

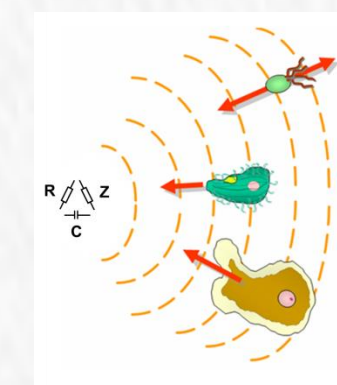
Comparative study on the effectiveness of bortezomib in combination with alpha-lipoic acid and thiamine in melanoma and myeloma cell lines

A. Takács¹, E. Lajkó¹, I. Istenes², L. Kőhidai¹, O. Láng¹



¹ Impedimetry MERILL Core Facility/Chemotaxis Research Group, Department of Genetics Cell- and Immunobiology, Semmelweis University, Budapest (Hungary)

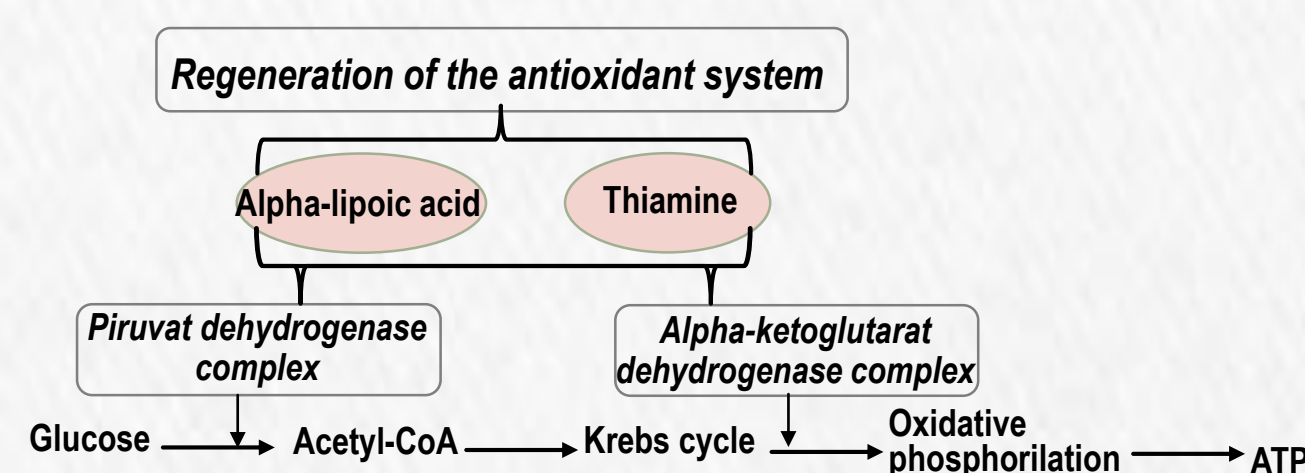
² 1st Department of Internal Medicine, Semmelweis University, Budapest, Hungary
angela.takacs1@gmail.com



Introduction

Bortezomib, a proteasome inhibitor chemotherapeutic agent, is utilized in the treatment of multiple myeloma and nowadays of melanoma, too [1]. It induces apoptosis through oxidative and ER stress-mediated up-regulation of NOXA, a pro-apoptotic protein and blocking the NF-KB pathway. One of the most common and dose-limiting side-effects of bortezomib is bortezomib-induced peripheral neuropathy (BIPN). The first line agents to treat BIPN are neuroprotective antioxidants, e.g. alpha-lipoic acid and thiamine [2]. Alpha-lipoic acid and thiamine are natural compounds, that serve as coenzymes in several metabolic pathways producing biological energy, ATP. Unfortunately, the evidence is presented that the antioxidants, e.g. ascorbic-acid decrease the chemotherapeutic effect of bortezomib [2].

(bortezomib: bort; ascorbic-acid: Cv; alpha-lipoic acid: aL; thiamine/ vitamine B1: B1 v)



Objectives

- the primary objectives of this study were to:
 - determine the effect of bortezomib on the proliferation of suspension myeloma cells and adherent melanoma cells
 - determine the influence of the alpha-lipoic acid and thiamine on the inhibitory effect of bortezomib
- the secondary objectives of this study were to:
 - verify the concentration course effect
 - analyze the apoptotic effects by flow cytometry

Conclusion

Bortezomib is **cytotoxic** in all the tested cell lines (IC₅₀: WM35 – 2,1 * 10⁻⁷ M; A2058 – 1,6 * 10⁻⁷ M). It **induces apoptosis** and **arrests** the cell cycle of melanoma cells in **G2/M** phase.

