Hijacking Ubiquitin E3 Ligases Using PROTAC Technology to Effectively Degrade BRD4 and Achieve Anti-tumor Efficacy

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251st American Chemical Society National Meeting

March 13, 2016
## Degrader: New Drug Discovery Approach

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Mode of Action</th>
<th>Selectivity</th>
<th>Affinity/active site requirement</th>
<th>Intracellular Access</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Drug Discovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small Molecules</td>
<td>Antagonist/Agonist</td>
<td>Low to High</td>
<td>Yes</td>
<td>High</td>
<td>All Routes</td>
</tr>
<tr>
<td>Peptides</td>
<td>Antagonist/Agonist</td>
<td>High</td>
<td>Yes</td>
<td>Low to Possible</td>
<td>i.v. / s.c.</td>
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<tr>
<td>Biologics</td>
<td>Antagonist/Agonist</td>
<td>High</td>
<td>Yes</td>
<td>Low</td>
<td>i.v. / s.c.</td>
</tr>
<tr>
<td>Arvinas</td>
<td>PROTACs</td>
<td>Degrader</td>
<td>No</td>
<td>High</td>
<td>All Routes</td>
</tr>
</tbody>
</table>

Degradation over inhibition

- Higher and longer pharmacological effect without requiring continuous high exposure
- Applicable to targets without active site/low affinity ligands (non-druggable targets)
PROTACs Hijack E3 Ubiquitin Ligases to Degrade Target Protein

- PROTAC (Proteolysis Targeting Chimeras) is composed of two ligands connected with a linker.
- Upon tertiary complex formation, E3 ligases transfer ubiquitin to target protein surface lysine and set target protein for degradation via proteasome machinery.
- PROTAC is released and continues target protein degradation process.

Cereblon (CRBN) E3 Ligase Ligand: IMiDs

- Immunomodulatory drugs (IMiDs): thalidomide, lenalidomide and pomalidomide
- Cereblon was identified in the study of teratogenicity of thalidomide (Science, 2010)
- Cereblon forms an E3 ubiquitin ligase complex with damaged DNA binding protein 1 (DDB1), cullin 4A (CUL4A) and regulator of cullins 1 (ROC1), a family of CRLs
- Binding of IMiDs to CRBN leads to recruitment of IKZF1 and IKZF3 and consequent degradation via ubiquitin proteasome system (UPS)

Ligands of VHL E3 Ligase

- The von Hippel Lindau (VHL) protein binds to adaptor protein EloB/C and Cullin2-Rbx1 to form a member of CRL2 as an E3 ligase
- Primary substrate of VHL is the hypoxia induced factor 1α (HIF1α), a transcription factor related to hypoxic response
- HIF1α is degraded via UPS following hydroxylation of proline by prolyl hydroxylases which leads to recruitment by VHL
- VHL ligands were identified in the study of inhibition VHL and HIF1α protein-protein interaction

Leu-Ala-Pro(OH)-Tyr-Ile

\[ \text{Ki} = 2.4 \, \mu\text{M} \]

\[ \text{Kd} = 0.19 \, \mu\text{M} \]


C.M. Crews, et al. WO 2013/106646
Representative Example of Building PROTACs and Identifying Degrader Hits

<table>
<thead>
<tr>
<th>Connector Length</th>
<th>D&lt;sub&gt;max&lt;/sub&gt;</th>
<th>DC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</td>
<td>&gt;1000</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>86%</td>
<td></td>
<td>71</td>
</tr>
<tr>
<td>96%</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>96%</td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>96%</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>
BRD4: Key Epigenetic Cancer Target

- Elevated expression of Myc transcription factors occurs frequently in human cancers and is associated with tumor aggression & poor outcome

- Inhibition of BRD4 – a member of the BET family – is a strategy to target MYC
  - BET inhibitors abrogate MYC transcription and block tumor growth

- BET inhibitors selectively disrupt numerous additional tumor oncogene super-enhancers

- Multiple BET inhibitors currently in clinical trials for cancer

- BRD4 tightly binds acetylated histones via its BET bromodomains

- JQ1 competes with this binding & displaces BRD4 from chromatin

Valent and Zuber, Cell Cycle 13, 689-90, 2014

Hijacking CRBN E3 Ligase to Degrade BRD4

OTX-15 based to recruit BRD4

Linker

Pomalidomide based to recruit CRBN

OTX-015

ARV-825 (R = H), active degrader; R = Me, inactive degrader

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Affinity to BD1 and BD2 of BRD4</th>
<th>c-Myc ELISA IC$_{50}$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BD1 $K_d$ (nM)</td>
<td>BD2 $K_d$ (nM)</td>
</tr>
<tr>
<td>ARV-825</td>
<td>90</td>
<td>28</td>
</tr>
<tr>
<td>JQ1</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>OTX-015</td>
<td>14</td>
<td>3.5</td>
</tr>
<tr>
<td>dBET1</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>


Synthesis of ARV-825

1. HO-O-O-O-O-O-OH → TsCl, TEA → TsO-O-O-O-O-O-O-Os
2. HO-O-O-O-O-O-OH → K₂CO₃, DMF, 50 °C → O₂N-Ph-O-O-O-O-O-Os
3. NaN₃, EtOH, 80 °C → O₂N-Ph-O-O-O-O-N₃
4. PPh₃, THF/H₂O → O₂N-Ph-O-O-O-O-NH₂
5. DIPEA, DMF, 90 °C → F-screening
6. Fe, NH₄Cl, EtOH/H₂O → NH₂
7. JQ1 carboxylic acid, HATU, DIPEA, DMF → ARV825
BRD4 Degrader: Longer Lasting Effect on c-Myc Suppression

