Pirfenidone sensitizes NSCLC cells to the antitumor effect of Vinorelbine

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Introduction

Drug repurposing in cancer emerged as a strategy to identify antitumor potential in drugs clinically approved for the treatment of other diseases, offering new treatment possibilities especially for cancers with limited therapeutic alternatives [1], as the non-small cell lung cancer (NSCLC). Currently, Vinorelbine, an anti-mitotic drug, is used for the treatment of advanced NSCLC; however, it is often associated with high toxicity levels [2]. Hence, the combination of Vinorelbine with repurposed drugs would allow to reduce its concentration and thus the associated toxicity. Pirfenidone, an approved anti-fibrotic drug, appears as a possible repurposing contestant as it has been previously demonstrated to sensitize different cancer cells to the effect of some anti-cancer agents [3].

Aim

This study aimed to assess the potential off-target effect of Pirfenidone as a chemosensitizer of NSCLC cells to Vinorelbine, in order to reduce Vinorelbine's concentration and toxicity.

Methodologies

- Sulforhodamine B Assay;
- Trypan Blue Exclusion Assay;
- Cell Cycle Profile analysis by Flow Cytometry;
- BrdU Incorporation Assay.

Results

Table 1 – The GI50 values of NCI-H460 cell line treated with Vinorelbine and Pirfenidone, determined with the SRB assay.
Results are presented as mean ± SEM of 3 independent experiments.

Cancer Cell Line	GI_{50} (nM) Vinorelbine at 48h	GI_{50} (mM) Pirfenidone at 48h
NCI-H460	6.9 ± 0.46	1.94 ± 0.03

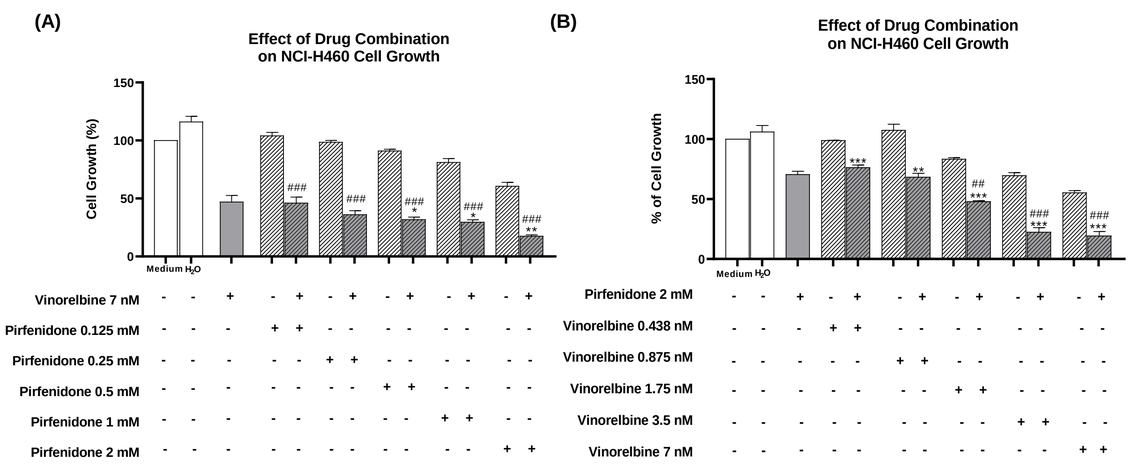

Figure 1 – Effect of the combination treatment of Vinorelbine and Pirfenidone in NCI-H460 cells. Determination of the % of cell growth of the sensitive NCI-H460 cell line, treated with drug combinations consisting of **(A)** Vinorelbine at 7 nM with different concentrations of Pirfenidone; **(B)** Pirfenidone at 2 mM with decreasing concentrations of Vinorelbine, using the SRB assay. Data represents the mean ± SEM of 3 independent experiments. * p < 0.05, ** or ## p < 0.01, *** or ### p < 0.001, when comparing the effect of the drug combination with Vinorelbine (*) or Pirfenidone (#) on their own.

