FAK Inhibition Re-sensitizes Platinum-resistant Serous Ovarian Cancer #2679

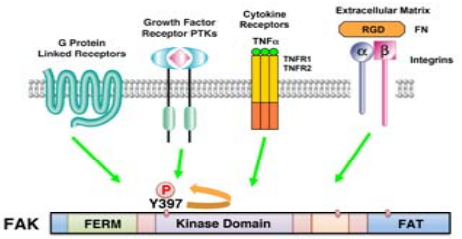
Lisa M. Bean[1#], Florian J. Sulzmaier[1#], Isabelle Tancioni[1], Sean Uryu[1], Christine Jean[1], Xiao Lei Chen[1], Elizabeth G. Kleinschmidt[1], Kristen M. Anderson[1], Edward A. Cordasco[1], Joshua Axelrod[1], Vihren N. Kolev[2], Jonathan A. Pachter[2], Dwayne G. Stupack[1] and David D. Schlaepfer[1]

[1]UC San Diego, Moores Cancer Center, La Jolla CA 92093 [2]Verastem Inc. Needham, MA 02494 [#]Authors contributed equally

Abstract

Focal adhesion kinase (FAK), an intracellular tyrosine kinase, has been linked to mesothelioma and breast cancer stem cell (CSC) survival. Here, we find that FAK activation is elevated in platinum (CP)-resistant ovarian cancer cells and that FAK tyrosine phosphorylation is increased after CP treatment of CP-sensitive ovarian cancer cells. Nanomolar levels of FAK inhibitor (VS-4718) selectively blocked CP-resistant ovarian carcinoma methylcellulose colony growth. Oral VS-4718 administration to mice reduces CP-resistant orthotopic tumor burden with a concomitant decrease in tumor-associated aldehyde dehydrogenase (ALDH) activity, a marker of ovarian CSCs. Residual ovarian tumor cells from VS-4718-treated mice exhibit reduced ALDH activity and secondary tumor initiating capacity. CRISPR-mediated FAK knockout or VS-4718 treated ovarian carcinoma cells exhibit diminished Oct-4 transcription factor and ALDH-1A1 CSC-associated protein marker expression. Co-administration of VS-4718 with CP-taxol chemotherapy reduced CP-resistant tumor burden and exhibited additive inhibitory effects on ovarian carcinoma spheroid growth. As CP activates FAK and that FAK activity sustains ovarian carcinoma CSC phenotypes, our results support the testing of FAK inhibitors in combination with CP to prevent recurrent and chemo-resistant ovarian cancer.

FAK is activated (Y397 phosphorylation) by multiple cell receptors



(Adapted from Sulzmaier et al., *Nature Cancer Reviews* 14:598, 2014)

Elevated FAK expression is a poor prognostic marker for ovarian cancer patients

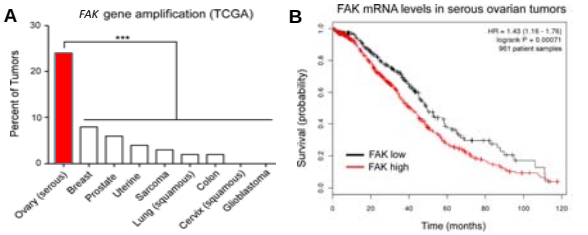
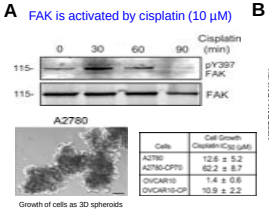


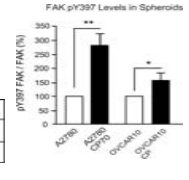
Fig. 1 Elevated FAK gene (*Ptk2*) amplification in ovarian cancer and Kaplan–Meier analyses of FAK mRNA levels with overall patient survival. A) The cBio Cancer Genomics Portal (http://www.cbioportal.org/public-portal/) was queried for percentage of *Ptk2* gene amplification in The Cancer Genome Atlas (TCGA) with different cancers and significance determined by the Chi squared test (***p<0.001). B) The Kaplan–Meier Plotter (http://www.kmplot.com/ovar) was queried to evaluate Affymetrix microarray expression of FAK mRNA levels in 961 annotated serous ovarian cancer patient tumor samples. Selections were: overall survival (follow up threshold of 10 years), split patients by median, stage (all), histology (serous), grade (all), debulk (all), and chemotherapy treatments (all). High levels of FAK expression (red) are associated with decreased patient survival (logrank P = 0.0007) and the Hazard ratio (with 95 % confidence intervals) is shown. (Results published in Ward et. al., *Clinical & Experimental Metastasis* 30: 579, 2013)

