Inhibition of Rho-associated Coiled-coil Containing Protein Kinases with Belumosudil Mesylate Shows Anti-tumor and Immune Modulatory Properties in Models of Multiple Myeloma

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Introduction:

Myeloma patient outcomes have continuously improved with the introduction of proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs), monoclonal antibodies (mAbs) recognizing cell surface proteins and, most recently, B-cell maturation antigen (BCMA)-targeted bispecifics and chimeric antigen receptor (CAR)-guided T-cells. However, the prognosis in triple-class refractory (TCR) and quadruple-class refractory (QCR) remain poor indicating a persistent need for novel agents.

Rho-associated coiled-coil containing protein kinases (ROCK1 and ROCK2) may represent novel potential targets since ROCK1 has been associated with a plasma cell leukemia-like transcriptional profile and as a myeloma dependency. Moreover, ROCK2 mediates expression of Interferon regulatory factor 4 (IRF4) and c-MYC, as well as Th17 responses that may contribute to an immune suppressive microenvironment.

Methods:

To examine the central hypothesis that targeting ROCK1/2 could be a novel and effective approach to myeloma therapy, we performed pre-clinical studies with the ROCK1/2 inhibitor belumosudil mesylate single agent or in combination with mAB Isatuximab using drug-naïve and drug-resistant myeloma cell lines *in vitro*.

Also, *in vivo* studies with immune correlates were performed in the Vk*MYC immune-competent syngeneic myeloma model, which has been reported to have a 67% positive predictive value for clinical efficacy in humans.

Results:

Figure A.

Belumosudil reduces myeloma cell viability

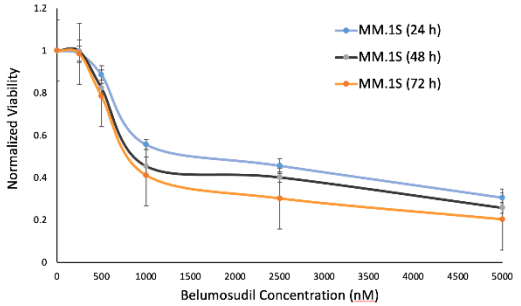


Figure B.

Belumosudil induces myeloma cell apoptosis

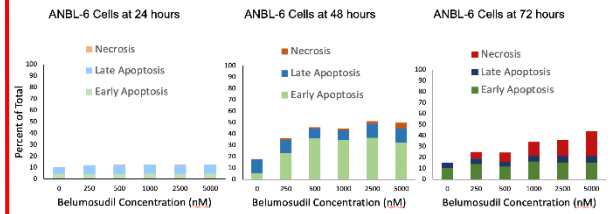


Figure C.

Activity in Vk*MYC mice

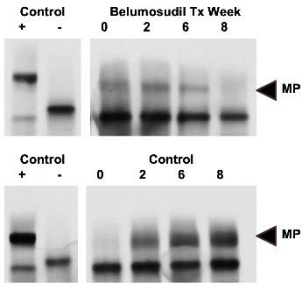
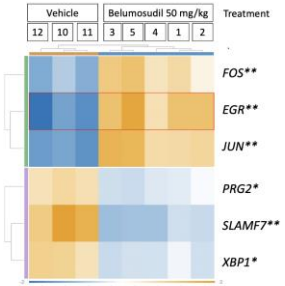


Figure D.

Immune correlates in Vk*MYC mice



Genes whose expression is increased (yellow) or decreased (blue).

Figure E.

Belumosudil in combination with Isatuximab induced myeloma cell apoptosis

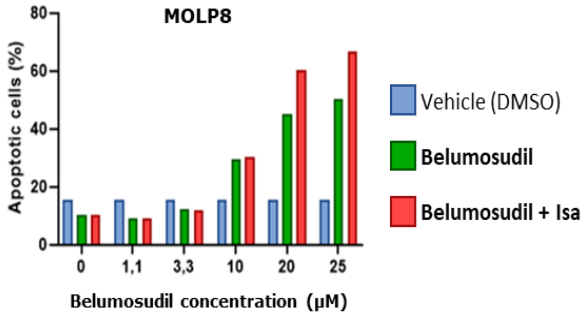
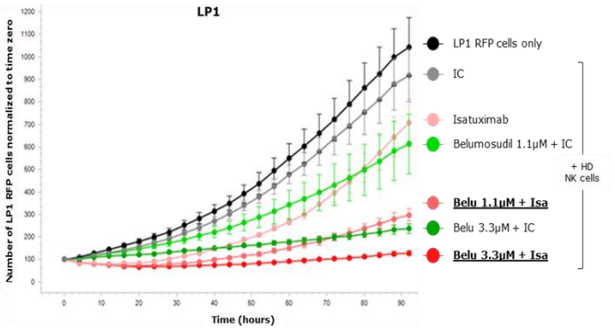


Figure F.

Belumosudil in combination with Isatuximab induced killing of myeloma cells in presence of NK-cells



Discussion:

Belumosudil mesylate as a single agent reduced the viability (A), induced early and late apoptosis (B) of myeloma cell lines in clinically relevant concentration- and time-dependent manner. Furthermore, we demonstrated that dosing of belumosudil *in vivo* at 50 mg/kg twice weekly for eight weeks in Vk*MYC mice with paraprotein levels consistent with active myeloma produced a consistent decline in disease burden as determined by serum protein electrophoresis monitoring (**C**) compared to vehicle controls. NanoString analysis of tumor samples from these mice (**D**) showed down-regulation of *XBP1* (X-box binding protein 1) and *SLAMF7* (signaling lymphocytic activation molecule family member 7), consistent with the anti-tumor efficacy of belumosudil mesylate. In addition, up-regulation was seen of c-*JUN* and c-*FOS*, consistent with promotion of T-cell activation and clonal expansion, as well as effector differentiation of antigen-activated $CD8^+$ T cells.

Notably, when used in combination with the CD38 mAb isatuximab (**E**) and in the presence of NK cells (**F**), belumosudil enhanced myeloma cell killing and prevented isatuximab-induced loss of CD38 expression.

Conclusions:

These pre-clinical *in vitro* and *in vivo* data support the hypothesis that targeting of ROCK1 and ROCK2 with belumosudil mesylate may be a promising strategy for relapsed/refractory myeloma and provide a rationale for its translation to the clinic. Currently, clinical trials of single-agent belumosudil and in combination with dexamethasone are approved, pending patient enrollment.

Disclosures:
Cervoni-Curet F. is part of ASH-Sanofi Fellows Advisory Board; FNCervonil@mdanderson.org