

# Comparative Evaluation of Cellular Responses to Proton Flash Irradiation in B16-F10 Melanoma and L929 Fibroblast cells

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## INTRODUCTION

Melanoma is an aggressive malignant disease originating from the uncontrolled proliferation of melanocytes. Radioterapy remains the most effective treatment approach for this condition [1]. Proton radiotherapy is an innovative cancer treatment method that, due to specific mechanisms of interaction with matter and linear energy transfer (LET), has the ability to target the tumor volume without affecting surrounding healthy tissues [2]. To increase tumor control, the FLASH method is being developed, which involves administering treatment doses with dose rates greater than 40 Gy/s [3].

The aim of this study is to determine the effects induced by high proton dose rates (1500, 1000, 250 and 50 Gy/s) with doses of 1 and 3 Gy, on B16-F10 melanoma cells and L929 fibroblast cells by determining cell viability, clonogenic capacity and evaluating the genotoxicity induced following treatment.

## METHODS AND MATERIALS

B16-F10 melanoma cells and L929 mouse epithelial fibroblast cells were purchased from ATCC (Virginia, USA). The cells were grown in DMEM + 10% fetal bovine serum (FBS) culture medium in 150 mL culture vessels.

To perform the cell viability test using the MTS assay, sterile irradiation chambers were prepared, in which cells were seeded on Mylar foils 24 h before irradiation, at cell densities of 5,000 cells/chamber.

For the clonogenic assay, 24h before irradiation, cells were seeded in irradiation chambers at a density of 600 cells/chamber. After irradiation, the Mylar foils were transferred to 6-well plates and allowed to grow for the equivalent of 6 doubling times.

To analyze treatment-induced genotoxicity, the Micronucleus assay was performed. 24 h before irradiation, 30,000 cells were seeded in each irradiation chamber.

The samples were irradiated at the Tandetron particle accelerator with an energy of 3 MeV. The administration of doses of 1 and 3 Gy of protons with dose rates of (1,500, 1,000, 250 and 50) Gy/s was performed at an energy of 1.95 MeV.

## RESULTS

At 1 Gy and 50 Gy/s, L929 cells showed greater protection than B16 melanoma cells. At 3 Gy, L929 cell viability decreased at high dose rates (1,000 and 1,500 Gy/s) but increased significantly at lower dose rates (250 and 50 Gy/s). In contrast, B16 cell viability decreased as the dose rate decreased, resulting in enhanced sparing of L929 cells, particularly at 250 and 50 Gy/s.

The clonogenic assay results show a dose dependent reduction in colony forming efficiency for both cell lines. At 3 Gy, L929 clonogenic capacity increases with decreasing the dose rate while clonogenic capacity of B16-F10 cells decreases with decreasing the dose rate.

In B16-F10 cells, the increase in frequency of micronuclei appearance after irradiation with a dose of 1 Gy at the maximum rate suggests increased genotoxicity compared to control samples. With a decrease in the dose rate, the frequency of micronuclei increases.

At 3 Gy, the number of micronuclei is greater compared to samples irradiated with a dose of 1 Gy for 1500, 1000 and 250 Gy/s, a result that is correlated with the increase in the lesions suffered at the cellular DNA level. For the dose rate of 50 Gy/s, a decrease in the frequency of micronuclei occurred.

## CONCLUSIONS

The results indicate a dose and dose rate-dependent response in both cell lines, with differential response between L929 and B16-F10. At low dose rates, L929 shows a relative protection response compared to B16-F10, confirmed by viability and clonogenic assay. Micronucleus analysis suggests variations in genotoxicity depending on dose and dose rate in B16-F10 melanoma cells. Data for L929 are preliminary and will be completed with further studies.

