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In vitro evaluation of myelosuppressive effects of the ATRi tuvusertib and ATMi lartesertib (M4076), alone and in combination

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CONCLUSIONS

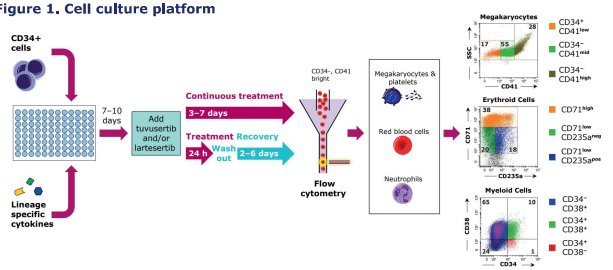
- **Phenotypic and functional changes induced by tuvusertib and lartesertib *in vitro* were consistent with clinical observations in monotherapy studies[1,2]**
- **Erythroid, myeloid, and megakaryocytic cell culture models may be used to better understand and predict the hematological effects of monotherapies and combinations in the clinic**
- ***In vitro* washout experiments may help support the concept of intermittent dosing regimens and warrant further investigation**

INTRODUCTION

- In cancer therapy, drug combinations enhance efficacy and reduce resistance development, but may also increase the risk of toxicity
- ATR and ATM genes have a synthetic lethal relationship in cancer[3] where an ATMi potentiates the efficacy of an ATRi *in vitro* and *in vivo*, offering a potential therapeutic approach in combination[4]
- DDRis induce on- and off-target adverse events including hematological and gastrointestinal toxicities[5], which impact the therapeutic window
- Tuvusertib and lartesertib are potent, selective, orally administered ATRi and ATMi, respectively.[1,2,6–9] Their combination is currently being evaluated in patients with advanced solid tumors in the DDRiver Solid Tumors 320 study[8] (see poster CT063[9])
- The objective of this *in vitro* investigation was to evaluate the hematopoietic effect of tuvusertib and lartesertib, alone and in combination (both intermittent and continuous), on erythroid, megakaryocytic and myeloid bone marrow cell lineages

METHODS

Figure 1. Cell culture platform

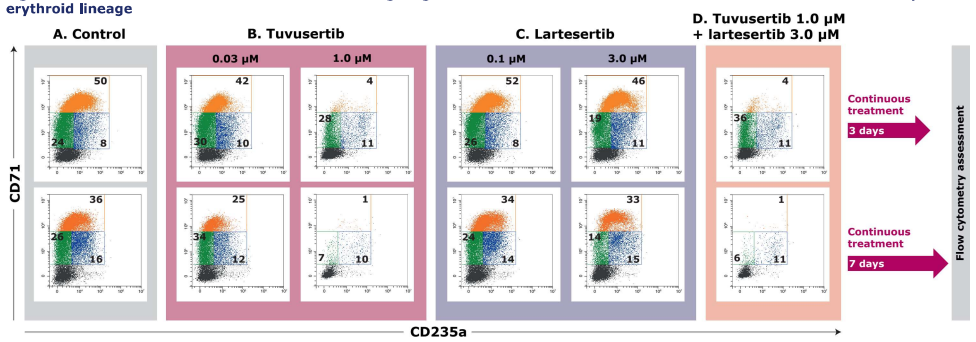


- Mobilized peripheral blood CD34+ cells were plated for differentiation into three hematopoietic lineages for 10 (RBCs) or 7 (neutrophils and platelets) days (**Figure 1**)
- After *in vitro* differentiation, the lineages were cultured in presence of tuvusertib (0.003 to 3 µM) and/or lartesertib (0.01 to 10 µM), encompassing the unbound plasma concentration range evaluated in phase 1 studies of these agents and their combination[1,2,8,9]
- Cells were evaluated in triplicate by flow cytometry on day 3 and 7 of drug exposure for markers of different lineages
- To simulate a drug holiday, cells were exposed to tuvusertib, lartesertib and their combination for 24 h, washed out and further cultured as per assay conditions

RESULTS

- Continuous *in vitro* treatment with tuvusertib as a single agent inhibited the maturation of the hematopoietic erythroid lineage in a concentration- and time-dependent manner (**Figure 2A–B**)
- The effect of lartesertib is less pronounced on all hematopoietic lineages when compared to tuvusertib monotherapy (**Figure 2B–C**).
- Effects with a combination of both drugs appear to be primarily driven by tuvusertib concentrations (**Figure 2D**)
- Similar effects were are also observed in myeloid and megakaryocytes lineages (data not shown)

