Poster # LB152

ACR-368 synergizes with PD-L1 blockade by coordinated activation of adaptive and innate immunity pathways to achieve robust anti-tumor efficacy

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Introduction

- ACR-368 is a potent and selective CHK1/2 inhibitor with demonstrated durable clinical activity which is currently being evaluated as monotherapy and in combination regimens in Acrivon Therapeutics' ongoing registrational-intent Phase 2 endometrial cancer clinical trial.
- Preclinical studies indicate that ACR-368 activates multiple anti-tumor immune responses, supporting its combination with immune checkpoint inhibitors (ICIs).
- ACR-368 in combination with anti-PD-L1 led to complete tumor regression in a syngeneic colorectal cancer mouse model and long-lasting immune memory driven by anti-tumor innate and adaptive immune responses.

Potent synergy of ACR-368 with anti-PD-L1 and induction of durable immune memory mediated by CD4+ and CD8+ cells

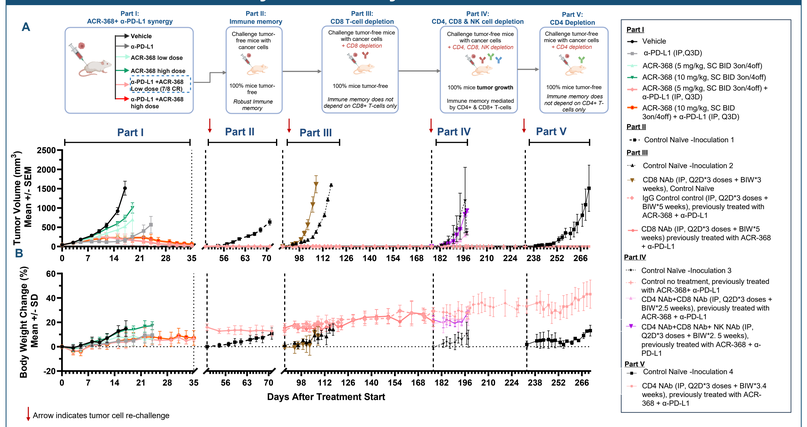

Figure 1: Complete tumor regression and durable immune memory mediated by adaptive immunity. (A) In a syngeneic colorectal cancer mouse model, ACR-368 monotherapy resulted in 74% tumor growth inhibition and anti-PD-L1 resulted in complete tumor regression in 2/8 mice. Robust synergy was observed with the combination of ACR-368 with anti-PD-L1, which led to complete tumor regression in 7/8 mice (Part I), and generated durable immune memory persisting beyond 200 days after four sequential tumor re-challenges (Part II-V). Systematic depletion of immune cell subpopulations (Parts III–V) showed that the absence of either CD4+ or CD8+ T-cells alone did not lead to tumor growth upon rechallenge, while co-depletion of both resulted in tumor formation, indicating that immune memory is driven by adaptive immune responses. (B) The combination of ACR-368 and anti-PD-L1 was well tolerated, consistent with non-overlapping toxicities.

ACR-368 activates ssDNA/dsRNA sensing and innate immune signaling pathways

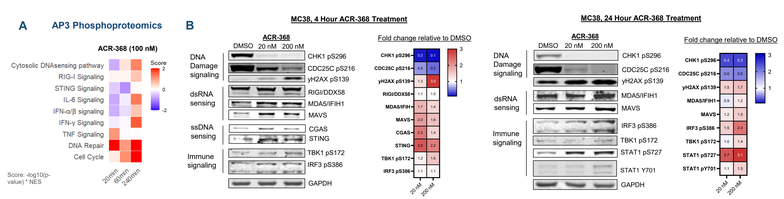

Figure 2: Activation of innate immune signaling and nucleic acid sensing pathways *in vitro*. (A) Acrivon Predictive Precision Proteomics (AP3) analysis of MC38 cells showed a time-dependent increase in phosphorylation of proteins associated with multiple innate immune signaling and DNA damage pathways after ACR-368 treatment vs DMSO. (B) Western blot analysis of selected markers in ACR-368 treated cells (20nM, 200nM) showed reduction in CHK1 activity accompanied by an increase in DNA damage, ssDNA/dsRNA sensing (4h), and subsequent activation of innate immunity pathways (24h).

