Novartis Institutes for Biomedical Research

From Molecule to Patient: A Pharma Perspective
A New Science of Therapeutics for Undruggable Targets

Jay Bradner, M.D. | Novartis Institutes for BioMedical Research

American Society for Clinical Pharmacology & Therapeutics
Washington, D.C. | March 15, 2019

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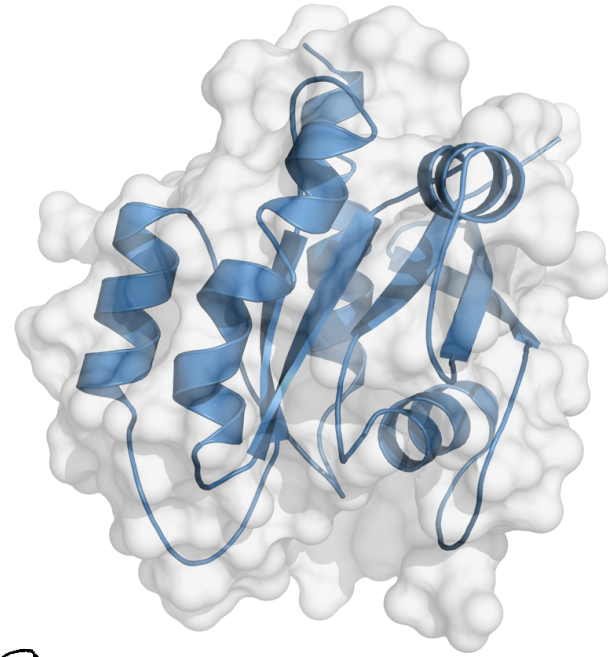
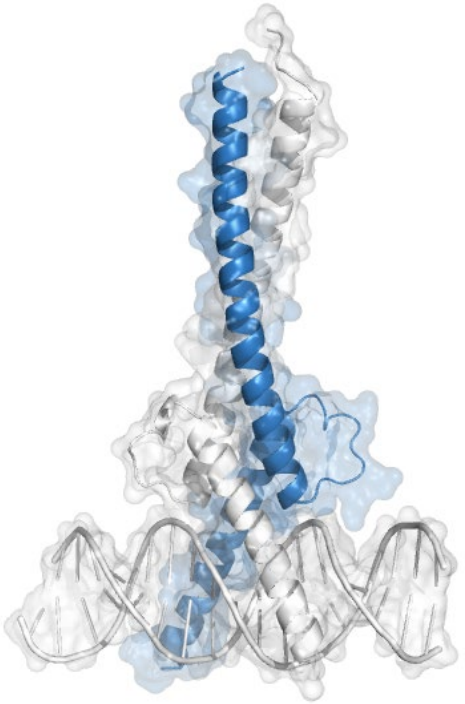
Silverstein, “The Giving Tree”

