**Supplementary Materials and Methods**

**Cell Cultures**

SK-Mel-28 and PC3 cells were maintained in Eagle's minimal essential medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and L-Glutamine (2 mM for SK-Mel-28; 4 mM for PC3). CHLA-20 and CHLA-90 cells were maintained in Iscove’s Modified Dulbecco’s Medium (IMDM) supplemented with either 10% or 20% fetal bovine serum (FBS) and 1% insulin-transferrin-selenium basal solution (Thermo Fisher Scientific, Waltham, MA). SK-N-SH and CHP-134 cells were grown in Eagle's minimal essential medium (EMEM) supplemented with 10% FBS. SH-SY5Y and SK-N-Be2 cells were cultured in a 50% EMEM and 50% F-12 nutrient basal media mixture supplemented with 10% FBS. In addition, all human neuroblastoma cells were cultured at 37°C in a humidified incubator at 5% CO2 and supplemented 100U/mL penicillin and 100mg/mL streptomycin.

**Cell viability assays**

*In vitro* cell survival assays were measured using the colormetric based Cell Titer96 AQueous Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI). The CellTiter 96® AQueous Assay is composed of a novel tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS] and an electron coupling reagent, phenazine methosulfate. Cells were plated in 96-well plates at 2000 - 4000 cells per well, incubated at 37 °C overnight and then treated with various concentrations of TR100 alone, ATM-3507 alone, TR100 and ATM-3507 plus various chemotherapy drugs. The cell viability was performed on day 3-5. For the dosing schedule optimization experiment, TR100 or vincristine was added at day 1 with the other being added at day 2 or both TR100 and vincristine were added on day 1 and plates were read at day 5. Results are presented as percentage of survival cells compared with controls. All cell viability assays were run in quadruplicate and the data shown are representative of at least 2 independent experiments. Inhibitory concentration (IC)50 values were calculated as drug concentrations necessary to inhibit 50% growth compared to untreated control cells.

**Antibodies**

The Anti-Poly-ADP-Ribose Polymerase (PARP) polyclonal antibody (*11835238001*) used in Fig. 6C was obtained from Roche (Indianapolis, IN) and in Fig. 6D the antibodies (9542) and anti- Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) antibody (14C10) were purchased from Cell Signaling Technologies (Danvers, MA). The polyclonal secondary antibodies: Alexa Fluor® 568 conjugated goat anti-Mouse IgG (H+L) and Alexa Fluor® 488 conjugated goat anti-Rabbit IgG (H+L) and were obtained from Life Technologies (Grand Island, NY). The monoclonal anti-alpha-tubulin antibody produced in mouse was purchased from Sigma-Aldrich. The rabbit polyclonal anti-Aurora B antibody (ab2254) was obtained from Abcam (Cambridge, MA).

**Pharmacokinetic study of ATM-3507**

ATM-3507 pharmacokinetic studies were performed by Jubilant Biosys Limited (Karnataka, Inda). Briefly, 54 female Balb/C mice were dosed orally with 30 mg/kg ATM-3507. Mice were then anesthetized using isoflurane at scheduled time points (0.5, 2, 4, 8, 12, 24, 48 and 72 h post dose). Blood was collected (0.1 mL) by retro-orbital puncture, heparinized in a 0.5 mL blood tube, mixed and stored on ice until processed. Whole blood samples was then centrifuged. Plasma was harvested, transferred to a micro-centrifuge tube and then stored at -20ºC until analyzed for ATM-3507 by LC-MS-MS.

**Supplementary Figures**

****

**Figure S1.** Western blot of tropomyosin isoforms in neuroblastoma cells used in this project.

****

**Figure S2.** Effect of combining tropomyosin and microtubule inhibitors on cell growth. **A, B,** CHLA-20 cells were treated with vincristine alone or combined with TR100 or ATM-3507 and analyzed by MTS assay. Three dimensional displays suggest synergy for each drug that alone had modest activity but in combination resulted in zero survival.

**Figure S3. A**, **B**, Effect of TR100 or ATM-3507 in combination with paclitaxel in CHLA-20 neuroblastoma cells. **C**, Effect of TR100 in combination with doxorubicin in CHLA-20 neuroblastoma cells. We observed some synergy with paclitaxel but effects with doxorubicin were additive.

**Figure S4.** Cytotoxicity of CHLA-20 cells treated with different dosing regimens of TR100 plus VCR. **A**, TR100 and VCR were administrated simultaneously on day 1. **B**, TR100 was administrated on day 1 and VCR was added on day 2. **C**, VCR was administrated on day 1 and TR100 was added on day 2. Cell survival was assessed on day 5 by MTS.

**Figure S5.** Effect of combining TR100 and vincristine on tumor growth. **A**, Treatment schemas used for *in vivo* studies. Analysis of **B,** tumor growth and **C**, animal survival with monotherapies or combinations of intravenous (IV) vincristine and intraperitoneal (IP) TR100 (**B, C**; n=5).

****

**Figure S6.** Weights of animals from Fig. 4 and S5

****

**Figure S7.** The combination of tropomyosin inhibitors plus vincristine did not impact the microtubule network of cells in interphase. **A-F**, Images of the microtubule network (green) and nuclei (blue) in CHLA-20 cells. Cells have been treated with vehicle control **A**; low dose TR100 (3.1 µM) **B**; low dose vincristine (10 nM) **C**; low dose TR100 plus low dose vincristine **D**; low dose ATM-3507 (3 µM) **E**; or low dose ATM-3507 plus low dose vincristine **F**. Scale bar = 10µm.

**Figure S8.** The combination of TR100 and paclitaxel induces apoptosis in CHLA-20 neuroblastoma cells. **A**, Western blot showing cleaved PARP with only with combination treatment. **B**, Western blot showing cleaved PARP with TR100 that was reduced with the pan-caspase inhibitor. **C**, The effect of combination therapy on cell survival was partially reversed using a pan-caspase inhibitor.

**Table S1.** IC50concentrations for TR100 and ATM-3057 in a panel of neuroblastoma cell lines.

|  |  |  |
| --- | --- | --- |
| **Cell Line** | **TR100 (M)** | **ATM-3507 (M)** |
| CHLA-20 | 4.40 ±1.82\* | 4.99 ±0.45 |
| CHP134 | 3.02 ±0.70 | 3.83 ±0.67 |
| CHLA-90 | 5.99 ±0.83 | 6.84 ±2.37 |
| SK-N-BE(2) | 3.99 ±0.83 | 5.00 ±0.42 |

\*IC50 values and standard deviation determined from 3 independent experiments.

**Table S2.** Pharmacokinetic analysis after single dosing of ATM3507/Dexolve intravenously at 30mg/kg in non-tumor bearing immunocompetent mice (Balb/C, n=3).

|  |  |
| --- | --- |
| **PK Parameter** | **ATM-3507 (IV 30mg/kg)** |
| T1/2 (h) | 5.01 |
| C0 (ng/ml) | 6853 |
| Cmax (ng/ml) | 5758 ±782 |
| Cmax (µM) | 9.42±1.23 |
| AUC 0-t (ng.h/mL) | 14548 |
| AUC 0-∞(ng.h/mL) | 14782 |
| CL (mL/min/Kg) | 33.8 |
| Vd (L/Kg) | 7.23 |