A Bi-Functional Mechanism of Action: Activating the NLRP3 Inflammasome in Macrophages and Triggering Apoptosis in Cancer Cells via HK2/VDAC Modulator

AACR 2018
Abstract #: 1725

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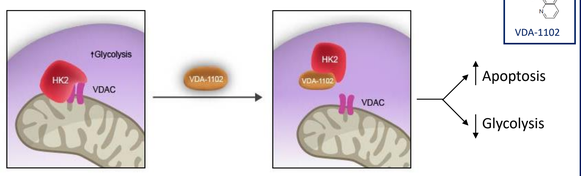
BACKGROUND

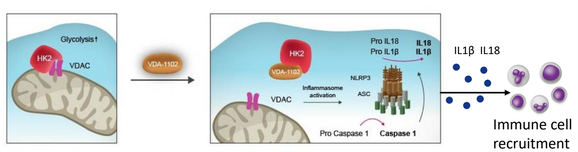
- Metabolic re-programming to aerobic glycolysis, known as the Warburg effect, allows cancer cells an efficient conversion of glucose to biomass and energy required for rapid cell growth and proliferation.
- Similar metabolic re-programming occurs in some activated immune cells.
- A key enzyme in glycolysis is hexokinase (HK), which catalyzes the first step of glucose metabolism.
- Many cancer types express high levels of HK2, whereas the HK1 isozyme is ubiquitously expressed in normal cells.
- In cancer cells, HK2 attaches to the outer mitochondrial membrane via interaction with the VDAC1 channel.
- VDAC1/HK2 association results in apoptosis prevention and a high rate of glycolysis, by blocking pro-apoptotic signals and allowing a continuous flux of mitochondrial ATP to HK.
- Transient high HK2 expression, and binding to VDAC1, is also found in a variety of activated immune cells to support their changing metabolic needs.
- It has been recently published that detachment of HK2 from VDAC is one of the first events leading to NLRP3-inflammasome activation, resulting in IL-1β and IL-18 secretion from macrophages (Cell 2016, 166:624).
- VDA-1102 is a novel small-molecule HK2 modulator that selectively detaches HK2 from VDAC1, triggering apoptosis in cancer cells while promoting an anti-tumor immune responses.

VDA-1102 Mechanism of Action (MOA)

Effects of VDA-1102 on Cancer Cells:



Effects of VDA-1102 on Macrophages:

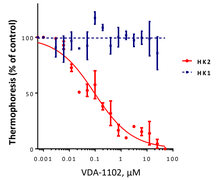


RESULTS

MOA of VDA-1102 (Biochemical)

VDA-1102 Selectively Detaches HK2 from VDAC

Dissociation of recombinant HK proteins from purified VDAC

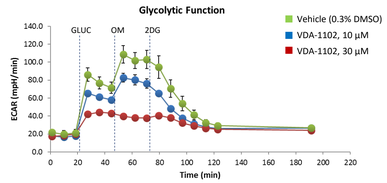


HK2 IC_{50} = 0.09 μM
HK1 IC_{50} >> 10 μM

MST assay (Microscale Thermophoresis): VDAC-HK complexes were allowed to bind for 10 min at RT, then VDA-1102 was added for 10 min and MST was recorded

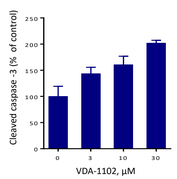
Effects of VDA-1102 on Cancer Cells

VDA-1102 Reduces Glycolysis in Cancer Cells



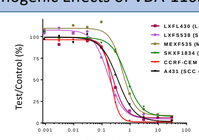
Mouse colorectal cancer cells (CT26) were seeded in Seahorse plates and were pre-treated with VDA-1102 for 3 hours. During extracellular flux analysis, cells were sequentially treated with glucose (GLUC), oligomycin (OM), and 2-deoxyglucose (2DG)

VDA-1102 Induces Apoptosis in Cancer Cells



Human squamous cell carcinoma A431 cells were treated with VDA-1102 for 6 hours. Apoptosis was evaluated by PathScan® Cleaved Caspase-3 sandwich ELISA

Anti-Clonogenic Effects of VDA-1102 on Human Tumors

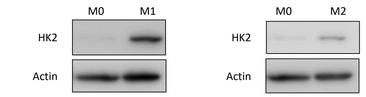


Tumor model	IC50 (μM)
LXFS538	0.15
CCRF-CEM	0.20
LXFL430	0.24
A431	0.36
MEXF535	0.54
SKXF1834	0.74

VDA-1102 inhibits anchorage-independent growth and colony formation of patient-derived tumors and cell lines in semi-solid medium. Assays were performed between 11-14 days.

