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Targeted Protein Degradation Therapeutics

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

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Degrader: New Drug Discovery Approach

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

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

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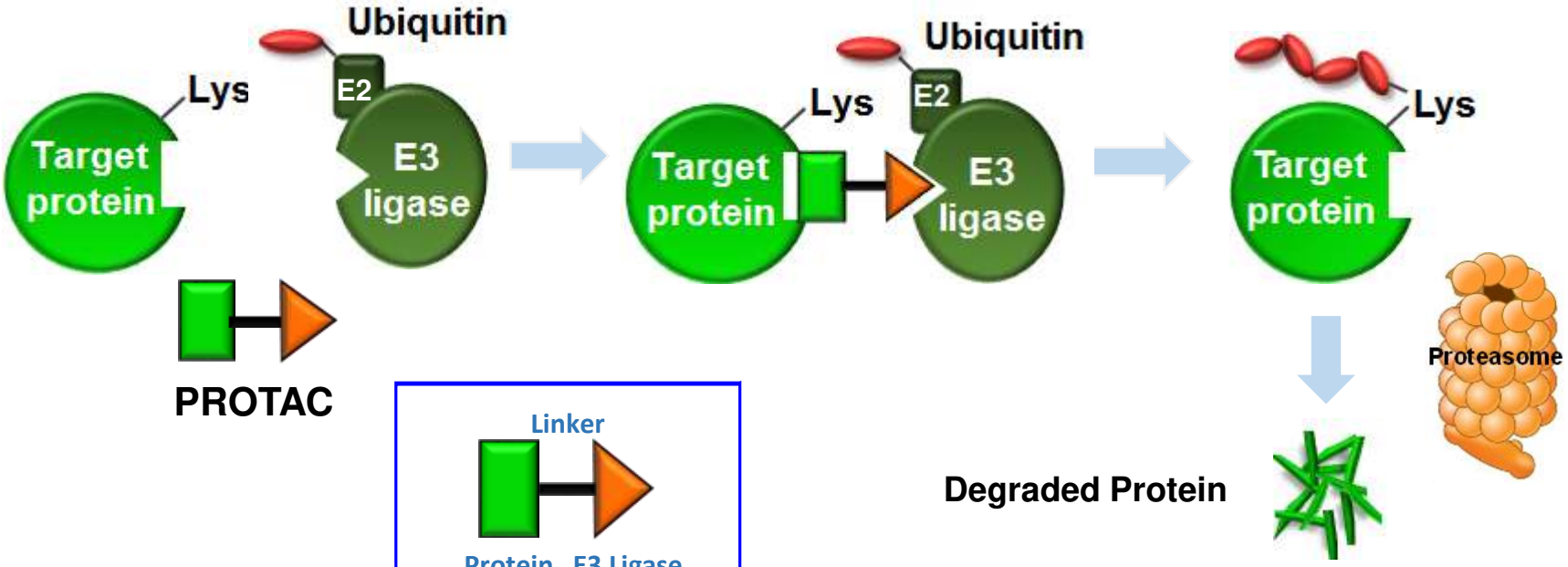
- PROTAC (Proteolysis Targeting Chimeras) is composed of two ligands connected with a linker
- Upon tertiary complex formation, E3 ligases transfer ubiquitin to target protein surface lysine and set target protein for degradation via proteasome machinery
- PROTAC is released and continues target protein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

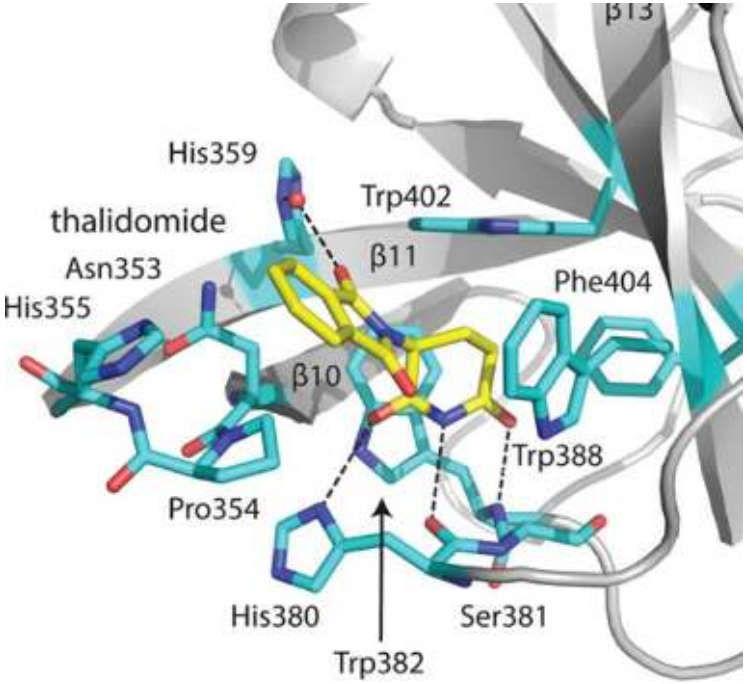


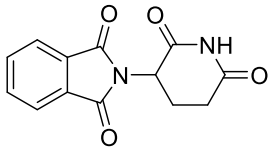
C. M. Crews, *et al*. J. Bio. Chem. *285*, 11057, *2010*

C. M. Crews, et al. ACS Chem. Biol. *3*, 677, *2008*

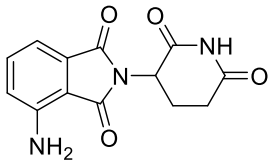
Cereblon (CRBN) E3 Ligase Ligand: IMiDs

- 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)

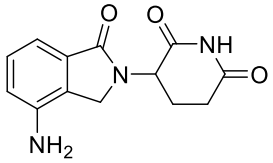




Thalidomide



Pomalidomide



Lenalidomide

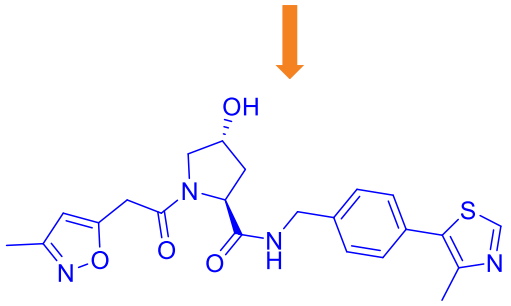
E. S. Fischer, *et al*. Nature *512*, 49-53, *2014*

