

IN VITRO STUDY OF THE EFFECTS OF CERALASERTIB (AZD6738) ON THE TUMOR MICROENVIRONMENT CELL POPULATIONS

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Background Recent clinical trials have shown the potential therapeutic benefit of combining durvalumab (anti-PD-L1) with the ataxia telangiectasia and rad3-related (ATR) protein inhibitor ceralasertib (AZD6738).^{1 2} Here, we characterized the effect of ceralasertib on T cells, tumor cells, and cancer-associated fibroblasts (CAFs) *in vitro* to understand how ceralasertib may synergize with anti-PD-L1 in modulating various aspects of tumor immune biology.

Methods To determine the effect of ceralasertib on CD8 T-cells, melanoma dissociated tumor cells (DTCs) or CD3/CD28-stimulated healthy donor (HD) CD8 T-cells, were cultured in the presence or absence of ceralasertib at 6 μ M (concentration determined by pRAD50 EC50 (H-Score)). Changes to the phenotype of CD8 T-cells were analyzed by multi-parameter flow cytometry, and polyfunctionality was assessed by single-cell intracellular proteomics (Isoplexis technology). Changes in markers of immunogenic cell death (ICD) and senescence were assessed to determine the effect of ceralasertib on tumor cells and CAFs using melanoma (SKMEL-2) and fibroblast (IMR-90) cell lines. Statistical analysis was done using paired student t-test.

Results Treatment of HD CD3/CD28-activated CD8 T-cells with 6 μ M ceralasertib for 3 days led to a reduction ($87.98\% \pm 2.34$ of DMSO control; n=5) of T central memory (TCM) CD8 T-cells (CD3⁺CD8⁺CD45RA⁻CCR7⁺). Similarly, the frequency of TCM CD8 T-cells from DTCs was reduced when compared to DMSO (p=0.0085; n=7). Ceralasertib treatment reduced proliferation of HD TCM CD8 T-cells (CD3⁺CD8⁺KI67⁺CD45RA⁻CCR7⁺) ($19.18\% \pm 2.67$ of DMSO control; n=5) and increased the frequency of CD8 T-cells expressing gH2AX, consistent with ceralasertib's mechanism of action on DNA damage. HD ceralasertib-treated CD3/CD28-activated CD8 T-cells demonstrated a higher Polyfunctional Strength Index (PSI), defined here as a greater number of cells secreting 2 or more of the following cytokines: GM-CSF, Granzyme B, IFN-g, MIP-1a, MIP-1b and TNF-a versus control-treated cells. Treatment of the melanoma cell line SKMEL-2 with ceralasertib for 3 days induced ICD (higher ATP, HMGB1, calreticulin and cleaved gasdermin D levels). Treatment for only 2 days and culture for an additional 5 days induced senescence (higher b-galactosidase activity, higher calreticulin and HLA Class I membrane surface expression). Interestingly, ceralasertib treatment of IMR-90 fibroblasts induced only senescence regardless of treatment length (2–7 days).

Conclusions Ceralasertib combines with anti-PD-L1 to enhance anti-tumor activity in clinical studies. *In vitro* experiments demonstrate that ceralasertib has pro-immunogenic effects on multiple cell types of the tumor microenvironment, which may contribute to the therapeutic benefit of ceralasertib when combined with durvalumab in patients.

REFERENCES

1. Kwon M, Kim G, Kim R, Kim KT, Kim ST, Smith S, Mortimer PGS, Hong JY, Loembé AB, Irurzun-Arana I, Koulai L, Kim KM, Kang WK, Dean E, Park WY, Lee

- J. Phase II study of ceralasertib (AZD6738) in combination with durvalumab in patients with advanced gastric cancer. *J Immunother Cancer*. 2022 Jul;**10**(7)
2. Kim R, Kwon M, An M, Kim ST, Smith SA, Loembé AB, Mortimer PGS, Armenia J, Lukashchuk N, Shah N, Dean E, Park WY, Lee. Phase II study of ceralasertib (AZD6738) in combination with durvalumab in patients with advanced/metastatic melanoma who have failed prior anti-PD-1 therapy. *J Ann Oncol*. 2022 Feb;**33**(2):193–203.

Ethics Approval All suppliers met AstraZeneca's HBS standards and provided informed consent.

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