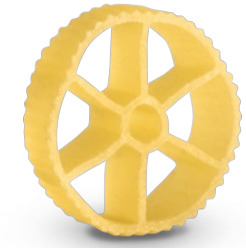


KRAS



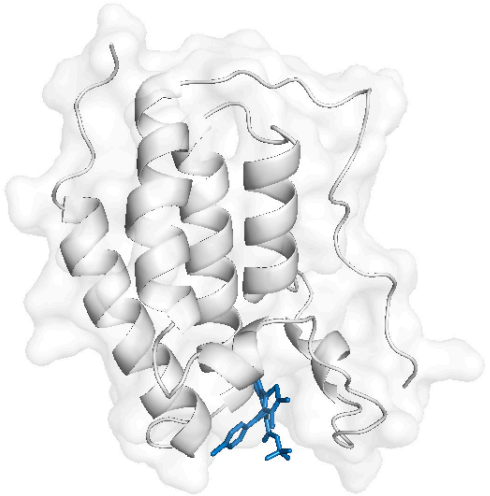
MYC

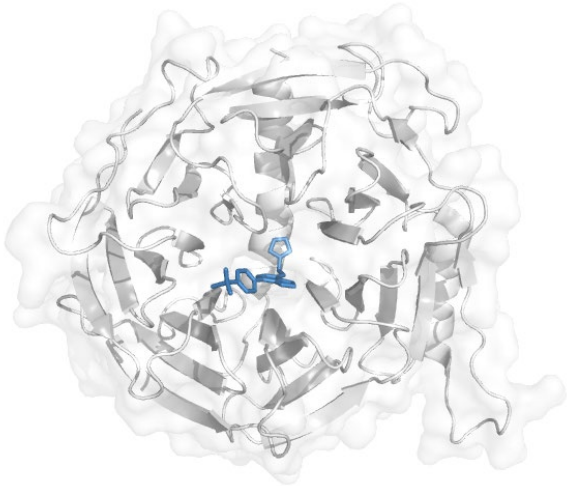
druggable

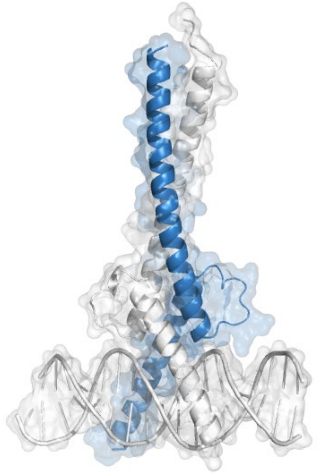


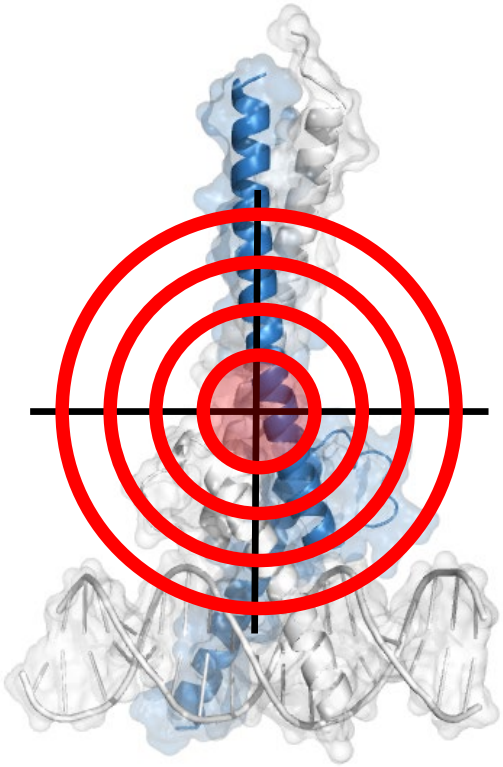
druggable

undruggable



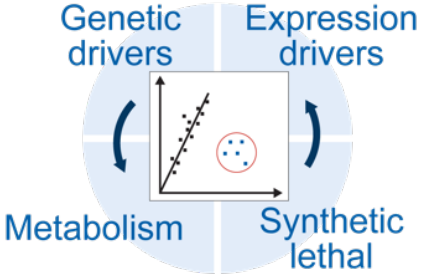






Hematologic Malignancies	Gene Regulatory Factor Alterations
Acute Promyelocytic Leukemia	RARα fusions
Myeloid Malignancies (AML, MPN, MDS)	TET2, DNMT3A, IDH1/2, ASXL1, Cohesin, PRC2
T-ALL	TAL1, NOTCH1, PRC2
B-ALL	CREBBP, CTCF
Mixed Lineage Leukemia	MLL rearrangement
Diffuse Large B-Cell Lymphoma	MYC, BCL6, ARID1A, MLL3, CREBBP, EP300
Burkitt lymphoma	MYC, ID3, SWI/SNF
Multiple Myeloma	MYC, TP53, NSD2, MAF, KDM6A, IRF4

