BCLXL and BCLXS as Resistance Markers for BCL2 Inhibitors in Melanoma

Felipe Dantas[1], Evelyn de Groot[2], Cesar Augusto Ruiz Elizondo[2], Renato Guerrieri[2], Michael A Davies[2], Y.N. Vashisht Gopal, Ph.D[2].

[1]University of Texas at Austin, Austin, TX

[2]Melanoma Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX

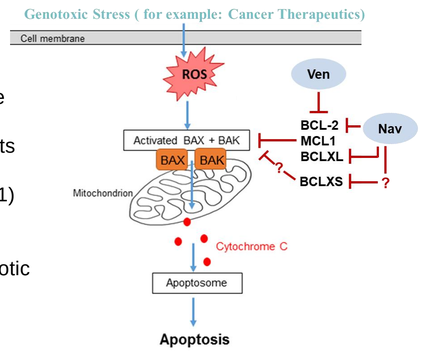
THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Background

- Cutaneous melanoma is the most aggressive form of skin cancer
- Immunotherapies and single-agent targeted therapies have high relapse rates
- 70% of cutaneous melanoma patients have increased expression of BCL2 family proteins (e.g. BCL2 and MCL1) that prevent apoptosis[1]
- Although BCLXL and BCLXS are respectively described as anti-apoptotic and pro-apoptotic proteins in other cancers, their roles are not well understood in melanoma

Figure 1. Apoptosis Pathway[2]



- BCL2 is a marker of chemotherapy treatment resistance and a marker of response to BCL2 inhibitors such as Venetoclax and Navitoclax - however, the role of BCLXL in treatment response is not known, especially in melanoma
- Shorter and potentially antagonistic isoform, BCLXS, has not been studied in melanoma
- Navitoclax targets BCL2 and BCLXL, while Venetoclax solely targets BCL2
- Navitoclax's action on BCLXS is unknown

Hypotheses

- BCLXL-upregulated cells will demonstrate a greater rate of growth than parental. BCLXS-upregulated cells will have the inverse effect, based on prior evidence in other cancers
- Navitoclax will induce greater cell death in BCLXL-upregulated cells, but not in BCLXS-upregulated cells
- BCLXL and BCLXS will have opposite effects on mitochondrial energetics, based on prior evidence of their opposite tumorigenetical effects on other cancers

Generating Cell Lines

- One BCLXL-upregulated cell line clone and one BCLXS-upregulated cell line clone were engineered from the low-BCL2-expressing melanoma cell line WM4257 via lentiviral constructs
- Clones were made to be puromycin resistant.

PDX-CDX Model	MAPK mutation	Resistant to BRAFi?	BCL2 Expression	MCL1 Expression	BCL-xL Expression	MITF Amplification
WM4257	*BRAFV600E*	Yes	Low	High	Med	Yes

Methods

Cell Cycle Analysis: Cells were seeded in 6-well plates overnight, then treated with the indicated doses of Navitoclax or Venetoclax. After 72 hours, cells were harvested, stained with propidium iodide, and cell cycle analysis was performed[3]

Cell Viability: Cells were seeded in 96 well plates then treated with Navitoclax and Venetoclax. Cell viability was evaluated after 72 hours (3 days)

Cell Growth: Cells were seeded in 96 well plates then cell growth was evaluated over a period of 5 days

Cell Energetics: Cells were seeded in Seahorse XF assay plates and mito stress test was performed following the manufacturer's instructions

Results

Cell cycle analysis of parental and BCLXL or BCLXS over-expressing cells

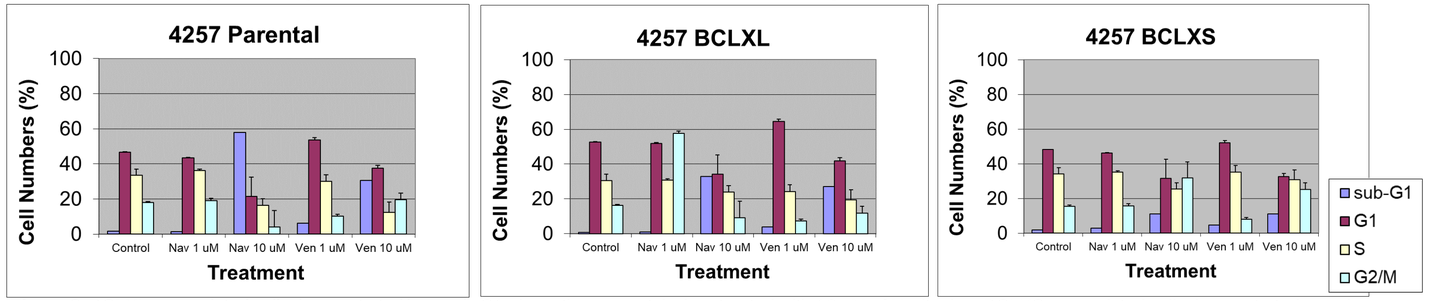


Figure 2. BCLXL-upregulated cells demonstrated less sub-G1 phase than parental when treated with 10 uM of Navitoclax. BCLXS-upregulated cells demonstrated even less sub-G1 phase than BCLXL-upregulated cells when treated with 10 uM of Navitoclax. The same patterns are seen with Venetoclax, with 10 uM causing less sub-G1 phase for BCLXL-upregulated cells and even less sub-G1 phase for BCLXS-upregulated cells. A G2/M spike was seen in BCLXL cells treated with 1 uM Navitoclax, however no similar spike was seen in any other sample or trial.