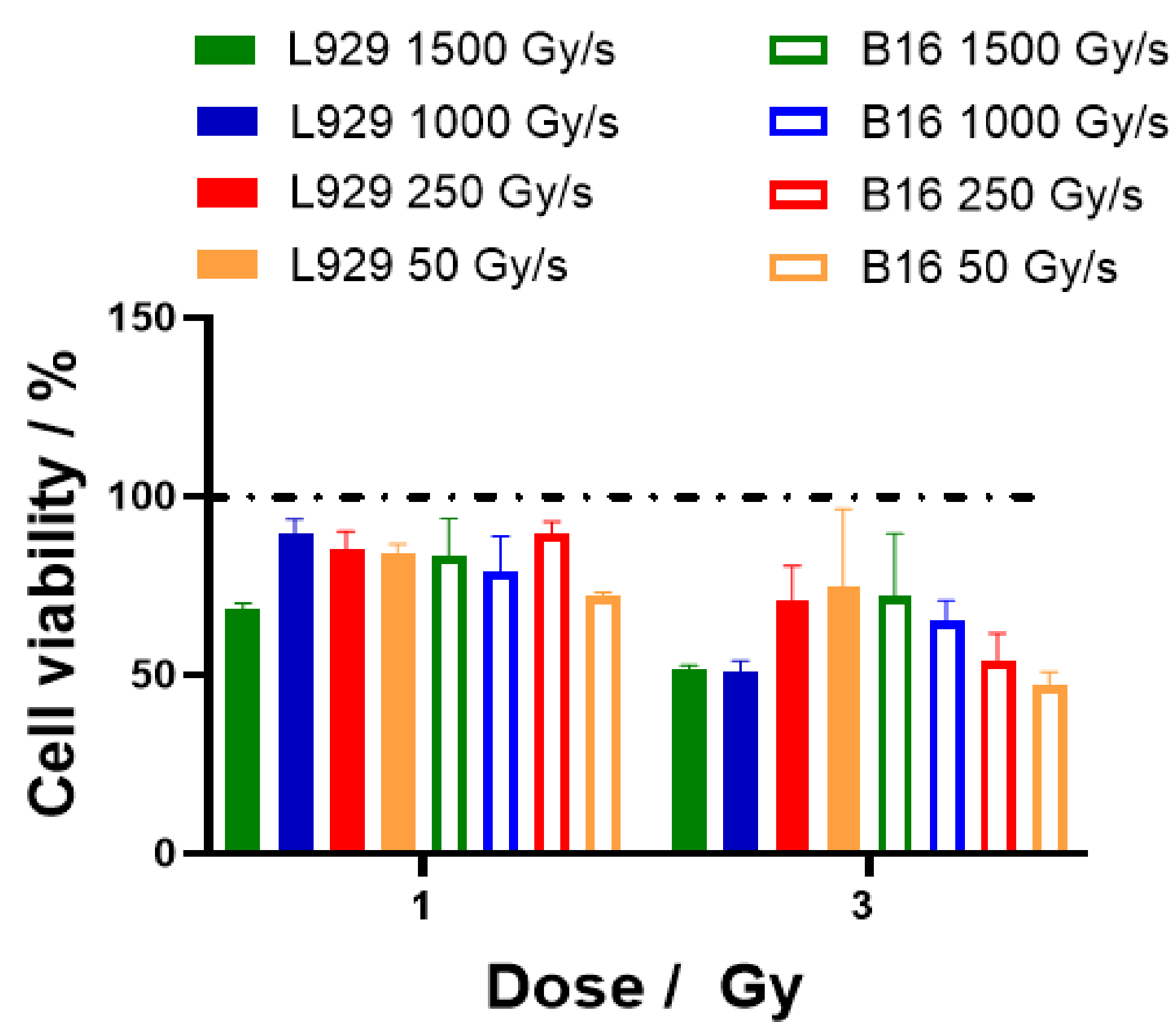


Chart 1. Viability Assay

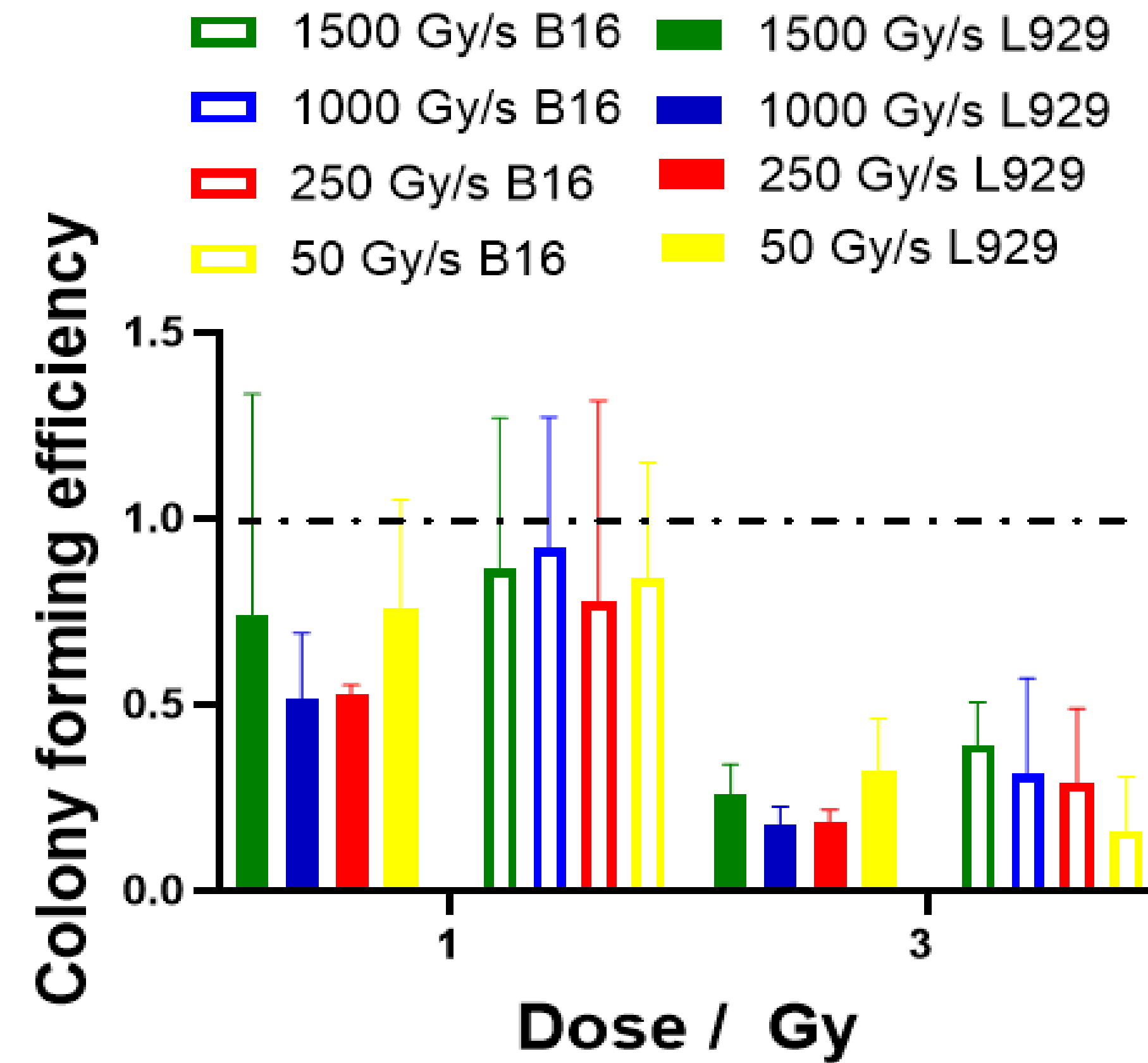


