PROTAC BRD4 Degraders Allow a More Effective Therapeutic Strategy than BRD4 Inhibitors

TAT Presentation

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CSO
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PROTAC Technology Platform
PROTACs Hijack E3 Ubiquitin Ligases to Degrade Target Protein

- PROTAC (Proteolysis Targeting Chimeras) has two connected ligands
- Upon tertiary complex formation, E3 ligases transfer ubiquitin to target protein surface lysine and targets protein for degradation via proteasome machinery
- PROTAC is released and continues target protein degradation process

# Degraders: A New Drug Discovery Approach

<table>
<thead>
<tr>
<th>Current Drug Discovery</th>
<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>Small Molecules</td>
<td>Antagonist/Agonist</td>
<td>Low to High</td>
<td>Yes</td>
<td>High</td>
<td>All Routes</td>
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</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>
<td></td>
</tr>
<tr>
<td>Arvinas</td>
<td>PROTACs</td>
<td>Degrader</td>
<td>No</td>
<td>High</td>
<td>All Routes</td>
<td></td>
</tr>
</tbody>
</table>

**Degradation versus inhibition**

- More robust and longer pharmacological effect without continuous high exposure
- Applicable to targets without active site/low affinity ligands (non-druggable targets)
PROTAC-Induced Target Ubiquitination

- RIPK2 poly-ubiquitination with various concentrations of PROTAC
- RIPK2 ubiquitination by PROTAC is concentration-dependent, time-dependent and stereospecific

Catalytic in vivo protein knockdown by small-molecule PROTACs
PROTAC-Induced Target Ubiquitination

- Sub-stoichiometric PROTAC concentrations result in RIPK2 ubiquitination, showing the catalytic nature of PROTAC activity

Catalytic in vivo protein knockdown by small-molecule PROTACs
Bondeson DP ….. Crews CM
Nat Chem Biol. 2015;11:611-7
BRD4 Program
BRD4: Key Epigenetic Cancer Target

- BRD4 inhibition is a strategy to target MYC and other oncogenes
  - BET inhibitors abrogate MYC transcription and block tumor growth
  - BET inhibitors selectively disrupt tumor oncogene super-enhancers
- Several BET inhibitors are currently in Phase 1 clinical trials for cancer
  - GSK, Constellation, Tensha, Oncoethix/Merck, Abbvie, Gilead, Bayer, Incyte, Forma, BMS
  - All derived from benzodiazepine (JQ-1) and have pan-BET activity

BRD4 Inhibitors: Phase 1 Clinical Activity

- Inhibitors are cytostatic in preclinical models
- Activity has been modest in Phase 1 clinical trials
- OTX015 \(^1\)
  - Advanced leukemia (N=41; pts dosed 14d on / 7d off)
    - 2 complete remissions (CRs), 1CRI \(^2\)
    - Dose-limiting toxicity (DLT): Diarrhea & asthenia
  - Other hematologic malignancies (N=45; pts dosed continuously)
    - 2 CRs, 1 partial response (PR); all in diffuse large B cell lymphoma (DLBCL)
    - DLT: Primarily thrombocytopenia; diarrhea in 1 patient, neutropenia in 1 patient
- Constellation lymphoma trial\(^3\) (N=47; pts dosed 14d on / 7d off)
  - 2 CRs in DLBCL, 1 PR in follicular lymphoma
  - DLT: Thrombocytopenia & diarrhea in 1 patient each
  - Maximum tolerated dose not yet reached

Conclusion: Room for improved clinical activity

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2. Complete remission with incomplete recovery of platelets.
VHL- and Cereblon-Based PROTACs Degrade BRD4

VHL-Based PROTAC

DC$_{50}$: 1 nM  
$D_{\text{max}}$: 95%

Cereblon-Based PROTAC

DC$_{50}$: 200 pM  
$D_{\text{max}}$: 95%

Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4  
BRD4 PROTAC Degradation is Proteasome-Dependent

VHL-Based PROTAC

<table>
<thead>
<tr>
<th></th>
<th>A763</th>
<th>MG132</th>
<th>Carfilzomib</th>
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<td>-</td>
<td>+</td>
<td>+</td>
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</table>

BRD4

Actin

PROTAC A763: 300 nM
Proteasome inhibitors:
MG132: 5uM
Carfilzomib: 5uM

Cereblon-Based PROTAC

<table>
<thead>
<tr>
<th></th>
<th>A825</th>
<th>MG132</th>
<th>Carfilzomib</th>
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<tr>
<td></td>
<td>-</td>
<td>+</td>
<td>++</td>
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BRD4

Actin

PROTAC A825: + (10nM); ++ (100nM)
Proteasome inhibitors:
MG132 5 uM
Carfilzomib: 5uM
BRD4 PROTACs More Effective *in vitro* than Inhibitors
Molecular Understanding Provides Clinical Opportunity

