Pre-Clinical Evaluation of a Camsirubicin Analog MNPR-202 in Diffuse Large B Cell Lymphoma (DLBCL)

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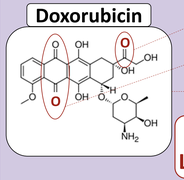
Background

MNPR-202: Promising DNA Damaging Response Drug Candidate

The standard R-CHOP treatment for DLBCL has a high relapse risk because dose intensity cannot be maintained due to Doxorubicin (Dox) cardiotoxicity.

Camsirubicin, a novel analog of Dox engineered to reduce cardiotoxicity, has shown no signs of irreversible heart damage across two Phase 1 trials (one ongoing) and a Phase 2 trial.

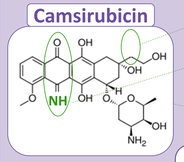
Doxorubicin



- Calcium disruption via C13-OH doxorubicinol
- Redox cycling at the C5 quinone
- Inhibition of topoisomerase IIβ

Cardiotoxic Lifetime-Dose Limitation

Camsirubicin

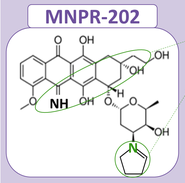


- C13 deoxygenation prevents doxorubicinol
- C5 imination eliminates quinone redox cycling
- Selectivity for topoisomerase IIα over IIβ

Toxicity ↓
Maximum Dose ↑
Cumulative Dose ↑

MNPR-202 is a camsirubicin analog that retains the non-cardiotoxic backbone but is modified at other sites with the intent to evade doxorubicin drug resistance mechanisms.

MNPR-202



- Retains non-cardiotoxic backbone of camsirubicin
- Amine modification reduces drug efflux

Drug Resistance ↓

Here, we report on pre-clinical studies with **MNPR-202** in blood cancer cell lines. Its analog, camsirubicin, is presently in a Phase 1b dose-escalation clinical trial.

Previous and Ongoing Clinical Trials

Support for Camsirubicin's Toxicity Profile

- No irreversible drug-related clinical cardiotoxicity observed to date in any trial.
- In a prior Phase 2 trial, patients were dosed for up to 16-20 cycles at a dose level of 265 mg/m^2. The current dose level in the ongoing Phase 1b trial is at 520 mg/m^2 and continues to escalate.

MNPR-202: Previous *in vitro* Study in Solid Tumors

In vitro IC50's of Doxorubicin and MNPR-202 (μM)

Cell Line	Cell Type	Dox	MNPR-202	Dox/MNPR-202
RD	Rhabdomyosarcoma	0.43±0.05	0.44±0.13	1
SW-982	Synovial sarcoma	4.3±1.5	0.45±0.14	9.6
SW-872	Liposarcoma	0.35±0.89	0.57±0.23	0.6
T47D	Breast Ductal Carcinoma	10.3±7.8	0.82±0.56	12.6
MES-SA^{DoxS}	Uterine Fibrosarcoma	0.55±0.08	0.76±0.16	0.7
MES-SA^{DoxR}	Uterine Fibrosarcoma	7.8±1.8	0.66±0.003	11.8

MNPR-202 has a similar cytotoxic potency to doxorubicin, and retains potency even in doxorubicin-resistant solid tumor cell lines.

Present Study Shows Similar Potency in Blood Cancers

Viability of DLBCL Cell Lines

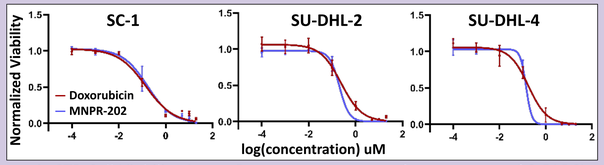


Figure 1. *CTB Cell Viability Assay. Dose-response curves for treatment of 3 DLBCL cell lines with Dox or MNPR-202 for 48 h.*

MNPR-202 displays a similar cytotoxic potency to doxorubicin, even in blood cancer cell lines (lymphoma).

MNPR-202 Induces Increased Apoptosis

Apoptosis Assay by Flow Cytometry

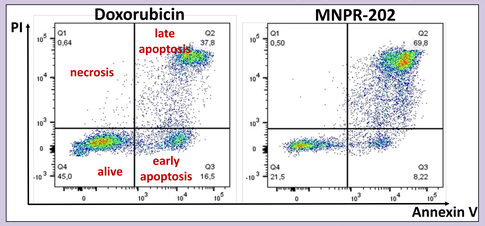


Figure 2. *Flow cytometry plots of SC-1 cell line using Annexin V-PI staining after treatment with Dox or MNPR-202 (0.4 μM) for 48 h.*

MNPR-202 demonstrates increased apoptosis in lymphoma cells compared to doxorubicin.

