



cells, bortezomib had an apoptotic effect, which was significantly decreased only in melanoma cells after the incubation with the bortezomib + antioxidants.

In conclusion, a good correlation between the apoptotic and the cell cycle arresting effect of bortezomib was found as well as the antagonistic effect of the antioxidant to bortezomib activities proved to be mediated by these mechanisms. Based on our results the combination therapy of bortezomib and low dose of antioxidants could be offered for the treatment of BIPN in multiple myeloma.

Keywords: bortezomib, alpha lipoic acid, thiamine, melanoma, impedimetry

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A CONVENIENT METHOD TO PRE-SCREEN CANDIDATE GUIDE RNAS FOR CRISPR/Cas9 GENE EDITING BY NHEJ-CLONING A "SELF-CLEAVING" GFP-EXPRESSION PLASMID

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The efficacies of guide RNAs (gRNAs), the short RNA molecules used by *Streptococcus pyogenes* Cas9 nuclease to determine its sequence specificity vary dramatically, thus the selection of appropriate target sites is critical for most applications. Existing methods to test the efficiency of a given spacer sequence commonly measure the sequence alterations acquired during the repair of the cleaved DNA. The advantage of PCR based methods are that they examine the mutations caused by Cas9 in their genomic context, but are relatively labour intensive. On the other hand, reporter assays that enable the use of flow cytometry are simple, making genomic DNA isolation and PCR amplification unnecessary, but they generally do not measure the sequence alterations caused by Cas9 in their genomic environment, rather on a fluorescent protein coding plasmid and furthermore they require extra cloning steps. Here, we present a simple method for experimentally pre-test the activities of various gRNAs targeting the genome by combining the advantages of the PCR based and fluorescent assays. Our flow cytometry based approach is convenient, measures the modifications by Cas9 in their genomic context and requires no extra cloning steps when this method is applied.