

Leveraging p53 and MCL-1 as Therapeutic Targets in Small Cell Lung Cancer

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Background:

Small cell lung cancer (SCLC) is a neuroendocrine carcinoma known for its acquired chemoresistance. While chemoresistance is common after platinum-based strategies, lurbinectedin, or bispecific T-cell engagers, a need remains for novel therapeutic strategies in relapsed SCLC. Importantly, SCLC harbors near ubiquitous alterations in *TP53*, a historically undruggable target. New approaches with p53 reactivators, APR-246 and rezatapopt, can stabilize the conformation of mutant p53 to induce apoptosis. Here, we seek to explore their use, alone, and in combinations, for SCLC.

Methods:

For drug response, 1,000 cells were incubated for 24 hours (h) at 37°C. APR-246 (0.39-50 µM), rezatapopt (0.078-10 µM), or DMSO was added for 120 h, prior to evaluation of cell viability (CellTiter-Glo®). H82 and H524 were seeded in 384-well plate using Bravo automated liquid handling platform. Following 24 h, a 500-drug library screen was performed with 4 doses (0.1, 0.5, 2.5, 10 µM) of compounds +/- 2 µM APR-246 using Echo650. After 5 days, cell viability was measured for luminescence. To evaluate APR-246 effects, 1 X 10⁶ cells were incubated for 24 h, exposed to drug (1 or 5 µM APR-246, or DMSO) and harvested at 24 or 48 h. To determine the effects of MCL-1 knockdown, 1 X 10⁶ cells were reverse transfected with 50 nM pooled small interfering RNA (siRNA) against MCL-1 (or control) for 48 h and harvested. For western blot, standard lysate

preparation was used. Membranes were incubated in Every Blot Blocking Buffer with 1:500-1000 primary antibody (0.75 h) followed by 1:5000 secondary Ab (0.5 h). Four SCLC lines were chronically exposed to increasing doses (up to 75 μM) of APR-246 to generate "APR-R". RNA sequencing (RNA-seq) was performed on extracted RNA with an Illumina array.

Results:

Within a panel of SCLC, most SCLC lines demonstrated sensitivity to APR-246 as a single agent (IC_{50} range, 1.04-3.77 μM), compared to a p53 homozygous null line H1299 (IC_{50} >50 μM). In the context of *TP53* alterations, no difference in APR-246 IC_{50} was observed ($P = 0.23$). Across neuroendocrine (NE) subtypes (SCLC-A, SCLC-N, and SCLC-P), no difference in APR-246 sensitivity was seen ($P = 0.86$). Similarly, H748, a p53 Y220C mutant SCLC line, is sensitive to both rezatapopt (IC_{50} , 0.39 μM) and APR-246 (IC_{50} , 1.52 μM). Proteomics of H82 and H524 APR-R (compared to parental lines) both demonstrated overexpression of BCL-2, midkine (MDK), and ARMC2. RNA-seq confirmed transcriptional activation of BCL-2 and MDK in APR-R versus parental lines. Interestingly, an ASCL1+ SCLC H146 which is less sensitive to APR-246 (IC_{50} , 6.28 μM) was found to have overexpression of BCL-2 protein, when compared to other SCLC tested. A 500-drug screen in conjunction with APR-246 demonstrated a class effect with APR-246 and MCL-1 inhibitors (MCL-1i), wherein transient loss of MCL-1 by siRNA knockdown induced apoptosis and decreased p53 expression.

Conclusions:

MCL-1i with p53 reactivators is a promising therapeutic combination for SCLC, independent of NE subtype or *TP53* alteration. Mechanistic understanding of how BAX/BAK homeostasis may regulate this response in SCLC is underway.