

# FAK/PYK2 Inhibitors VS-6063 and VS-4718 Enhance Immune Checkpoint Inhibitor Efficacy

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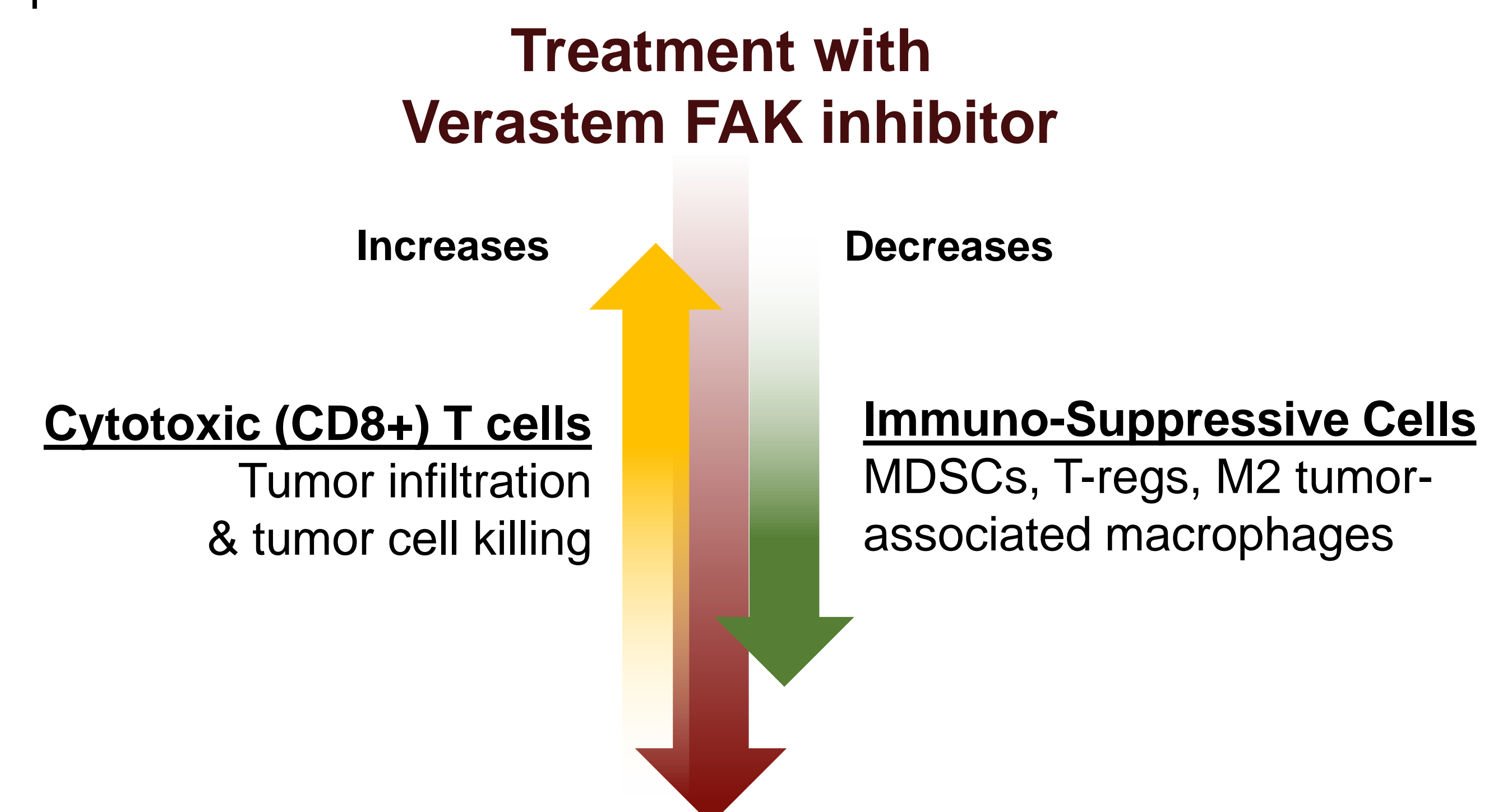
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## ABSTRACT

