Effects of MST-312 long-term treatment in the MDR Phenotype of Non-Small Cell Lung Cancer

Villarinho, N.J.[1], Vasconcelos, F.C.[1], Mazzoccoli, L. [1], Maia, R. C.[1], de Oliveira, D.M.[2], **Lopes, G.P.F.[1]**

[1]Programa de Hemato-Oncologia Molecular, Coordenação de Pesquisa, INCA, RJ, Brazil.
[2]Departamento de Patologia, UnB, DF, Brazil.

BACKGROUND

In Brazil, lung cancer is the second more incident type of cancer in men and fourth in women. Immortalization by telomeres elongating is one important step in tumorigenesis. A treatment strategy consists in the use of telomerase inhibitors. However, a long-term use of these inhibitors may result in acquired resistance characterized by distinctive mechanisms such as alternative lengthening of telomere (ALT) and the multiple drug resistance (MDR).

AIMS

Our aim was to evaluate proliferation, morphology and MDR phenotype during a long-term treatment with MST-312 using a NSCLC in vitro model.

METHODS

Subtoxic concentration of MST-312 for long-term culture was obtained by cultivating H460 human cells with increasing concentrations of telomerase inhibitor for 72 hours. Cellular viability was assessed by Acid Phosphatase assay. Doubling Time (DT) was obtained by cultivating cells for 120h. Cells were counted by trypan blue exclusion every 24h. Both results were obtained using the software Graphpad Prism 5.0 (Windows). For the long-term treatment, cells were cultivated with subtoxic concentration of MST-312 or DMSO during a period of time based on its DT. Cells morphology were documented by microscopy. MDR was evaluated by activity and expression of P-glycoprotein, MRP1 and BCRP efflux pumps using flow cytometry.

RESULTS

Ic50 telomerase inhibitor and subtoxic concentrations were 2.57µM and 2µM, respectively. DT and time of treatment achieved was 17.52 hours and 52 days, respectively. After 120h of treatment, cells cultivated with MST-312 presented vacuoles figures and protoplasmic cytoplasm with undefined borderlines were compared to cells cultivated with DMSO (vehicle). Activity of BCRP was increased after 2 weeks of MST-312 treatment, but normalized on the following weeks.

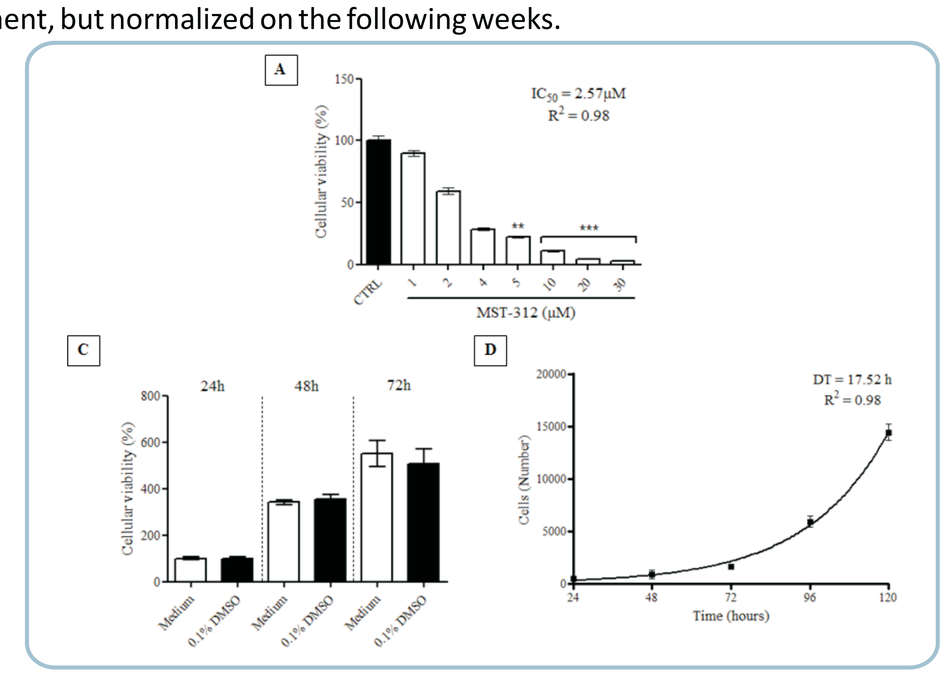

Figure 1: Effects of short-term treatment on lung cancer cellular viability. (A) Analysis of cellular viability performed by the Acid Phosphatase Assay after 72h of treatment with MST-312. Data are expressed as median with ranges and relative to the control (*P < 0.05, ** P < 0.01, *** P < 0.001; Kruskal-Wallis, followed by Dunn's post-test; N=4). (C) Absence of toxic effect of DMSO under the same conditions (N=4). (D) Determination of DT for H460 cells (N=3).