Ligands of VHL E3 Ligase

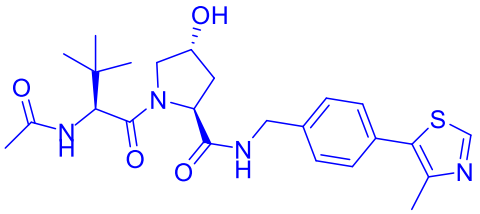
- The von Hippel Lindau (VHL) protein binds to adaptor protein EloB/C and Cullin2-Rbx1 to form a member of CRL2 as an E3 ligase
- Primary substrate of VHL is the hypoxia induced factor 1α (HIF1α), a transcription factor related to hypoxic response
- HIF1α is degraded via UPS following hydroxylation of 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

C.M. Crews, et al. Oncogene 27, 7201, 2008



Ki = 2.4 μM

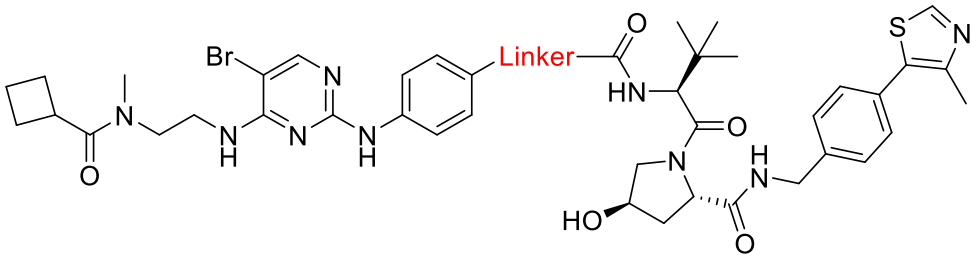


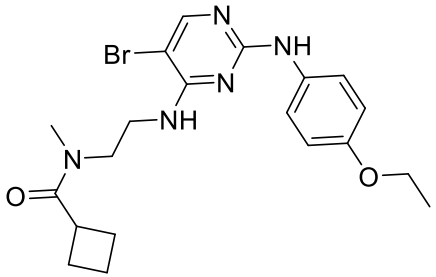
Kd = 0.19 μM

C.M. Crews, et al. Angew. Chem. Int. Ed. 51, 11463, 2012

C.M. Crews, et al. WO 2013/106646

Representative Example of Building PROTACs and Identifying Degrader Hits

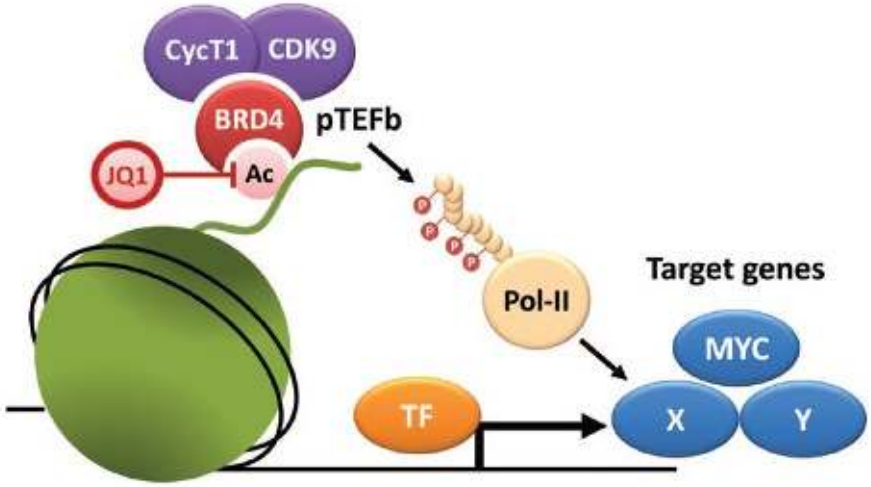




Connector Length	D_{max}	DC_{50} (nM)
	ND	>1000
	ND	>1000
	86%	71
	96%	12
	96%	29
	96%	25

BRD4: Key Epigenetic Cancer Target

- Elevated expression of Myc transcription factors occurs frequently in human cancers and is associated with tumor aggression & poor outcome[1]
- 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

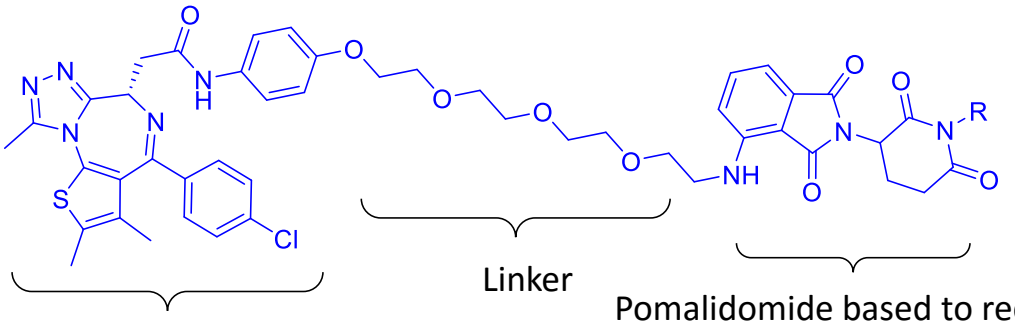


- BRD4 tightly binds acetylated histones via its BET bromodomains
- JQ1 competes with this binding & displaces BRD4 from chromatin[1]

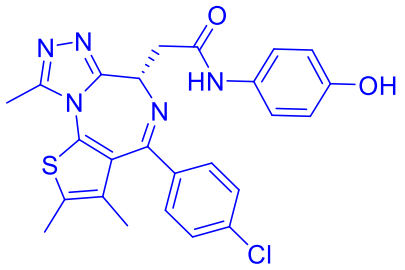
Valent and Zuber, Cell Cycle 13, 689-90, 2014

[1]J. E. Bradner, et al. Nature 468, 1067-1073, 2010

Hijacking CRBN E3 Ligase to Degrade BRD4



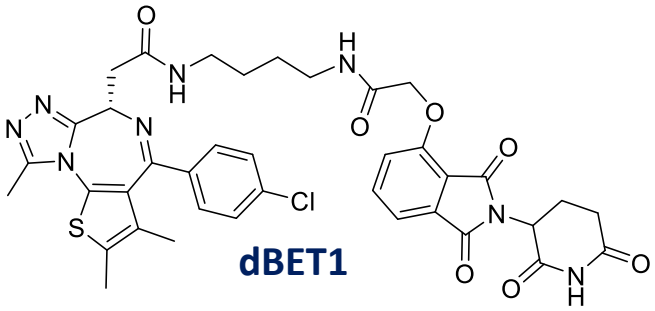
OTX-15 based to recruit BRD4 | Linker | Pomalidomide based to recruit CRBN