Figure 2: Effects of tuvusertib and lartesertib as single agents and in combination on the *in vitro* maturation of the hematopoietic erythroid lineage

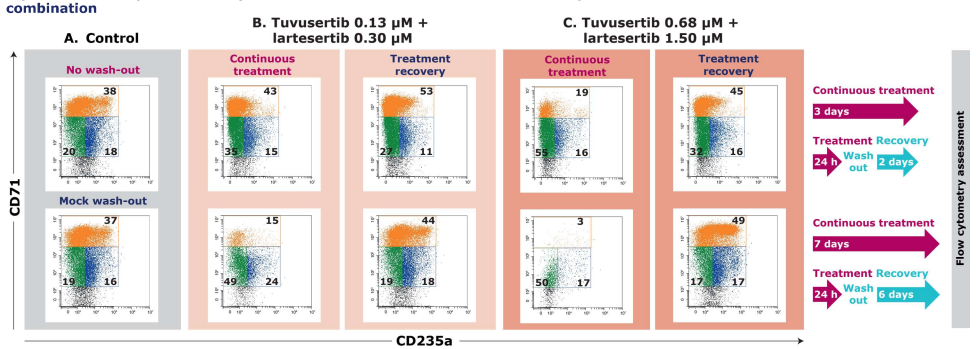


■ CD71^{high}; ■ CD71^{low} + CD235a^{neg}; ■ CD71^{low} + CD235a^{pos}

Human erythroid lineage cells were continuously exposed to tuvusertib, lartesertib, or their combination at various concentrations. Flow cytometry assessments were conducted on days 3 and 7 after treatment start. Control shows viable cells on day 3 and day 7 after treatment with solvent. Bold black numbers indicate the percentage of the specific cell populations within the gates.

- When *in vitro* exposure to the combination of tuvusertib and lartesertib was limited to 24 h followed by wash-out, maturation of the erythroid lineage was progressively restored within 7 days from drug withdrawal (**Figure 3A–C**)

Figure 3. Recovery of human erythroid cell maturation in culture after interruption of treatment with tuvusertib and lartesertib in combination



■ CD71^{high}; ■ CD71^{low} + CD235a^{neg}; ■ CD71^{low} + CD235a^{pos}

Human erythroid lineage cells were exposed to tuvusertib + lartesertib in combination at various concentrations for 24 h, washed out and further cultured, or continuously treated. Flow cytometry assessments were conducted on days 3 and 7 after the treatment start. Control shows viable cells on day 7 after treatment with solvent. Bold black numbers indicate the percentage of the specific cell populations within the gates.

Abbreviations: ATM, ataxia telangiectasia-mutated; **ATMi**, ATM inhibitor; **ATR**, ataxia telangiectasia-mutated and Rad3-related; **ATRi**, ATR inhibitor; **d**, day; **DDR**, DNA damage response; **DDRi**, DNA damage response inhibitors; **h**, hour; **RBCs**, red blood cells

References: 1. Yap T, et al. *Clin Cancer Res* 2024 (epub; doi:10.1158/1078-0432.CCR-23-2409); **2.** Siu LL, et al. *Cancer Res* 2023;83:CT171; **3.** Kantindze OL, et al. *Trends Cancer* 2018;4:755–68; **4.** Turchick A, et al. *Mol Cancer Ther* 2023;22:859–72; **5.** Martorana, et al. *Cancers* 2022;14:953; **6.** Zimmermann A, et al. *MCT* 2022;21:859–70; **7.** Zimmermann A, et al. *Cancer Res* 2022;82:2588; **8.** https://clinicaltrials.gov/study/NCT05396833 (accessed 26 March 2024); **9.** Siu L, et al. *AACR* 2024; Poster CT063.

Disclosures: Anup Zutshi and Karthik Venkatakrishnan are employees of EMD Sereno. Yongzhao Huang and Emer Clarke are employees of Discovery Life Sciences, Seattle, WA, USA. Ioannis Gounaris is an employee of Merck Serono Ltd., Feltham, UK, an affiliate of Merck KGaA, Darmstadt, Germany. Anna Tafuri is an employee of the healthcare business of Merck KGaA, Darmstadt, Germany.

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