Although durable responses to single agent immune checkpoint inhibitors have been reported, additional approaches are needed to extend this therapeutic benefit to a greater proportion of cancer patients. Accordingly, substantial efforts are ongoing to identify agents that can augment T cell-mediated killing of tumor cells and potentiate the effects of checkpoint inhibitors. Focal Adhesion Kinase (FAK) and the closely related family member PYK2 are potentially valuable targets due to the roles of these protein kinases in regulating key cellular populations in the tumor microenvironment. In addition to the potency of the small molecule FAK/PYK2 inhibitors VS-6063 and VS-4718 to target cancer stem cells, we have also reported that these agents inhibited monocyte-derived macrophages, decreased IL-6 production from macrophages in vitro, and reduced tumor-associated macrophages in xenograft models. We now report that VS-6063 and VS-4718 dose-dependently stimulate proliferation of CD8+ cytotoxic T cells isolated from healthy donors. This is in distinct contrast to other protein kinase inhibitors, such as the SRC inhibitor dasatinib, which potently impair the proliferation of CD8+ cytotoxic T cells.

Based on the observed enhancement of CD8+ T cells and previously noted inhibition of tumor-associated macrophages, we investigated whether FAK/PYK2 inhibitors would potentiate the anti-tumor efficacy of an anti-PD-1 monoclonal antibody in syngeneic mouse tumor models. Mice bearing established MC38 colorectal tumors were treated with VS-4718 in combination with an anti-PD-1 antibody. Combination of VS-4718 with anti-PD-1 extended the median overall survival (OS) to 42 days relative to 21, 25 and 28 day median OS with vehicle control, single agent anti-PD-1 and single agent VS-4718, respectively. Moreover, on day 56, 30% of mice treated with the VS-4718/anti-PD-1 combination were alive in contrast to the vehicle control, single agent VS-4718, and single agent anti-PD-1 groups in which no mice survived. Analysis of MC38 tumors at day 12 of treatment revealed a significant decrease in Tregs in the VS-4718/anti-PD-1 combination group, relative to vehicle control, providing a mechanistic understanding for the enhanced efficacy of this combination.

These data provide a rationale for clinical trials in cancer patients to test whether a FAK/PYK2 inhibitor in combination with an immune checkpoint inhibitor could increase the breadth of responsive tumor types, increase the number of responders, and confer a more durable anti-tumor response.

