Acquired *NRG1* fusion as a mechanism of secondary resistance to EGFR therapy in *EGFR*-mutated lung cancer

Juan Luis Gomez Marti[1,2], Tom Zhang[2], Rachel Lai[2], Marissa S. Mattar[2], Maria Poplawska[3], Naveen Menon[3], Sabina Hajiyeva[1], Stephen Machnicki[4], Marc Ladanyi[2,5], Romel Somwar[2,5] and Morana Vojnic[6]

1. Department of Pathology and Laboratory Medicine, Lenox Hill Hospital, New York, NY, 10075

2. Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, 10065

3. Division of Hematology/Oncology, Lenox Hill Hospital, New York, NY, 10075

4. Department of Radiology, Lenox Hill Hospital New York, NY, 10075

5. Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, 10065

6. Rutgers Cancer Institute, New Brunswick, NJ, 08901

Background. EGFR tyrosine kinase inhibitors (TKIs) have transformed the treatment landscape of lung adenocarcinomas (LUAD) with *EGFR* mutations and propelled a new era for targeted therapies. Although durable responses can often be achieved, resistance inevitably develops, despite newer EGFR antagonists. Common resistance mechanisms include histological transformation, on-target *EGFR* mutations, off-target alterations/amplifications (*MET*, *BRAF*, *RET*, *FGFR*, etc.), and mutations in downstream pathways (KRAS, PIK3CA). Here, we investigated the role of *NRG1* rearrangements as an acquired mechanism of resistance to osimertinib in clinical settings.

Methods. Patient samples were sent to Caris Life Sciences for RNA and DNA sequencing on clinical diagnostic platforms. Isogenic cell lines were generated by lentiviral transfer of cDNAs into two cell lines with EGFR ex19del (HCC827 and PC9). Gene expression was confirmed by RT-PCR followed by Sanger sequencing of PCR amplicons. Cell growth was evaluated by time course studies in the absence or presence of 100 nM osimertinib.

Results. A LUAD patient with EGFR ex19 del and CNS metastases was placed on osimertinib but developed disease progression after 15 months of therapy. Biopsy of a recurrent lung lesion was sent for mRNA sequencing and found the original EGFR ex19 del and a new *TNFRSF10B::NRG1* fusion. Since *NRG1* fusions are known to act through HER3-HER2 complexes, the patient was treated with afatinib monotherapy, which

resulted in a partial response lasting almost 12 months. Subsequently, progression of disease prompted another biopsy revealing small cell transformation, at which stage the therapy was changed to platinum-based chemotherapy with immunotherapy. The patient succumbed to disease complications four months later. To model this patient's mechanism of resistance to EGFR TKI treatment, *TNFRSF10B::NRG1* cDNA was transduced into HCC827 and PC9 cells. Expression of mRNA was confirmed by RT-PCR and Sanger sequencing. Growth of HCC827 cells expressing an empty vector (HCC827-EV) was completely inhibited after treatment with 100 nM osimertinib. In contrast, treatment of HCC827-NRG1 cells with 100 nM osimertinib had no effect on cell growth.

Conclusions. While acquired resistance to EGFR TKI is established, novel resistance mechanisms are continually being discovered. Here, we present a clinical case with *in vitro* validation of an acquired *TNFRSF10B::NRG1* fusion as a mechanism of resistance to EGFR TKI. This further supports the importance of rebiopsy and resequencing at each step of disease progression in oncogene-driven LUAD. Further preclinical studies exploring combination therapies, including zenocutuzumab with osimertinib are currently being investigated. Zenocutuzumab has recently been FDA-approved for lung, pancreatic, and cholangiocarcinoma with NRG1 rearrangements.