Pediatric Preclinical Testing Program (PPTP) Evaluation of BMN 673, an Inhibitor of Poly-ADP Ribose Polymerase (PARP), with Temozolomide (TMZ)

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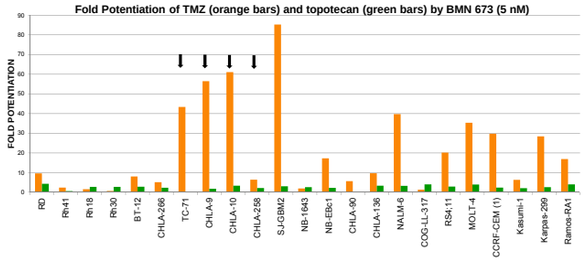
BMN 673

- BMN 673 is a potent inhibitor of PARP-1 and -2 that inhibits intracellular PAR formation at a concentration of 2.5 nM (Shen Y, et al. CCR 2013 19:5003-5015).
- PARP-1 and -2 are activated by DNA damage and play a critical role in the base excision DNA repair (BER) pathway.
- PARP inhibitors selectively kill cancer cells that are deficient in BRCA-1 or BRCA-2 function.
- PARP inhibitors enhance the cytotoxicity of DNA damaging agents by preventing cancer cells from repairing DNA damage.
- BMN 673 induces cytotoxicity by tightly trapping PARP at sites of DNA damage.
- Prior reports have documented selective sensitivity of Ewing sarcoma cell lines to PARP inhibitors.
- BMN 673 is in clinical evaluation and has shown substantial single agent anti-tumor activity in deleterious germline BRCA ovarian cancer and breast cancer.

BMN 673 IN VITRO COMBINATION TESTING AGAINST PPTP CELL LINES

- PARP inhibitors have been shown to act both by inhibition of PARP enzymatic activity and by "trapping" PARP to DNA at sites of single strand breaks creating lesions that induce cytotoxicity during DNA replication. Either method could potentiate the activity of chemotherapy agents administered concurrently.
- The ability of BMN 673 (10 nM) to potentiate the activity of TMZ and topotecan against PPTP cell lines was evaluated.
- BMN 673 potentiation of TMZ activity (median 10-fold lowering of IC_{50}) was far greater than its potentiation of topotecan activity (2.8-fold).
- BMN 673 potentiation of TMZ showed histotype selectivity, with a median 50-fold potentiation for Ewing cell lines (noted by arrows) compared to 9.6-fold for non-Ewing lines.
- BMN 673 potentiation of topotecan showed no histotype selectivity.



BMN 673 + TMZ COMBINATION TESTING: EWING FOCUS AND TWO DOSING REGIMENS

- Regimen B: single agent TMZ at 30 mg/kg/d x 5d.
- Regimen C: single agent BMN 673 at 0.25 mg/kg BID x 5d.
- For the BMN 673 + TMZ combination, two strategies were tested: one with a higher dose of TMZ (30 mg/kg/d x 5d) and the other with a higher dose of BMN 673 (0.25 mg/kg BID x 5d).
  - For TMZ at 30 mg/kg/d, the tolerable BMN 673 dose was 0.1 mg/kg BID (Regimen D).
  - For BMN 673 at 0.25 mg/kg BID, the tolerable TMZ dose was only 12 mg/kg/d, (Regimen E).
- The observation period was 12 weeks following one cycle of therapy.

