Novel nanoparticle formulation of Sabizabulin (VERU-111) for pancreatic cancer treatment

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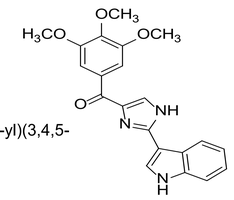
INTRODUCTION

- Pancreatic cancer (PanCa) is the fourth most common cause of deaths among both men and women in the US with an overall survival rate of just 5%.
- The management of PanCa, is exceptionally difficult due to the extremely poor response to available therapeutic modalities.
- Microtubules are dynamic structures composed of α−β tubulin heterodimers that are required for many aspects of cellular functions, including mitosis. This has made them an attractive target for the development of anti-cancer drugs.
- Aberrant expression of specific β-tubulin isotypes, particular βIII-tubulin is important clinically in tumor aggressiveness and resistance to chemotherapy.
- VERU-111 **(Fig. 1)**, an orally bioavailable, small molecule inhibitor has showed anti-cancer activity against a variety of cancers including pancreas.
- Herein, we have developed and characterized novel nanoformulation of VERU-111 (MNP-VERU-111) which showed better potential therapeutic efficacy in *in vitro* and ectopic xenograft mouse model.



(2-(1H-indol-3-yl)-1H-imidazol-4-yl)(3,4,5-trimethoxyphenyl) methanone

Figure 1:Chemical structure of VERU-111

METHODS

***In vitro* model system:** Pancreatic cancer cells (Panc1, AsPC1, and HPAFII).

MTS and colony formation assays: To investigate the effect of MNP-VERU-111 on cell proliferation and clonogenic potential of pancreatic cancer cells.

Invasion and Migration Assay : To investigate the effect of VERU-111 or MNP-VERU-111 on migration and invasion using BD Matrigel-coated chamber wells, and invasion chamber for 24 hrs.

Xenograft study: To investigate the effects of VERU-111 or MNP-VERU-111 on the growth of pancreatic cancer cells xenograft derived tumor in athymic nude mice.

Flow cytometry: To investigate the effect of VERU-111 or MNP-VERU-111on Cell cycle and apoptosis analysis in pancreatic cancer cells.

Western blot analysis: To investigate the effects of VERU-111 or MNP-VERU-111 on the protein levels of β-tubulin isotype, cell regulatory and apoptotic proteins in pancreatic cancer cells.

Quantitative real time PCR (qRT-PCR): To evaluate the effect of VERU-111 or MNP-VERU-111 on the mRNA expressions of β-tubulin isotype and EMT markers in pancreatic cancer cells.

Immunohistochemistry: To determine the effect of VERU-111 or MNP-VERU-111 on expression of PCNA, βI, βIII βIVa/b tubulin, E-Cadherin and Vimentine in excised xenograft tumors.

Confocal microscopy: To investigate the effect of VERU-111 or MNP-VERU-111 on the expression of βIII- tubulin in excised xenograft tumors.

REFERENCES

Kashyap VK, Wang Q, Setua S, Chauhan N, Chowdhury P, Nagesh PKB Kumari S, Yallapu MM, Miller DD, Li W, Jaggi M, Hafeez BB, Chauhan SC (2019).Therapeutic efficacy of a novel βIII/βIV-tubulin inhibitor (VERU-111) in pancreatic cancer. J Exp Clin Cancer Res. 23;38(1):29

RESULTS

Generation and characterization of unique formulation (MNP-VERU-111) for pancreatic cancer treatment

