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

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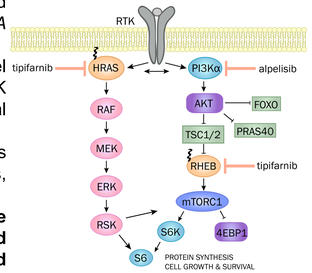
Abstract #1120

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

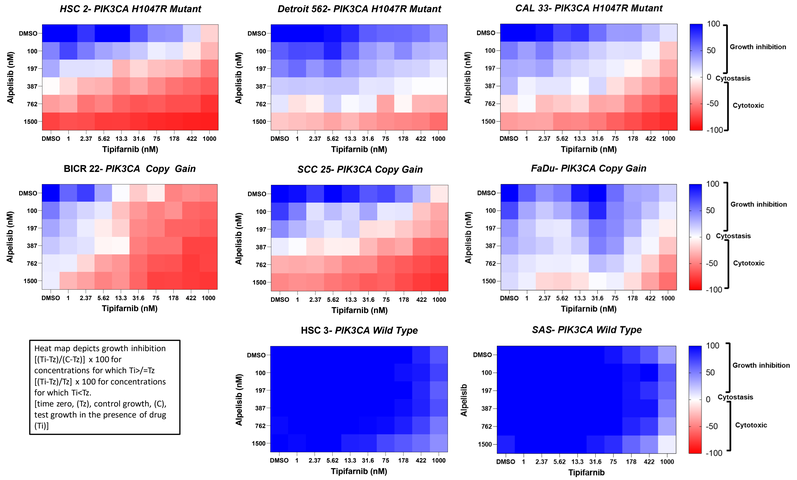
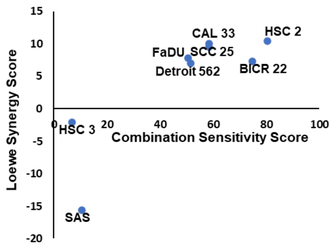


Figure 1. Tipifarnib and the PI3Kα inhibitor alpelisib specifically inhibit the growth of *PIK3CA*-dysregulated HNSCC cell line models. Heat maps indicate the degree of growth inhibition or cytotoxicity induced by tipifarnib, alpelisib, or the combination in HNSCC cell lines cultured as 3D tumor spheroids.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

Tipifarnib and alpelisib synergize in *PIK3CA*-altered cell lines



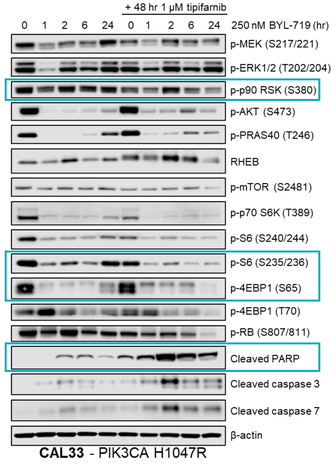
Cell line	Combination Sensitivity Score	Loewe Synergy Score	PIK3CA Status
HSC 2	80.3	10.4	H1047R
CAL 33	58.3	10	H1047R
Detroit 562	51.5	7	H1047R
SCC 25	58.3	9.5	Copy Gain
FaDU	50.4	7.8	Copy Gain
BICR22	74.8	7.35	Copy Gain
HSC 3	6.9	-2.1	WT
SAS	10.6	-15.6	WT

Loewe Synergy Score:
>+10 **Synergistic**
+10 to -10 **Additive**
<-10 **Antagonistic**

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, calculated using SynergyFinder R package (Zheng, et al. *Genomics Proteomics Bioinformatics* 2022). Higher scores indicate higher sensitivity or synergy.

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

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 defarnesylation. 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).



Depletion of RHEB expression phenocopies tipifarnib treatment

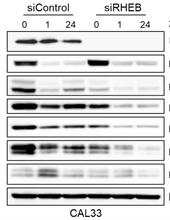


Figure 4. Depletion of expression of the obligately farnesylated mTOR activator RHEB phenocopies tipifarnib treatment. CAL33 cells were treated with small interfering RNAs to knock down RHEB expression (vs. control non-targeting pool) for 48 hours prior to addition of alpelisib (BYL-719). Cells were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

Tipifarnib blocks RHEB's localization to lysosomes

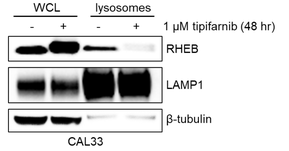


Figure 5. Tipifarnib treatment markedly diminishes the lysosomal localization of RHEB. Active RHEB localizes to lysosomes to regulate mTOR activity. Density gradient ultracentrifugation was utilized to extract lysosomes from CAL33 cells treated with DMSO or tipifarnib. Lysosomes were lysed and subjected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

Tipifarnib-alpelisib combination ablates growth of *PIK3CA*-dysregulated patient-derived HNSCC xenografts

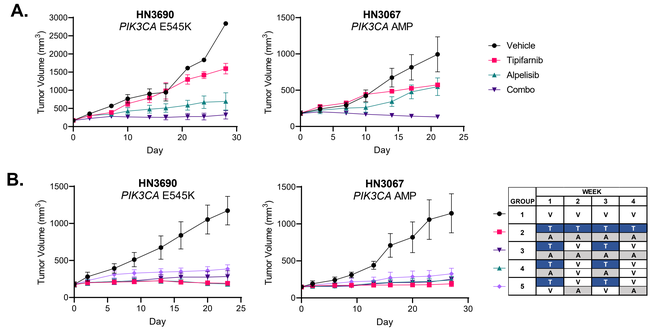


Figure 6. Combined, synchronous tipifarnib-alpelisib treatment robustly inhibits the growth of *PIK3CA*-dysregulated HNSCC patient derived xenograft models. A. Growth of HNSCC PDX models harboring indicated *PIK3CA* alterations, treated with vehicle, tipifarnib (60mg/kg BID), alpelisib (40 mg/kg QD), 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.

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)