Thiamine is barely cytotoxic, but **antagonised** the effect of 100 and 300 ng/mL bortezomib on **myeloma** cell lines.

Alpha-lipoic acid is **cytotoxic** on **myeloma** cells and **reversed** the cytotoxic effect of bortezomib. This character of alpha-lipoic acid was more pronounced in case of higher concentrations of bortezomib.

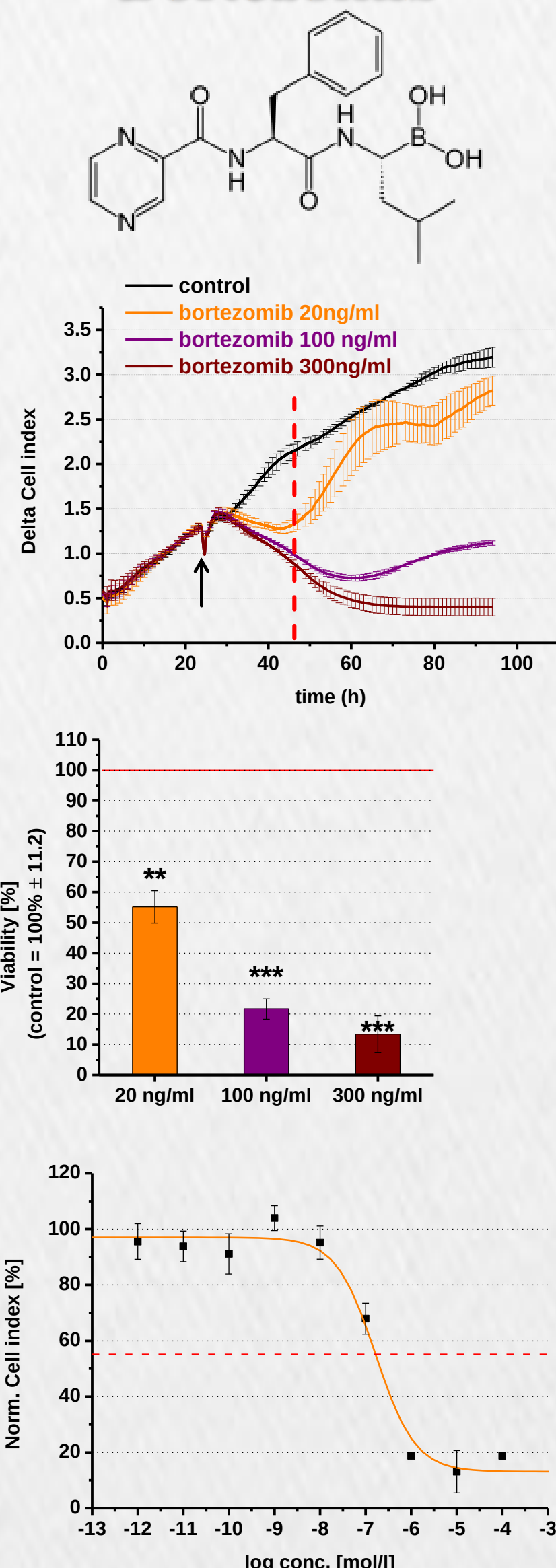
The antioxidants have **no apoptotic** effect in these model cells; however, did **not decrease** the number of **annexin** positive cells induced by 300 ng/mL bortezomib.

Based on our results the combination therapy of **bortezomib** and **low dose of antioxidants** could be **offered** in the treatment of **melanoma**, but **not** in **multiple myeloma**.

