among youth consumers. In March 2021, Puff Bar announced a return to market with "tobacco-free nicotine" after ceasing sales following an FDA warning letter in July 2020. But synthetic nicotine retains the same chemical properties as tobacco-derived nicotine and the same risks for addiction and abuse. The Food and Drug Administration (FDA) maintains synthetic nicotine products will be regulated on a case-to-case basis, suggesting "closed system devices" containing synthetic nicotine may not be regulated as tobacco products. DISCUSSION/SIGNIFICANCE: The growing popularity of disposable ENDS among youth is problematic. Awareness of strategic regulatory defiance (i.e., Puff Bar), will bring light to industry sales tactics. To develop comprehensive data on disposable ENDS use by young adults, an anonymous survey of college students will be conducted.

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### Therapeutic Class Labeling of EGFR Companion Diagnostics (CDxs)

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OBJECTIVES/GOALS: Review all approved companion diagnostics (CDxs) labeling for therapeutic class language. Examine the regulatory pathways of CDx products whose labels contain therapeutic class labeling. Develop recommendations for pharmaceutical industry professionals on best practices in the co-development of CDxs and oncology therapeutic products. METHODS/STUDY POPULATION: Literature discussing companion diagnostics was reviewed from EBSCOhost, PubMed, and OVID. The Intended Use language within CDx labels on the Food and Drug Administrations "List of Cleared or Approved Companion Diagnostic Devices (In Vitro and Imaging Tools)" website were reviewed on November 1, 2021 for therapeutics class language. For CDx products with therapeutic class label language, the regulatory history was evaluated to determine the development approach taken to achieve the language. RESULTS/ANTICIPATED RESULTS: A total of 45 CDxs were identified, of which only 2 contained therapeutic class labeling, both of which were devices for the identification of epidermal growth factor receptor (EGFR) mutations for the treatment of non-small cell lung cancer (NSCLC). Three additional EGFR CDxs were approved; however, they did not contain therapeutic class labeling. The first CDx was the Cobas EGFR Mutation Test V2, which received therapeutic group label language as an update on October 27, 2020; however, prior to the therapeutic class labeling, three oncology products were named in the Intended Use: Tarceva (erlotinib), Tagrisso (osimertinib), and Iressa (gefitinib). The second CDx to incorporate therapeutic class labeling was the ONCO/Reveal Diagnostic Lung and Colon Cancer Assay upon initial approval on July 30, 2021. DISCUSSION/SIGNIFICANCE: EGFR CDxs are the first to shift towards therapeutic class labeling. Indication, molecular alterations, and mechanism of action of the approved therapeutic class products, number of products approved, as well as CDx analytical and clinical validation influence class label relevance. Discussions with the FDA are encouraged early in development.

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