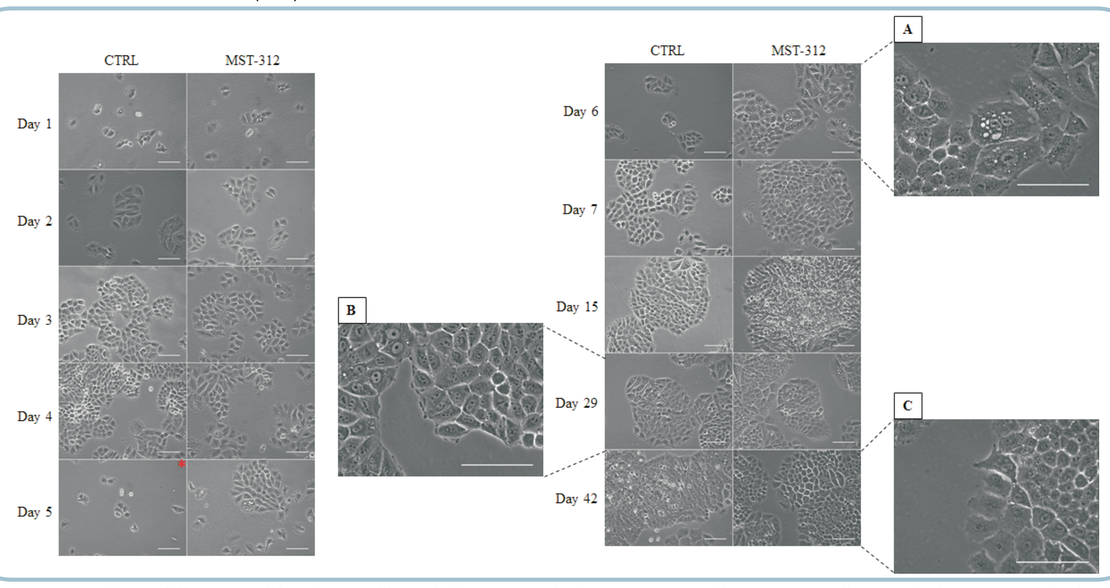

Figure 2: H460 shows different proliferation profile and phenotypes during treatment with MST-312. Following the beginning of the long-term treatment, both cultures (control and MST-312 treated cells) were registered by contrast-phase microscopy each 24h during treatment. The red asterisk (*) indicates when the control was tripsinized due to confluence above 80%. (A) Formation of vacuoles observed after 6 days. (B) Borderline phenotype of control. (C) MST-312 treated cells undefined border phenotype. Scale bar 100µm.

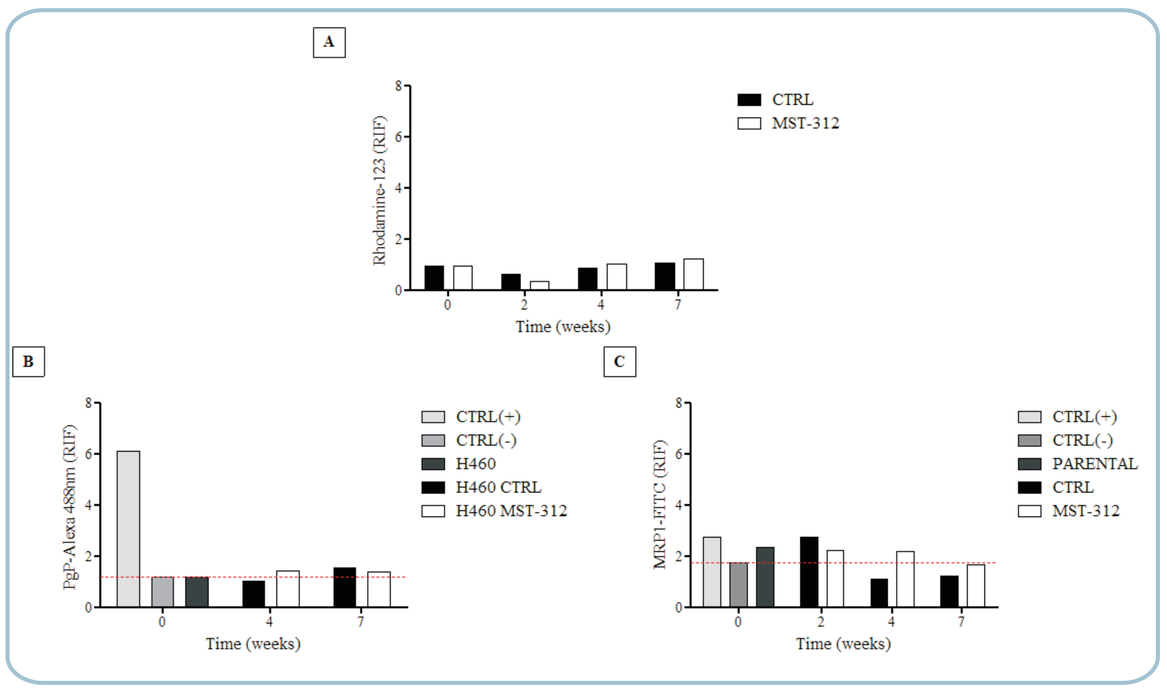

Figure 3: Effect of MST-312 on activity and expression of PGP and MRP1. (A) Activity of both ABC- transporter proteins was accessed by flow cytometry throughout the treatment using its fluorescent substrate (Rhodamine-123) and its modulator (Ciclosporin A). (B) Pgp expression in K562-Lucena human leukemia cell line was used as positive control (CTRL(+)) and K562, as negative control (CTRL(-)). (C) MRP1 expression in A549 human lung cancer cell line as positive control (CTRL(+)) and K562, as negative control (CTRL(-)). Red dotted lines indicates the lowest values observed on each negative control. RIF: Ratio intensity fluorescence.

