VS-5584 a Dual mTOR and PI3K Inhibitor has Antitumor Activity in Multiple *in vivo* Xenograft Tumor Models and Enhanced Efficacy in Combination with Cisplatin or Docetaxel

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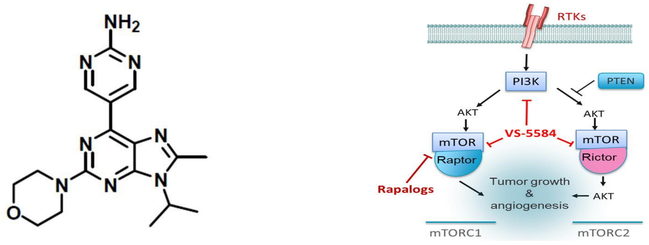
ABSTRACT

Verastem is developing VS-5584, a potent and selective dual inhibitor of the mammalian target of rapamycin complexes 1 and 2 (mTOR) and Class I phosphatidylinositide 3-kinases (PI3K), for the treatment of cancer. The PI3K/mTOR signaling pathway is a key regulator of cancer progression and in the survival of cancer stem cells (CSCs). VS-5584 has been shown to be an equipotent inhibitor of all four human Class I PI3K isoforms and the mTOR kinase, and PI3K signaling has been implicated in the maintenance of CSCs in solid tumors. In multiple orthogonal *in vitro* assays, VS-5584 has shown to preferentially target CSCs and exhibited significant antiproliferative activity across multiple cancer cell lines. Furthermore, oral administration of VS-5584 has been shown to reduce CSCs in xenograft models. The *in vivo* antitumor efficacy of once daily and intermittent oral administration of VS-5584 was evaluated in multiple xenograft tumor models representing small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), triple negative breast cancer (TNBC) and mesothelioma. Once daily (qd) treatment with VS-5584 demonstrated potent anti-tumor activity, with mean percentage tumor growth inhibition (TGI) ranging from 40% to 97% ($P < 0.05$). In these models, TGI was dose-dependent with dosages at and above 15 mg/kg showing good antitumor activity ($P < 0.05$). Interestingly, tumor regression (TR) was observed in 33% (3/9) of mice bearing H69 (SCLC) tumors, 60% (6/10) of mice bearing MDA-MB-468 (TNBC) tumors and 50% (5/10) of mice bearing NCI-H226 mesothelioma tumors. This significant antitumor activity was generally observed at well-tolerated dosages. In studies exploring intermittent dosing schedules, efficacy and tolerability were similar or better with a qd5 days on/2 days off or Monday, Wednesday, Friday schedule compared to continuous daily dosing. We also explored the efficacy of VS-5584 in combination with either cisplatin or docetaxel. In these studies, VS-5584 plus either cisplatin or docetaxel showed significant TGI compared to cisplatin alone in H69 SCLC and docetaxel alone in A549 NSCLC xenograft models ($P < 0.05$). This potent *in vivo* antitumor activity in xenograft models of NSCLC, SCLC, TNBC and mesothelioma suggests that VS-5584 has the potential for anticancer activity across a variety of cancer types. Intermittent dosing with VS-5584 was sufficient to achieve good efficacy while minimizing side effects, thus allowing a broader therapeutic window compared to qd dosing. VS-5584 in combination with either cisplatin or docetaxel had enhanced antitumor activity compared to two chemotherapeutic agents in two *in vivo* models of lung cancer. VS-5584 safety is being evaluated in a Phase 1 clinical trial assessing intermittent dosing in subjects with advanced non-hematologic malignancies or lymphoma.

INTRODUCTION

Figure 1. Structure & Selectivity Profile of VS-5584



Targeting Cancer Stem Cells for a Durable Clinical Response

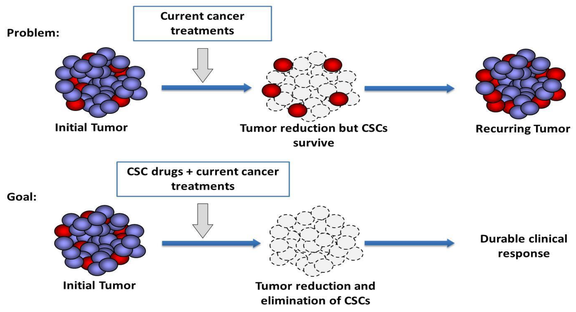


Table 1. VS-5584 is a Potent Inhibitor of Recombinant Human PI3Kα, PI3Kβ, PI3Kγ, and PI3Kδ mTOR Kinases, and the H1047R Mutant Form of PI3Kα with Low nM IC_{50}

