INTRODUCTION

• Activation of the MAPK pathway has been associated with a more immunosuppressive tumor microenvironment.
• Preclinical studies have demonstrated that targeting BRAFV600E or KRASG12D with agents such as dabrafenib or AMG101 leads to a more pro-inflammatory TME.
• As a downstream node in the MAPK pathway, the inhibition of ERK1/2 (ERK) is an attractive therapeutic option for potentially overcoming acquired resistance and bypassing signaling, with several ERK inhibitors under investigation in the clinic.
• Inhibition of ERK nuclear translocation by dual-mechanism ERK inhibitors has been reported to lead to an increase in expression of interferon response genes in vitro.
• We have recently described the discovery of ASTX029, a novel, dual-mechanism ERK inhibitor, through fragment screening and subsequent optimisation by structure-based drug design (SBDD).
• ASTX029 is currently undergoing clinical development in a Phase 1 trial in advanced solid tumors (NCT03302075).
• Here we describe the immunomodulatory effects of ASTX029 in BRAF- and KRAS-mutant models.

2. ASTX029 promotes pro-inflammatory TME gene expression changes in the CT-26 murine KRAS-mutant colorectal cancer model

Synergistic:
- CT-26 tumors were grown in female BALB/c mice and treated for 10 days with vehicle or ASTX029.
- AT studied by nSolver (reference tissue, spleen).

2.1. Gene expression changes in tumors were determined using the mouse PanCancer IQ 360™ Gene Expression Panel of 770 genes. Pathway analysis of gene expression changes by nSolver™ software demonstrated changes in several pathways, including an increase in interferon signaling markers.

2.2. To further investigate the changes in gene expression observed in the myeloid and lymphoid compartments, protein levels of 31 markers were assessed in FFPE sections of CT-26 tumors using nCounter GeoMx Digital Spatial Profiling. Sections were stained for markers, PanCK and CD45 before selection of regions of interest (ROIs) for analysis. Representative images and ROIs shown.

3. ASTX029 increases T cell marker expression in CT-26 tumors

3.1. Surface expression of HLA Class I and surface HLA Class I is measured in CT-26 murine melanoma cell lines following treatment.

3.2. The Mel 195 cell line was selected for further analysis. Surface profiling revealed upregulation of PD-L1 at higher concentrations of ASTX029. There was also a dependent increase in gene expression of melanoma antigens gp100 and MART-1.

4. Treatment with ASTX029 induces changes in the myeloid compartment in CT-26 tumors

4.1. To investigate the effects of ASTX029 on the myeloid compartment, we performed a CD45+ segment volcano plot to assess changes in the myeloid compartment.

4.2. Analysis of CD45+ changes demonstrated myeloid cell phenotype on treatment with ASTX029, with significant increase in MHC II levels, consistent with increased antigen presentation, and a significant decrease in Ly6G/Ly6C and CD14 levels.

SUMMARY AND CONCLUSIONS

• Treatment with ASTX029 leads to increased antigen presentation and antigen-specific T cell mediated killing in BRAF-mutant melanoma cell lines.
• ASTX029 inhibits gene expression changes consistent with increased antigen presentation and a more pro-inflammatory TME with increased interferon signaling in the CT-26 KRAS-mutant colorectal cancer model.
• Changes in myeloid cell phenotype in CT-26 tumors following treatment with ASTX029 were detected by digital spatial profiling. Future studies will further define these phenotypic changes and expand the results beyond this model.
• These data will aid rational combination of ASTX029 with other tumor-directed or immunomodulatory agents as part of the ongoing clinical development of ASTX029.

Please see the related poster #CT108 Losuff et al., A first-in-human Phase I study of ASTX029, a dual-mechanism inhibitor of ERK1/2, in melanoma and solid tumors.