OTX-015

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

Molecules	Affinity to BD1 and BD2 of BRD4		c-Myc ELISA IC_{50} (nM)
	BD1 K_d (nM)	BD2 K_d (nM)	
ARV-825	90	28	0.3
JQ1	12	10	38
OTX-015	14	3.5	52
dBET1	ND	ND	200

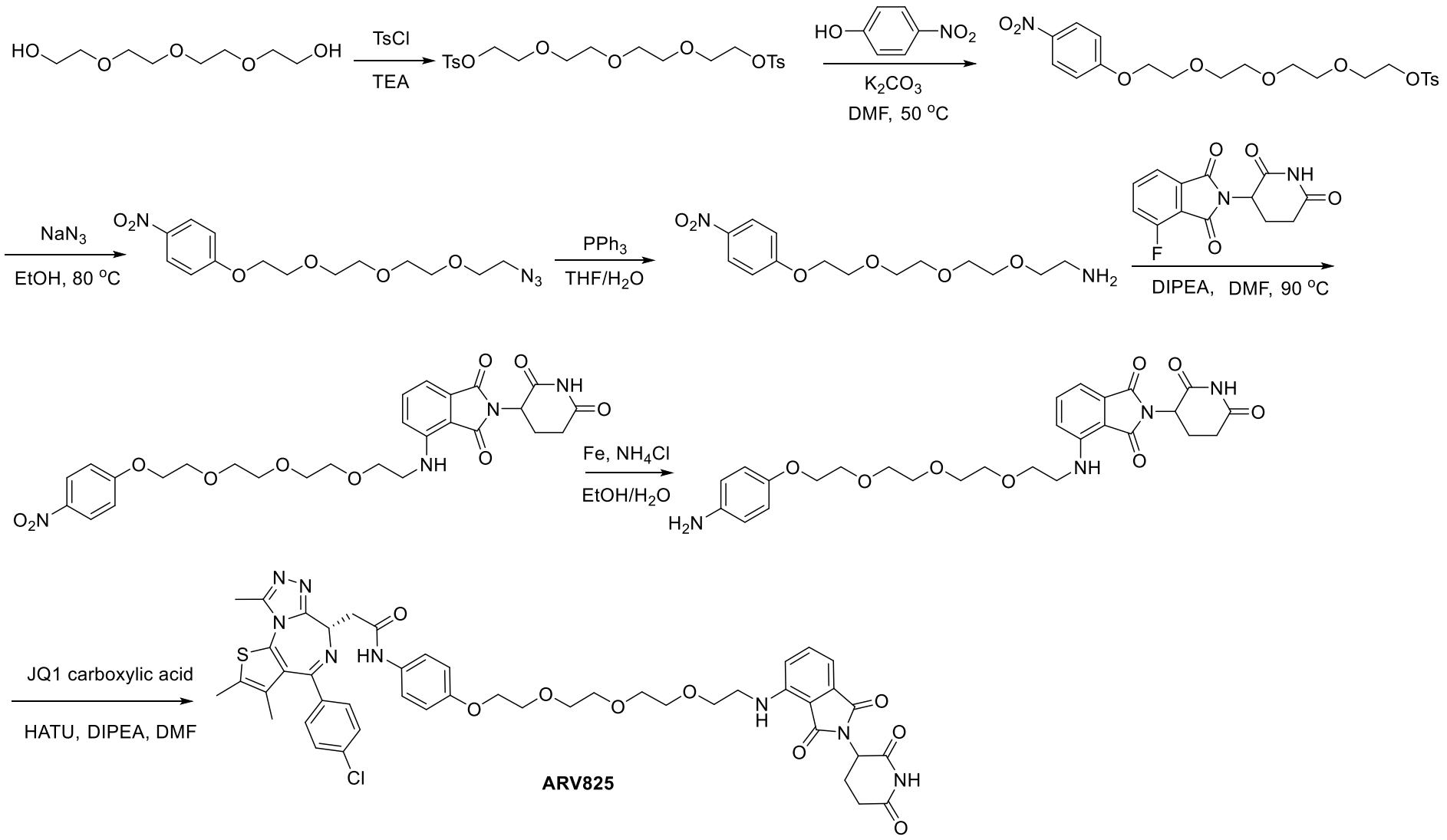


dBET1

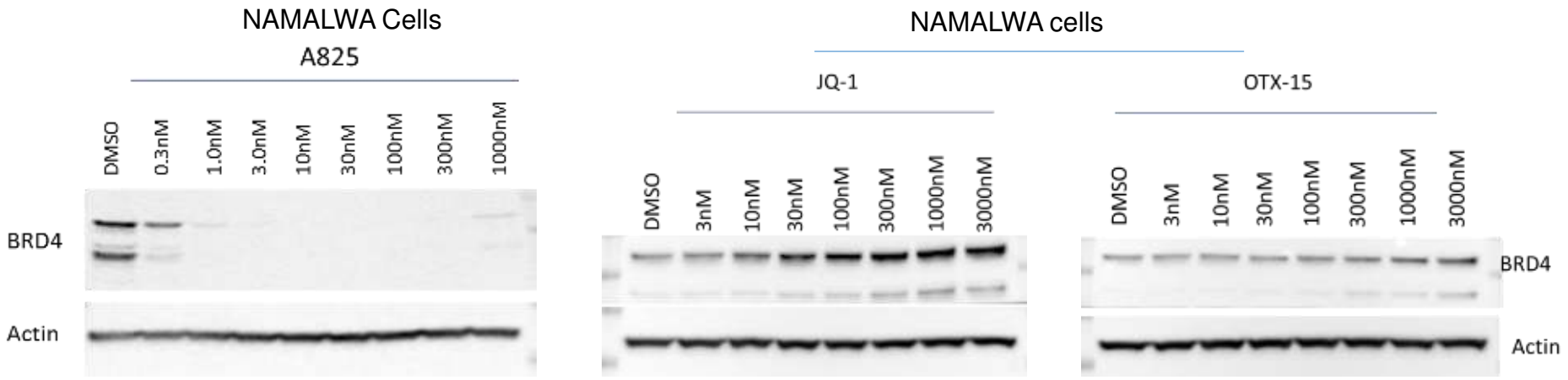
J. Lu, with C. M. Crews, et al., Chem. Biol. 22, 755-763, 2015

