The oral CHK1 inhibitor, SRA737, synergizes with immune checkpoint blockade in small cell lung cancer (SCLC)

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BACKGROUND

- Small cell lung cancer (SCLC) is the most lethal form of lung cancer, accounting for 15% of lung cancers in the United States with a five-year survival rate <5%
- We previously demonstrated overexpression of DNA damage response (DDR) proteins, including PARP1 and CHK1, in SCLC cell lines and patient tumors (1,2) suggesting increased reliance on these proteins for tumor cell survival.

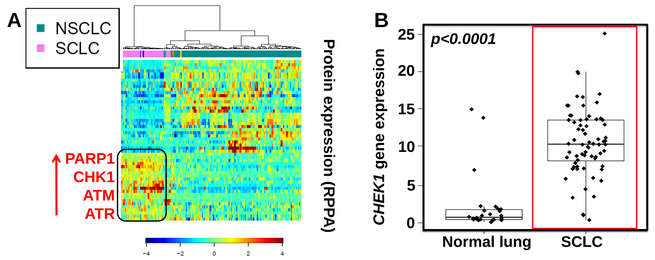

Figure 1. Higher expression of CHK1 in SCLC cell lines and patient tumors. (A) Protein expression analysis was conducted on SCLC and NSCLC lines by reverse-phase protein array (RPPA). DNA damage response (DDR) proteins (CHK1, PARP, ATM, ATR) were among the top proteins displaying higher expression in the SCLC vs NSCLC lines suggesting increased reliance on these proteins for tumor survival (1). **(B)** *CHEK1* gene is significantly higher in SCLC tumors as compared to normal lung (2,3).

- Despite the high mutation load in SCLC tumors, PD-1/PD-L1 blockade as a monotherapy (e.g. nivolumab) is active in only a minority (about 10%) of patients.
- SRA737 is a potent and selective oral CHK1 inhibitor currently being tested in clinical trials as monotherapy and in combination with low dose gemcitabine in a range of solid tumor indications with defined genetic backgrounds predicted to be sensitive to CHK1 inhibition.
- Emerging evidence of synergy between inhibitors of DDR proteins and immune checkpoint blockade, provided strong rationale to explore the potential of SRA737 in combination with anti-PD-L1 in preclinical models of immunotherapy-refractory SCLC.

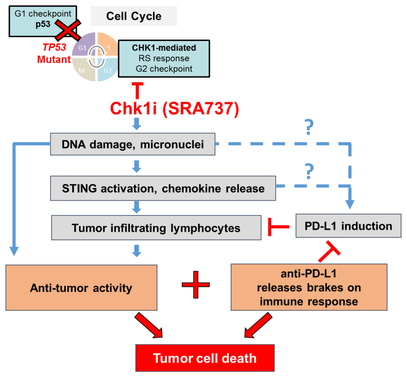

Figure 2. Potential synergy between SRA737 and anti-PD-L1. Tumors with dysregulated cell cycle rely on CHK1 activity to manage replication stress and avoid progression through the cell cycle with DNA damage. CHK1 inhibition leads to elevated replication stress (RS) and induction of DNA damage, triggering STING pathway activation and the production of immune-recruiting chemokines with coincident upregulation of PD-L1 expression in the tumors. Combined inhibition of CHK1 and PD-L1 is hypothesized to maximize tumor cell killing through a combination of intrinsic anti-tumor activity of SRA737 and recruitment of tumor infiltrating lymphocytes coupled with release of immune checkpoint blockade via anti-PD-L1 antibody.

REFERENCES

1. Byers LA et al., Cancer Discovery, 2012.
2. Sen T et al., Cancer Research 2012.
3. Sen T et al., Translational Lung Cancer Research 2018.
4. Li T and Chen ZJ. Journal of Experimental Medicine 2018.
5. Bronger H et al., British Journal of Cancer 2016.
6. Araujo JM et al., Scientific Reports 2018.

ACKNOWLEDGEMENTS

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RESULTS

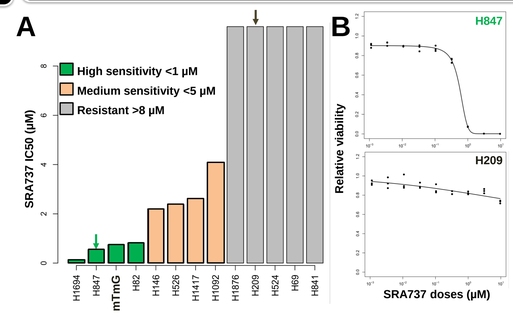

Figure 3. SRA737 shows a range of single agent anti-tumor activity in SCLC cell lines *in vitro*. (A) Bar graph depicting IC50 values for the CHK1 inhibitor (CHK1i) SRA737 across 13 human derived SCLC cell lines. mTmG mouse SCLC cell line was derived from GEMM triple knock-out (*Trp53, Rb1* and *p130*) (B) Representative dose response curves of one sensitive (H847) and one resistant (H209) cell line.