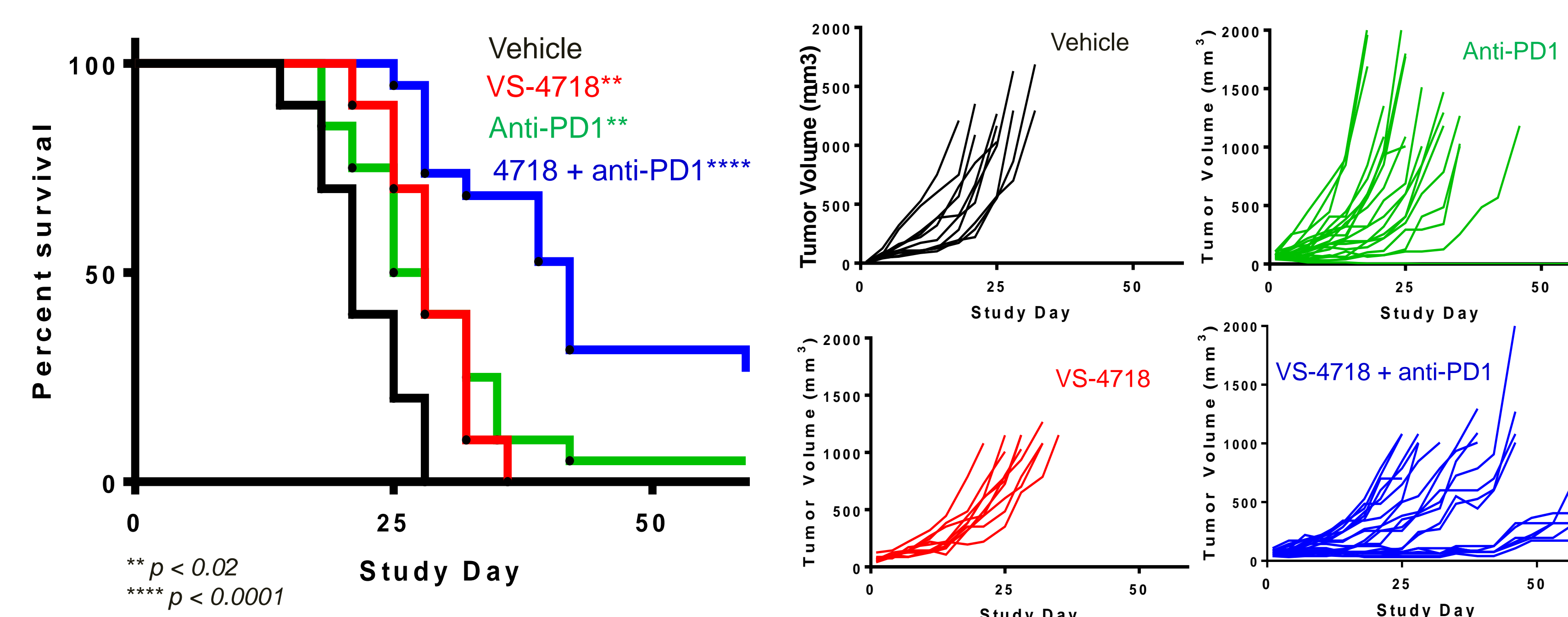


**More favorable tumor microenvironment for enhanced efficacy of Immuno-Oncology therapeutics**

**FIGURE 1: Roles of FAK/PYK2 inhibitor in tumor micro-environment**

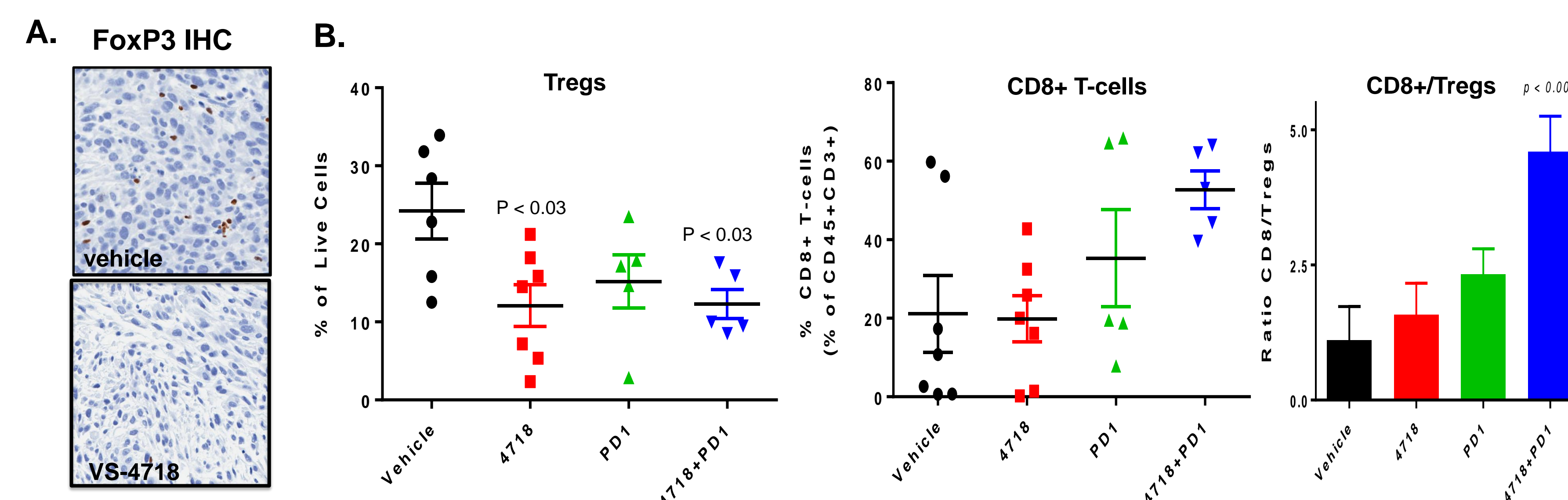