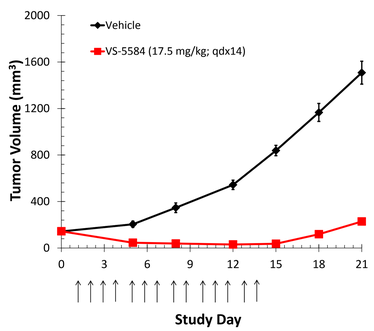
Biochemical Potencies (IC_{50}, nM)					
mTOR	PI3Kα	PI3Kαmut H1047R	PI3Kβ	PI3Kγ	PI3Kδ
3.4	2.6	3.3	21	2.7	3

VS-5584 was selective for these kinases among a panel of over 400 kinases (Hart et al., Mol Cancer Ther. 2013 Feb;12(2):151-61.)

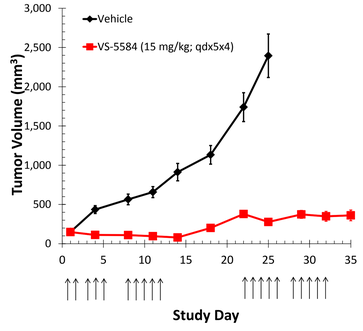
RESULTS

Figure 2. Intermittent Dosing Schedules (qdx5 or tiw) are as Effective as Continuous Daily Dosing (qd) in SUM159 TNBC Xenograft Model

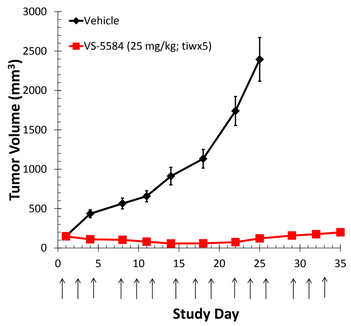
A. VS-5584 Daily Dosing



B. VS-5584 5 Days on/2 Days Off Dosing



C. VS-5584 Three Times a Week Dosing



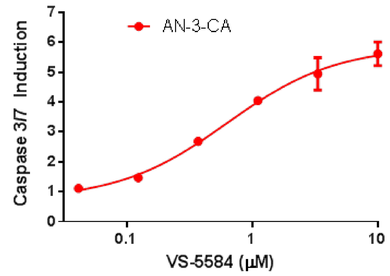
A. Female NOD SCID mice (n = 10) were injected with 2 X 10^6 SUM159 TNBC cells SC in the right flank. Mice were treated orally (PO) with VS-5584 (17.5 mg/kg; qdx14) when the mean tumor volume reached approximately 150 mm^3 (TGI = 84.9%; $P < 0.001$).

B. Female NOD SCID mice (n = 10) were injected with 2 X 10^6 SUM159 TNBC cells SC in the right flank. Mice were treated PO with VS-5584 (15 mg/kg; qdx5x4) when the mean tumor volume reached approximately 150 mm^3 (TGI = 84.4%; $P < 0.001$).

C. Female NOD SCID mice (n = 10) were injected with 2 X 106 SUM159 TNBC cells SC in the right flank. Mice were treated PO with VS-5584 25 mg/kg; tiwx5) when the mean tumor volume reached approximately 150 mm^3 (TGI = 94.9%; $P < 0.001$).

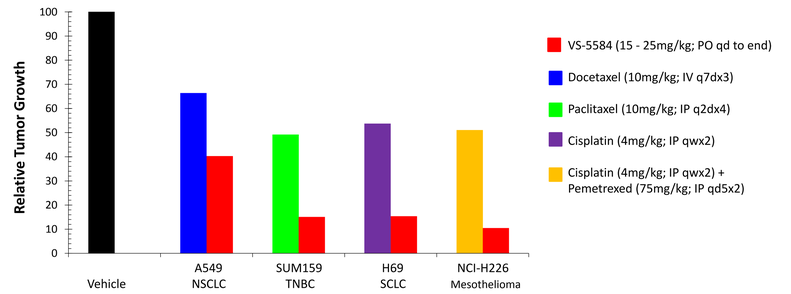
Arrows indicate dosing days. Data are presented as mean ± SEM.