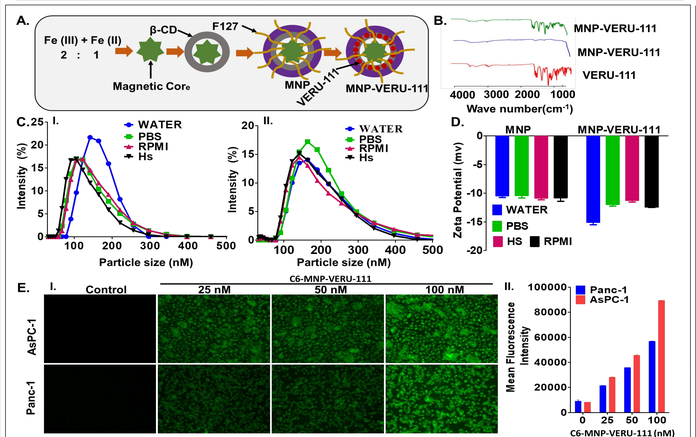


Figure 2: Generation and pre-screening of MNP-VERU-111 formulation. A. Representative structure of NP formulations containing various layers of polymers/stabilizers to achieve optimum particle size for EPR effect. **B.** FT-IR spectra of VERU-111, MNP and MNP-VERU-111. **C.** Line graph showing the size of MNP-VERU-111 in Human Serum (HS), DMEM, and RPMI media. **D.** Bar graph indicating Zeta potential of MNP and MNP-VERU-111 in HS, DMEM, and RPMI media. **E.** Cellular uptake of 6-coumarin labelled NPs. Representative images showing uptake of MNP-VERU-111 in PanCa cells (AsPC-1 and Panc-1) **(I)**. Bar graph represents fluorescence intensity of Coumarin-6 dye **(II)**.

MNP- VERU-111 exhibits more anti-cancer efficacy against than free VERU-111

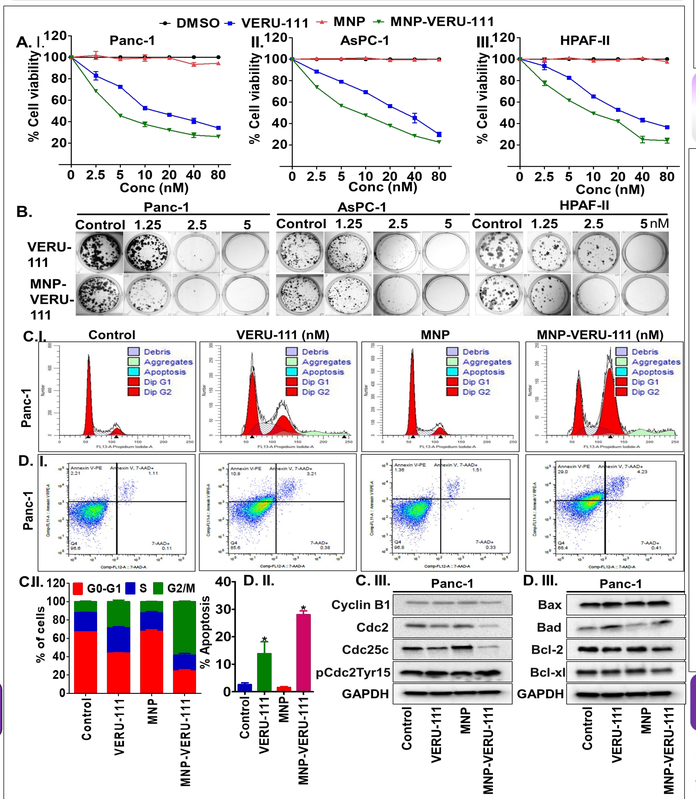


Figure 3: Effect of MNP-VERU-111 on proliferation, clonogenicity cell cycle and apoptosis in PanCa cells. Effect of VERU-111 and MNP-VERU-111 on cell proliferation (**A**), colony formation (**B**), cell cycle arrest **(C)** apoptosis **(D)** in PanCa cells. Asterisks represent significant (p<0.05) effect of MNP-VERU-111 compared to free VERU-111 (p<0.05).