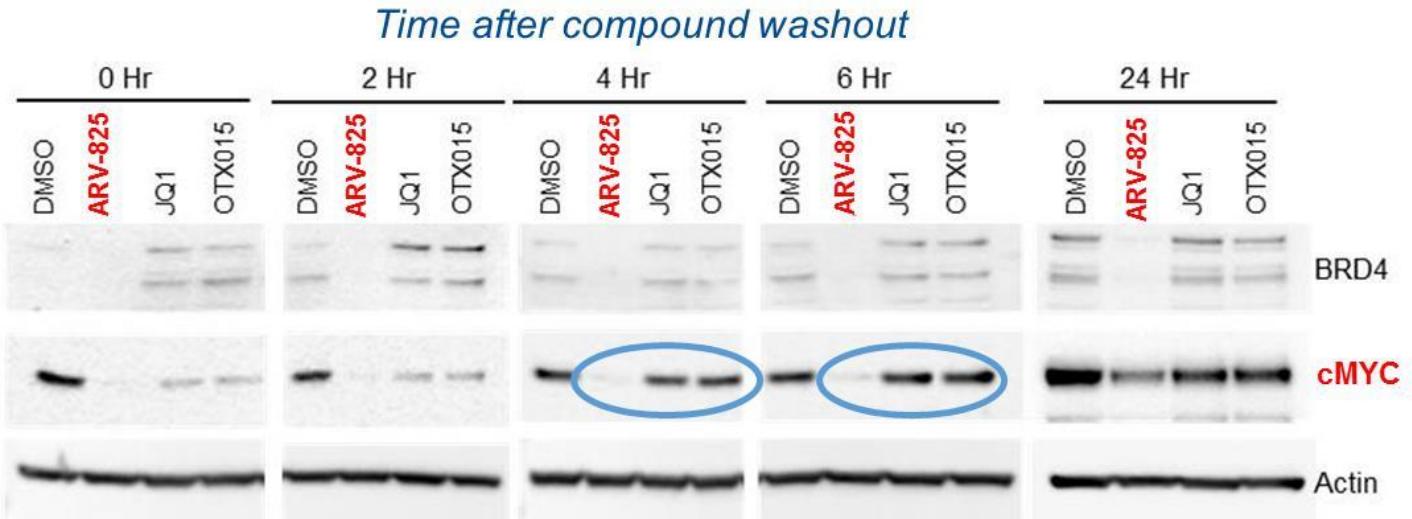
G. E. Winter, with J. E. Bradner, et al., Science 348, 1376-1381, 2015

Synthesis of ARV-825



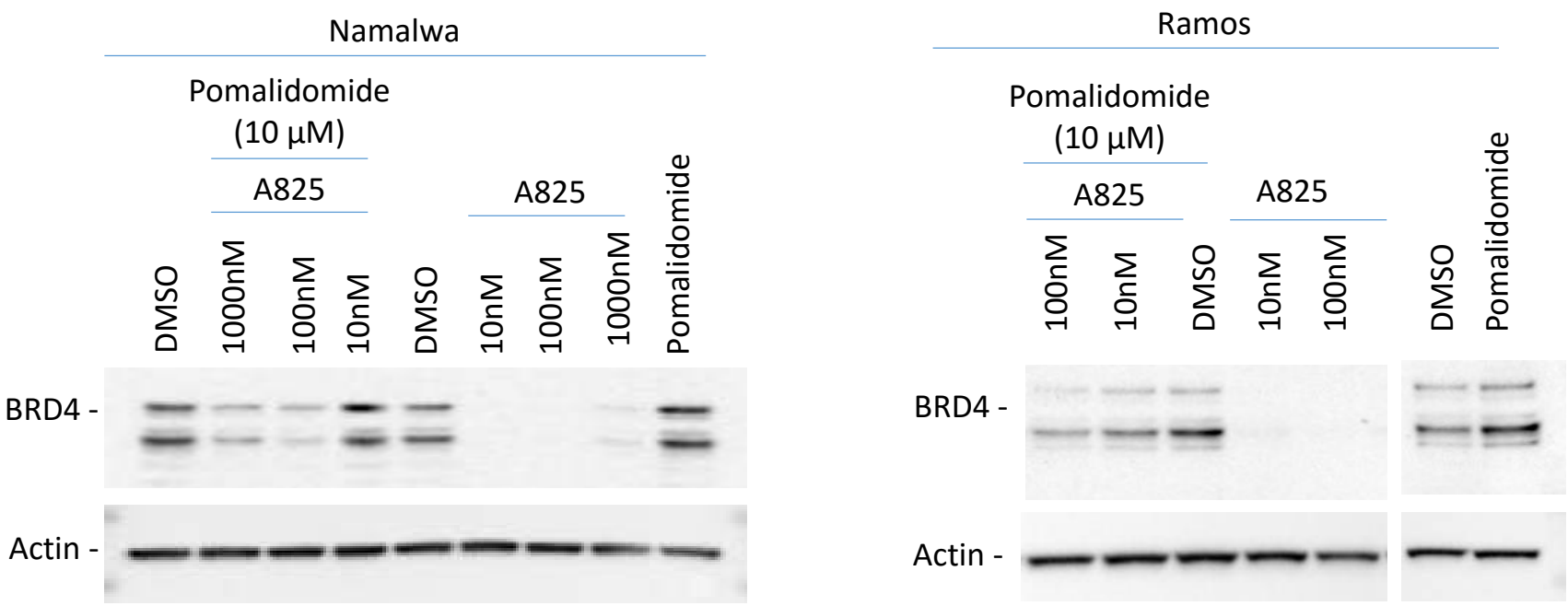
BRD4 Degrader: Longer Lasting Effect on c-Myc Suppression