## RESULTS

### Combination of VS-4718 + anti-PD1 improves survival of MC38 colorectal syngeneic tumor-bearing mice



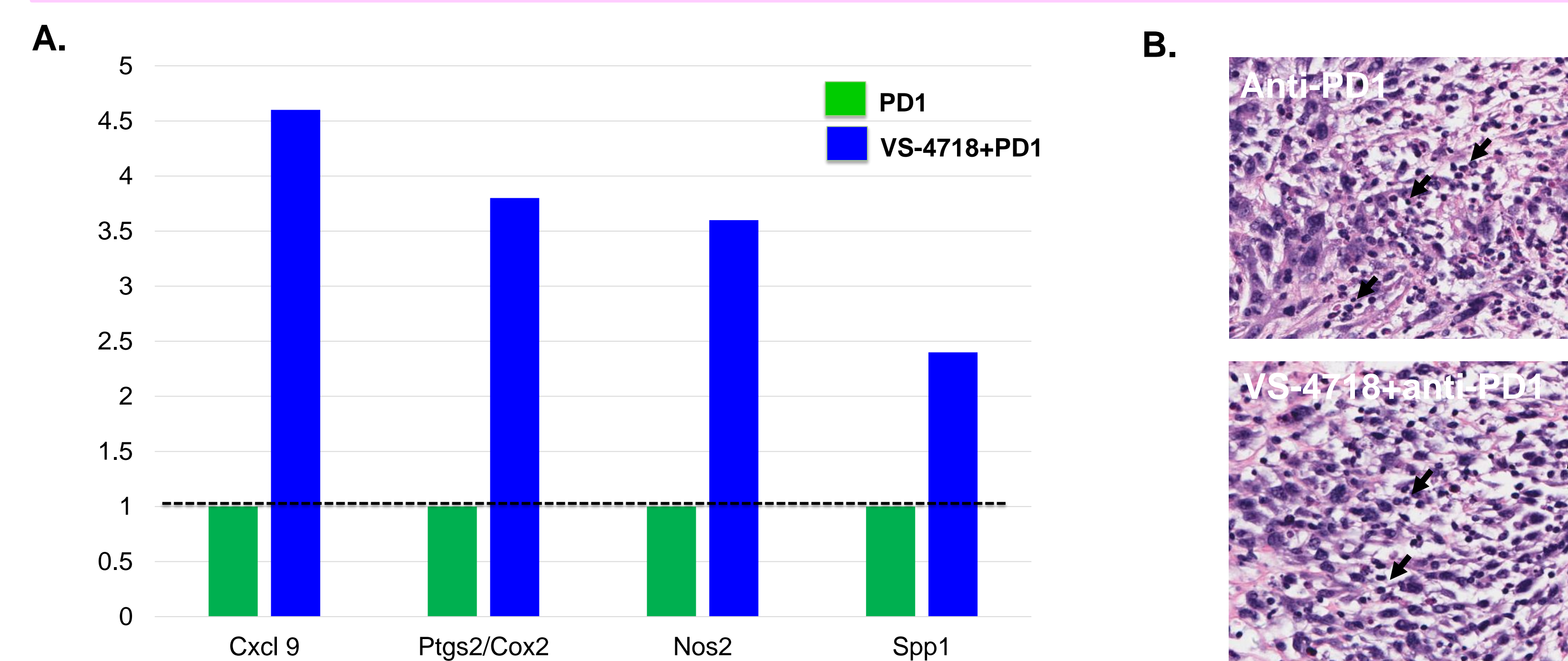
**FIGURE 2:** Syngeneic MC38 tumors were randomized once tumors reached 50-100mm<sup>3</sup> and treated with either vehicle, VS-4718 (50 mg/kg, BID), anti-PD1 (clone RMP1-14, 5 mg/kg on days 1, 4, 8, 11) or VS-4718 + anti-PD1 until tumors reached 1000mm<sup>3</sup> as a surrogate survival endpoint

