FAK and PI3K/mTOR Inhibitors Target Cancer Stem Cells: Implications for SCLC Treatment Strategies

Abstract #1525

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ABSTRACT

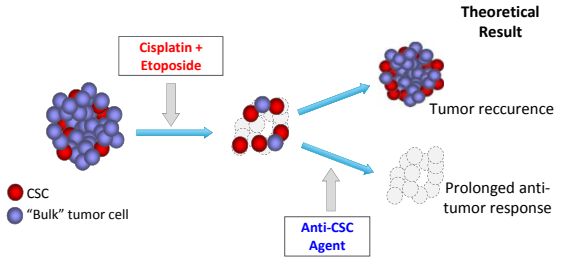
Small cell lung cancer (SCLC) is an extremely aggressive cancer with poor outcome. In most patients with SCLC, tumors initially respond to first line chemotherapy, but subsequently experience aggressive recurrence that may be attributed to the presence of a cancer stem cell (CSC) population with tumor-initiating and metastatic potential. Inhibitors of FAK and PI3K/mTOR have been shown to preferentially target CSCs in preclinical models of other cancers, where the evidence indicates that CSC populations are increased following chemotherapy. FAK and PI3K/mTOR inhibitors may be particularly valuable in a SCLC setting, where CSCs may be strong mediators of recurrence. Tumors of patients with SCLC are generally not resectable, such that tumor biopsies are not available for CSC evaluation. Therefore, it will be important to be able to monitor CSC markers by blood-based testing. A drug-resistant population of SCLC CSCs (side population, SP) can be monitored by enhanced ability to exclude Hoechst 33342 dye. Additionally, SCLC CD133+ cells have been demonstrated to possess CSC properties and presence of CD133+ cells in SCLC has been linked to poor prognosis.

Our data indicate that cisplatin and etoposide, first line chemotherapy for SCLC, enrich for side population (SP) cells, suggesting that chemotherapy is not effective against CSCs and should be combined with anti-CSC agents for more durable response. Here we investigate the antitumor activity of FAK and PI3K/mTOR inhibitors in SCLC xenograft models *in vivo*. Both a FAK inhibitor (VS-4718) and PI3K/mTOR inhibitor (VS-5584) were found to enhance the antitumor activity of chemotherapy in an NCI-H69 SCLC xenograft model. Additionally, oral administration of VS-5584 reduced the proportion of CSCs *in vivo* in an NCI-H841 SCLC tumor model as evidenced by a decrease in the percentage of SP cells and approximately 70-fold reduction in *in vivo* tumor-initiating capability. The combination of cisplatin/etoposide chemotherapy and FAK or PI3K/mTOR inhibitors strikingly extended the time to tumor regrowth. In sequential administration, VS-5584 significantly delayed tumor regrowth following cessation of cisplatin treatment in H69 xenograft tumors and a SCLC patient-derived xenograft (PDX) model. The preferential targeting of CSCs in preclinical SCLC models provides an important rationale for clinical development of FAK and PI3K inhibitors, where combination with chemotherapeutic agents or single agent activity post chemotherapy may potentially delay the relapse and improve outcome for patients with small cell lung cancer.

INTRODUCTION

Rationale: The majority of SCLC patients have objective responses to first line chemotherapy, however new tumors quickly relapse after cessation of treatment. Relapse may be attributable to the presence of a CSC population that if treated, may prolong a tumor response. Previously we have shown that FAK (VS-4718) and PI3K/mTOR (VS-5584) inhibitors target CSCs in multiple cancer types.

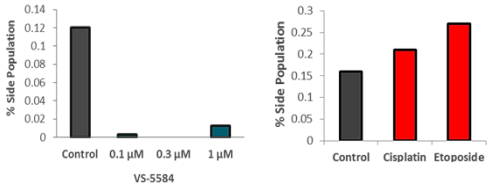
Fig 1: Importance of targeting cancer stem cells for a durable response



Schema for anti-CSC agent use following treatment of SCLC with cisplatin/etoposide therapy. Standard of care chemotherapy reduce "bulk" disease burden, but enriches for CSCs, such that disease is poised to recur. Use of a FAK (VS-4718) or PI3K/mTOR (VS-5584) inhibitors to target CSCs after chemotherapy may potentially increase duration of anti-tumor response.

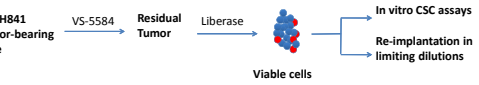
RESULTS

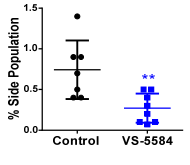
Fig 2: VS-5584 but not chemotherapy decreases SCLC CSCs *in vitro*.



SCLC H69 cells were cultured under hypoxic conditions and treated with VS-5584, cisplatin or etoposide for 2 days. Cells were incubated with Hoechst 33342 dye and the percentage of CSCs (Side Population) was determined by FACS.