Line	Tumor Type	Treatment Group	Median Time to Event	P-value	EFS T/C	Median RTV End of Study	Tumor Volume T/C	CR at 6 Weeks	Group Response
KT-10	Wilms	TMZ (Reg B)	21.8	0.084	1.2	>4	0.64	No	SD
KT-10	Wilms	BMN 673 (Reg C)	> EP	**<0.001**	**> 5.2**	>4	0.01	**Yes**	CR
KT-10	Wilms	Regimen D	> EP	**<0.001**	**> 5.2**	0.0	0.01	**Yes**	MCR
KT-10	Wilms	Regimen E	> EP	**<0.001**	**> 5.2**	0.0	0.00	**Yes**	MCR
SK-NEP-1	Ewing	TMZ (Reg B)	8.9	0.389	0.8	>4	1.06	No	PD1
SK-NEP-1	Ewing	BMN 673 (Reg C)	10.1	0.916	1.0	>4	0.95	No	PD1
SK-NEP-1	Ewing	Regimen D	32.3	**<0.001**	**3.1**	>4	0.09	No	PR
SK-NEP-1	Ewing	Regimen E	> EP	**<0.001**	**> 8.6**	0.0	0.08	**Yes**	MCR
TC-71	Ewing	TMZ (Reg B)	10.7	0.368	1.0	>4	1.15	No	PD1
TC-71	Ewing	BMN 673 (Reg C)	12.2	**0.016**	1.2	>4	0.82	No	PD1
TC-71	Ewing	Regimen D	> EP	**<0.001**	**> 8.8**	0.0	0.01	**Yes**	MCR
TC-71	Ewing	Regimen E	> EP	**<0.001**	**> 8.8**	0.0	0.01	**Yes**	MCR
CHLA258	Ewing	TMZ (Reg B)	10.2	0.242	1.1	>4	0.83	No	PD1
CHLA258	Ewing	BMN 673 (Reg C)	11.8	**0.034**	1.2	>4	0.68	No	PD1
CHLA258	Ewing	Regimen D	> EP	**<0.001**	**> 9.6**	0.0	0.01	**Yes**	MCR
CHLA258	Ewing	Regimen E	> EP	**<0.001**	**> 9.6**	>4	0.01	**Yes**	CR
ES6	Ewing	TMZ (Reg B)	25.1	**0.039**	1.3	>4	0.76	No	PD1
ES6	Ewing	BMN 673 (Reg C)	20.6	0.845	1.1	>4	0.92	No	PD1
ES6	Ewing	Regimen D	35.9	**<0.001**	1.9	>4	0.32	No	PD2
ES6	Ewing	Regimen E	31.6	**<0.001**	1.7	>4	0.47	No	PD2
ES4	Ewing	TMZ (Reg B)	15.8	**0.001**	1.4	>4	0.67	No	PD1
ES4	Ewing	BMN 673 (Reg C)	14.3	**0.032**	1.3	>4	0.84	No	PD1
ES4	Ewing	Regimen D	81.5	**<0.001**	**7.1**	>4	0.00	**Yes**	CR
ES4	Ewing	Regimen E	77.4	**<0.001**	**6.8**	>4	0.00	**Yes**	CR
EW5	Ewing	TMZ (Reg B)	9.9	0.507	1.1	>4	0.76	No	PD1
EW5	Ewing	BMN 673 (Reg C)	8.9	0.941	1.0	>4	0.96	No	PD1
EW5	Ewing	Regimen D	28.6	**<0.001**	**3.3**	>4	0.19	No	PR
EW5	Ewing	Regimen E	24.0	**<0.001**	**2.7**	>4	0.19	No	CR
ES-2	Ewing	TMZ (Reg B)	12.7	0.567	1.1	>4	1.10	No	PD1
ES-2	Ewing	BMN 673 (Reg C)	14.0	0.712	1.2	>4	0.85	No	PD1
ES-2	Ewing	Regimen D	19.2	**0.002**	1.6	>4	0.42	No	PD2
ES-2	Ewing	Regimen E	30.3	**<0.001**	**2.6**	>4	0.16	No	CR
