Discovery and Characterization of BMN673 (Talazoparib), a Novel, Highly Potent, and Orally Efficacious Poly(ADP-ribose) Polymerase-1,2 Inhibitor, as an Anticancer Agent

BioMarin Pharmaceuticals

BioMarin is a global biotechnology company that develops and commercializes innovative therapies for people with serious and life-threatening rare disorders (established in 1997)

Products on the Market:

- Naglazyme® (GALSULFASE)
- Vimizim® (elosulfase alfa)
- Kuvan® (sapropterin dihydrochloride) Tablets
- Aldurazyme® (Laronidase)
BioMarin’s Current Pipeline

<table>
<thead>
<tr>
<th>MOLECULE/INDICATION</th>
<th>PRECLINICAL TESTING</th>
<th>PHASE 1</th>
<th>PHASE 2</th>
<th>PHASE 3</th>
<th>BLA/ NDA/MAA</th>
<th>COMMERCIALIZATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALDURAZYME® FOR MPS I</td>
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<td>NAGLAZYME® FOR MPS VI</td>
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<td>KUVAN® FOR PKU (GLOBAL, EXCEPT JAPAN)</td>
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<td>FIRDAPSE® FOR LEMS (EU)</td>
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<td>REVEGLUCOSIDASE ALPHA FOR POMPE DISEASE</td>
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<td>CERLIPONASE ALPHA (BMN 190) - TTP1 FOR CLN2 DISEASE</td>
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<td>BMN 270 AAV-FACTOR VIII VECTOR FOR HEMOPHILIA A</td>
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<td>BMN 250 GILT rhNAGLU FOR SANFILIPPO SYNDROME / MPS IIIIB</td>
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- Worldwide rights of Talazoparib were sold in 2015 to Medivation Inc.
- $410M in upfront payment; $160M upon achievement of regulatory and sales based milestones
- Single-digit percentage royalties
What is PARP?

Poly (ADP-ribose) polymerase (PARP) is a family of proteins involved in a number of cellular processes involving mainly DNA repair and programmed cell death.

PARP Activation

DNA damage

PARP binding to DNA SSB

PARP activation through auto-modification and the modification of chromatin

PARP dissociation and PAR degradation by PARG

Repair of DNA SSB

Genotoxic Stress

Accepter Proteins

NAD^+ PARPs

Accepter Proteins

Branched elongation

Linear elongation
PARP Inhibitors Mode of Action

2 Mechanisms of Action

1. PARP1 inhibited cells accumulate unrepaired SSBs → DSBs when encountered by the replication machinery. If HR repair is disabled, cells reroute to alternative low fidelity DNA repair pathways, thus hastening genomic instability and cell death.

2. PARP Inhibitors compete with NAD+ at the catalytic site of PARPs inhibiting their enzymatic function and preventing synthesis of PAR----→ “PARP1-trapping model”
Current PARP Inhibitors

I. PARP Inhibitors in Clinical Trials

- Niraparib (formerly MK4827)
  Tesaro

- Rucaparib (AG-014699)
  Clovis Oncology

- Olaparib (AZD2281)
  Astra Zeneca
  Phase 3 in adjuvant and advanced germline BRCAm breast cancer

- CEP9722
  Teva Pharma

- Veliparib (ABT888)
  Abbvie
  * Phase 3 in neoadjuvant in combination with carboplatin and standard therapy in triple negative breast cancer
  * Phase 2/3 in advances setting as combination therapy in germline BRCAm breast cancer

II. OTHERS

- Nicotinamide

- 3-Aminobenzamide (3-AB)

- Iniparib (BSI-201)
Discovery of the Tetrahydropyridophthalazinone Scaffold

A.

\[
\begin{align*}
\text{NO}_2 \xrightarrow{10\% \text{ Pd/C, 1 atm}} & \text{NH}_2 \\
\text{EtOAc, rt, 2h} & \xrightarrow{\text{PhCHO, MeONa, ethyl propionate, reflux, 18h}} \text{NH}_2 \\
& \xrightarrow{\text{H}_2\text{NNH}_2, \text{H}_2\text{O, reflux}} \text{R}
\end{align*}
\]

B.