Fig 3: VS-5584 targets SCLC CSCs *in vivo*





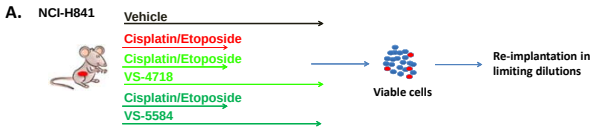
C. Limiting dilutions

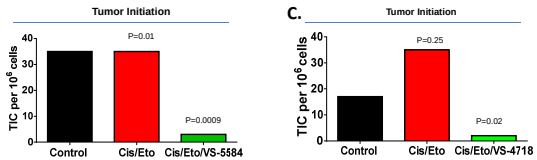
# Cells	Control	VS-5584
1,000,000	4(4)	2(4)
100,000	2(3)	0(3)
10,000	2(3)	0(3)
1,000	1(3)	0(3)
TIF	1/24,000	1/1,615,000
P		$5x10^{-6}$

VS-5584 reduced TIF by 67 fold.

Mice bearing NCI-H841 SCLC tumors were treated with either vehicle or 20 mg/kg VS-5584 thrice weekly for 3 weeks. A. Experimental outline. B, C. Cells were dissociated from tumors and subject to side population analysis by FACS (C) or implanted in secondary mice in limiting dilution assay (D). Figure 3D shows the number of mice that developed tumors relative to the number of mice tested (in parenthesis) at each dilution. Tumor initiating frequency (TIF) was calculated by ELDA software (http://bioinf.wehi.edu.au/software/elda/).

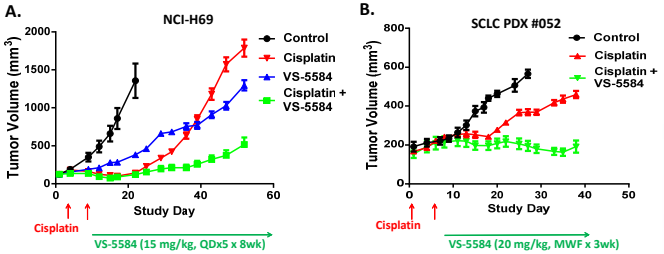
Fig 4: VS-5584 and VS-4718 inhibit CSCs in combination with chemotherapy in vivo

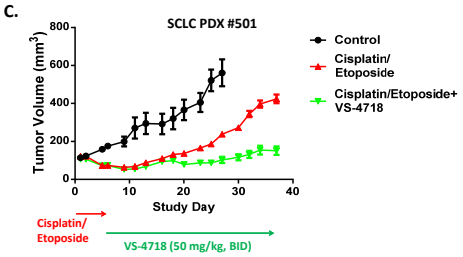




Mice bearing NCI-H841 SCLC tumors were treated with either vehicle; cisplatin (at day 1, 4mg/kg)/ etoposide (at day 2,3,4; 8mg/kg) or cisplatin/etoposide/VS-5584 (20 mg/kg on MWF dosing schedule for 18 days) or cisplatin/etoposide/VS-4718 (50 mg/kg, BID for 18 days). A. Experimental outline. B. and C. Tumor initiating cells (TIC) were measured by limiting dilution *in vivo* re-implantation assay.

Fig 5: VS-5584 and VS-4718 delay tumor regrowth after chemotherapy in SCLC models





A. Mice bearing NCI-H69 xenografts were treated with vehicle control, cisplatin (weekly dosing of 5 mg/kg cisplatin for 2 weeks), VS-5584 (oral gavage at 15 mg/kg on a QDx5 schedule for 8 weeks), alone or in combination. B. Mice bearing SCLC PDX #052 tumors were treated with vehicle control, cisplatin (weekly dosing of 5 mg/kg cisplatin for 2 weeks) or cisplatin followed by VS-5584 (oral gavage at 20 mg/kg on MWF dosing schedule for 3 weeks). C. Mice bearing SCLC PDX #501 tumors were treated with vehicle, cisplatin (at day 1, 4 mg/kg), etoposide (at day 2,3,4; 8mg/kg) or cisplatin/etoposide followed by VS-4718 (oral gavage at 50 mg/kg, BID).

SUMMARY

- **Both VS-5584 and VS-4718 reduce SCLC CSCs *in vivo* when used in combination with chemotherapy.**
- **VS-5584 and VS-4718 delay tumor regrowth seen after treatment with cytotoxic agents in SCLC xenograft models.**
- **VS-5584 is currently being evaluated in a Phase 1 clinical trial in patients with solid tumors or lymphoma NCT01991938.**
- **VS-4718 is currently being evaluated in a Phase 1 clinical trial in patients with solid tumors NCT01849744.**
- **The preferential targeting of CSCs in preclinical SCLC models provides an important rationale for clinical development of PI3K/mTOR and FAK inhibitors in combination with chemotherapy to potentially delay the relapse and improve outcome for patients with SCLC.**