Figure 3. VS-5584 Induces Apoptosis



AN-3-CA human endometrial cancer cells were treated with VS-5584 for 20 h followed by Caspase-3/7 assay. Data are presented as mean ± SEM.

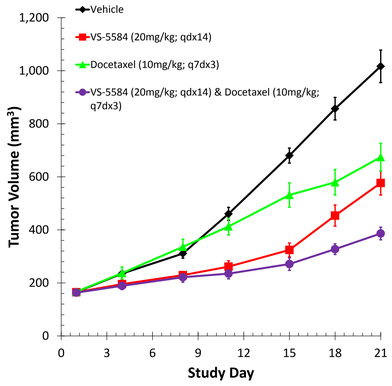
Figure 4. VS-5584 has Enhanced Antitumor Activity Compared to Standard Chemotherapeutic Agents in Xenograft Models of NSCLC, TNBC, SCLC and Mesothelioma



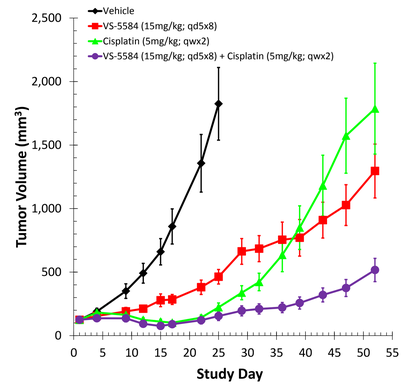
Female immunodeficient mice (n = 9 or 10) were injected with A549 NSCLC, SUM159 TNBC, H69 SCLC or NCI-H226 mesothelioma cells SC in the right flank. When mean tumor volume was approximately 150 mm^3, mice were treated PO with VS-5584, IV with docetaxel, IP with paclitaxel, IP with cisplatin or IP with a combination of cisplatin + pemetrexed. Data are presented as relative tumor growth compared to vehicle ($P < 0.05$, VS-5584 vs. vehicle and VS-5584 vs. chemotherapeutic agent in each model).

Figure 5. VS-5584 in Combination with Either Cisplatin or Docetaxel has Enhanced Antitumor Activity

A. A549 NSCLC



B. H69 SCLC



A. Female BALB/c nude mice (n = 10) were injected with 5 x 10^6 A549 NSCLC cells SC in the right flank. Mice were treated PO with VS-5584 (20 mg/kg; qdx14), IV with docetaxel (10 mg/kg; q7dx3) or combination of both when the mean tumor volume reached approximately 150 mm^3 (TGI; VS-5584 = 43.3%, docetaxel = 33.7% or combination = 62.0%; $P < 0.001$; VS-5584 + docetaxel combination showed significant tumor growth inhibition compared to docetaxel or VS-5584 alone, $P < 0.01$). Data are presented as mean ± SEM.

B. Female athymic nude mice (n = 10) were injected with 5.0 x 10^6 H69 SCLC cells SC in the right flank. Mice were treated PO with VS-5584 (15 mg/kg; qd5x8), IP with cisplatin (5 mg/kg; qwx2) or combination of both when the mean tumor volume reached approximately 150 mm^3 (TGI; VS-5584 = 80.0%, cisplatin = 94.2% or combination = 96.7%; $P < 0.05$). Data are presented as mean ± SEM.

SUMMARY

- **Based on the single and combination activity observed across a broad panel of cancer models, VS-5584 has the potential for broad antitumor activity across a variety of different cancer types**
- **VS-5584 in combination with standard chemotherapeutic agents had enhanced antitumor activity compared to chemotherapeutic agents alone in the models tested**
- **Intermittent dosing with VS-5584 resulted in significant antitumor activity that was similar to or better than a once daily continuous dosing regimen. This may be due to induction of apoptosis by VS-5584**
- **These preclinical data provide the rationale for intermittent dosing of VS-5584 being evaluated in an ongoing Phase 1 dose escalation trial (NCT01991938) with the goal of achieving an optimal therapeutic window**

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