ACR-368 activates DNA damage responses and induces apoptotic cell death *in vivo*

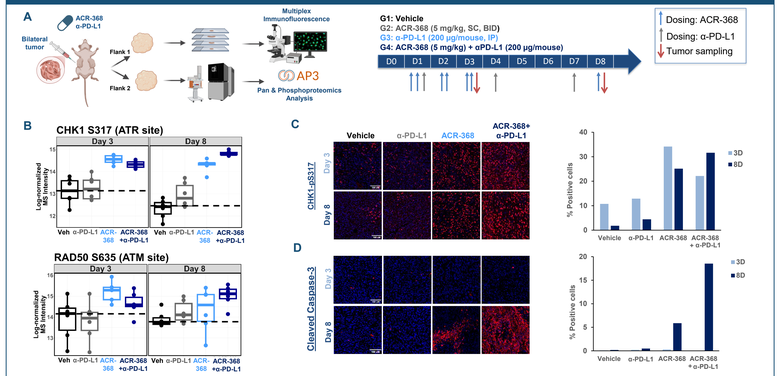

Figure 3: Robust induction of DNA damage response signaling by ACR-368 *in vivo*. (A) Mice with bilateral syngeneic tumors were treated with vehicle, ACR-368 (5 mg/kg), anti-PD-L1 (200 μg/mouse), or the combination. Tumors from either flank were collected for immunostaining (IF) or AP3 profiling. AP3 analysis confirms drug activity as indicated by the strong induction of ATM (RAD50-S635) and ATR (CHK1-S317) –phosphorylation sites at early and late timepoints (lower panels). (C) Spatial IF analysis of Day 3 and Day 8 samples confirms ACR-368 driven ATR activation and subsequent apoptosis in Day 8, as indicated by cleaved caspase-3 staining (D).

Enhanced innate immune signaling and up-regulation of immune pathways by ACR-368 and anti-PD-L1 combination

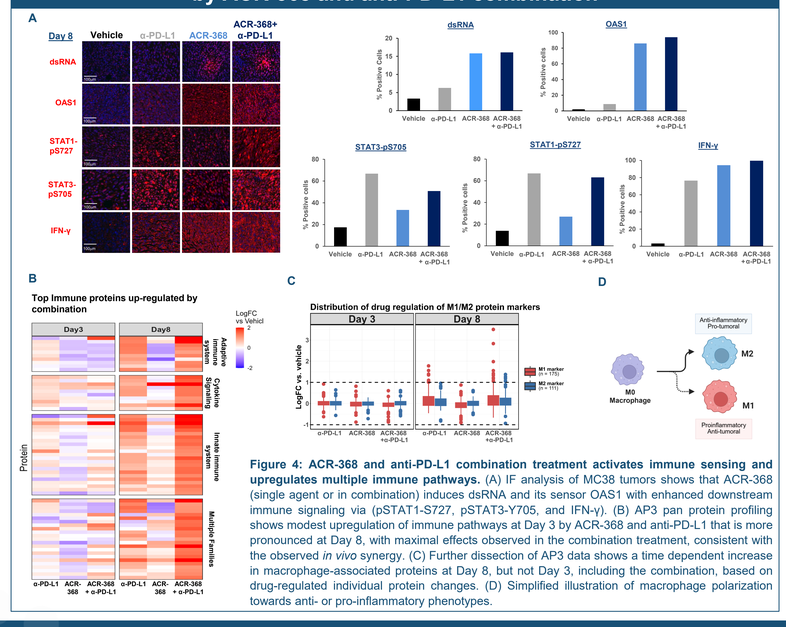

Figure 4: ACR-368 and anti-PD-L1 combination treatment activates immune sensing and upregulates multiple immune pathways. (A) IF analysis of MC38 tumors shows that ACR-368 (single agent or in combination) induces dsRNA and its sensor OAS1 with enhanced downstream immune signaling via (pSTAT1-S727, pSTAT3-Y705, and IFN-γ). (B) AP3 pan protein profiling shows modest upregulation of immune pathways at Day 3 by ACR-368 and anti-PD-L1 that is more pronounced at Day 8, with maximal effects observed in the combination treatment, consistent with the observed *in vivo* synergy. (C) Further dissection of AP3 data shows a time dependent increase in macrophage-associated proteins at Day 8, but not Day 3, including the combination, based on drug-regulated individual protein changes. (D) Simplified illustration of macrophage polarization towards anti- or pro-inflammatory phenotypes.