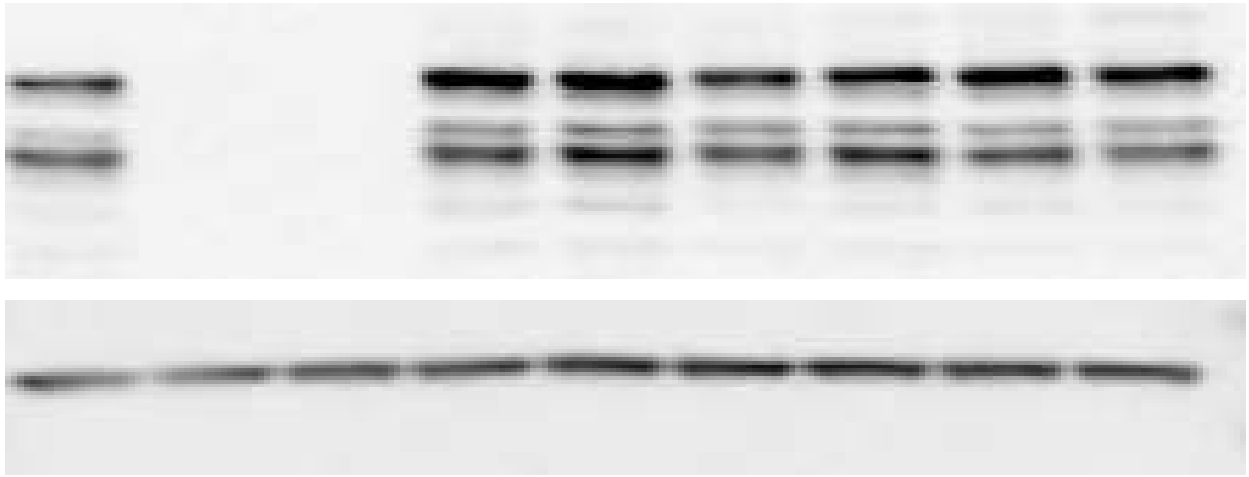
Namalwa cells treated with ARV-825 (0.1 μM), JQ1 (1 μM), OTX (1 μM) for 24 h, followed by 3 washes

Degradation by ARV-825 Is Dependent on Cereblon Binding

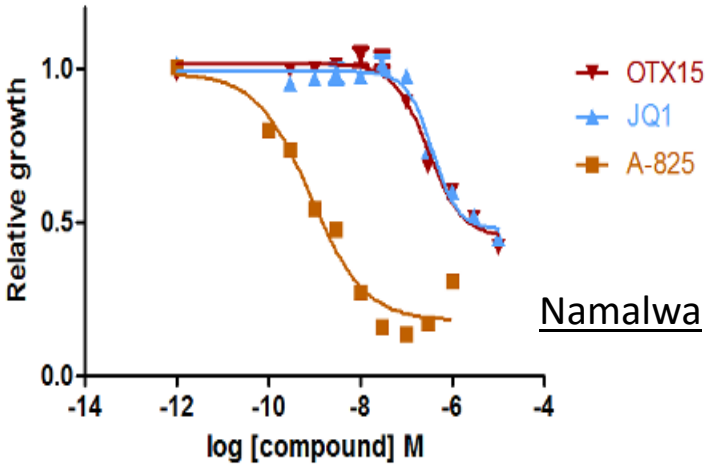


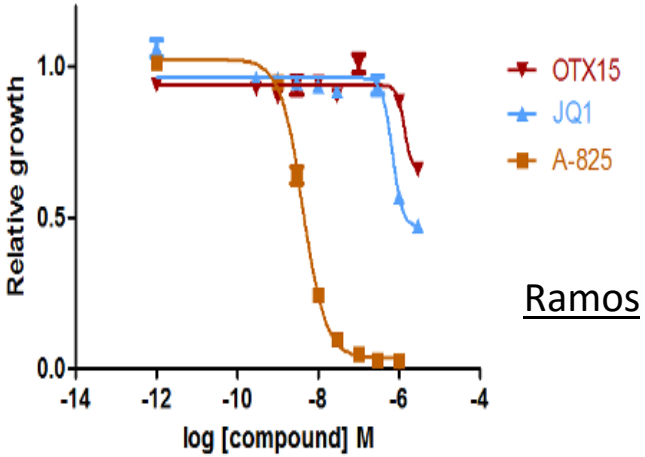
Degradation by ARV-825 Is via UPS

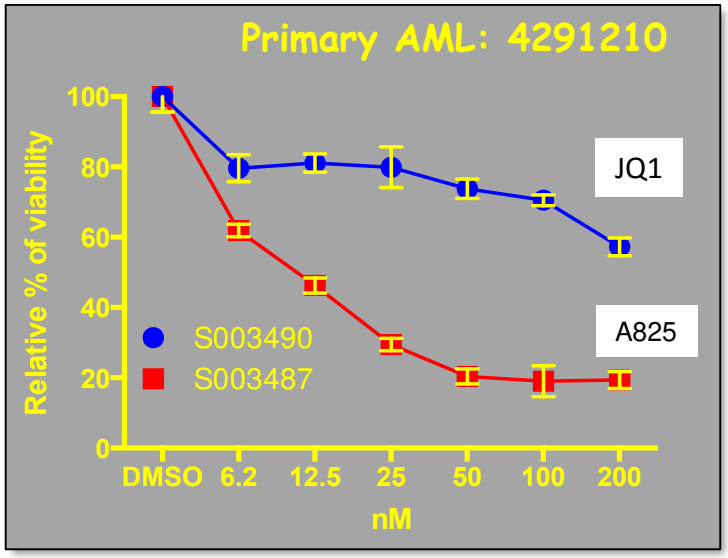
DMSO	+							
A825 (10 nM)		+		+		+		
A825 (100 nM)			+		+		+	
MG132 (5 µM)				+	+			+
Carfilzomib (5 µM)						+	+		+