HK Levels in Bone Marrow-Derived Macrophages (BMDM)

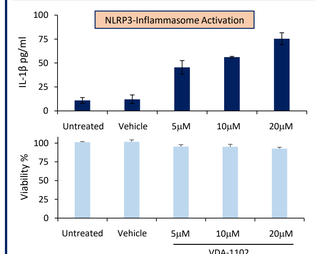
HK2 protein levels are up-regulated in activated macrophages



Naïve BALB/c BMDM (M0) were stimulated either with IL-4 (M2) or with LPS (M1). HK2 protein levels were detected by WB

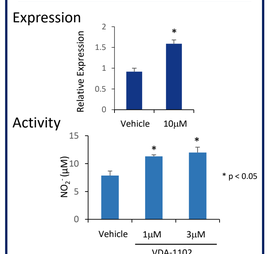
VDA-1102 Activates M1 Macrophages *In Vitro*

VDA-1102 Induces Secretion of IL-1β from BMDM without Affecting Viability



BMDM were stimulated with IFN-γ and LPS and treated for 16 hr with VDA-1102. IL-1β was determined with ELISA kit

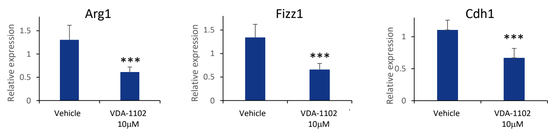
VDA-1102 Increases iNOS Expression and Activity



BMDM were stimulated with LPS and treated with VDA-1102. qPCR was used for iNOS gene expression analysis (24 hr); NO levels (72 hr).

VDA-1102 Suppresses M2 Macrophage *In Vitro*

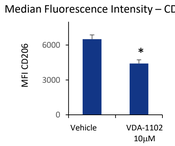
VDA-1102 Reduces M2 Marker Gene Expression

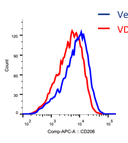


BMDM were stimulated with IL4 and treated with VDA-1102 for 72 hr. qPCR was used for gene expression analysis. *** p < 0.001

VDA-1102 Reduces the M2 Marker CD206

Median Fluorescence Intensity – CD206



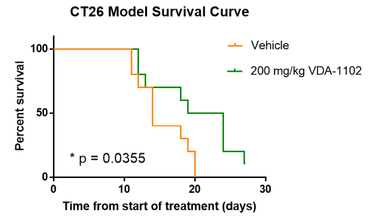


BMDM stimulated with IL-4 and treated with VDA-1102 for 72 hr. FACS analysis was performed for CD206 cellular expression. * p < 0.05

VDA-1102 Efficacy *In Vivo*

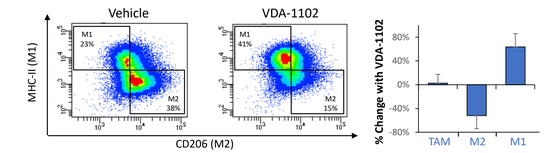
VDA-1102 Inhibits Tumor Growth and Increases Survival in a Mouse Model

CT26 colorectal cancer syngeneic mouse model



Treatment started when tumor volume was 50-100mm³ (6 days after tumor inoculation).
VDA-1102 was administered SC following IP injection of BNPP (a mouse esterase inhibitor) 30 min prior to VDA-1102 injection

VDA-1102 Shifts Macrophages from M2 to M1 Phenotype in CT26 Model



FACS analysis and quantification of $CD11b^+F4/80^+$ (TAM) cell population in CT26 tumors on day 14 post implantation (n=2). Expression of CD206 (M2) and MHC-II in $CD11b^+F4/80^+$ cell population. The bars show the % change in each cell population in VDA-1102-treated group relative to the vehicle-treated control

SUMMARY

- VDA-1102 is a novel selective small-molecule HK2 modulator that targets cancer cells as well as the innate immune system.
- VDA-1102 stimulates multiple anti-tumor mechanisms: triggers apoptosis within cancer cells, increases anti-tumor immune responses (activating NLRP3-inflammasome in M1 macrophages) and decreases immune suppression (suppressing M2 macrophages).
- Our findings support further development of VDA-1102 to evaluate its potential as an anti-cancer therapy, both as a monotherapy and in combinations.