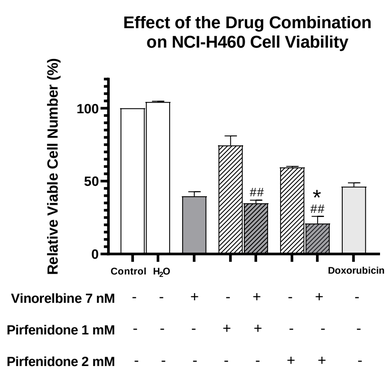

Figure 2 – **Viable cell number of NCI-H460 cells determined using the trypan blue exclusion assay.** Cells were incubated with Vinorelbine and/or Pirfenidone for 48 h. Doxorubicin was used as positive control. Data are expressed as mean ± SEM from 3 independent experiments (n=3). * p<0.05; ## p<0.01, when comparing the effect of the drug combinations with Vinorelbine (*) or Pirfenidone (#) on their own.

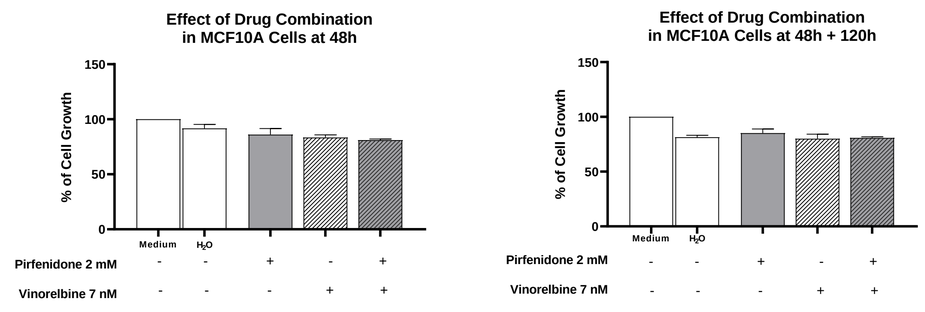

Figure 3 – Effect of the drug combination on non-tumorigenic cells. Determination of the % of cell growth of the MCF10A non-tumorigenic breast cell line treated with the drug combination consisting of Vinorelbine at 7 nM and Pirfenidone at 2 mM, after (A) 48 h treatment or (B) 48 h treatment plus an additional 120 h without drug treatment, using the SRB assay. Results represent mean ± SEM from 3 independent experiments (n=3).