MNPR-202 Affects DNA Damage Response (DDR)

Elevated DNA Damage and Cell Death Response

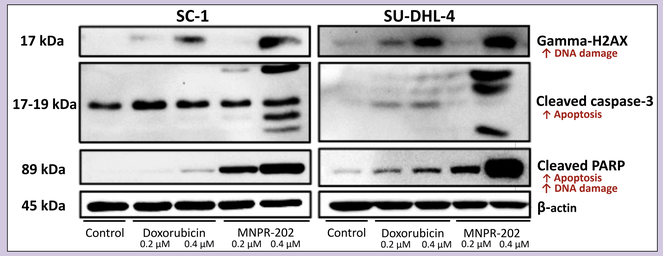


Figure 3. *Western blots for apoptosis and/or DNA damage markers gamma-H2AX, cleaved caspase-3, and cleaved PARP for 2 DLBCL cell lines. Cells were treated for 24 h.*

MNPR-202 demonstrates increased DNA damage in lymphoma cells compared to doxorubicin.

MNPR-202 vs Dox: Unique Immune Activation Profile

Immune Response Gene and Protein Expression Analysis

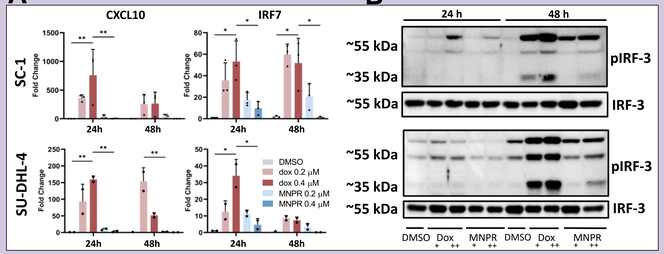


Figure 4. *(**A**) qPCR results for immunomodulatory innate immune response genes CXCL10 and IRF7 in 2 DLBCL cell lines. Fold change relative to 24h DMSO-treated control. (**B**) Western blots for pIRF-3 and IRF3 in the same cell lines treated with Dox or MNPR-202 (+ = 0.2 μM; ++ = 0.4 μM).*

MNPR-202 has a distinct profile of innate immune activation.

MNPR-202 vs Dox: Differential Gene Expression Analysis

RNA-Sequencing of DLBCL Cell Lines

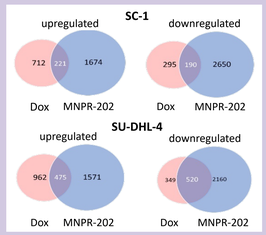


Figure 5. *Venn Diagrams showing numbers of up-regulated, down-regulated, and overlapping genes in 2 DLBCL cell lines treated with Dox or MNPR-202.*

- There are numerous non-overlapping genes between Dox and MNPR-202.
- MNPR-202 works under distinct cellular regulatory pathways.

MNPR-202 vs Dox: Synergy Screening

A combination drug screen with 183 compounds was performed, revealing distinct differences in the synergy profile between doxorubicin and MNPR-202 with other compounds.
Expanded synergy profiling on two representative drugs shown below:

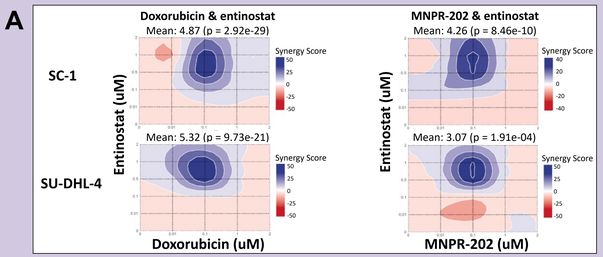


Figure 6. *2D contour maps showing relationships between Dox or MNPR-202 and (**A**) entinostat or (**B**) volasertib. The synergy scores were calculated via the HSA method. A high or low synergy score defines synergy or antagonism, respectively.*

MNPR-202 demonstrates a more favorable synergy profile with volasertib compared to doxorubicin. Hence, MNPR-202 may be superior in certain combination regimens.

Conclusions

- Dox and MNPR-202 have similar cytotoxic potency in cell lines, and MNPR-202 is possibly superior in certain Dox-resistant cell lines.
- MNPR-202 demonstrates more DNA damage and apoptosis, and it shows a unique immune activation profile compared to doxorubicin, likely working through **distinct cellular pathways**.

Future Directions

- Evaluate drug resistance pathways in response to MNPR-202.
- *In vivo* studies to examine cardiotoxicity profile of MNPR-202.
- *In vivo* studies to evaluate suitability of MNPR-202 vs doxorubicin for immunotherapy combinations.

Citations & Acknowledgments: