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Combined inhibition of ATR and ATM with tuvusertib and lartesertib (M4076) impacts the tumor microenvironment

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CONCLUSIONS

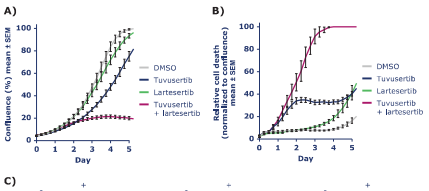
- **Combined inhibition of ATR and ATM with tuvusertib and lartesertib *in vitro* enhances cell death compared to either treatment alone and activates the cGAS-STING signaling pathway**
- **Treatment with tuvusertib and lartesertib induces the expression and secretion of pro-inflammatory cytokines such as IL2 and IL6 *in vitro* in MC38 cells and transiently increases the expression of PD-L1**
- **In the MC38 tumor model *in vivo*, the combination of tuvusertib and lartesertib alters the TME with dynamic changes in PD-L1 expression, infiltration of CD8+ T-cells, NK cells and macrophages**

INTRODUCTION

- ATR and ATM are two of the main kinases of the DDR, resolving replication stress and DNA double strand breaks, respectively
- Tuvusertib[1,2] and lartesertib (M4076)[3,4] are highly selective and potent inhibitors of ATR and ATM which are currently being evaluated as combination treatment in patients with advanced solid tumors[5]
- Recent work has demonstrated that the addition of ATM inhibitors enhances the cytotoxicity and *in vivo* anti-tumor efficacy of ATR inhibitors by abrogating the ATR inhibitor-mediated G1 cell cycle arrest and enhancing chromosomal damage[6]
- As a result of DDR inhibition, damaged chromosome fragments can end up in the cytosol where they are detected by cytosolic nucleic acid sensors such as cGAS, resulting in the induction of inflammatory signaling pathways and an anti-tumor immune response[7,8]
- To better understand how this could be exploited therapeutically, we assessed the influence of combined pharmacological ATR and ATM inhibition on the TME in the MC38 syngeneic mouse model

RESULTS

Figure 1. Combined inhibition of ATR and ATM enhances cytotoxicity and activates the cGAS-STING signaling pathway

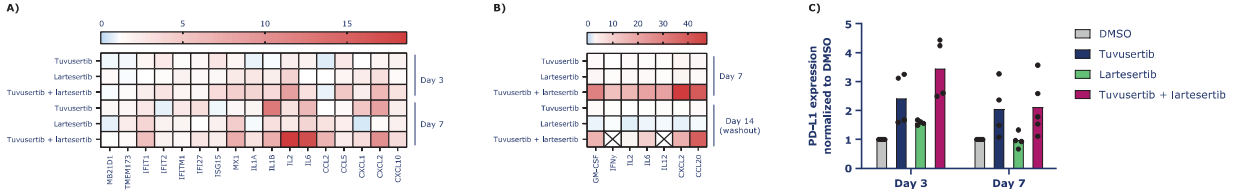


A) Incucyte analysis of cell growth and **B)** cell death of MC38 cells upon single treatment with DMSO, tuvusertib 50 nM, lartesertib 1 µM, or the combination. **C)** Western blot analysis of MC38 cells at day 7 after single treatment with the inhibitors.

RESULTS

- Gene expression of inflammatory cytokines increases upon combination treatment (**Figure 2A**) and also translates into increased cytokine secretion (**Figure 2B**)
- Combination treatment increases PD-L1 gene expression on day 3 which is however followed by a decrease on day 7 (**Figure 2C**)

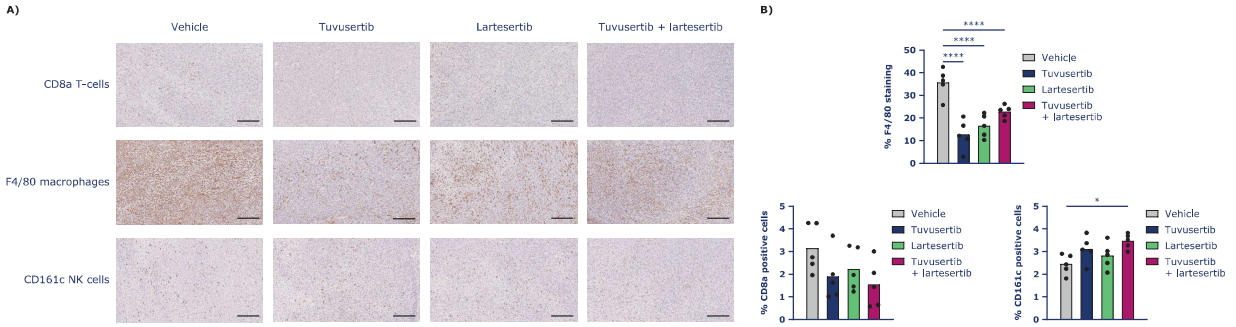
Figure 2. Treatment with tuvusertib + lartesertib increases PD-L1 expression and results in cytokine secretion



A) RT-qPCR analysis at day 3 and day 7 after a single treatment of MC38 cells with tuvusertib 50 nM, lartesertib 1 µM, or the combination. Expression normalized to housekeeping gene GAPDH and DMSO. **B)** Meso Scale Discovery cytokine secretion assay (Meso Scale Diagnostics LLC, Rockville, MD, USA) performed at day 7 after one-time treatment of MC38 cells as indicated above. After 7 days, inhibitors were washed away, and cytokine secretion was again tested on day 14. **C)** RT-qPCR analysis of PD-L1 (CD274) expression normalized to housekeeping gene GAPDH and DMSO. X = not detected.

- In the syngeneic mouse model MC38 continuous combination treatment decreases both CD8+ T-cells and macrophages in tumors but increases NK cells (**Figure 3A,B**)

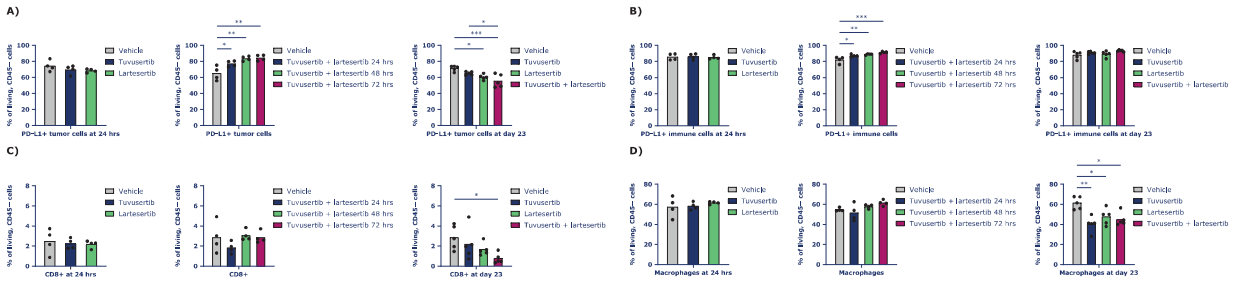
Figure 3. Immune cell infiltration in MC38, tumors is altered *in vivo* upon treatment with tuvusertib and lartesertib



A) IHC staining was performed on sections from MC38 tumors grown in immunocompetent C57BL/6J mice. Mice were treated daily for a period of 23 days with tuvusertib 25 mg/kg, lartesertib 100 mg/kg, or the combination. **B)** The total IHC-positive stained cell count as a percentage of total cell nuclei in viable tumor area (CD8a/CD161c) or the percentage area of total viable tumor area (F4/80) was quantified using a Deep Learning algorithm. Scale bars are 250 µm, *p<0.05, ****p<0.0001, one-way ANOVA with Tukey-Kramer multiple comparison test.

- Initial changes in the TME that occur upon short-term treatment (daily treatment for up to 72 hrs) with tuvusertib + lartesertib are reversed upon prolonged daily treatment (23 days, **Figure 4 A-D**)

Figure 4. Changes in TME upon treatment with tuvusertib and lartesertib are dependent on the duration of treatment



FACS immunoprofiling of MC38 tumors from C57BL6/J mice treated daily with tuvusertib 25 mg/kg, lartesertib 100 mg/kg, or the combination. **A)** PD-L1 expression on living, CD45− tumor cells. **B)** PD-L1 expression on living, CD45+ immune cells. **C)** Percentage of CD8+ T-cells in MC38 tumors, gated from living, CD45−, CD3+ cells. **D)** Percentage of F4/80+ macrophages, gated from living, CD45−, CD11b+ cells. *p<0.05, **p<0.01, ***p<0.001, one-way ANOVA with Tukey-Kramer multiple comparison test.

Abbreviations: ANOVA, analysis of variance; **ATM**, ataxia telangiectasia mutated; **ATR**, ataxia telangiectasia and Rad3-related protein kinase; **cGAS-STING**, cyclic GMP–AMP cyclase–stimulator of interferon genes; **DDR**, DNA damage response; **DMSO**, dimethyl sulfoxide; **FACS**, fluorescence-activated cell sorting; **IHC**, immunohistochemistry; **NK**, natural killer; **PD-L1**, programmed death ligand 1; **RT-qPCR**, reverse transcription quantitative polymerase chain reaction; **SEM**, standard error of the mean; **TME**, tumor microenvironment.

References: 1. Zimmermann A, et al. *Cancer Res* 2022;82:2588; **2.** Yap TA, et al. *Clin Cancer Res* 2024;doi: 10.1158/1078-0432.CCR-23-2409; **3.** Zimmermann A, et al. *Mol Cancer Ther* 2022;21:859–70; **4.** Stakyte A, et al. *Nat Struct Mol Biol* 2021;28:789–98 **5.** ClinicalTrials.gov NCT05396833 (last accessed 04 March 2024); **6.** Turchick A, et al. *Mol Cancer Ther* 2023;22:859–72; **7.** Sen T, et al. Cancer Discov 2019;9:646–61; **8.** Tang Z, et al. Clin Cancer Res 2021;27:4898–909.

Disclosures: Karin Laaber, Julia Jabs and Astrid Zimmermann are employees of the healthcare business of Merck KGaA, Darmstadt, Germany. Brian Elenbaas is an employee of EMD Serono. Audrey Turchick and Lyubomir T. Vassilev were employees of EMD Serono at the time of study.

Acknowledgements: This study was sponsored by the healthcare business of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945). Editorial assistance was provided by Mario Pahl of Bioscript Group, Macclesfield, UK, and funded by the healthcare business of Merck KGaA, Darmstadt, Germany.

Presented at the American Association for Cancer Research Annual Meeting 2024 | April 5–10, 2024 | San Diego, CA, USA