MNP-VERU-111 more effectively inhibits β-tubulin isotypes in PanCa cells

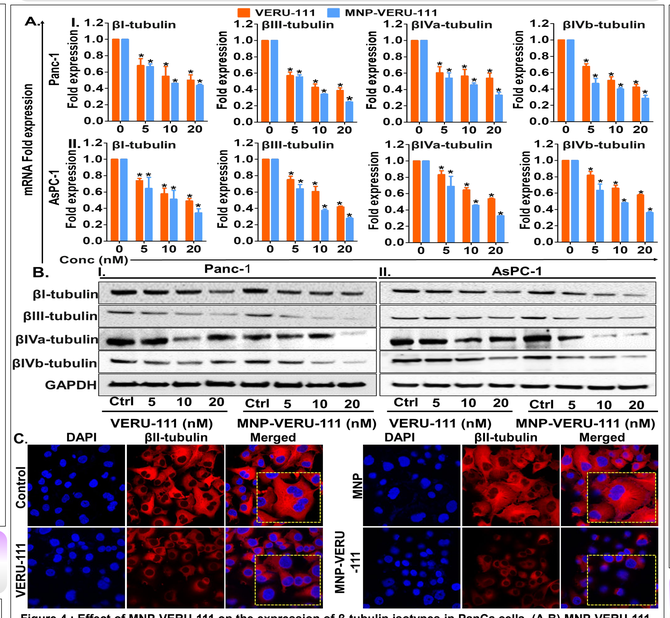


Figure 4 : Effect of MNP-VERU-111 on the expression of β-tubulin isotypes in PanCa cells. (A-B) MNP-VERU-111 significantly downregulates the expression of βI, βIII βIVa and βIVb- tubulin as determined by qRT-PCR and Western blot analyses. MNP-VERU-111 disrupts the βIII-tubulin microtubule network in PanCa cells (**C**).

MNP-VERU-111 more efficiently inhibits metastatic phenotype of PanCa cells

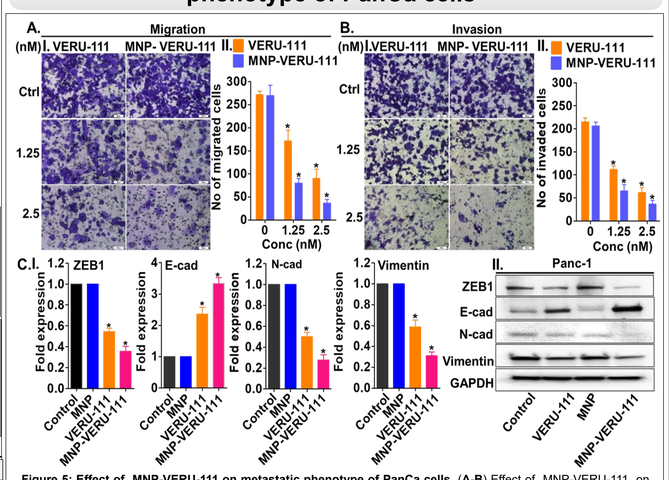


Figure 5: Effect of MNP-VERU-111 on metastatic phenotype of PanCa cells. (**A-B**) Effect of MNP-VERU-111 on migration (**A**) invasion (**B**) of PanCa cells as determined by commercial available kit. **(C)** Effect of MNP-VERU-111 on mRNA expression of EMT markers (ZEB1, E-Cad, N-Cad, and Vimentin) as determined by qRT-PCR **(C)** Western blot analysis in PanCa cells. Asterisks represent significant level (p<0.05) of MNP-VERU-111 as compared to VERU-111.

MNP-VERU-111 restores the expression of miR-200c *via* targeting βIII-tubulin and ZEB1