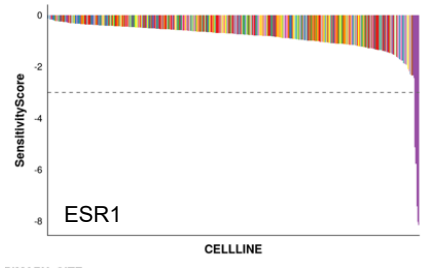
Project Drive



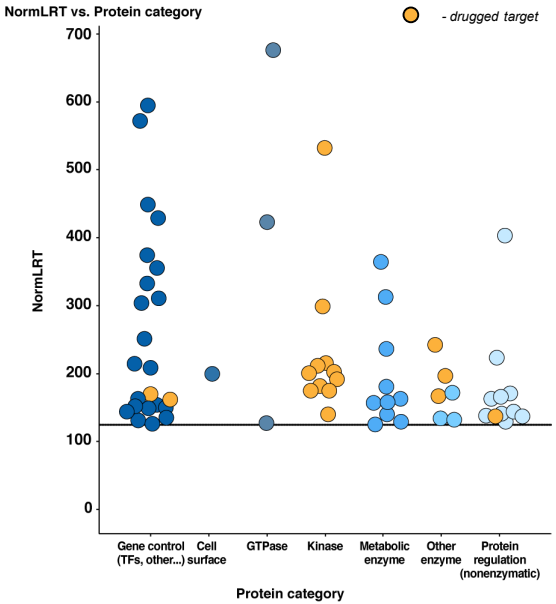
Functional Genomics Screen
400 cell lines | 20 Cancer Types
8000 genes | 20 shRNAs per gene
Mutation | Copy Number | Expression

MacDonald et.al., Cell 2017

Outlier Analysis



Undruggability



https://oncologynibr.shinyapps.io/drive/

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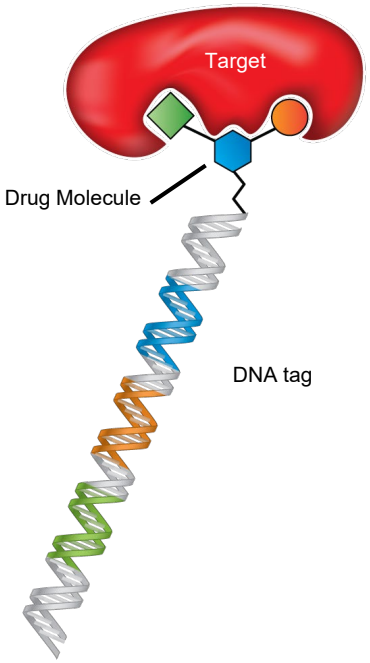
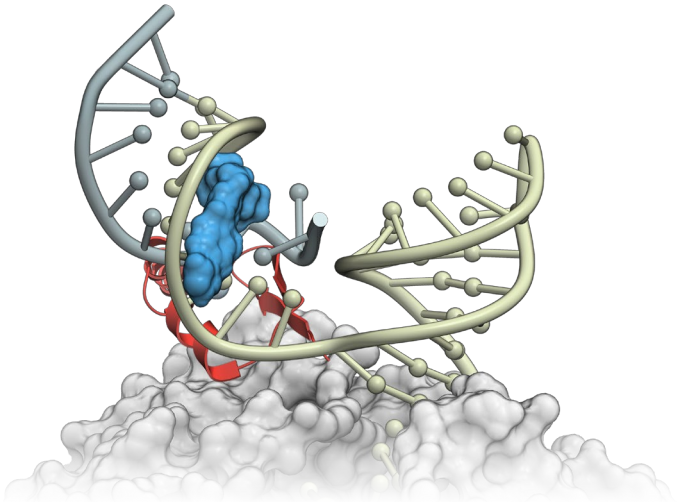
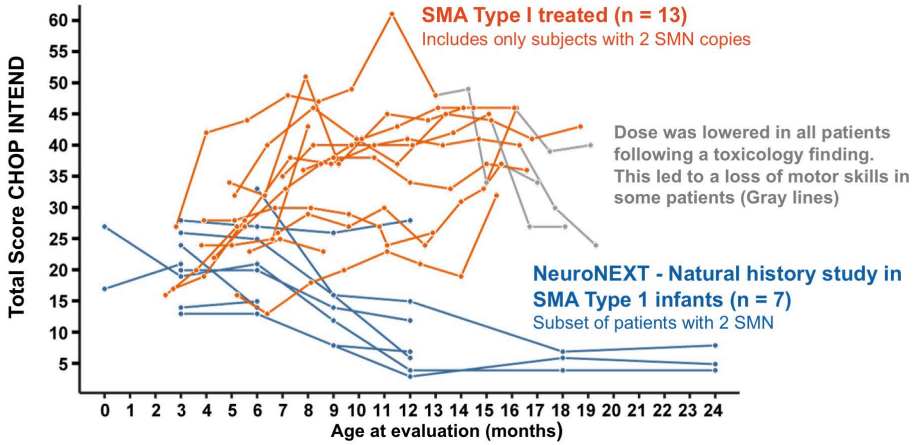
A New Science of Therapeutics

