*The On-Target MRTX1133 Resistance Mutation KRAS(R68S) Acts by Enhancing Lung Adenocarcinoma Cell Fitness*

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MRTX1133 represents a breakthrough as the first clinical-stage drug targeting KRAS G12D, a mutation driving a significant portion of lung adenocarcinomas (LUADs). However, as with most targeted therapies, on-target resistance mutations (as well as activation of potentially other MRTX1133 resistance pathways) are an anticipated hurdle. This study utilizes the LentiMutate system to discover and characterize on-target resistance mechanisms against MRTX1133. KRAS plays a critical role in cancers, with mutations found in roughly 20-30% of all cases, particularly enriched in aggressive cancers like pancreatic, colorectal, and lung cancers. Functioning as a molecular switch, KRAS cycles between inactive (GDP-bound) and active (GTP-bound) states. Oncogenic mutations, like G12D, lock KRAS in a constitutively active GTP form, promoting uncontrolled cell proliferation. Initially deemed "undruggable" due to a lack of clear binding pockets, RAS has been successfully targeted through various approaches, including the inducible switch 2 pocket (SW2P) near the nucleotide binding site. We employed LentiMutate, a rapid and unbiased method for discovering on-target drug resistance mutations, and after discovery, validated each putative resistance mutation for their ability to confer MRTX1133 resistance. We identified and validated KRAS R73P and R68S as the most prevalent mutations conferring primary resistance against MRTX1133. Notably, R73P mutations haven't been observed with other SW2P binders, while R68S has, indicating potential MRTX1133 specificity for this resistance mutation. Interestingly, molecular dynamics simulations did not predict a significant decrease in inhibitor binding for these mutations. To elucidate this finding, we performed biochemical and functional characterization. These mutations were observed to decrease both intrinsic and GAP-mediated GTP hydrolysis, mimicking the behavior of RAS oncogenes. Additionally, R68S evaluation in cellular models revealed activation of MAPK signaling. These findings suggest that R68S contributes to drug resistance, at least partially, by enhancing cell fitness. This biologic activity of a drug resistance mutation thus has major potential implications for the development of future strategies to combat drug resistance mechanisms in KRAS G12D-driven cancers, including lung cancers. (SPORE P50 CA070907).