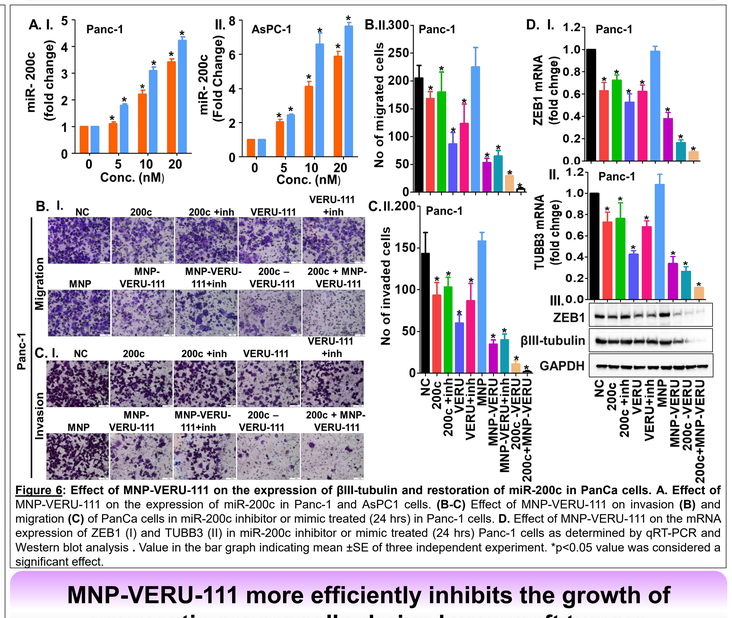


Figure 6: Effect of MNP-VERU-111 on the expression of βIII-tubulin and restoration of miR-200c in PanCa cells. A. Effect of MNP-VERU-111 on the expression of miR-200c in Panc-1 and AsPC1 cells. **(B-C)** Effect of MNP-VERU-111 on invasion **(B)** and migration **(C)** of PanCa cells in miR-200c inhibitor or mimic treated (24 hrs) in Panc-1 cells. **D.** Effect of MNP-VERU-111 on the mRNA expression of ZEB1 (I) and TUBB3 (II) in miR-200c inhibitor or mimic treated (24 hrs) Panc-1 cells as determined by qRT-PCR and Western blot analysis . Value in the bar graph indicating mean ±SE of three independent experiment. *p<0.05 value was considered a significant effect.

MNP-VERU-111 more efficiently inhibits the growth of pancreatic cancer cells-derived xenograft tumors

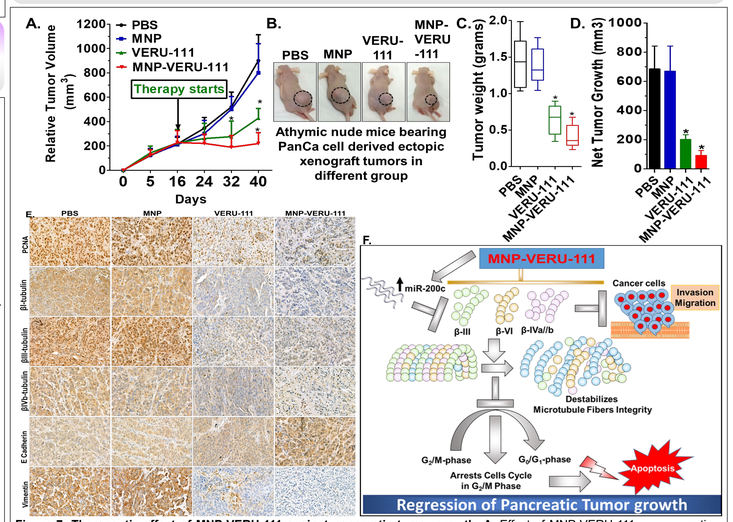


Figure 7: Therapeutic effect of MNP-VERU-111 against pancreatic tumor growth. A. Effect of MNP-VERU-111 on pancreatic tumor growth in ectopic xenograft tumors. PanCa cells (2X10^6) were injected subcutaneously and various indicated treatment of free and nanoformulation of VERU-111 was started when tumor reach a targeted volume of approximately 200 mm^3. Line bar graph representing the tumor volume of indicated groups. **(B)** Representative pictures of tumor bearing mice of indicated groups at day 40. **(C-D)** Box plot and bar graphs are showing the weight **(C)** and volume (D) of excised tumors at day 40 of different treatment groups. **(E)** Effect of free and MNP-VERU-111 on the expression of indicated proteins in excised tumors. **(F)** Schematic representation is showing the molecular mechanism of VERU-111 to inhibits the growth of pancreatic cancer.

CONCLUSION

Our results demonstrate that MNP-VERU-111 is a new therapeutic modality which show more superior therapeutic efficacy than free VERU-111. We suggest that MNP-VERU-111 could be used as a new therapeutic modality for the treatment of pancreatic cancer alone or in combination with current therapeutic regimens.

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