**Introduction and background**

The MAPK pathway is a major driver of malignant progression, particularly in cancers arising from mutations in pathway components. We have previously reported that KO-947, a potent and selective inhibitor of ERK2, with extended target residence time and favorable pharmacokinetic properties, displays robust single-agent activity in PDX models of adenocarcinomas with RAS/RAF mutations and in squamous cell carcinomas (SCCs) that lack mutations in MAPK pathway components. Here, we report that focal amplification of chromosome 11 between bands q13.3 and q13.4 is a feature of several tumor types, including ESCC and HNSCC. TCGA genomic analyses revealed selectivity to KO-947 in head and neck (HNSCC) and esophageal SCC (ESCC) PDX models.

Recurring amplification of chromosome 11 between bands q13.3 and q13.4 is a feature of several tumor types, including ESCC and HNSCC. TCGA genomic analyses revealed that amplification of the cyclin D1 locus was most closely linked to incidences of >50% in ESCC and ~20% in HNSCC, although higher frequencies were seen in some subtypes, such as pharyngeal and HPV-negative SCC. The 11q13 amplicon commonly contains about a dozen genes, including CCND1, ANO1, and the recently described calcium-dependent chloride channel ANC1.

**Summary of PDX campaigns with KO-947**

PDX campaigns were conducted at Crown Bio. Groups of three nu/nu or NOD/SCID mice were inoculated SC with low-passage patient-derived xenografts and tumors were allowed to become established to a volume of ~250mm³ before initiation of therapy with oral KO-947 at 300mg/kg weekly.

**Results**

ERK blockade inhibited tumor growth to some extent in almost every model, illustrating the fundamental role of the MAPK pathway in the growth of both ADCs and SCCs, but the majority of response observed were in squamous cell carcinoma models, especially ESCC and HNSCC. Although response rates were high, KRAS and BRAF mutations are extremely rare in SCCs, suggesting that other genetic factors likely underlie the observed dependence on MAPK pathway signaling in these tumor types.

**Cytogenetics**

- The 11q13 amplicon contains at least ten co-amplified genes.
- Not all genes in the 11q13 amplicon are overexpressed in responding ESCC models.
- Cyclin D1, ANO1 and FADD genes are co-amplified in SCCs.

**Model for ERK-dependent oncogenic cooperativity between amplified 11q13 genes**

- KO-947, a potent and selective inhibitor of ERK2 currently in Phase I clinical development, displays robust antitumor activity in PDX models of head and neck and esophageal squamous cell carcinoma.
- 11q13 amplification may be a promising marker for patient enrichment in SCC, as response rates in ESCC and HNSCC PDX models were increased in the amplified subpopulation.
- The 11q13 amplicon contains multiple potential oncogenes, including three (CCND1, ANO1, FADD) that are MAPK-associated, and one (FGF3) that is likely to drive 11q13-amplified SCC cells in the MAPK pathway (i.e., ERK addiction).
- 11q13-amplified SCC models that failed to respond to KO-947 commonly displayed paradoxical low levels of the 11q13 gene product ANO1, suggesting that ANO1 may play a key role in ERK dependency in SCCs.

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