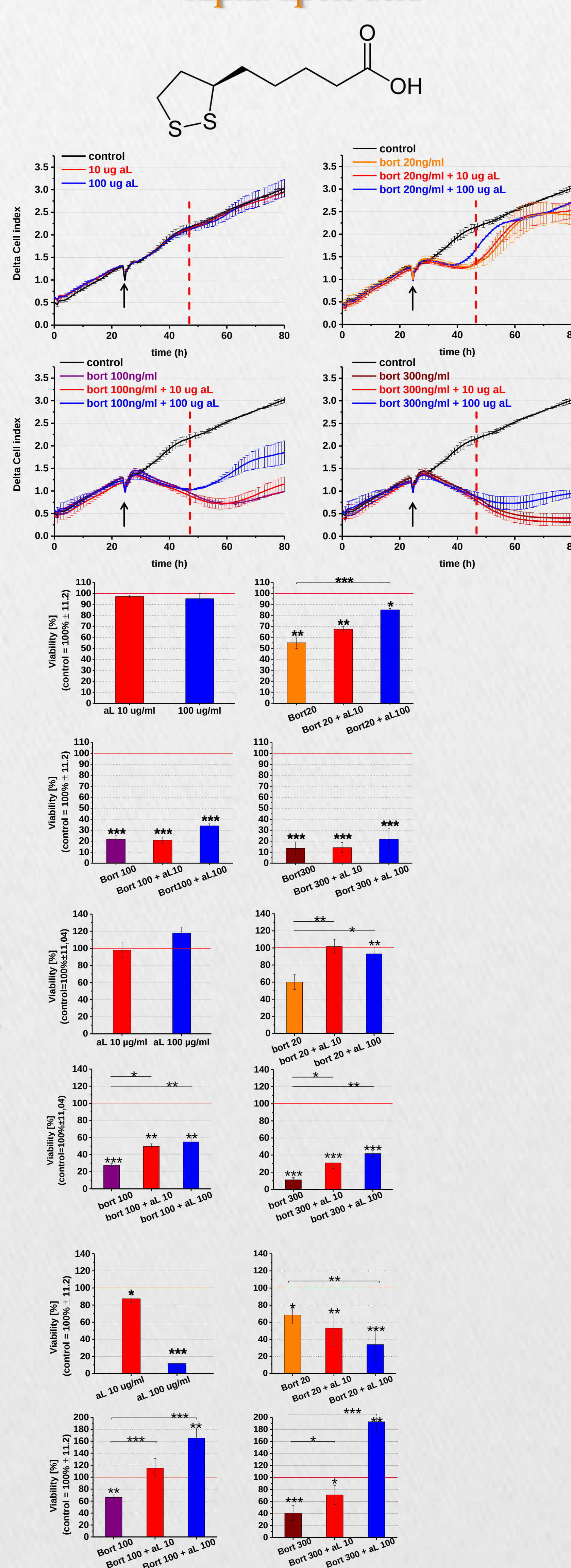
	Primer melanoma WM35	Metastatic melanoma A2058	Multiple Myeloma U266
Alpha-lipoic acid	loss of effectiveness	at low concentration ponderable	loss of effectiveness
Thiamine	ponderable	ponderable	loss of effectiveness