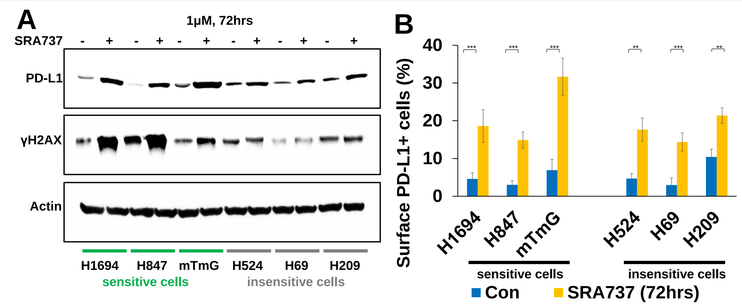

Figure 4: SRA737 treatment induces PD-L1 protein levels and surface expression in SCLC cell lines. (A) Western blot analysis of 3 sensitive (H1694, H847, mTmG) and 3 resistant (H524, H69, H209) cell lines that were treated with SRA737 (1 μM, 72 hours). SRA737 treatment increased the protein expression of γH2AX (DNA damage marker) in sensitive but not in resistant cell lines. However, SRA737 treatment induced the protein expression of PD-L1 in all cell lines irrespective of the sensitivity to SRA737. (B) Flow cytometry of 3 sensitive (H1694, H847, mTmG) and 3 resistant (H524, H69, H209) cell lines that were treated with SRA737 (1 μM, 72 hours) showed increased surface expression of PD-L1 in all cell lines irrespective of the sensitivity to SRA737. *** p<0.001, **p<0.01.

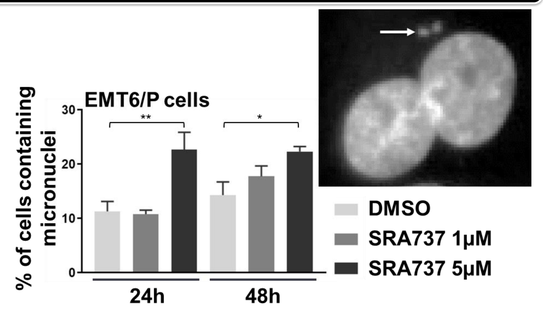

Figure 5. SRA737 induces micronuclei formation *in vitro*. DNA damage-inducing therapies and targeted DDR inhibitors have been demonstrated to induce micronuclei, which in turn activate innate immune signaling pathways (e.g. STING, interferon) (4). EMT6/P mouse mammary cells were treated with DMSO or SRA737 at 1uM or 5uM for either 24h or 48h. The cells were fixed and stained with Hoechst DNA dye. Plates were imaged using high content microscopy. The means and standard deviations of the percent of cells harboring at least one micronucleus are shown. Representative image shown with white arrow denoting micronuclei. * p<0.05, **p<0.01.

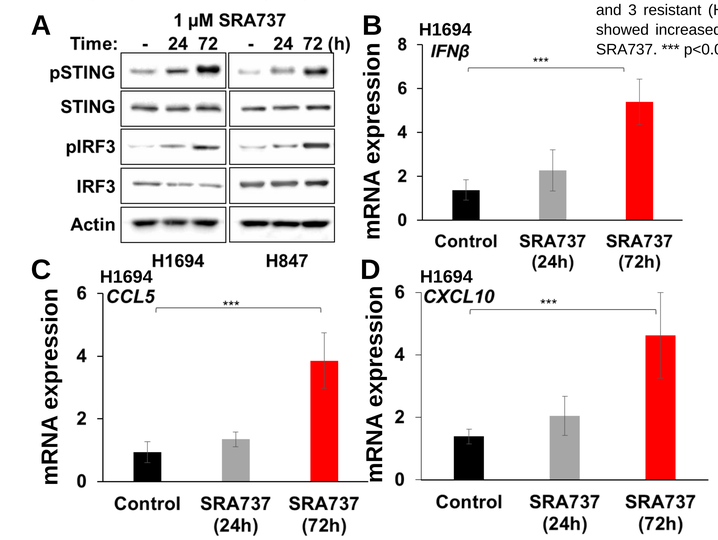

Figure 6: SRA737 induces STING and Type I interferon signaling, resulting in upregulation of lymphocyte-recruiting chemokines in SCLC cell lines *in vitro*. (A) Immunoblots of markers in the STING pathway including total and phospho STING (S366), total and phospho IRF3 (S396) in lysates collected from SCLC cell lines treated with SRA737. Actin served as a loading control. (B-D) Quantitative PCR (qPCR) measurement of innate immune signaling factors *IFNβ* (B), *CCL5* (C) and *CXCL10* (D) mRNA expression in SCLC cell lines 24 and 72 hours after SRA737 treatment. GAPDH served as internal control for mRNA expression. These immunomodulatory effects have been correlated with anti-tumoral immune responses (5,6). *** p<0.001.