DELs | A Bigger Haystack



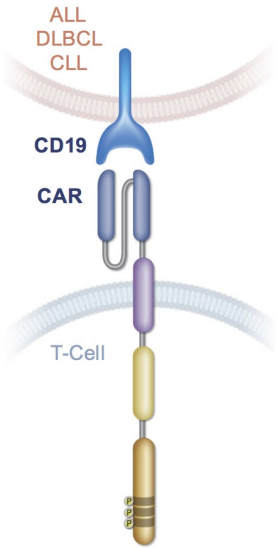
RNA Glue | LMI070 in Spinal Muscular Atrophy

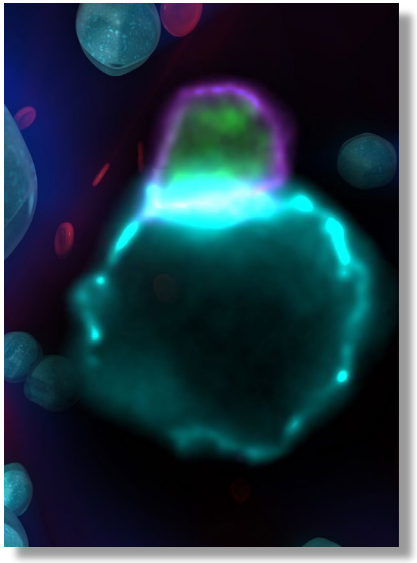


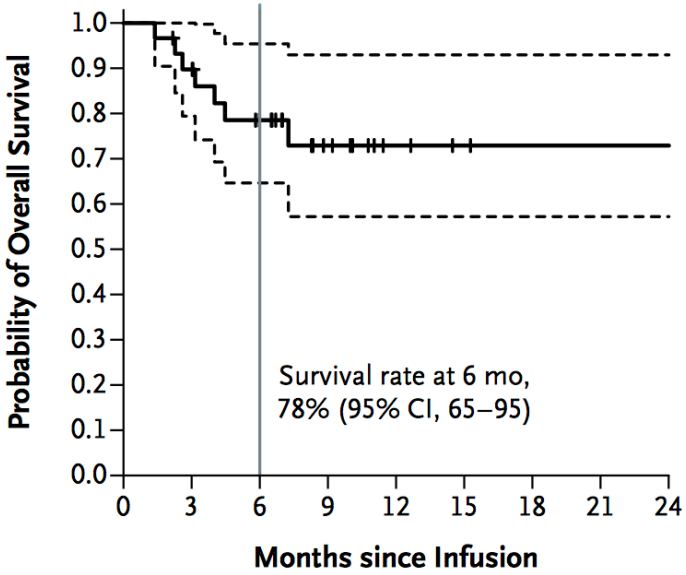


Source: Kolb et al NeuroNEXT, graciously provided prior to publication

Advanced Cell Therapy | CART

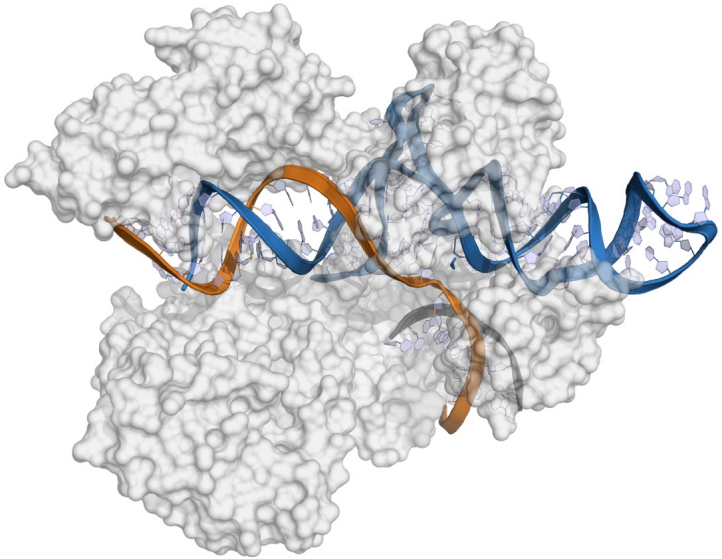