- BRD4 PROTAC shows greater apoptosis than OTX015 in lymphoma

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**Daudi Burkitt Lymphoma**

<table>
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<tr>
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<th>OTX015</th>
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<tr>
<td>ARV-825-0.3uM</td>
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<tr>
<td>DMSO control</td>
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</tr>
<tr>
<td>A686-0.3uM</td>
<td></td>
</tr>
<tr>
<td>A686-1.0uM</td>
<td></td>
</tr>
<tr>
<td>A686-3.0uM</td>
<td></td>
</tr>
<tr>
<td>A686-10.0uM</td>
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**Western Blot:**
- BRD4
- Full-length PARP
- Cleaved-PARP
- MYC
- Actin
BRD4 PROTAC Has Sustained Effects in Cells

BRD4 Degradation and MYC Suppression – NAMALWA Burkitt’s Lymphoma Cells
BRD4 PROTAC Has Sustained Effects in Vivo

BRD4 Degradation and MYC Suppression – 22RV1 Prostate Cancer Tumors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Timepoint</th>
<th>Plasma (nM)</th>
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<tbody>
<tr>
<td>A2104 6mpk</td>
<td>8h</td>
<td>335</td>
</tr>
<tr>
<td></td>
<td>24h</td>
<td>60</td>
</tr>
<tr>
<td>A2148 6mpk</td>
<td>8h</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>24h</td>
<td>ND</td>
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Low Plasma PK
A Degrader with Differential Biology Could Provide Therapeutic Advantages

- Preclinical data show that PROTACs, vs. BET inhibitors, have:
  - Enhanced potency
  - Better durability of effect
  - More pronounced apoptosis / cytotoxic effects
  - Lack of rebound BRD4 up-regulation
  - Greater effects on tumors in the presence of stroma compared to cytotoxic agents (e.g., Ara-C)

- Intermittent dosing possible; tumor regressions observed in xenografts

- MoA & preclinical literature suggest broad clinical application
  - Examples:
    - DLBCL, AML
    - Prostate, ovarian
BRD4 PROTACs Are Apoptotic in Primary AML Cells

- RNAi screen identifies BRD4 as a therapeutic target in AML
  \[Nature\ 478, 524–528\]
  - JQ1 has broad activity in diverse AML subtypes, partially due to effect on MYC
  - Collaboration with MD Anderson Cancer Center, Dr. Borthakur

Superior effect of BRD4 PROTAC over inhibitor in primary AML samples

Data from Dr. Gautam Borthakur’s Lab

Acute Myelogenous Leukemia (primary patient cells)

BRD4 PROTAC ARV-825 Overcomes Stroma-Mediated Protection

Collaboration with MD Anderson Cancer Center, Dr. Borthakur

Protein Expression Analysis by CyTOF

*Surface protein

*CXCR4
BRD4 PROTACs Have Superior Activity to Inhibitors in Prostate Cancer Cell Lines

ARV-771: Active BRD4 PROTAC
ARV-766: Inactive BRD4 PROTAC
ARV-056: VHL Ligand
JQ1: BRD4 inhibitor
OTX015: BRD4 inhibitor
Enzalutamide: AR Antagonist
**Cell Growth Inhibition and c-MYC Suppression in 22Rv1 Prostate Cancer Cells**

- PROTACs are unique compared to BETi for effects on prostate cells
BRD4 PROTAC Provides Robust Degradation of BRD4 \textit{In Vivo}

- Tumor Model: 22RV-1
- Mice: NU/NU
- Time point: 8h
- Route: IV
BRD4 PROTAC Provides Superior \textit{in vivo} Efficacy in 22Rv1 Prostate Cancer Model
BRD4 PROTACs Demonstrate Robust Anti-Proliferative Activity in Ovarian Cancer Cells

ARV-771, ARV-885: Active BRD4 PROTACs
ARV-766: Inactive BRD4 PROTAC
OTX015: BRD4 inhibitor
BRD4 PROTACs Have Superior Pro-Apoptotic Activity in DLBCL Cells Compared to BETi’s
BRD4 PROTACs Provide Robust Tumor Regression in DLBCL Tumors

- Tumor regressions observed on Q3D schedule
- CB17 SCID mice
- 25 mpk OTX015 dosing arm discontinued due to deaths
Pathway to the Clinic

- BRD4 PROTACs with differential biology identified
  - Driving towards a clinical candidate

- Robust preclinical efficacy efficacy observed
  - Heme malignancies: BRD4 PROTACs more apoptotic than BRD4 inhibitors – Acute myelogenous leukemia (AML), diffuse large B-cell lymphoma (DLBCL), multiple myeloma
  - Solid tumors: Between 5x-10,000x more sensitive to PROTACs than to BRD4 inhibitors, which by themselves are relatively inactive – Prostate, ovarian and lung cancers

- Fully exploiting the competitive potential of PROTAC technology
  - Induce apoptosis – vs. stasis seen with inhibitors
  - Induce tumor regression – vs. growth inhibition seen with inhibitors
  - Anticipate Phase 1 to start 2017
Acknowledgements

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  - Kevin Coleman – Biology Lead
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  - Martha Altieri
  - Hanqing Dong
  - Jing Wang
  - Xin Chen
  - Andy Crew

- **Yale**
  - Craig Crews – Arvinas Founder
Targeted Protein Degradation Therapeutics