\[
\begin{align*}
\text{NH}_2 \xrightarrow{\text{PhCHO, MeONa, ethyl propionate, reflux, 18h}} & \text{N} \\
& \xrightarrow{\text{H}_2\text{NNH}_2, \text{H}_2\text{O, reflux 3h}} \text{R}
\end{align*}
\]

Parp1 IC\textsubscript{50} 6.1 nM
Gl\textsubscript{50} 300 uM
SAR study

7: R = H
7a: R = F
7b: R = Cl

21a: R = F, R₁ = A
21b: R = F, R₁ = B
21c: R = F, R₁ = C
21d: R = H, R₁ = B
21e: R = H, R₁ = A
21f: R = H, R₁ = C
21g: R = H, R₁ = E
21h: R = H, R₁ = G
21i: R = Cl, R₁ = B
21j: R = H, R₁ = J
21k: R = H, R₁ = K
21l: R = H, R₁ = S

22a-f: R₃ = H, R₁ = B, R₂ = L (a), A (b), S (c), J (d), M (e), N (f)
22g: R = H, R₁ = T, R₂ = C
22h-j: R = H, R₁ = A, R₂ = O (h), P (i), G (j)
22l-n: R = H, R₂ = B, R₁ = S (l), K (m), J (n)
22o-r: R = H, R₁ = C, R₂ = Q (o), N (p), M (q), J (r)
22s-t: R = H, R₂ = J, R₁ = E (s), G (t)
23a-b: R = F, R₁ = A, R₂ = B (a), C (b)
23c-d: R = F, R₁ = B, R₂ = J (c), M (d)
23e-f: R = F, R₁ = C, R₂ = J (e), M (f)
23g: R = Cl, R₁ = B, R₂ = J

48a: R₁ = 4-CHO, R₂ = H
49a: R₁ = H, R₂ = 3-CHO
50a: R₁ = 3-CHO, R₂ = H
51a: R₁ = 4-CHO, R₂ = 4-F

25-27
51-57

A = 4-PhCH₂NMe₂, B = Ph, C = 4-Ph-F, D = 3-PhCH₂NMe₂, E = 4-PhCl, G = 4-PhCF₃, J = 1-Me-imidazol-2-yl, K = 4-PhCH(OMe)₂, L = i-Pr, M = 1-Me-1,2,4-triazol-5-yl, N = 4-Me-1,2,4-triazol-3-yl, O = 4-Ph-iPr, P = 4-PhMe, Q = thiazol-2-yl, S = 3-PhCH(OEt)₂, T = 4-PhCH(OEt)₂
Synthetic Route to Talazoparib

a. $R_1 = 4$-fluorophenyl
b. $R_1 = 4$-(acetamidophenyl)
c. $R_1 = pyrimidin-5$-yl
d. $R_1 = 1$-Me-pyrazol-5-yl
e. $R_1 = 4$-cyanophenyl
# In Vitro Activity and Metabolic Stability

PARP1 activity
Cellular PARylation assay → inhibition of intracellular PARP1/2
TMZ chemosensitization → ability to potentiate cell killing by temozolomide
Capan-1 cytotoxicity assay → single agent anticancer activity in BRCA2 mutation

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>PARP1-enzyme IC$_{50}$ (nM)</th>
<th>Cellular PAR Inhibition EC$_{50}$ (nM)</th>
<th>TMZ chemosensitization GI$_{50}$ (uM)</th>
<th>Capan-1 (BRCA2 mutant) EC$_{50}$ (uM)</th>
<th>rLM t$_{1/2}$ (min)</th>
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<tbody>
<tr>
<td>H</td>
<td>H</td>
<td>H</td>
<td>6.1</td>
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<tr>
<td>H</td>
<td>4-CH2NMe2</td>
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<td>5.85</td>
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<td>112</td>
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<td>4-CH2NMe2</td>
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<td>H</td>
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<tr>
<td>4-CH2NMe2</td>
<td>H</td>
<td>F</td>
<td>3.29</td>
<td>8.48</td>
<td>46</td>
<td>0.146</td>
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<tr>
<td>4-CH2NMe2</td>
<td>4-F</td>
<td>F</td>
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<td>94</td>
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<td>Rucaparib</td>
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<td>1.98</td>
<td>4.74</td>
<td>144</td>
<td>0.609</td>
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7/9/2016