ACR-368 in combination with anti-PD-L1 promotes pro-inflammatory macrophage phenotypes and enhances cytotoxic innate and adaptive immune responses

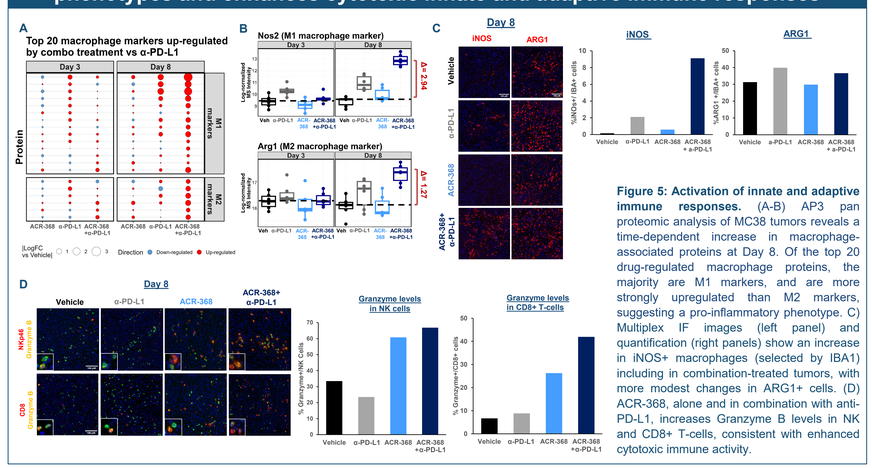

Figure 5: Activation of innate and adaptive immune responses. (A-B) AP3 pan proteomic analysis of MC38 tumors reveals a time-dependent increase in macrophage-associated proteins at Day 8. Of the top 20 drug-regulated macrophage proteins, the majority are M1 markers, and are more strongly upregulated than M2 markers, suggesting a pro-inflammatory phenotype. C) Multiplex IF images (left panel) and quantification (right panels) show an increase in iNOS+ macrophages (selected by IBA1) including in combination-treated tumors, with more modest changes in ARG1+ cells. (D) ACR-368, alone and in combination with anti-PD-L1, increases Granzyme B levels in NK and CD8+ T-cells, consistent with enhanced cytotoxic immune activity.

ACR-368 is associated with reduced T-cell exhaustion marker expression in combination with anti-PD-L1

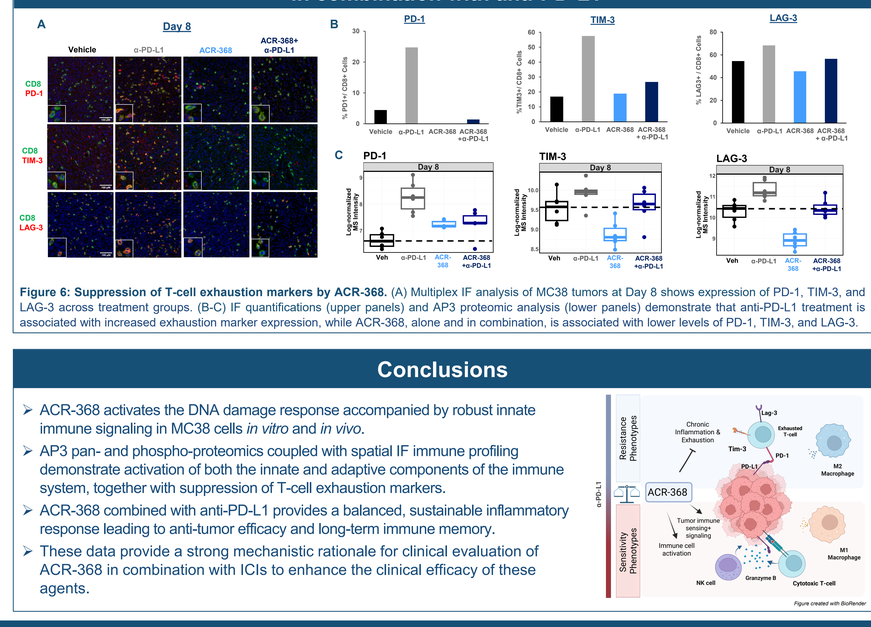

Figure 6: Suppression of T-cell exhaustion markers by ACR-368. (A) Multiplex IF analysis of MC38 tumors at Day 8 shows expression of PD-1, TIM-3, and LAG-3 across treatment groups. (B-C) IF quantifications (upper panels) and AP3 proteomic analysis (lower panels) demonstrate that anti-PD-L1 treatment is associated with increased exhaustion marker expression, while ACR-368, alone and in combination, is associated with lower levels of PD-1, TIM-3, and LAG-3.

Conclusions

- ACR-368 activates the DNA damage response accompanied by robust innate immune signaling in MC38 cells *in vitro* and *in vivo*.
- AP3 pan- and phospho-proteomics coupled with spatial IF immune profiling demonstrate activation of both the innate and adaptive components of the immune system, together with suppression of T-cell exhaustion markers.
- ACR-368 combined with anti-PD-L1 provides a balanced, sustainable inflammatory response leading to anti-tumor efficacy and long-term immune memory.
- These data provide a strong mechanistic rationale for clinical evaluation of ACR-368 in combination with ICIs to enhance the clinical efficacy of these agents.

ACRIVON THERAPEUTICS