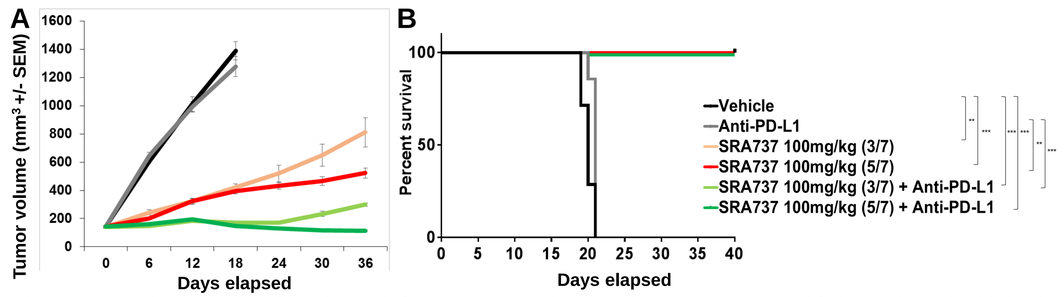

Figure 7: SRA737 in combination with anti-PD-L1 induces tumor regressions in an immune competent model of SCLC (mTmG). Triple knockout mTmG (*Trp53, Rb1* and *p130*) SCLC cells were implanted into the flank of B6129F1 mice. The mice were treated for three weeks with either IgG (control), SRA737 (100mg/kg, either 3/7 or 5/7 days), anti-PD-L1 (300ug, 1/7 days) or the combination. (A) Tumor growth and (B) survival curve show that while anti-PD-L1 antibody treatment was largely ineffective, SRA737 significantly delayed tumor growth (at Day 21: T/C=0.30 for 3/7 days & T/C=0.28 for 5/7 days). Combination treatment with SRA737 and anti-PD-L1 demonstrated remarkable anti-tumor efficacy, resulting in stable disease following SRA737 schedule of 3/7 days (T/C=0.12) and tumor regressions following SRA737 schedule of 5/7 days (T/C=0.1). *** p<0.001, **p<0.01.

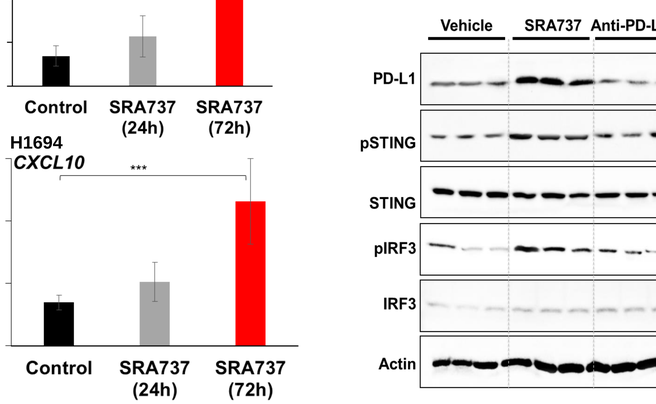

Figure 8: SRA737 induces STING signaling and PD-L1 induction in an immune competent model of SCLC (mTmG). Triple knockout mTmG (*Trp53, Rb1* and *p130*) SCLC cells were implanted into the flank of B6129F1 mice. Mice were treated for one week with either IgG (control), SRA737 (100mg/kg, 5/7 days), anti-PD-L1 (300ug, 1/7 days) or the combination, followed by tumor harvesting and analysis of protein and mRNA. Corresponding tumor lysate immunoblots (A) for markers of the STING pathway, including total and phospho STING (S366), total and phospho IRF3 (S396). Quantitative PCR (qPCR) measurement of mRNA expression of *IFNβ* (B), *CCL5* (C) and *CXCL10* (D) in the corresponding tumors. *** p<0.001.

CONCLUSIONS

- We demonstrate that inhibition of Chk1 by SRA737 treatment results in micronuclei formation and an induction of STING and Type I interferon signaling, leading to expression of immune cell-recruiting chemokines in cell culture as well as in the tumors of an immune competent murine model of SCLC.
- SRA737 induces PD-L1 expression in SCLC cells *in vitro* as well as in the tumors isolated from SRA737-treated animals.
- SRA737 inhibits tumor growth and synergizes with an immune checkpoint blockade agent, anti-PD-L1 antibody, to induce tumor regression in an anti-PD-L1 refractory SCLC model.
- This promising combination strategy warrants clinical investigation in SCLC and other cancer types that are refractory to immunotherapy.