A FAK is activated by cisplatin (10 μM)

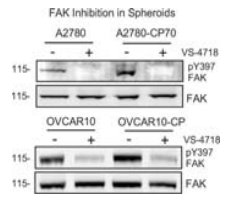


Cells	Cell Growth Cisplatin IC50 (μM)
A2780	12.6 ± 5.2
A2780-CP70	62.2 ± 8.7
OVCAR10	1.4 ± 0.6
OVCAR10-CP	10.9 ± 2.2

B Elevated FAK pY397 in cisplatin (CP)-resistant cells



C FAK Y397 phosphorylation is sensitive to VS-4718



VS-4718
FAK-Pyk2 selective ATP-competitive inhibito in clinical trial testing

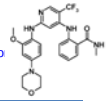
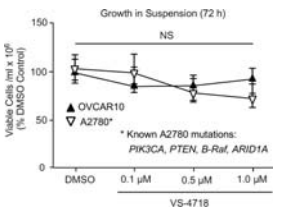
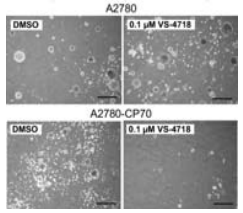


Fig. 2. Increased FAK Y397 phosphorylation after cisplatin treatment and in cisplatin-resistant ovarian cancer cells. (A) Serum-starved spheroids were stimulated with 10 μM cisplatin for the indicated times prior to cell lysis and immunoblotting for pY397 FAK and total FAK by LICOR. Table: Growth of ovarian carcinoma cells in cisplatin (72 h) and determination of IC50 values. B) FAK activation in spheroids from cisplatin-sensitive (A2780 and OVCAR10) and cisplatin-resistant (A2780-CP70 and OVCAR10-CP) spheroids as determined by the ratio of pY397 FAK to total FAK by immunoblotting. A2780 and OVCAR10 values set to 100. C) Lysates from DMSO (control) or VS-4718 (1 μM for 1 h) treated cells grown as spheroids were immunoblotted for pY397 FAK and total FAK.

A Parental OVCAR10 and A2780 cell growth in suspension is resistant to VS-4718



B VS-4718 prevents A2780-CP70 but not A2780 growth as spheroids



C

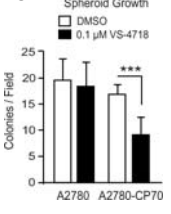


Fig. 3. VS-4718 FAK inhibition selectively prevents CP-resistant ovarian carcinoma spheroid growth. A) Anchorage-independent growth of parental OVCAR10 and A2780 cells in the presence of VS-4718 (0.1 to 1.0 μM). Values are means ± SD and expressed as percent of DMSO control. B) Representative phase contrast images of A2780 or A2780-CP70 colony growth in methylcellulose with DMSO (control) or 0.1 μM VS-4718 after 12 days. Scale is 2.5 mm. C) Quantitation of methylcellulose spheroid colonies in DMSO or 0.1 μM VS-4718. Values are means ± SEM of 3 independent repeats (*** p < 0.001).

Mouse tumor model testing of cisplatin-paclitaxel plus VS-4718 FAK Inhibitor therapy

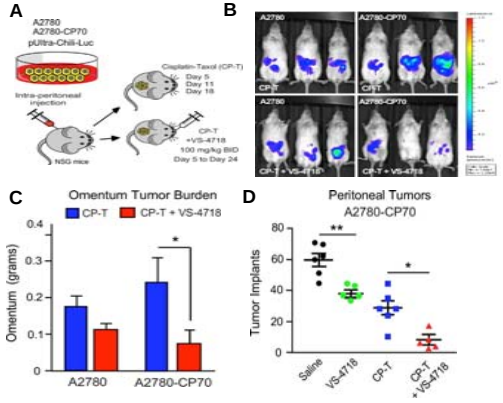
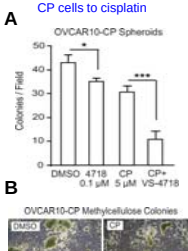


Fig. 4. VS-4718 FAK inhibition re-sensitizes cisplatin-taxol chemotherapy on cisplatin-resistant tumors. (A) Experimental tumor model schematic. A2780 and A2780-CP70 transduced with pUltra-Chili-Luc were injected intraperitoneally into NSG mice and randomized at Day 5 into cisplatin-taxol (CP-T, 3mg/kg cisplatin and 2mg/kg paclitaxel administered by IP injection) or CP-T with VS-4718 (100 mg/kg, BID oral gavage) treatment groups with euthanization at Day 24. (B) Tumor burden was indirectly visualized by luciferase activity by bioluminescent imaging. (C) Mean omentum-associated tumor mass ± SEM (n=5, * p < 0.05) from each treatment group. (D) Mean number of peritoneal tumor implants ± SEM (* p < 0.05, ** p <0.01) from each treatment group as measured using Image J of dTomato fluorescence obtained via Olympus OV100 imaging.

