Tipifarnib potentiates the antitumor effects of PI3Kα blockade in HNSCC via convergent inhibition of mTOR activity

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BACKGROUND

• The PI3K pathway is the most frequently activated pathway in HNSCC; ~30% of tumors harbor PIK3CA mutation or amplification
• Feedback reactivation of PI3K or compensatory parallel pathways limits the single agent efficacy of PI3K inhibitors, necessitating development of rational combination strategies
• Tipifarnib, a farnesyltransferase inhibitor, blocks hyperactivated growth factor signaling at multiple nodes, including HRAS and RHEB

• Here, we utilize cell line and PDX models to evaluate the therapeutic potential of combined tipifarnib and PI3Kα inhibitor alpelisib in the PIK3CA-dysregulated subset of HNSCC

RESULTS

Tipifarnib and alpelisib inhibit spheroid growth of PI3Kα-dysregulated HNSCC cell line models

Figure 1. Tipifarnib and the PI3Kα inhibitor alpelisib specifically inhibit the growth of PIK3CA-dysregulated HNSCC cell line models. Heat maps indicate the degree of growth inhibition or cytotoxicity induced by tipifarnib, alpelisib, or the combination in HNSCC cell lines cultured as 3D tumor spheroids. PIK3CA status as indicated above heat map, copy gain defined as >2.5 copies.

Tipifarnib and alpelisib synergize on PIK3CA-altered cell lines

Tipifarnib blunts mTOR and RSK reactivation following alpelisib treatment and induces apoptosis

Depletion of RHEB expression phenocopies tipifarnib treatment

CONCLUSIONS

• Tipifarnib and alpelisib synergize to induce cytotoxicity in PI3Kα-dysregulated cell lines and tumor stasis or regression in PIK3CA-mutant/amplified PDX models
• Tipifarnib blocks the compensatory reactivation of MAPK and mTOR signaling that follows single-agent alpelisib treatment
• RHEB inhibition via tipifarnib allows for durable blockade of mTOR activity and induction of apoptosis when combined with alpelisib
• The combination of tipifarnib and alpelisib holds potential for the treatment of recurrent/metastatic HNSCCs harboring dysregulated PIK3CA and is being evaluated in the recently initiated KURRENT clinical trial (NCT04997902)

Figure 2. Tipifarnib and alpelisib have synergistic activity in cell lines harboring PIK3CA mutation or copy gain. Loewe synergy score is plotted against combination sensitivity score. Heat maps indicate the degree of growth inhibition or cytotoxicity induced by tipifarnib, alpelisib, or the combination in HNSCC cell lines cultured as 3D tumor spheroids. PIK3CA status as indicated above heat map, copy gain defined as >2.5 copies.

Figure 3. Combined tipifarnib and alpelisib treatment inhibits mTOR and RSK more potently and durably than alpelisib alone and strongly induces apoptosis. Immunoblots of indicated MAPK/PI3K pathway components and apoptotic markers in PIK3CA-mutant CAL33 cells treated with alpelisib (BYL-719) for 0, 1, 2, 6, and 24 hours in the absence or presence of tipifarnib (48-hour treatment). Shift in RHEB mobility is indicative of deamorylation. Combination treatment results in stronger inhibition of mTOR activity/target phosphorylation (p70 S6K, S6, 4EBP1), cell cycle arrest (RB phosphorylation), and cell death (PARP and caspase cleavage).

Figure 4. Depletion of expression of the obligately farnesylated mTOR activator RHEB phenocopies tipifarnib treatment. CAL33 cells were treated with small interfering RNAs to knock down RHEB expression (vs. control non-targeting pool) for 48 hours prior to addition of alpelisib (BYL-719). Cells were collected and lysed after 0, 1, or 24 hours of alpelisib treatment and immunoblot analysis performed to assess mTOR activity levels.

Figure 5. Tipifarnib treatment markedly diminishes the lysosomal localization of RHEB. Active RHEB localizes to lysosomes to regulate mTOR activity. Density gradient ultracentrifugation was utilized to extract lysosomes from CAL33 cells treated with DMSO or tipifarnib. Lysosomes were lysed and subjected to immunoblot analysis alongside whole-cell lysate (WCL). LAMP1 is a lysosome-specific marker.

Figure 6. Combined, synchronous tipifarnib-alpelisib treatment robustly inhibits the growth of PIK3CA-dysregulated HNSCC patient-derived xenograft models. A. Growth of HNSCC PDX models harboring indicated PIK3CA alterations, treated with vehicle, tipifarnib (60 mg/kg BID), alpelisib (40 mg/kg OD), or the combination. B. Impact of varied four-week dosing schedules on the growth of PIK3CA-mutant/amplified PDX tumors. Tipifarnib (T) and alpelisib (A) were administered over the course of study as indicated in table.