BRD4 PROTACs Are Apoptotic in BL and AML Cells

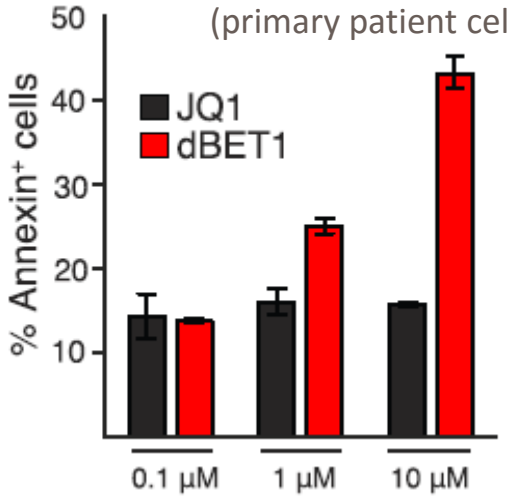






Data from Dr. Gautam Borthakur's Lab at MDACC

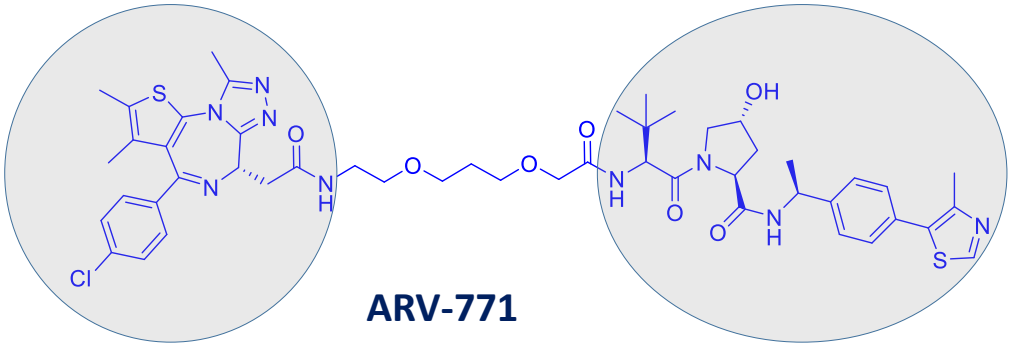
Acute Myelogenous Leukemia
(primary patient cells)



Winter et al. *Science* (2015) 348,:1376-1381

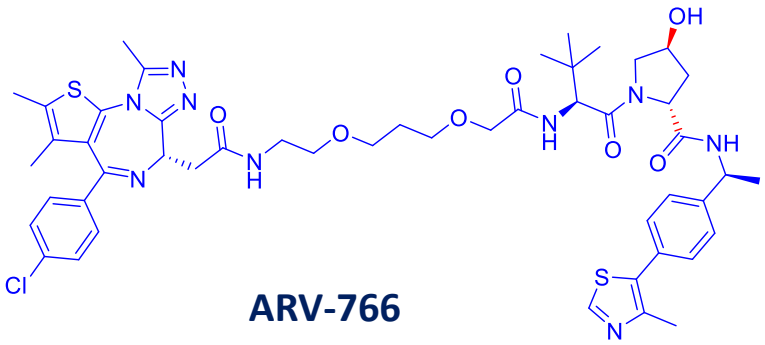
Identification of Active BRD4-VHL PROTAC

