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Custom AmpliSeq™ targeted resequencing to identify novel clincopathologic correlates in early-stage lung adenocarcinoma: A Yale lung cancer biorepository study

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The 5-year survival for Stages I/II lung adenocarcinoma (L-ADC) (60,500 US cases annually) following curative-intent complete resection and where indicated, adjuvant chemotherapy, ranges from 36% (Stage II) to only 80% (Stage IA). Although evaluation of EGFR and KRAS somatic mutations in early-stage L-ADCs is now common, clinicopathologic correlates and associations with prognosis are incompletely understood and the correlates of less common driver mutations and frequent passenger mutations (example STK11, p53) are only emerging. To better characterize the clinicopathologic correlates of L-ADC somatic mutations, we have coupled an ongoing prospective cohort study of early-stage L-ADC patients treated with curative-intent surgery at Yale-New Haven Hospital with a robust, novel next-generation DNA sequencing and analysis pipeline. Eligible participants are enrolled into the Yale Lung Cancer Biorepository, a Biobank that couples biospecimen best practices for both fresh and formalin-fixed materials with comprehensive clinico-epidemiologic annotations for each participant both at intake and at regular follow-up. Genomic DNA from both tumor and germ line is prepared using the Ambion Recover All DNA preparation kits and quantified using RNAse P standard. Ten ng is subsequently used for each of 3 pools from a customly constructed Ion Torrent AmpliSeq™ panel that targets 93 genes where the published literature supports a significant role for somatic mutations in L-ADC biology. Following emulsion PCR and sequencing on the Ion Torrent PGM™ next-generation DNA sequence, resulting BAM files are processed through a novel front-end bioinformatics pipeline that specifically adjudicates sequencing run to identify and eliminate from subsequent analyses runs not meeting specific qualifying criteria. Next, variants are aligned from matched germ line and tumor samples and among tumor-specific variants, the non-synonymous coding mutations are further pursued being organized according to functional roles within genes and pathways with these summary data arrayed into a data matrix for subsequent statistical analysis. This talk will focus on our uniquely comprehensive method, while highlighting results from our preliminary analyses.

Biography

Bonnie E Gould Rothberg is a Molecular Cancer Epidemiologist and Assistant Professor within the Department of Oncologic Research at the Yale Cancer Center with secondary appointments in the Departments of Chronic Epidemiology and Pathology at the Yale School of Medicine. She completed her MD (1994), MPH (2005) and PhD (2009) all from Yale University and joined the Yale faculty in 2011 following a 2-year Postdoctoral fellowship. Her lab is invested in leveraging Ion Torrent targeted-resequencing to identify both germ line and somatic genetic variation that are prognostic for early-stage cancers where, despite a curative-intent margin-free resection the median 5-year survival rates approach 50%. She has also built and still serves as the inaugural Medical Director for the Yale Lung and Gastrointestinal Cancer Biorepositories which combine biospecimen best practices with rigorous and robust clinico-epidemiologic annotations useful for evaluating gene-environment interactions that influence cancer outcomes. She has published over 30 manuscripts related to her work.

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