Chart 2. Clonogenic Assay

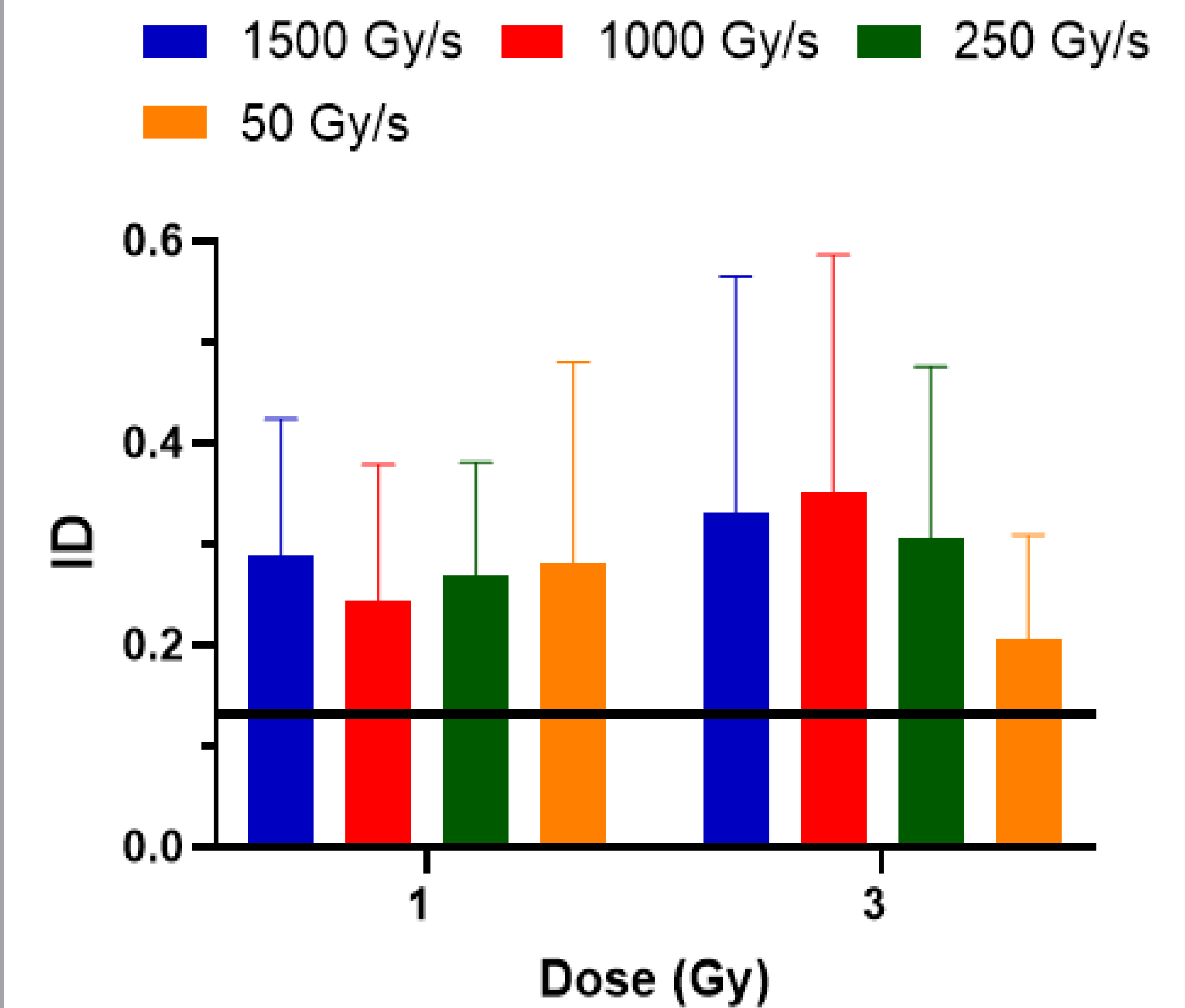


Chart 3. Micronucleus Assay

## REFERENCES

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