Abstract No. 1661

Immune modulation by the dual-mechanism ERK inhibitor, ASTX029, in MAPK-activated tumor models

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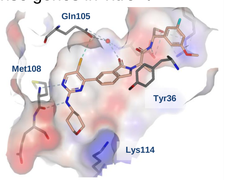
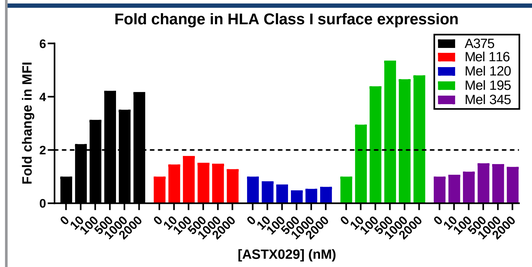
INTRODUCTION

- Activation of the MAPK pathway has been associated with a more immunosuppressive tumor microenvironment (TME) [1].
- Preclinical studies have demonstrated that targeting of $BRAF^{V600mut}$ or $KRAS^{G12C}$ with agents such as dabrafenib or AMG510 leads to a more pro-inflammatory TME [2,3].
- 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 bypass 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 [4].
- 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) [5].
- ASTX029 is currently undergoing clinical development in a Phase 1 trial in advanced solid tumors (NCT03520075).
- Here we describe the immunomodulatory effects of ASTX029 in BRAF- and KRAS-mutant models.

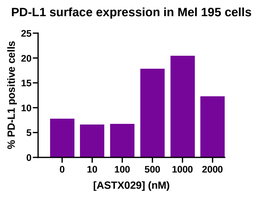


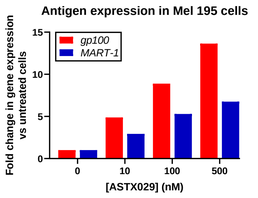
1. ASTX029 increases antigen presentation in human BRAF-mutant melanoma cell lines



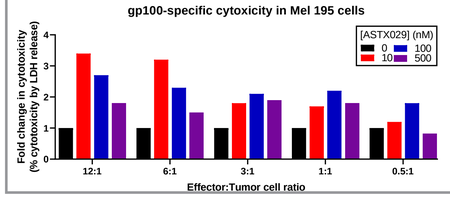
Surface expression of HLA Class I was measured in BRAF-mutant melanoma cell lines following treatment with ASTX029.

2 out of 5 cell lines (A375 and Mel 195) showed an increase of >2-fold in HLA Class I expression upon treatment.





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

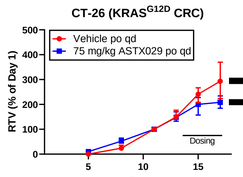


Treatment with ASTX029 caused an increase in antigen-specific T cell cytotoxicity following co-culture of Mel 195 cells with gp100-specific T cells at several ratios of effector to tumor cells.

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

Syngeneic CT-26 tumors were grown in female BALB/c mice and treated daily for 6 days with vehicle or ASTX029.

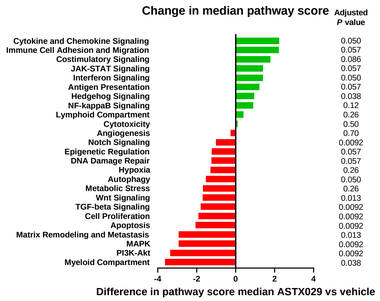
Median ± interquartile range, n =6 per group

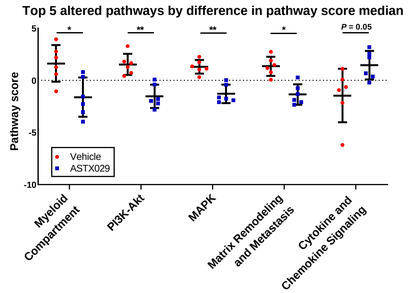


Tumors were collected on day 6 of dosing and divided, if large enough, for gene expression analysis and for FFPE preparation.

Vehicle samples: 6 for IO 360 NanoString® panel, 6 FFPE samples for GeoMx® analysis

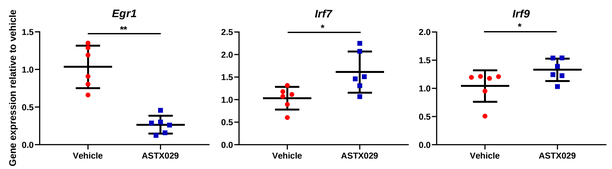
ASTX029 samples: 6 for IO 360 NanoString® panel, 5 FFPE samples for GeoMx® analysis





Mean ± SD and * = *P* <0.05, ** = *P* <0.01 vs vehicle, two-tailed Mann-Whitney U test adjusted by the Benjamini-Hochberg method

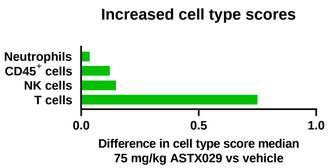
Gene expression changes in tumors were determined using the mouse PanCancer IO 360™ Gene Expression Panel of 770 genes. Pathway analysis of gene expression changes by nSolver™ software demonstrated changes in several pathways, including an increase in interferon signaling markers.