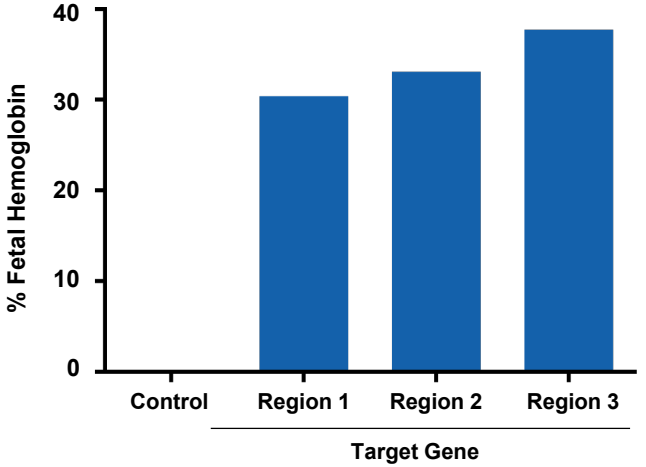




Grupp et al., NEJM 2014;371:1507-17

Advanced Cell Therapy | CRISPR





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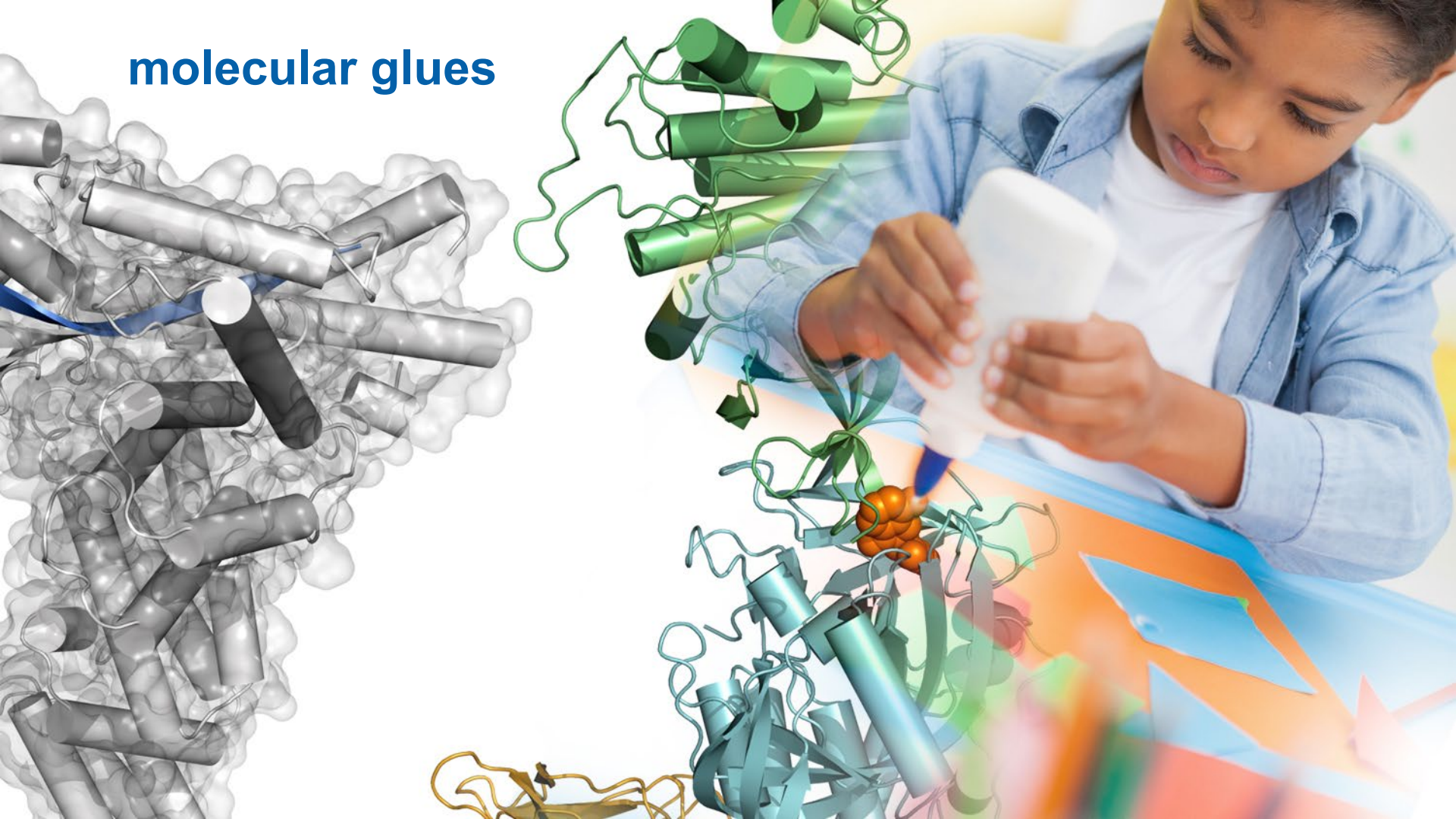
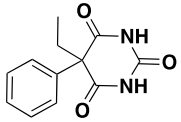
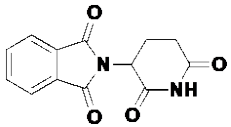
molecular glues