Tanja Krainz @ Wipf Group
# In Vitro Activity and Metabolic Stability

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<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>PARP1-enzyme IC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>Cellular PAR inhibition EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
<th>TMZ chemosensitization GI&lt;sub&gt;50&lt;/sub&gt; (uM)</th>
<th>Capan-1 (BRCA2 mutant) EC&lt;sub&gt;50&lt;/sub&gt; (uM)</th>
<th>rLM t&lt;sub&gt;1/2&lt;/sub&gt; (min)</th>
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<td>Phenyl</td>
<td>1-Me-imidazol-2-yl</td>
<td>H</td>
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<td>4-fluorophenyl</td>
<td>1-Me-imidazol-2-yl</td>
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<td>phenyl</td>
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<td>phenyl</td>
<td>1-Me-1,2,4-triazol-5-yl</td>
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<td>4-fluorophenyl</td>
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<td>44</td>
<td>0.071</td>
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<td>4-fluorophenyl</td>
<td>1-Me-1,2,4-triazol-5-yl</td>
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<td>2.14</td>
<td>5.48</td>
<td>19</td>
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<td>144</td>
<td>0.609</td>
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In Vitro Activity Comparison with other PARP Inhibitors

<table>
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<tr>
<th>Compound</th>
<th>PARP1 enzyme IC$_{50}$ (nM)</th>
<th>Cellular PAR inhibition EC$_{50}$ (nM)</th>
<th>TMZ chemosensitization GI$_{50}$ (nM)</th>
<th>MX-1 cell (BRCA1 mutant) EC$_{50}$ (nM)</th>
<th>Capan-1 cell (BRCA2 mutant) EC$_{50}$ (nM)</th>
<th>MRC-5 (normal) EC$_{50}$ (uM)</th>
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<tr>
<td>Veliparib</td>
<td>4.73</td>
<td>5.94</td>
<td>6203</td>
<td>ND</td>
<td>&gt;10000</td>
<td>&gt;10</td>
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<tr>
<td>Rucaparib</td>
<td>1.98</td>
<td>4.69</td>
<td>144</td>
<td>5.3</td>
<td>609</td>
<td>8.53</td>
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<td>Olaparib</td>
<td>1.94</td>
<td>3.57</td>
<td>237</td>
<td>23.2</td>
<td>259</td>
<td>5.83</td>
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<td>Nicaparib</td>
<td>8.05</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>650</td>
<td>ND</td>
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<td>Talazoparib (8S, 9R)-47</td>
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<td>2.51</td>
<td>4</td>
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<td>Talazoparib (8S, 9R)-47</td>
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<td>864</td>
<td>1807</td>
<td>Nd</td>
<td>1135</td>
<td>nd</td>
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**PARP catalytic inhibition vs. PARP trapping**

**Trapping:** Induction of allosteric conformational change in the enzyme, therefore stabilizing its association with damaged DNA → prevents DNA replication and transcription, killing cancer cells more effectively than catalytic inhibition alone.
In Vivo Antitumor Effect Single Agent Use

- Talazoparib as a single agent in immunodeficient mice bearing established subcutaneous MX-1 tumor xenografts
- Once daily oral administration of BMN673 for 28 consecutive days

- BMN673 well tolerated with no animal lethality or significant weight loss after treatment
Split-dosing Treatment

0.33 mg/kg/dose once daily vs. 0.165 mg/kg/dose twice daily for 28 days

➢ Tumor growth in the QD cohort eventually re-established after cessation of drug treatment
➢ Continuous suppression of PARP1/2 is required for a sustained antitumor effect
Anti-Tumor Effect in Combination with Cisplatin

- Ability to potentiate DNA damaging anticancer agents
- MX-1 tumor bearing mice treated with 8 consecutive daily oral doses of BMN673, olaparib or vehicle;
- Cisplatin was dosed intraperitoneally at 6mg/kg on day 3
Conclusions

- Unique and extensive binding interactions with PARP1 and PARP2 proteins.
- Excellent in vitro anti-tumor activity as a single agent in BRCA1/2 deficient cells.
- Mechanism of action via PARP trapping
- Excellent pharmacokinetic properties
- Excellent in vivo anticancer efficacy as a single agent and as a chemosensitization agent in BRCA1-deficient MX-1 breast cancer xenograft model