C206: Pediatric Preclinical Testing Program (PPTP) Evaluation of BMN 673, an inhibitor of Poly-ADP Ribose Polymerase (PARP), Alone and with Temozolomide (TMZ)

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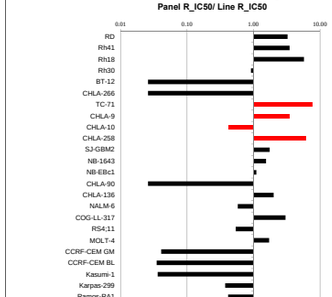
BMN 673

- BMN 673 is a potent inhibitor of PARP-1 and -2 that inhibits intracellular PAR formation at a concentration of 2.5 nM.
- 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 also enhance the cytotoxicity of DNA damaging agents by preventing cancer cells from repairing 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 ACTIVITY

- The median rIC_{50} value for the PPTP cell lines was 28.4 nM (96 hr exposure), with a range from 3.7 nM (TC-71, Ewing sarcoma) to >1.0 μM (3 cell lines).
- BMN 673 demonstrated cytotoxicity against some of the PPTP cell lines, with Relative In/Out% values approaching -100% for these cell lines
- The median rIC_{50} value for the 4 Ewing sarcoma cell lines (6.4 nM) was lower than the rIC_{50} for the non-Ewing sarcoma cell lines (40.1 nM) (p=0.048).

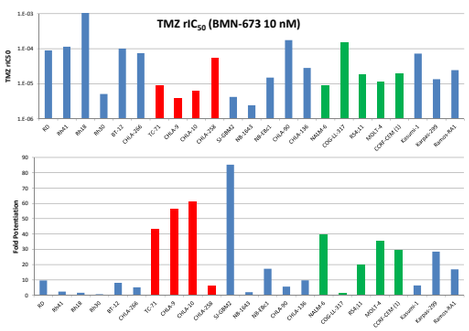
COMPARE-Like plot for BMN 673 against PPTP cell lines



BMN 673 AND TEMOZOLOMIDE (TMZ)

- 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.
- We hypothesized that a potent PARP inhibitor like BMN 673 could be a highly effective cytotoxic agent when combined with low doses of TMZ.
- The role of TMZ in the combination is to create DNA adducts (via N7, N3, and O6 methylation) that lead to single strand breaks during base excision repair and that in the presence of BMN 673 are converted to cytotoxic lesions.
- An in vitro prediction of this hypothesis is that the presence of BMN 673 will markedly reduce the concentration of TMZ required for cytotoxicity (see below).
- An in vivo prediction of the hypothesis is that BMN 673 will be highly effective when combined with low doses of TMZ (see far right).

BMN 673 AND TEMOZOLOMIDE (TMZ) IN VITRO



- BMN 673 (10 nM) was tested in combination with TMZ (0.3 μM to 1000 μM) at 96 hr exposure duration.
- The median rIC_{50} for TMZ in the presence of 10 nM BMN 673 was 20 μM, compared to a rIC_{50} of 374 μM for single agent TMZ (top figure).
- Three of 4 Ewing sarcoma cell lines had rIC_{50} values < 10 μM (red bars), as did 3 cell lines with low MGMT expression (Rh30, GBM2, and NB-1643).
- The median BMN 673 potentiating effect (ratio of TMZ rIC_{50} in the absence and presence of BMN 673) was greatest for Ewing sarcoma cell lines (50-fold) and acute lymphoblastic leukemia (ALL) cell lines (30-fold) (green bars), compared to a median 9-fold effect for the remaining cell lines (lower figure) .

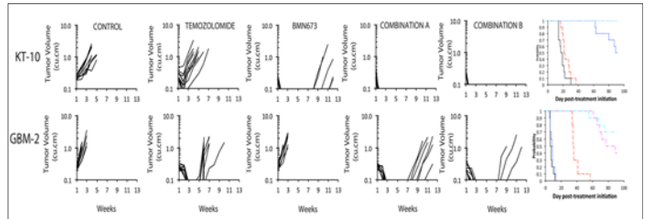
BMN 673 IN VIVO ACTIVITY

BMN 673 SINGLE AGENT TESTING RESULTS:

- BMN 673 was tested as a single agent at 0.33 mg/kg/day x 28 days (BID dosing M-F and SID dosing S-S).
- 33 of 35 solid tumor xenografts showed progressive disease to single agent BMN 673, including all 5 Ewing xenografts studied.
- All 8 acute lymphoblastic leukemia (ALL) xenografts showed progressive disease to BMN 673.
- Complete responses (CR) were observed for a Wilms tumor (KT-10) and a medulloblastoma model (BT-45).
- The two models with CRs to BMN 673 were the only two PPTP xenografts that showed maintained CRs to cisplatin.