### Decreased Tregs and increased CD8+ T cells with VS-4718 + anti-PD1 combination in MC38 tumors



**FIGURE 3:** Syngeneic MC38 tumors were randomized once tumors reached 50-100mm<sup>3</sup> and then treated with either vehicle, VS-4718 (50 mg/kg, BID), anti-PD1 (clone RMP1-14, 5 mg/kg on days 1, 4, 8, 11) or VS-4718 + anti-PD1 for 12 days at which point tumors were harvested and embedded in FFPE for staining Foxp3 by IHC (A) or processed live for flow cytometry (B).

### Increased inflammation with VS-4718 + anti-PD1 combination in MC38 tumors

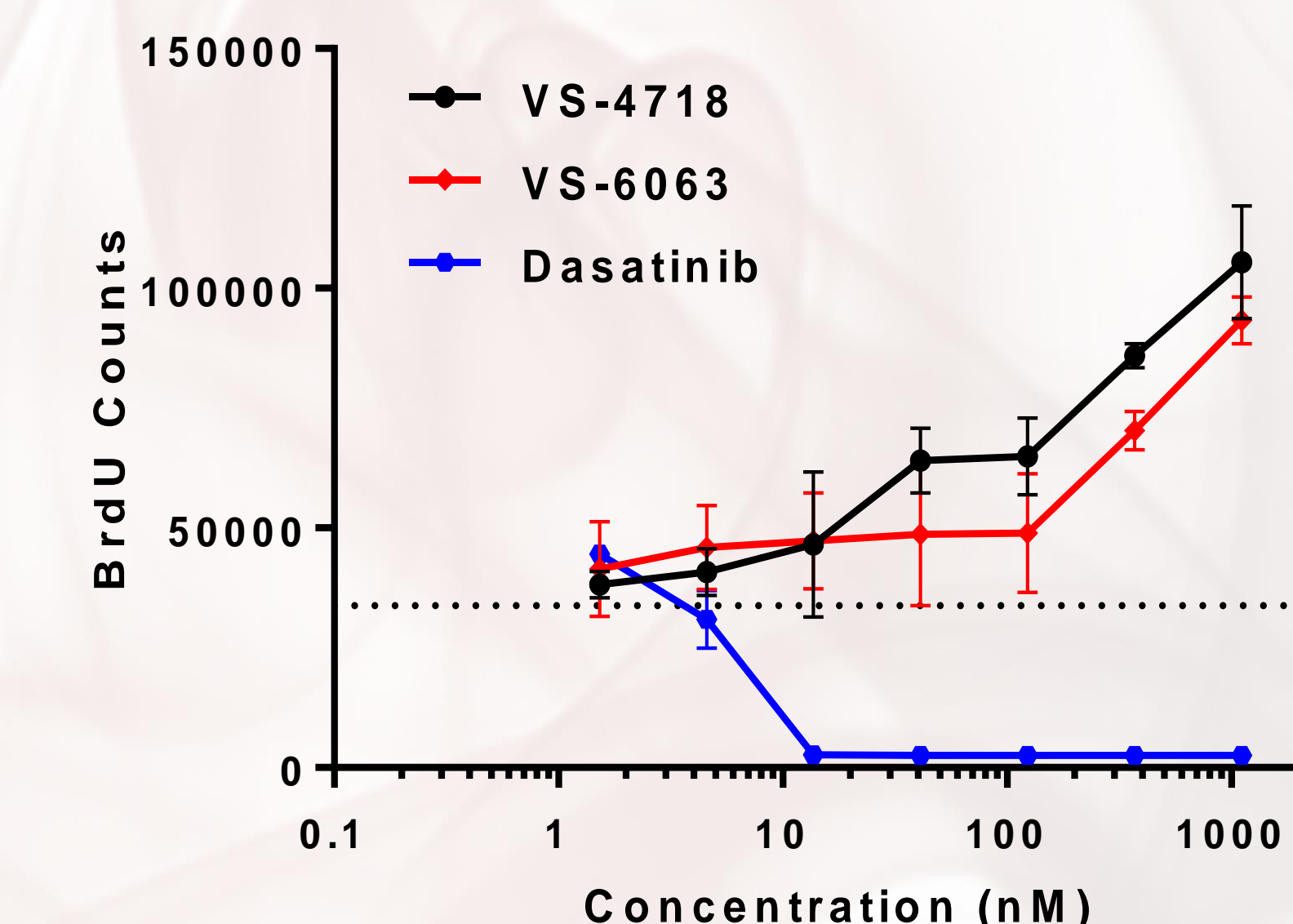


**Figure 4:** Syngeneic MC38 tumors were randomized once tumors reached 50-100mm<sup>3</sup> and then treated with either anti-PD1 (clone RMP1-14, 5 mg/kg on days 1, 4, 8, 11) or VS-4718 (50 mg/kg, BID) + anti-PD1 for 12 days at which point tumors were harvested in RNALater and analyzed for changes in inflammatory cytokines/chemokines by qPCR (A) or embedded in FFPE and stained with H&E (B).