Namalwa cells treated with ARV-825 (0.1 μM), JQ1 (1 μM), OTX (1 μM) for 24 h, followed by 3 washes
Degradation by ARV-825 Is Dependent on Cereblon Binding

<table>
<thead>
<tr>
<th>Namalwa</th>
<th>Ramos</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pomalidomide (10 µM)</strong></td>
<td><strong>Pomalidomide (10 µM)</strong></td>
</tr>
<tr>
<td>A825</td>
<td>A825</td>
</tr>
<tr>
<td>DMSO</td>
<td>100nM</td>
</tr>
<tr>
<td>BRD4 -</td>
<td>BRD4 -</td>
</tr>
<tr>
<td>Actin -</td>
<td>Actin -</td>
</tr>
</tbody>
</table>
Degradation by ARV-825 Is via UPS

<table>
<thead>
<tr>
<th></th>
<th>DMSO</th>
<th>A825 (10 nM)</th>
<th>A825 (100 nM)</th>
<th>MG132 (5 µM)</th>
<th>Carfilzomib (5 µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRD4 -</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin -</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
BRD4 PROTACs Are Apoptotic in BL and AML Cells

Data from Dr. Gautam Borthakur’s Lab at MDACC

Identification of Active BRD4-VHL PROTAC

**Molecules** | cMYC ELISA IC$_{50}$ (nM)  
--- | ---  
ARV-771 | 1.0  
ARV-766 | >1000  
JQ-1 | 38  
OTX-015 | 52
Synthesis of ARV-771

BocHN-F-Bromophenyl + 3-Thiophene

(1) Pd(OAc)$_2$, KOAc, 90 °C
(2) 4N HCl in MeOH

H$_2$N-Phenyl-Sulfide-HCl

(1) HATU, DIPEA, THF
(2) 4N HCl in MeOH

$\text{A}$

O-O-OH + tert-butyl-2-bromoacetate

tetra-butyl ammonium chloride

(1) Pd/C, MeOH
(2) tosyl chloride, pyridine

O-O-OH

(1) NaN$_3$, DMF, 70 °C
(2) PPh$_3$, THF, H$_2$O

(1) JQ-1 carboxylic acid
HATU, DIPEA, DMF
(2) HCOOH
(3) $\text{A}$, DIPEA, HATU, DMF

ARV-771
ARV-771 caused robust caspase 3/7 induction in 22rv1, VCaP and LnCaP95 prostate cancer cells
Correlation of c-Myc Suppression and Cell Growth Inhibition in 22RV1 Prostate Cancer Cells

- Total 46 VHL ligand based BRD4 PROTACs in the data set
- ARV-771: c-Myc IC_{50} = 1.0 nM, GI_{50} = 12 nM
- ARV-776 (epimer of A1771): c-Myc 10% inhibition @ 300 nM, GI 0% @ 1000 nM
Subcutaneous Dosing of ARV-771 Caused Tumor Regression in 22RV1 Xenograft Model

22RV-1 Tumor Size

- Vehicle QD. S.C.
- Vehicle QD. P.O.
- ARV-771 30mg/kg QD. S.C.
- 50MPK OTX015 QD. P.O.

80% TGI

Tumor regression

BRD4

MYC

Tubulin

Veh (n=9)

10MPK ARV-771 SC (n=9)
PROTACs: Extended or Beyond Rule of 5

- PROTACs are in the chemical space of extended rule of 5 (eRo5) or beyond rule of 5 (bRo5)
  - Extended Ro5
    • MW: 500-700 Da; clogP: 0-7.5; HBD ≤ 5; HBA ≤ 10; PSA ≤ 200 Å²; NRotB ≤ 20
  - Beyond Ro5 (MW >500, with at least one of the following)
    • MW 700-3000 Da; clogP < 0 or >7.5; HBD >5; HBA >10; PSA > 200 Å²; NRotB > 20

- Drugs and clinical candidates (N=475) with MW 500-30000 Da
  - eRo5: N=195, 71% oral; bRo5: N=280, 30% oral
  - Many macrocycles take advantage of intramolecular H-bonding


- All four FDA approved oral NS5A inhibitors (HCV) are not macrocycles and are in the chemical space of bRo5
  - Ledipasvir (MW 889); Ombitasvir (MW 894); Elbasvir (MW 882); Daclatasvir (MW 739)

- At Arvinas, we achieved PROTACs as development candidates across multiple target classes
Summary

- Demonstrated novel approach of hijacking natural E3 ligases to degrade target proteins using Arvinas’ PROTAC platform technology
- New rules are being established to expand the current knowledge of molecules beyond rule of 5
- BRD4 PROTACs are superior to BET inhibitors in suppressing cancer cell growth and inducing apoptosis
- PROTAC ARV-771 showed tumor regression activity in 22rv1 prostate xenograft efficacy study following subcutaneous dosing
- Multiple PROTACs achieved robust BRD4 degradation in tumors of 22rv1 xenograft model following single low dose delivered subcutaneously
- BRD4 degraders could provide a potential therapeutic approach to the treatment of multiple tumors
Acknowledgements

Arvinas
Kanak Raina
Jing Lu
Martha Altieri
Debbie Gordon
Ann Marie Rossi
Xin Chen
Jing Wang
Hanjing Dong
Kam Siu
Kevin Coleman
Jim Winkler
Andy Crew

Yale University
Craig M. Crews