Results

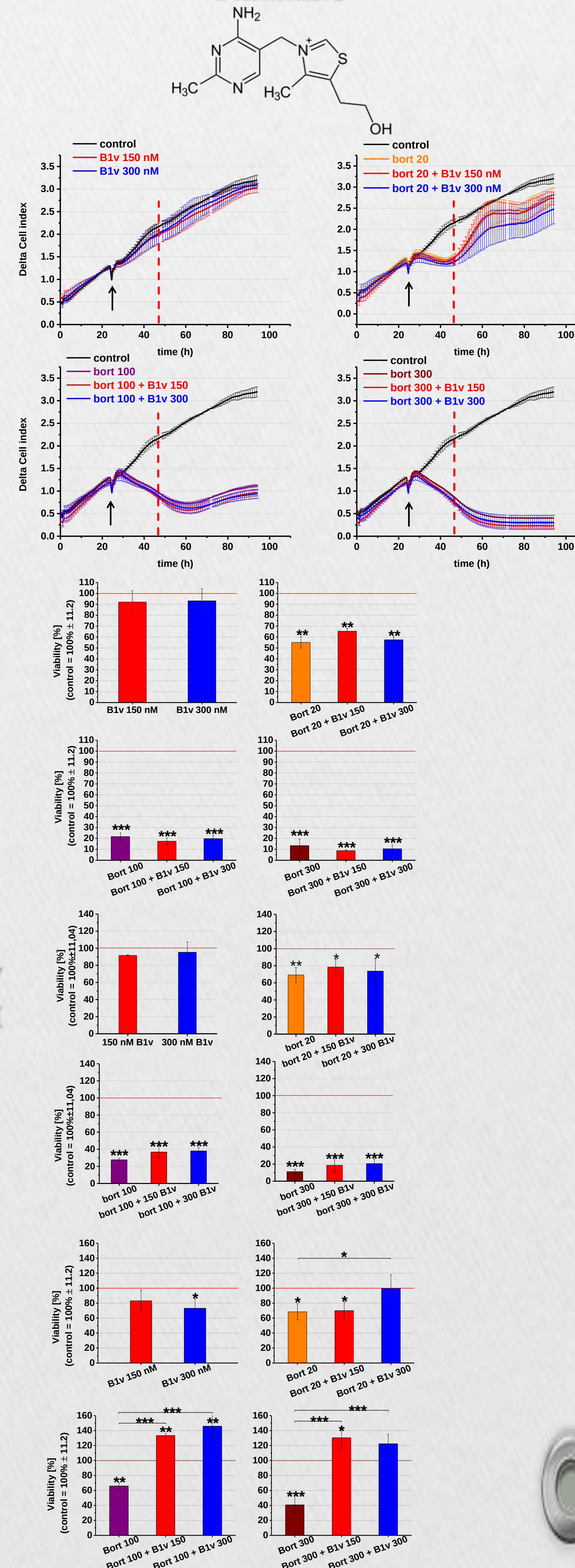
Bortezomib



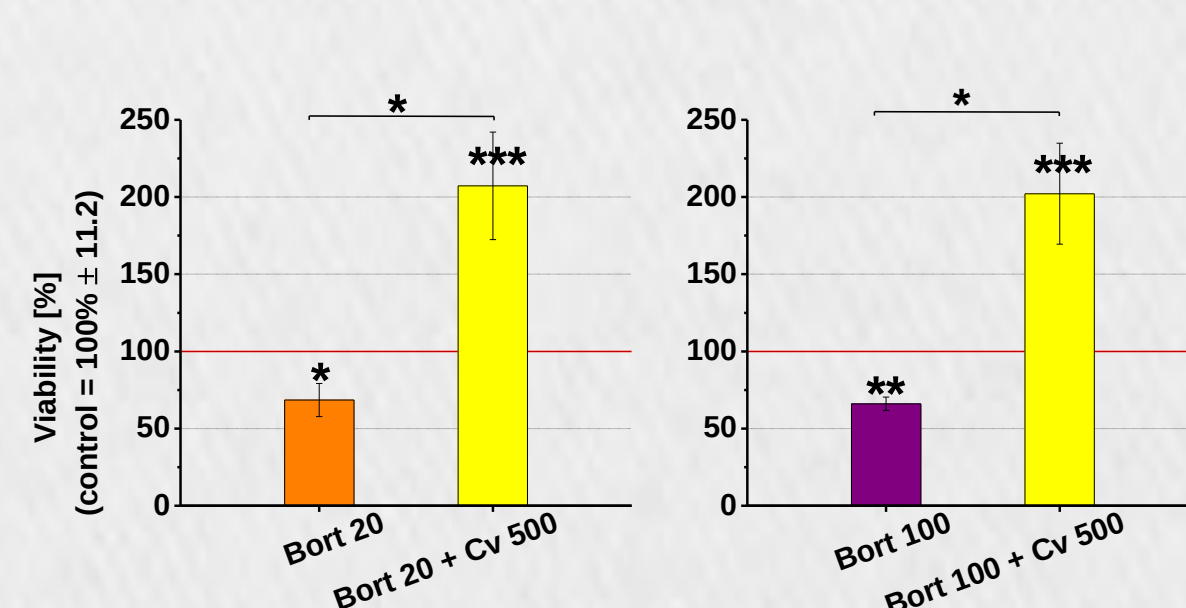
Alpha-lipoic acid



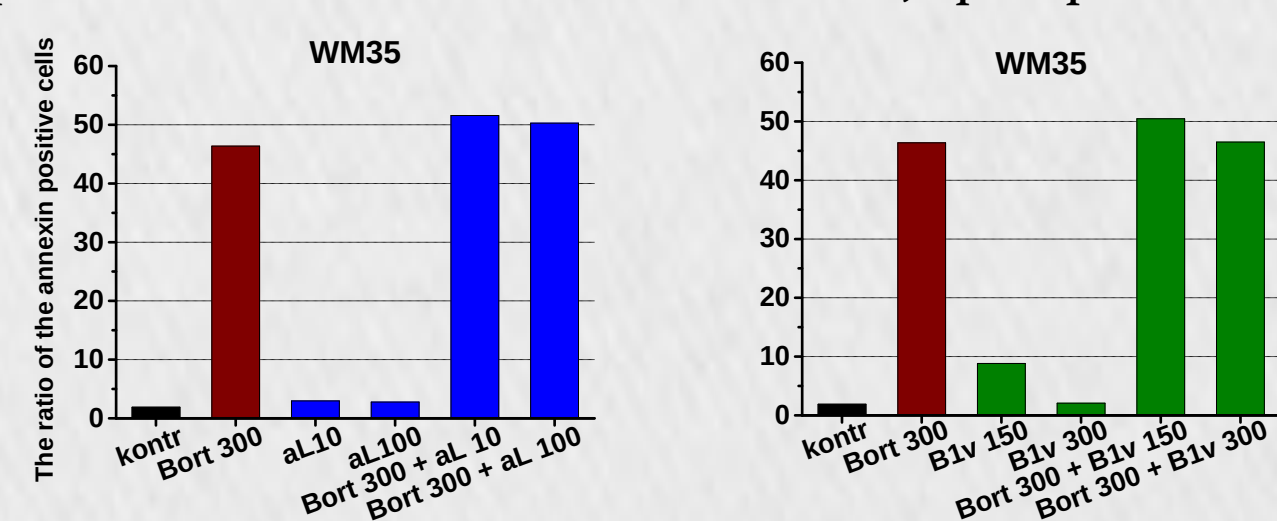
Thiamine



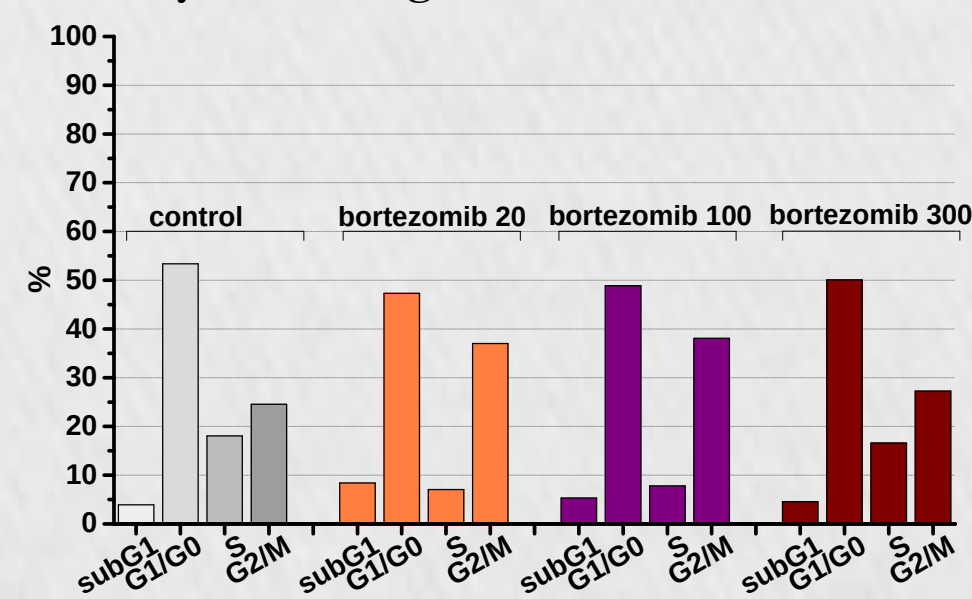
Ascorbic acid



The apoptotic effect of the combinations of bortezomib, alpha-lipoic acid and thiamine



The cell cycle arresting effect of bortezomib on WM35 cells



Methods

Model cells: primary melanoma cell line – WM35
metastatic melanoma cell line – A2058
myeloma cell line – U266

the tested concentrations were (based on therapeutic serum levels):

- bortezomib: 20, 100 and 300 ng/mL
- alpha-lipoic acid: 10 and 100 µg/mL
- thiamine: 150 and 300 nM

xCELLigence SP (ACEA):

- impedance-based system
- change of the cell index (delta cell index) is proportional with the number of the viable cells

Flow cytometry

- apoptosis assay by annexin V labelling
- cell cycle analysis by DNA designation with propidium iodide