Mean ± SD and * = *P* <0.05, ** = *P* <0.01 vs vehicle, two-tailed Mann-Whitney U test

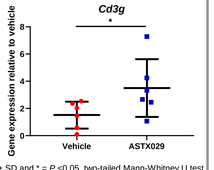
qRT-PCR analysis confirmed a significant decrease in expression of the negative regulator *Egr1* and an increase in expression of the interferon response genes *Irf7* and *Irf9* following treatment with ASTX029.

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



Cell type marker with greatest fold change

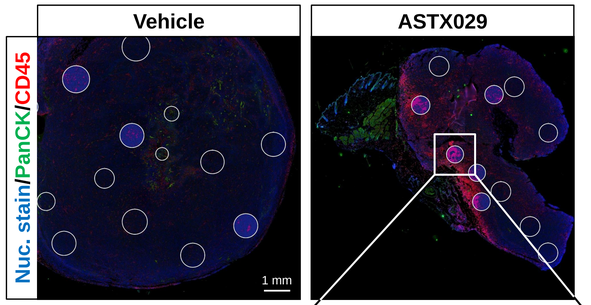
Gene	Cell Type	P value
Fcgr4	Neutrophils	0.59
Ptprc	CD45+ cells	0.36
Xcl1	NK cells	0.53
Cd3g	T cells	0.047

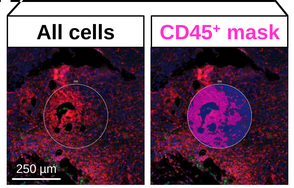


Mean ± SD and * = *P* <0.05, two-tailed Mann-Whitney U test

ASTX029 treatment resulted in increases in nSolver™ cell type score for several cell types, the largest increase being that for T cells. Treatment with ASTX029 induced a significant increase in the T cell marker gene *Cd3g*, which encodes a signaling component of the T cell receptor complex, as assessed by qRT-PCR.

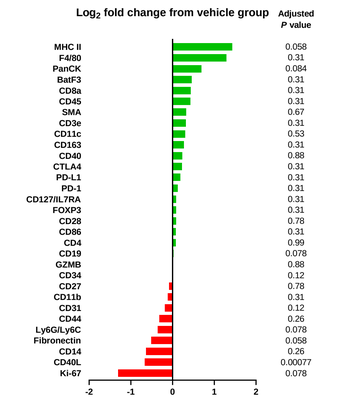
4. Treatment with ASTX029 induces changes in the myeloid compartment in CT-26 tumors





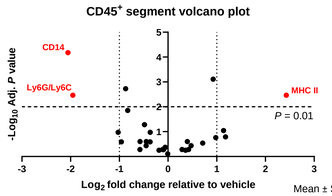
To further investigate the changes in gene expression observed in the myeloid and lymphoid compartments, protein levels of 31 markers were assessed in FFPE sections of CT-26 tumors using NanoString® GeoMx® Digital Spatial Profiling. Sections were stained for nuclei, PanCK and CD45 before selection of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

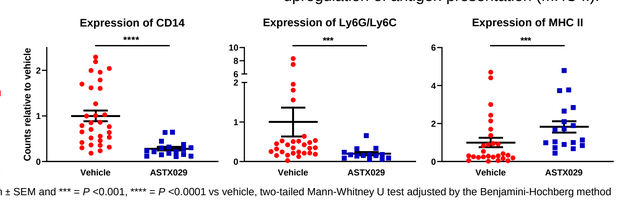
Regions were segmented for CD45 expression and selected for further analysis



P values calculated using two-tailed Mann-Whitney U test adjusted by the Benjamini-Hochberg method

Increased levels of CD45 following treatment suggest increased immune cell infiltration. Changes were consistent with changes in NanoString® pathway scores, such as downregulation of myeloid markers (CD14) and upregulation of antigen presentation (MHC II).





Mean ± SEM and *** = *P* <0.001, **** = *P* <0.0001 vs vehicle, two-tailed Mann-Whitney U test adjusted by the Benjamini-Hochberg method

Analysis of CD45+ regions demonstrated changes in myeloid cell phenotype on treatment with ASTX029, with a 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 induces 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 LoRusso et al., *A first-in-human, Phase 1 study of ASTX029, a dual-mechanism inhibitor of ERK1/2, in relapsed/refractory solid tumors.*

DO NOT POST

References:
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2. Hornet Moreno et al (2016). OncoImmunology 5:7; e1052212
3. Canon et al (2019). Nature 575; 217
4. Kidger et al (2020). Mol Cancer Ther 19; 525
5. Munck et al EORTC-NCI-AACR Virtual Symposium 2020, poster #187

Poster presented at AACR Annual Meeting 2021. April 10 - 15, 2021. (Poster available to download from Astex's website at www.astx.com)