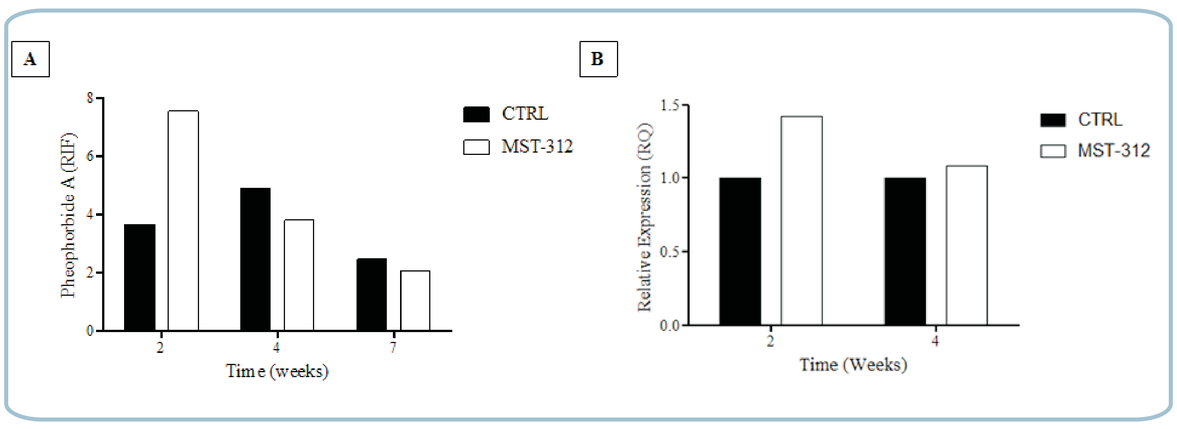

Figure 4: Transient activity and expression of ABCG2 in the beginning of MST-312 long-term treatment. (A) The activity of ABCG2 was accessed by flow cytometry throughout the treatment using its fluorescent substrate (Pheophorbide A) and its specific modulator (Fumitremorgin C). (B) Quantification of ABCG2 mRNA by RT-PCR.

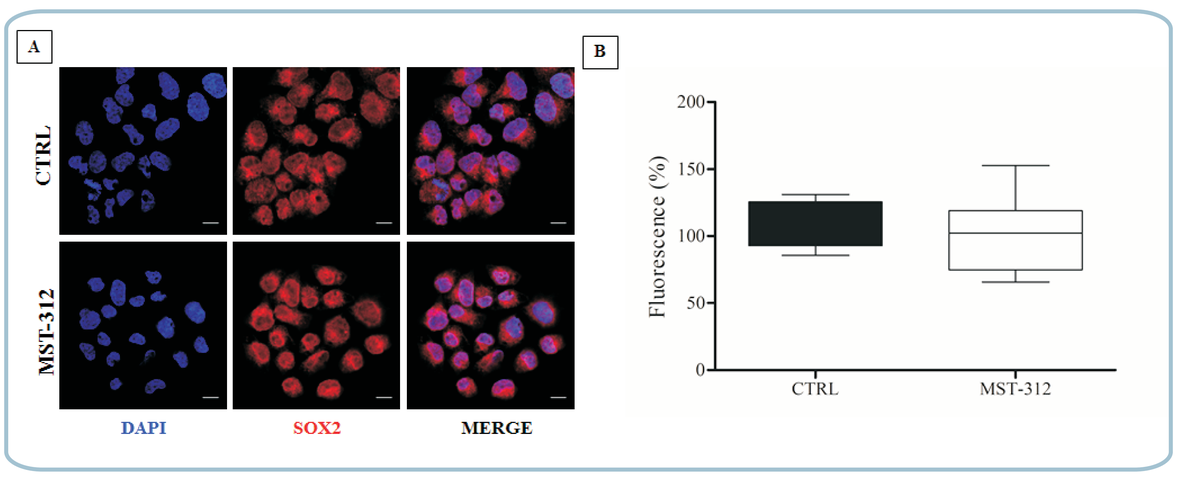

Figure 5: Six weeks treatment with MST-312 has no effect in the expression of SOX2. (A) Imnunofluorescence assay with nuclei staining (DAPI) and SOX2 staining on control and MST-312 treated cells. (B) The total fluorescence from >150 cells was compared. No significant difference was observed. Scale bar: 10mm.

CONCLUSION

Telomerase inhibitor long-term treatment promoted relevant morphology changes suggesting an invasiveness phenotype. MST-312 could be a substrate for BCRP as a transient resistance.

Keywords: Lung cancer, Telomerase Inhibitor, MDR
Supported By (Agências de Fomento): **FAPDF e Ministério da Saúde/INCA**

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos /INCA