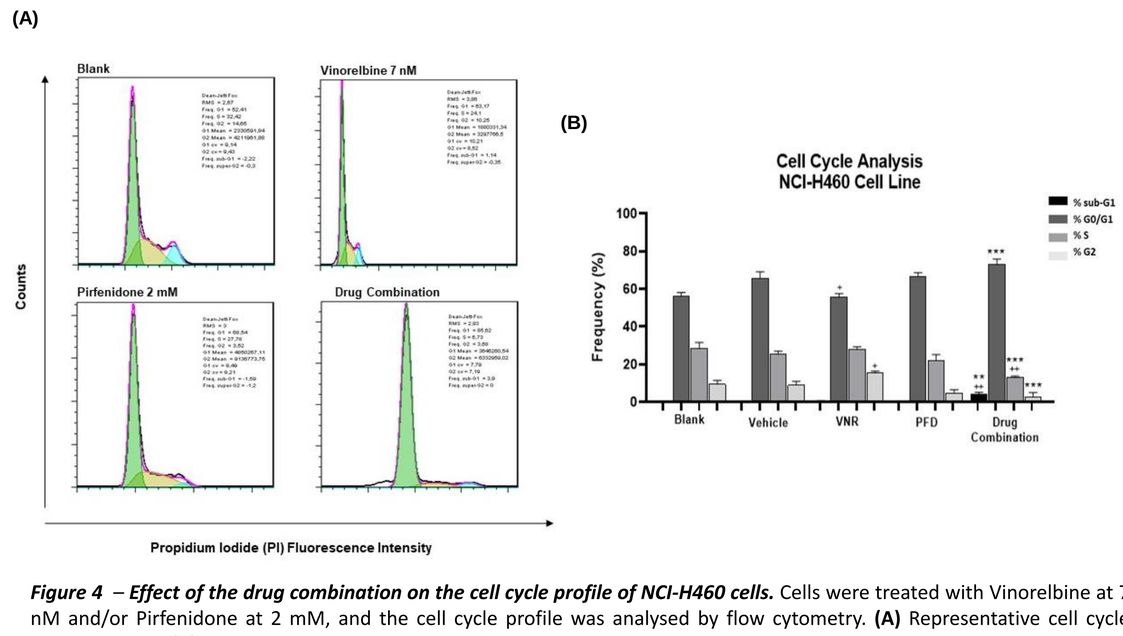

Figure 4 – ***Effect of the drug combination on the cell cycle profile of NCI-H460 cells.*** Cells were treated with Vinorelbine at 7 nM and/or Pirfenidone at 2 mM, and the cell cycle profile was analysed by flow cytometry. **(A)** Representative cell cycle histograms and **(B)** Frequency of cell cycle stages. Doxorubicin was used as positive control. Data represents mean ± SEM of 3 independent experiments. + p < 0.05; ** or ++ p < 0.01; *** p < 0.001, when comparing the effect of the treatment condition with Vinorelbine (*) or the vehicle (+).

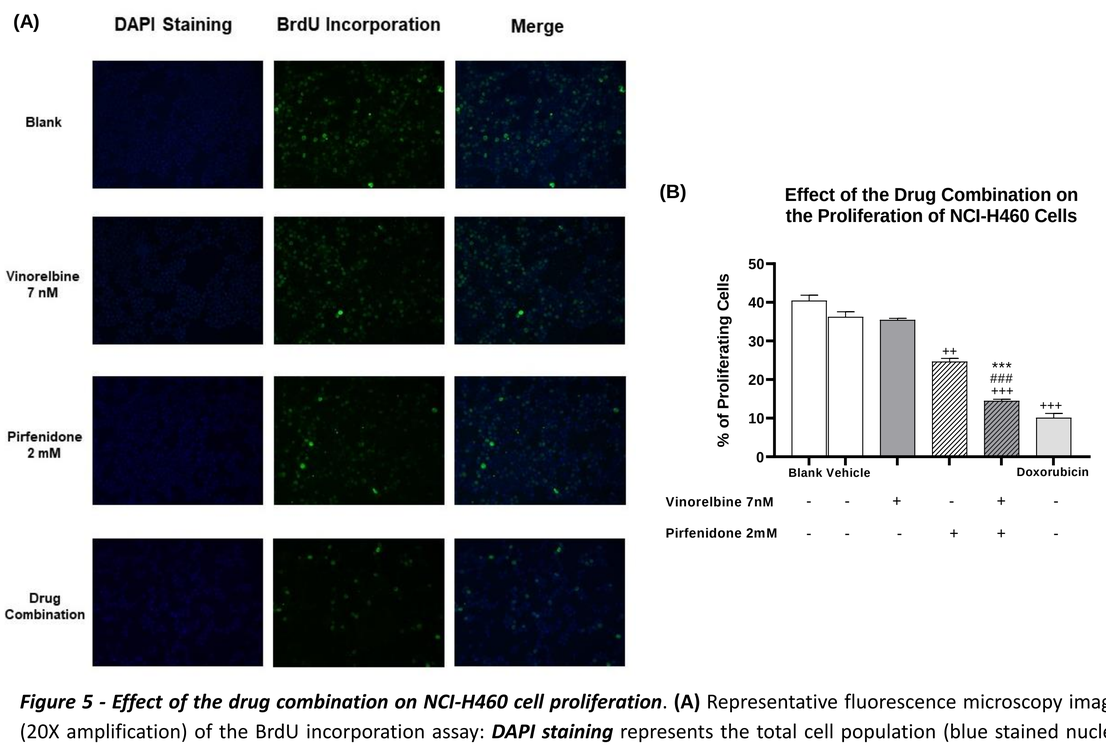

Figure 5 - Effect of the drug combination on NCI-H460 cell proliferation. **(A)** Representative fluorescence microscopy images (20X amplification) of the BrdU incorporation assay: ***DAPI staining*** represents the total cell population (blue stained nuclei); ***BrdU Incorporation*** represents proliferating cells (green); **(B)** % of Proliferating Cells. Results represent mean ± SEM of 3 independent experiments. Doxorubicin was used as a positive control. ++ p < 0.01; ***, ### or +++ p < 0.001, when comparing with Vinorelbine (*) , Pirfenidone (#) or the vehicle (+).

Conclusion

Results show that the drug combination decreases NSCLC cell growth and cell viability, promoting cell cycle arrest and reducing cellular proliferation. Future *in vitro* and *in vivo* studies will validate these results, to further evaluate the possibility of repurposing Pirfenidone for the adjuvant treatment of NSCLC in combination with Vinorelbine.

References

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