BRD4-binding moiety

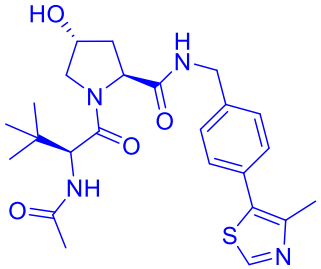


VHL-binding moiety

ARV-771



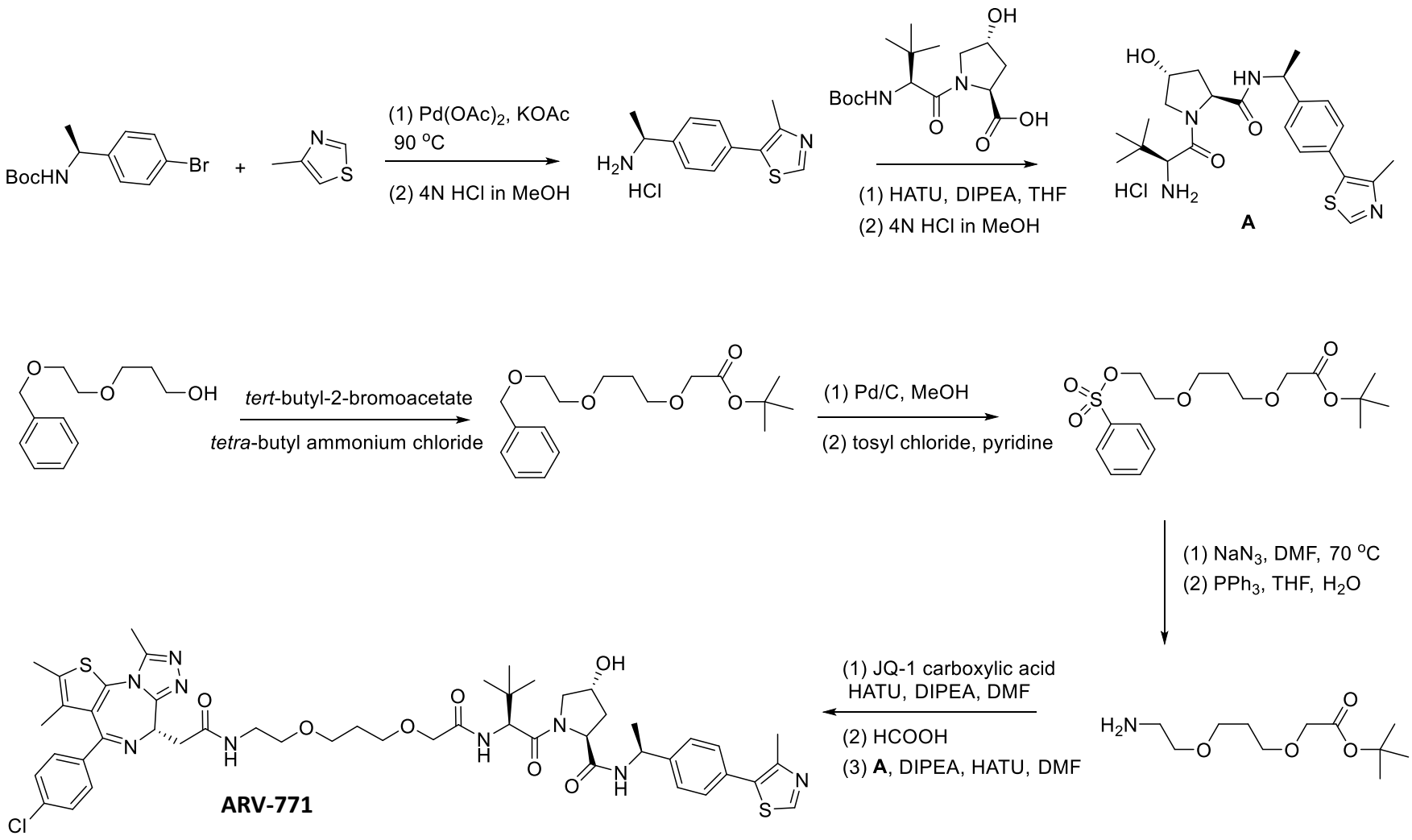
ARV-766



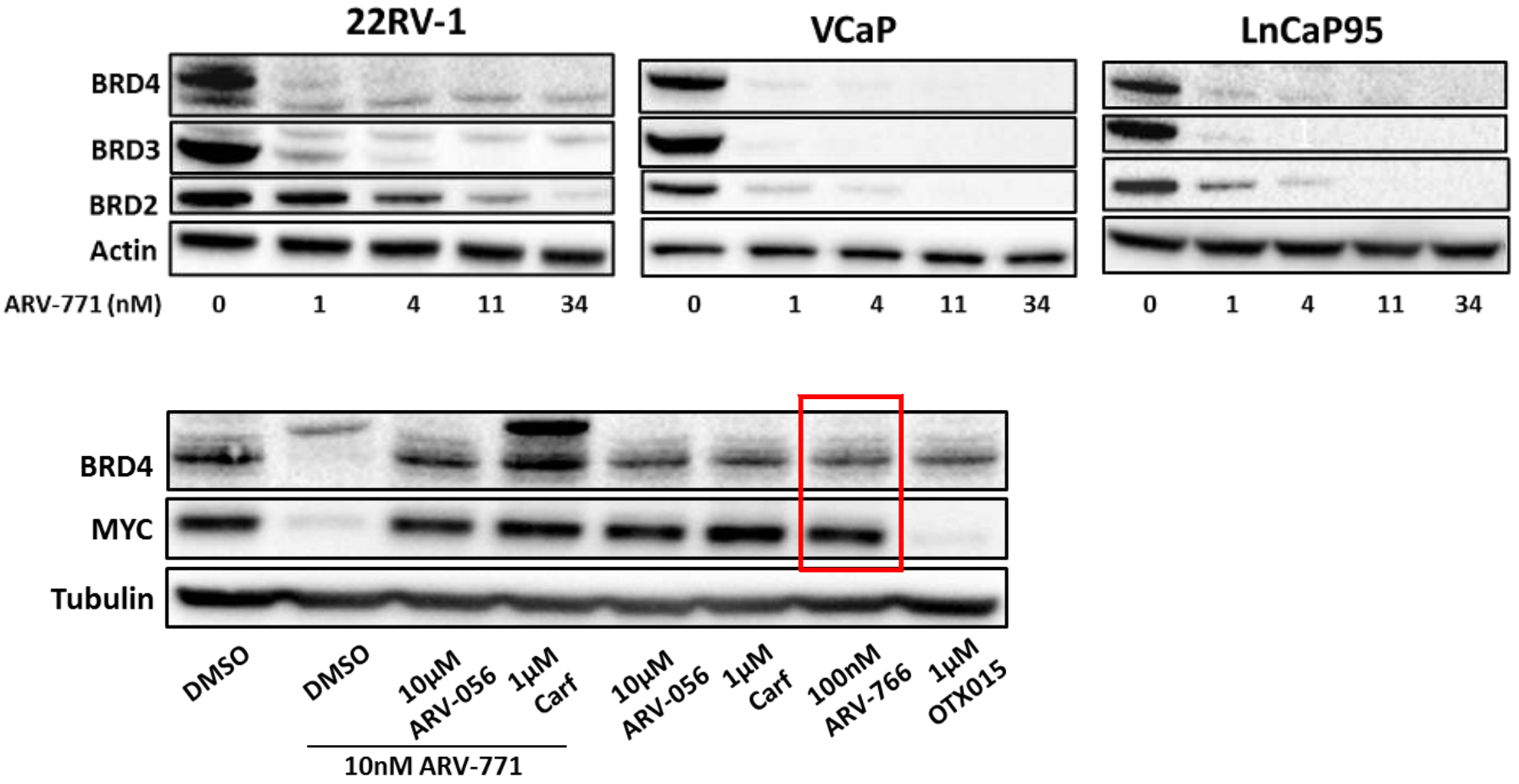
ARV-056

Molecules	cMYC ELISA IC_{50} (nM)
ARV-771	1.0
ARV-766	>1000
JQ-1	38
OTX-015	52

Synthesis of ARV-771

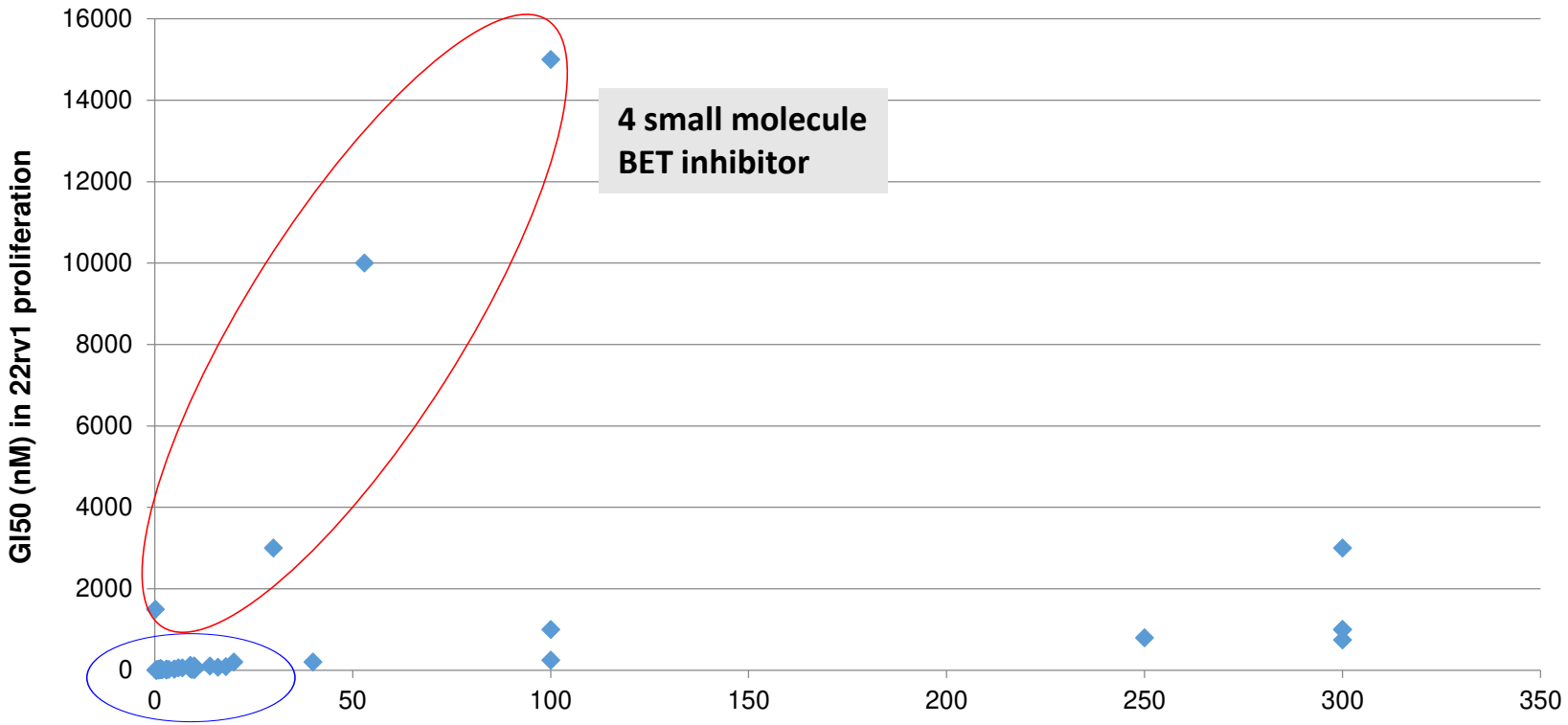


ARV-771 Degraded BRD4 in Prostate Cancer Cells



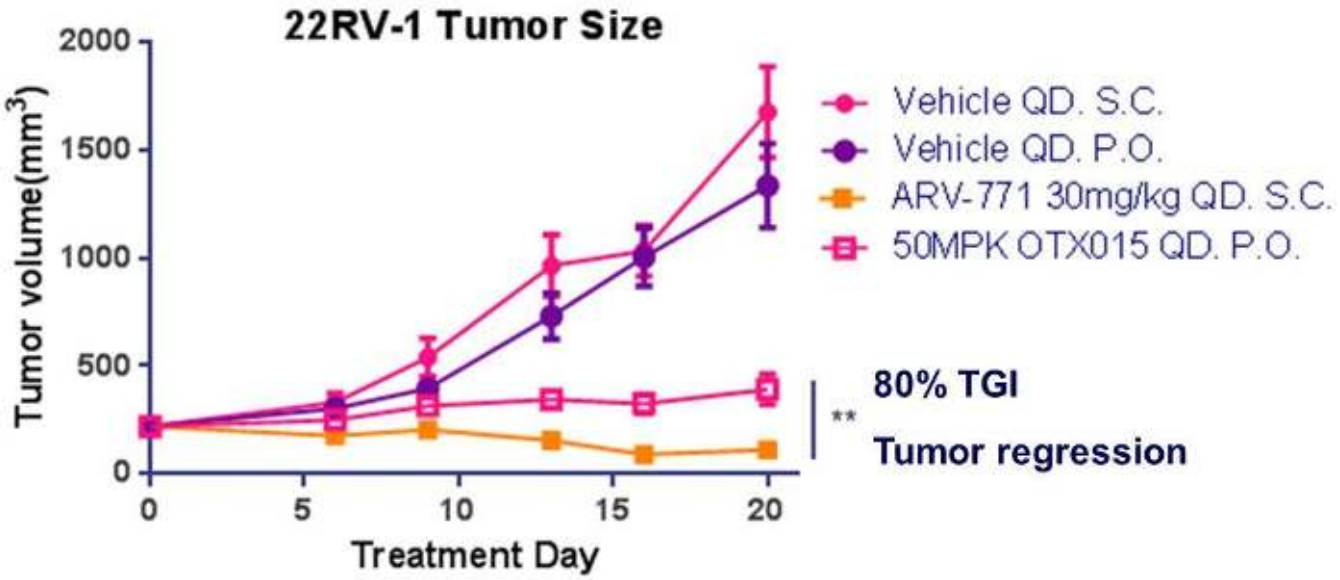
ARV-771 caused robust caspase 3/7 induction in 22rv1, VCaP and LnCaP95 prostate cancer cells

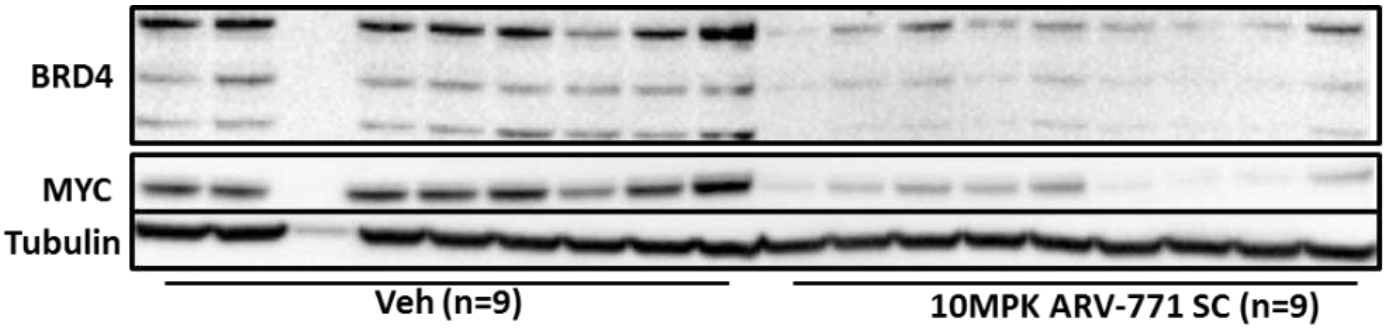
Correlation of c-Myc Suppression and Cell Growth Inhibition in 22RV1 Prostate Cancer Cells



- 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





PROTACs: Extended or Beyond Rule of 5

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- 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 $Å^2$; NRotB ≤ 20
  - Beyond Ro 5 (MW >500, with at least one of the following)
    - MW 700-3000 Da; clogP < 0 or >7.5; HBD >5; HBA >10; PSA > 200 $Å^2$; 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

J. Kihlberg, et al. J. Med. Chem. 2016, Ahead of press

- 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

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