### VS-4718 and VS-6063 enhance human CD8+ T cell proliferation

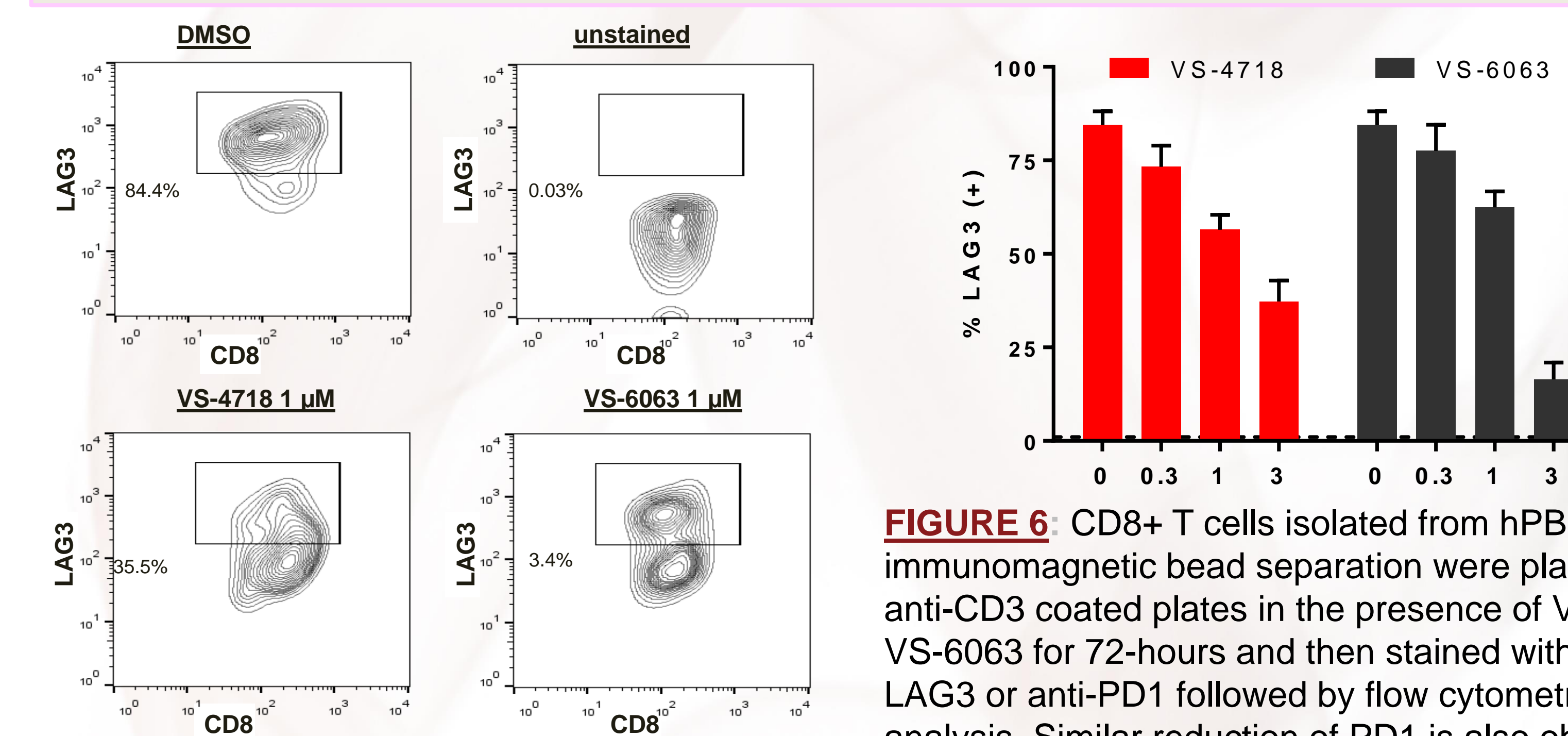
Cellular EC50 (nM) avg ± SD

	FAK	PYK2
VS-4718	34 ± 27	43 ± 40
VS-6063	21 ± 4	46 ± 37



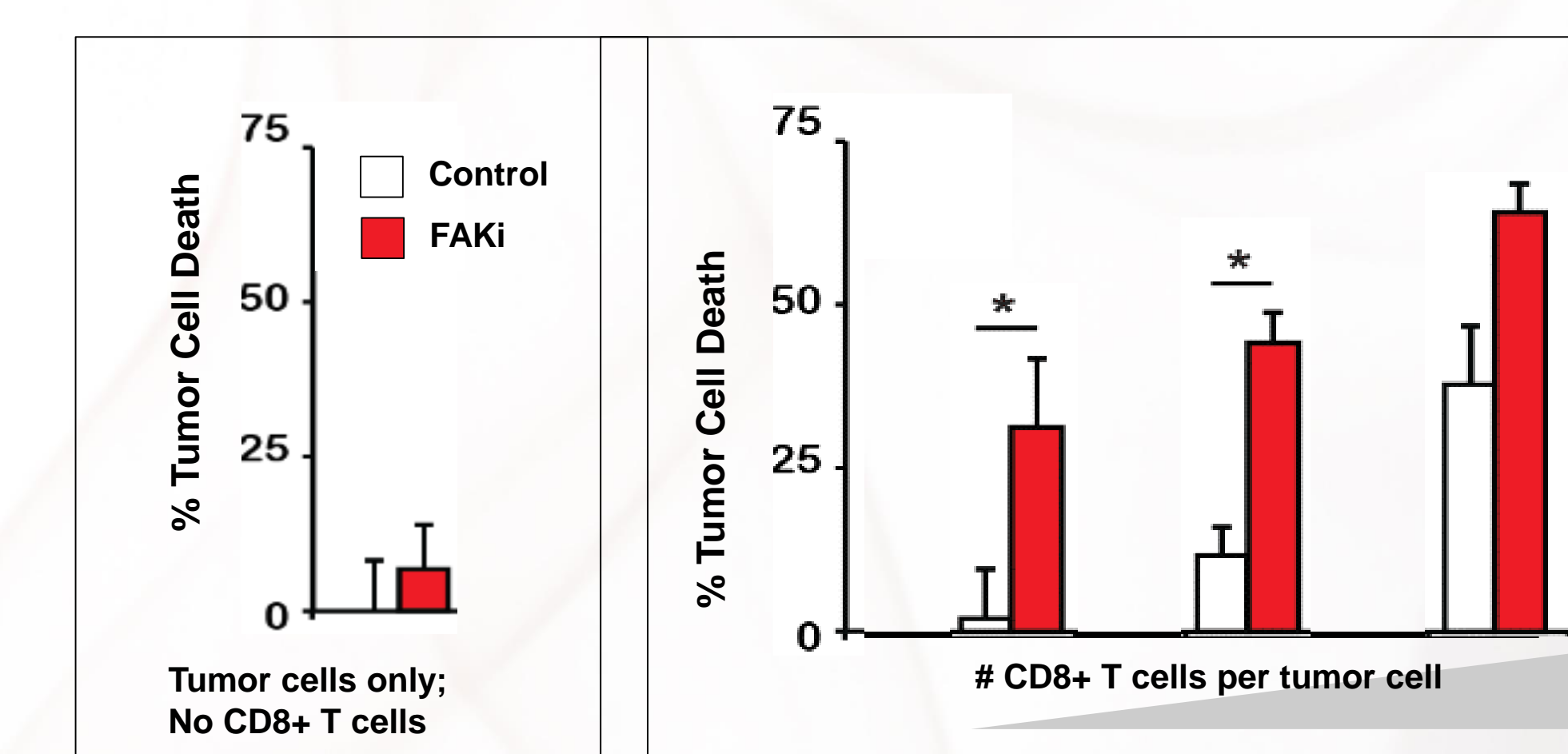
**FIGURE 5:** CD8+ T cells were isolated from fresh, healthy human PBMCs by negative immunomagnetic bead separation. Purified CD8+ T-cells were plated on CD3-coated plates in the presence or absence of VS-4718 or VS-6063 for 72 hours. Assay wells were pulsed with BrdU for the last 3-4 hours of culture and subjected to a BrdU-incorporation assay for the determination of actively proliferating cells. Data are presented as a fold-change of DMSO control wells.

### VS-4718 and VS-6063 decrease CD8+ T cell exhaustion markers



**FIGURE 6:** CD8+ T cells isolated from hPBMCs by immunomagnetic bead separation were plated on anti-CD3 coated plates in the presence of VS-4718 or VS-6063 for 72-hours and then stained with anti-LAG3 or anti-PD1 followed by flow cytometric analysis. Similar reduction of PD1 is also observed.

### FAK inhibitor enhances CD8+ cytolytic activity against cognate tumor cells



**FIGURE 7:** Antigen-specific CD8+ T-cells were co-cultured with cognate luciferized tumor cells at various E:T ratios in the presence of VS-4718 1μM on top of Matrigel. After 24 hours, cultured were analyzed for changes in luciferase as a measure of tumor cell viability.

## SUMMARY

- Combination of FAK inhibitor + anti-PD1 improves survival of MC38 tumor-bearing animals
- FAK inhibitor + anti-PD1 combination induces decreased Tregs and increased CD8+ T cells and inflammatory factors in the MC38 tumors
- FAK inhibitors increase CD8+ T cell proliferation, decrease CD8+ T cell exhaustion markers, and increase T cell-mediated tumor cell killing in vitro
- These data provide rationale for clinical trials with a FAK inhibitor in combination with an immune checkpoint inhibitor