Cell Viability after Single-Agent Treatment

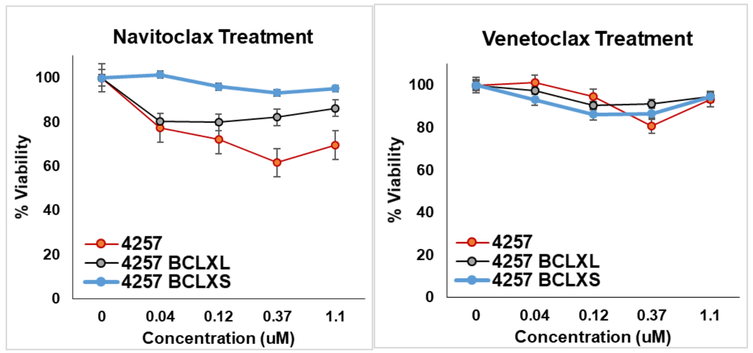


Figure 3. Parental 4257 cells were the most sensitive to Navitoclax. Interestingly BCLXS-upregulated cells were the least sensitive to Navitoclax, followed by BCLXL-upregulated cells. Venetoclax treatment did not induce significant differences in cell-line growth rates.

5-Day Cell Growth

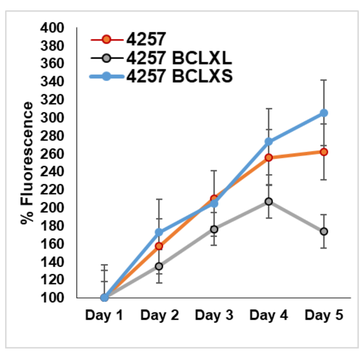


Figure 4. BCLXS-upregulated cells possessed greater rates of growth than parental and BCLXL-upregulated cells, going against the currently known role of BCL-XS as described in literature as a tumor suppressor.

Cell Energetic Profiles

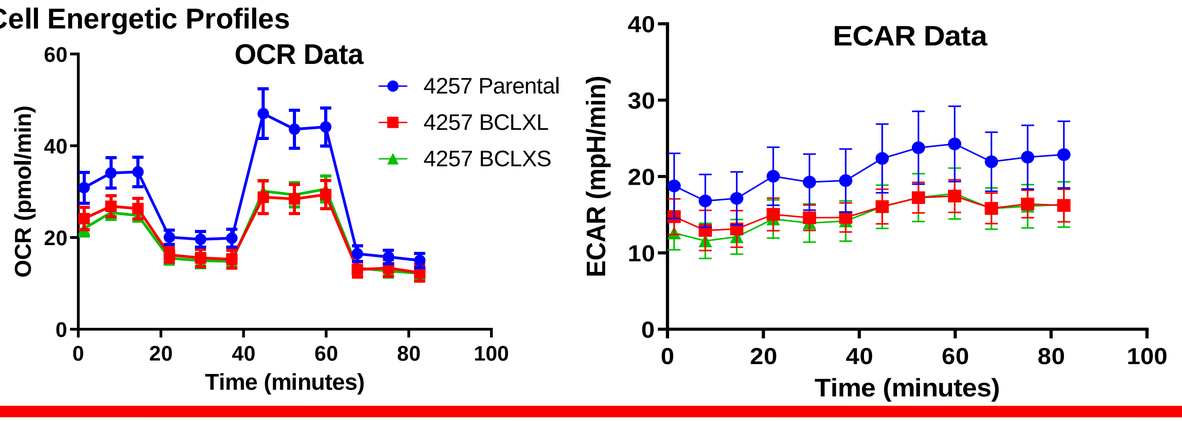


Figure 5. Both BCLXL-upregulated and BCLXS-upregulated demonstrated lower OCR (Oxygen Consumption Rate) and ECAR (Extracellular Acidification Rate) rates as compared to parental. As typical melanoma tumors' OCR levels are over 100, this assay is being re-run.

Conclusions

- BCLXL upregulation had a negative effect on cell growth as well as drug sensitivity relative to parental cells, going against its previously documented tumorigenic role in other cancers
- BCLXS upregulation had a positive effect on cell growth and a negative effect on drug sensitivity relative to parental cells going against its previously documented tumor suppressive role in other cancers
- BCLXS-upregulated cells had lower drug sensitivity than BCLXL-upregulated cells
- BCLXL upregulation caused a decrease in apoptosis following BCL2 inhibitor drug treatment, and BCLXS upregulation caused an even greater decrease
- BCLXL upregulation caused a decrease in cell respiration and glycolysis
- BCXLS upregulation also caused a decrease in cell respiration and glycolysis
- Considering the data, BCLXL and BCLXS likely play differential roles in cutaneous melanoma as compared to other cancers
- Results may be partially due to WM4257's low levels of BCL2 expression levels – research is needed on high-BCLX expression cell lines

Acknowledgements

- **Alice Liu, The University of Illinois at Chicago**
- **ITERT Summer Program**
- **Department of Translational Molecular Pathology**

References

[1] Rina, A et al. (2011). Born to be alive: a role for the BCL-2 family in melanoma tumor cell survival, apoptosis, and treatment. *Frontiers in Oncology.* 1, 34.

[2] Stevens, M & Oltean, S. (2019). Frontiers in Genetics. 10, 804

[3] Vashisht Gopal YN et al. (2014 Dec). Cancer Res. 1;74(23), 7037-47

Acknowledgements: This presentation is supported by the National Cancer Institute through the U54 CA096297/CA096300: UPR/MDACC Partnership for Excellence in Cancer Research Training Program. For further information, please contact Felipe Dantas at fwdantas1@hotmail.com.