EW8	Ewing	TMZ (Reg B)	9.1	**0.005**	1.3	>4	0.71	No	PD1
EW8	Ewing	BMN 673 (Reg C)	13.4	**<0.001**	**2.0**	>4	0.39	No	PD2
EW8	Ewing	Regimen D	15.7	**<0.001**	**2.3**	>4	0.44	No	PD2
EW8	Ewing	Regimen E	12.6	**<0.001**	1.9	>4	0.55	No	PD2
ES-3	Ewing	TMZ (Reg B)	10.3	**0.007**	1.2	>4	0.75	No	PD1
ES-3	Ewing	BMN 673 (Reg C)	10.7	**0.011**	1.2	>4	0.74	No	PD1
ES-3	Ewing	Regimen D	26.0	**<0.001**	**2.9**	>4	0.11	No	CR
ES-3	Ewing	Regimen E	18.3	**<0.001**	**2.1**	>4	0.28	No	CR
ES-7	Ewing	TMZ (Reg B)	18.3	**0.003**	1.6	>4	0.55	No	PD2
ES-7	Ewing	BMN 673 (Reg C)	15.7	**0.021**	1.4	>4	0.69	No	PD1
ES-7	Ewing	Regimen D	> EP	**<0.001**	**> 7.8**	0.0	0.01	**Yes**	MCR
ES-7	Ewing	Regimen E	> EP	**<0.001**	**> 7.8**	0.0	0.00	**Yes**	MCR
ES-8	Ewing	TMZ (Reg B)	7.1	0.110	1.2	>4	0.80	No	PD1
ES-8	Ewing	BMN 673 (Reg C)	10.7	**<0.001**	1.9	>4	0.43	No	PD2
ES-8	Ewing	Regimen D	38.7	**<0.001**	**6.7**	>4	0.03	No	CR
ES-8	Ewing	Regimen E	33.4	**<0.001**	**5.8**	>4	0.03	No	CR
Rh28	Alveolar RMS	TMZ (Reg B)	> EP	**<0.001**	**> 3.0**	0.0	0.48	**Yes**	MCR
Rh28	Alveolar RMS	BMN 673 (Reg C)	13.7	**0.003**	0.5	>4	1.56	No	PD1
Rh28	Alveolar RMS	Regimen D	> EP	**<0.001**	**> 3.0**	0.0	0.23	**Yes**	MCR
Rh28	Alveolar RMS	Regimen E	> EP	**<0.001**	**> 3.0**	0.0	0.01	**Yes**	MCR
Rh30	Alveolar RMS	TMZ (Reg B)	16.8	0.799	1.1	>4	0.91	No	PD1
Rh30	Alveolar RMS	BMN 673 (Reg C)	15.0	0.624	1.0	>4	0.95	No	PD1
Rh30	Alveolar RMS	Regimen D	50.0	**<0.001**	**3.2**	>4	0.09	No	CR
Rh30	Alveolar RMS	Regimen E	37.3	**<0.001**	**2.4**	>4	0.15	No	SD
Rh41	Alveolar RMS	TMZ (Reg B)	11.8	0.176	0.9	>4	1.09	No	PD1
Rh41	Alveolar RMS	BMN 673 (Reg C)	11.1	0.090	0.9	>4	1.14	No	PD1
Rh41	Alveolar RMS	Regimen D	11.3	0.072	0.9	>4	1.08	No	PD1
Rh41	Alveolar RMS	Regimen E	13.9	0.061	1.1	>4	0.70	No	PD1
GBM2	Glioblastoma	TMZ (Reg B)	35.3	**<0.001**	**5.4**	>4	0.21	No	CR
GBM2	Glioblastoma	BMN 673 (Reg C)	8.0	0.295	1.2	>4	0.88	No	PD1
GBM2	Glioblastoma	Regimen D	83.2	**<0.001**	**12.8**	>4	0.14	**Yes**	CR
GBM2	Glioblastoma	Regimen E	> EP	**<0.001**	**>14.0**	0.0	0.12	**Yes**	MCR