BMN 673 + TMZ COMBINATION TESTING:

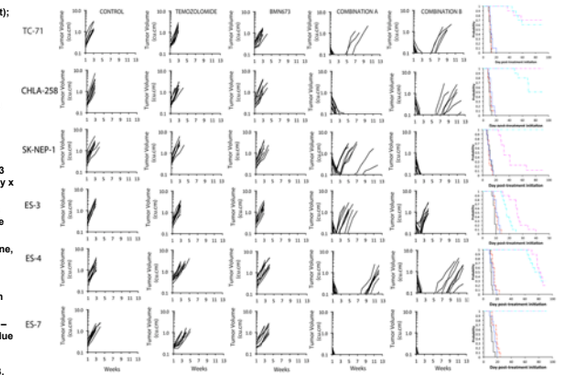
- For the BMN 673 + TMZ combination, two strategies were tested: one with a higher dose of TMZ (30 mg/kg/d x 5d) and the other with a higher dose of BMN 673 (0.25 mg/kg BID x 5d).
  - For TMZ at 30 mg/kg/d, the tolerable BMN 673 dose was 0.1 mg/kg BID (Combination A).
  - For BMN 673 at 0.25 mg/kg BID, the tolerable TMZ dose was only 12 mg/kg/d, (Combination B) .
  - For reference, standard TMZ doses range from 50 to 66 mg/kg/d.
- Initial combination testing focused on Ewing sarcoma xenografts (see right) and on xenografts responding to single agent BMN 673 (KT-10) or single agent TMZ (GBM2) (see below).



- For the MGMT negative glioblastoma xenograft GBM2, both BMN 673 + TMZ combinations induced CRs that were maintained at 6 weeks, and both were more active than TMZ as a single agent at 30 mg/kg/d.
- For the Wilms tumor xenograft KT-10 that was highly responsive to single agent BMN 673 (28 day schedule), both combination regimens induced CRs that were maintained through 12 weeks and both were superior to single agent BMN 673 (5 day schedule).
- See Figure Legend at upper right for explanation of treatments.

FIGURE LEGEND

- Control (no treatment);
- Temozolomide (30 mg/kg/d x 5d)
- BMN673 (0.25 mg/kg BID x 5 d)
- Combination A: Temozolomide 30 mg/kg /d and MN673 0.1 mg/kg BID; both daily x 5
- Combination B: Temozolomide 12 mg/kg /d and BMN673 0.25 mg/kg; both daily x 5.
- Each line represents the growth of a single tumor.
- For each xenograft line, 10 mice bearing SC tumors initiated treatment when the tumors were between 0.2–0.5 cm3.
- K-M Plots: black line – control, red – TMZ, blue – BMN-673, purple – combination A, light blue – combination B.



- Remarkably high activity was observed for the combinations, with 5 of 10 Ewing xenografts having CR responses maintained through at least 6 weeks for most treated animals (see figure above). Lines that did and did not meet this bench mark are listed below:
  - Maintained CRs at 6wks: TC-71, CHLA-258, SK-NEP-1, ES-4, ES-7.
  - Progressive disease by 6 wks: ES-3, and (ES-2, ES-6, ES-8, and EW-5 not shown).
- Maintained CRs 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.

CONCLUSIONS

- BMN 673 as a single agent induced CRs in only 2 of 43 models, both of which are also highly responsive to cisplatin.
- BMN 673 markedly potentiates the activity of TMZ in vitro, with potentiation being particularly notable for Ewing sarcoma xenografts with the level of potentiation exceeding 50-fold for some cell lines.
- 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 in a MGMT-negative GBM xenograft and in a Wilms tumor xenograft that was responsive to 28 days of single agent BMN 673.
- Additional xenografts are being tested to define the range of activity for the BMN 673 + low-dose TMZ regimen.
- Based on the PPTP results a pediatric phase 1 trial attempting to translate to the clinic the BMN 673 plus low-dose TMZ strategy is planned.

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This poster will be available at: http://pptp.nchresearch.org/presentations.html