VS-4718 sensitizes OVCAR10-CP cells to cisplatin



VS-4718 or FAK knockout decreases aldefluor activity and ALDH-1A1 expression

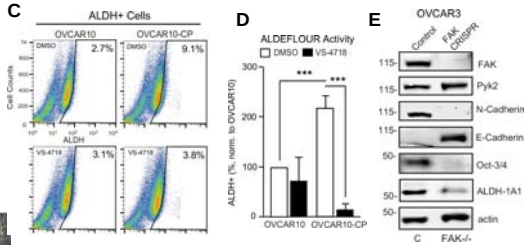
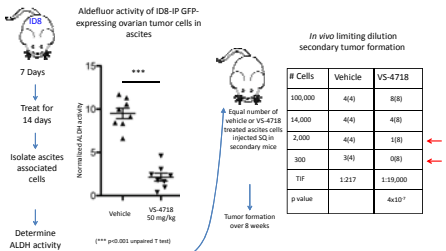


Fig. 5. FAK inhibition diminishes cisplatin-resistant cancer cell stem-like properties. (A) OVCAR10-CP colony growth in methylcellulose in the presence of DMSO, 0.1 μM VS-4718, 5 μM cisplatin, or 0.1 μM VS-4718 plus 5 μM cisplatin. Values are means ± S.E.M. (* p < 0.05, *** p < 0.001) from 3 independent repeats. (B) Representative phase contrast images of OVCAR10-CP colony growth in methylcellulose Scale is 2.0 mm. (C) Representative flow cytometry profiles of ALDH+ OVCAR10 or OVCAR10-CP cells grown as spheroids with or without VS-4718 (1 μM). *Not shown*, flow cytometry in the presence DEAB used to select ALDH+ gated group. (D) Percentage of ALDH+ OVCAR10 or OVCAR10-CP cells grown as spheroids with or without VS-4718 (1 μM). Values are means ± SEM (*** p <0.001) from 3 independent experiments and expressed as percent of OVCAR10. (E) FAK knockout (FAK-/-) in OVCAR3 cells created by CRISPR-Cas9 transduction and puromycin selection. Cell lysates were analyzed by FAK, Pyk2, N-Cadherin, E-cadherin, Oct-3/4, ALDH-1A1 immunoblotting. Actin was used as a loading control.

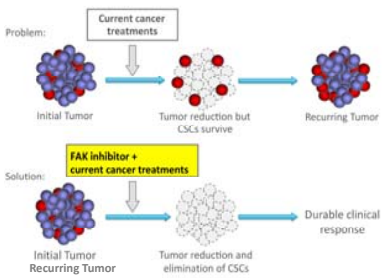
VS-4718 treatment reduces secondary ovarian tumor initiation frequency



# Cells	Vehicle	VS-4718
100,000	4(4)	8(8)
14,000	4(4)	4(8)
2,000	4(4)	1(8)
300	3(4)	0(8)
TIF	1:217	1:19,000
p value		4x10^-7

Fig. 6. FAK inhibition diminishes cancer cell stem-like properties as measured by secondary tumor initiation frequency. ID8-IP ovarian cancer cells were injected orthotopically into the bursal space of C57/BL6 mice and after 7 days, mice were randomized and treated with VS-4718 (50 mg/kg, BID) or vehicle. At Day 28, ascites-associated cells were collected and analyzed for ALDEFLOUR activity. Data points represent ALDH+ values from individual mice. Bars are means ± SEM (*** p <0.001). (B) VS-4718- or vehicle-treated ID8-IP tumors were dissociated into single cells and a defined number of viable cells were injected sub-cutaneously into nude mice to measure secondary tumor initiation frequency (TIF).

Non-stochastic model of ovarian tumor recurrence (spheroids and micro-metastases)



Summary

- FAK is activated by cisplatin treatment and elevated basal Y397 FAK phosphorylation occurs as a function of acquired cisplatin resistance.
- VS-4718 sensitizes cisplatin-resistant cells to platinum chemotherapy in vitro and in vivo.
- VS-4718 diminishes cisplatin-resistant cancer stem-like cell properties.
- FAK inhibitor combination with carboplatin-taxol chemotherapy may enable a more durable clinical response.