Domatinostat increases apoptosis, G2M cell cycle arrest and immunogenicity of Merkel cell carcinoma

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Introduction
Merkel cell carcinoma (MCC) is a rare, highly aggressive skin cancer prevalent in elderly and immunocompromised patients. Immune checkpoint inhibitors (ICI) like PD-1/PDL-1 blocking antibodies exert a strong clinical activity; however, primary or secondary resistance often occurs. Domatinostat (4SC-202), an orally available small molecule inhibitor targeting histone deacetylases (HDAC) class I, is currently in clinical evaluation to improve response to CPI (SENSITIZ, NCT03278665). In syngeneic tumor mouse models domatinostat treatment demonstrated immune-modulatory effects by increasing the intra-tumoral infiltration of cytotoxic CD8+ T cells (CTLs) and enhancing gene expression of a CPI response signature. Immune escape mechanisms described for MCC include low intra-tumoral infiltration by CTLs and reduced expression of antigen-presenting MHC class I molecules. Previous studies revealed that low MHC-I expression is reversible by epigenetic reactivation of genes encoding the antigen processing machinery (APM). Here, we present novel preclinical data about the efficacy and mode of action of domatinostat in MCC.

Domatinostat increases antigen presentation in MCC

Analysis of antigen presentation proteins in MCC cell lines MKL-1 and WaGa after domatinostat treatment. A) Immunostaining of APM-related proteins. Cells were treated with 2.5 µM domatinostat for 24h; viable cells were stained by fixed-purification via prepure of whole cell lysates. B) Flow cytometry analysis of HLA class I cell surface expression. Cells were treated with increasing doses of domatinostat (0.1–2.5 µM) for 24h. Stacked histograms show normalized counts of HLA-ABC population. Region of HLA-ABC positive cells is labelled. C) Quantification of HLA-ABC expression from flow cytometry analysis. Upper: mean fluorescence intensity; Lower: % HLA-ABC positive cells.

Domatinostat induces G2M arrest in MCC

Analysis of cell cycle effects of domatinostat in MCC cells. A) Single-cell RNA-seq analysis of WaGa cells. Cells were cultured under cell cycle phase and treatment condition. Cell cycle annotations were assigned by calculating signatures using markers genes identified by KnowCyc et al. 2015. Domatinostat-treated (2.5 µM, 24h) and untreated samples were separately aligned using cellranger and subsequently aggregated into one file. B) Cell cycle analysis by flow cytometry. After 24h treatment with domatinostat (2.5 µM) cells were pulse-labeled with BrdU/ BrdU. After 24h incubation to label BrdU was detected via a FITC-labeled anti-BrdU antibody. DNA was stained with 7-AAD. B) shows representative plots of the cell cycle analysis. C) Quantification of BrdU, represented as mean ± SD (n=3).

Conclusion
Global gene expression profiling by single cell RNA-seq revealed regulation of genes involved, among others, in apoptosis and antigen presentation. Importantly, domatinostat inhibited proliferation of MCC cell lines by induction of a G2M cell cycle arrest and apoptosis thus exerting a direct anti-tumoral effect in MCC. In viable cells, domatinostat increases their susceptibility to immune responses by elevating APM and MHC class I expression.

In summary, the HDACi domatinostat counters immune escape of MCC in multiple aspects, suggesting to combine domatinostat with checkpoint inhibitors (CPI) as a promising therapeutic strategy in MCC. Prospective clinical trials are needed to confirm this hypothesis.