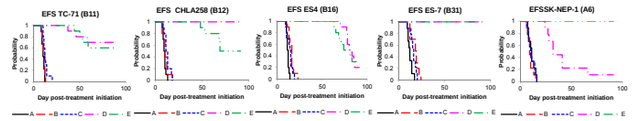
ADDITIONAL BMN 673 + TMZ COMBINATION TESTING

- For other xenografts, Regimen E (higher BMN 673 dose and low TMZ dose) was tested (see table below).
- The only xenografts with regressions (D645 and CHLA-79) express low MGMT and were previously shown to be responsive to single agent TMZ.
- The observation period was 6 weeks following one cycle of therapy.

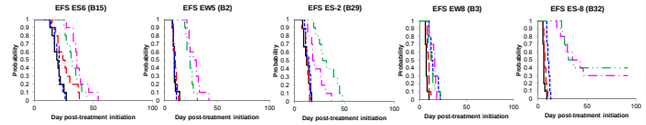
Line	Tumor Type	Median Time to Event	P-value	EFS T/C	Median RTV/CD45 at End of Study	Tumor Volume T/C	Group Response
D645	Glioblastoma	> EP	**<0.001**	**> 4.8**	0.0	0.12	MCR
NB-SD	Neuroblastoma	38.6	**<0.001**	**5.9**	>4	0.57	PD2
NB-1691	Neuroblastoma	15.1	**<0.001**	**2.8**	>4	0.25	PD2
NB-EBc1	Neuroblastoma	10.7	**<0.001**	**2.7**	>4	0.18	PD2
CHLA-79	Neuroblastoma	32.1	**<0.001**	**5.2**	>4	0.16	CR
NB-1643	Neuroblastoma	19.7	**<0.001**	**3.2**	>4	0.29	PD2
OS-1	Osteosarcoma	33.1	**<0.001**	1.5	>4	0.64	PD1
OS-2	Osteosarcoma	> EP	**<0.001**	> 1.7	2.7	0.42	PD2
OS-17	Osteosarcoma	29.7	**<0.001**	1.5	>4	0.63	PD1
OS-31	Osteosarcoma	38.5	**<0.001**	1.8	>4	0.39	PD2
ALL-2	ALL B-precursor	17.7	0.050	**2.1**	>25	.	PD2

- **Event:** 4-fold increase in tumor volume
- **> EP**: Time to event longer than the evaluation period (42 days)
- **EFS T/C**: Ratio of median time to event for treated and control animals.
- **RTV**: Relative tumor volume (ratio to day 1 tumor volume)
- **PD1 (Progressive Disease 1):** >25% ↑ in tumor volume, EFS T/C value ≤1.5;
- **PD2 (Progressive Disease 2):** >25% ↑ in tumor volume, EFS T/C value >1.5;
- **CR (Complete response):** disappearance of measurable tumor mass (< 0.10 cm^3)
- **MCR (Maintained CR):** tumor volume <0.10 cm^3 at the end of the study period

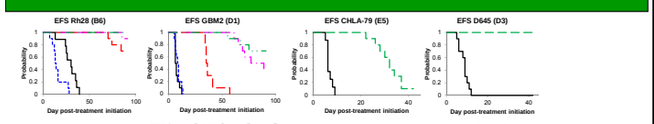
K-M CURVES FOR RESPONSIVE EWING LINES



K-M CURVES FOR LESS RESPONSIVE EWING LINES



K-M CURVES FOR TMZ-RESPONSIVE LINES



A: Control; **B**: TMZ 30 mg/kg x 5d; **C**: BMN 673 0.25 mg/kg BID x 5d;
D: BMN 673 0.1 mg/kg BID x 5d plus TMZ 30 mg/kg x 5d; **E**: BMN 673 0.25 mg/kg BID x 5d plus TMZ 12 mg/kg x 5d

CONCLUSIONS

- BMN 673 markedly potentiates the activity of TMZ in vitro with potentiation being particularly notable for Ewing sarcoma cell lines, some of which showed a level of potentiation exceeding 50-fold.
- BMN 673 potentiates the activity of topotecan in vitro to a much lesser extent than it does TMZ, and the potentiation of topotecan has no histotype-specificity.
- In vivo, BMN 673 plus TMZ was highly effective: maintained CRs (MCRs) were observed both for the regimen with higher dose TMZ and the regimen with low-dose TMZ, and in general responses to the two schedules were comparable. SK-NEP-1 responded better to the low-dose TMZ regimen, while CHLA-258 responded better to the higher-dose TMZ regimen.
- Highly synergistic activity for the BMN 673 + low-dose TMZ combination was observed for 5 of 10 Ewing sarcoma models, with maintained CRs noted in vivo at TMZ doses of 12 mg/kg (~20% of the standard TMZ preclinical dose).
- The BMN 673 + low-dose TMZ combination was also highly active against xenografts with low MGMT expression that are also responsive to single agent TMZ, including two MGMT-negative GBM xenografts (GBM2 and D645), a MGMT-negative rhabdomyosarcoma xenograft (Rh28), and a neuroblastoma xenograft (CHLA-79). The combination with low-dose TMZ (12 mg/kg) was as or more effective than single agent TMZ at 30 mg/kg for GBM2 and Rh28.
- The BMN 673 + low-dose TMZ combination is also highly active against the Wilms tumor xenograft (KT-10) that is responsive to single agent BMN 673.
- The non-Ewing lines evaluated that were not responsive to single agent BMN 673 or TMZ did not show tumor regressions to the BMN 673 plus TMZ combination.
- A pediatric phase 1 trial evaluating the BMN 673 plus low-dose TMZ strategy (ADVL1411) will activate in Q2 2014.

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