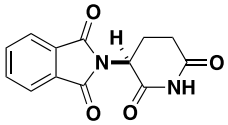




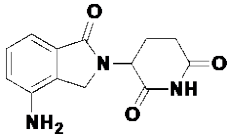
phenobarbital



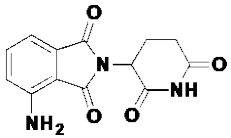
thalidomide
2006



(S)-thalidomide

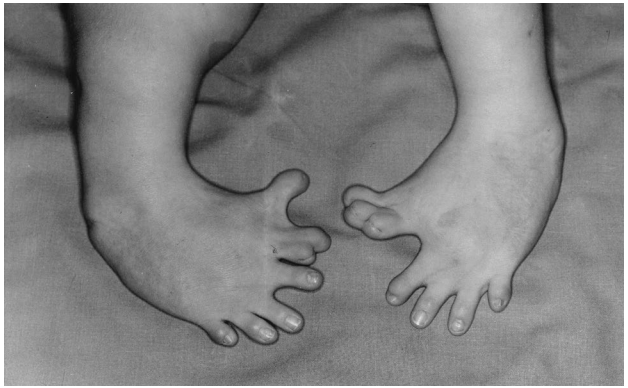


lenalidomide
(2005, 20066)



pomalidomide
(2013)

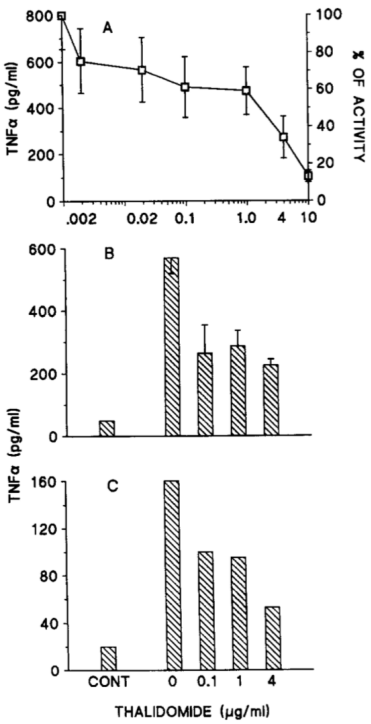
Dr. Heinrich Mückter



Rock Brynner and Trent Stephens, *Dark Remedy* 2001

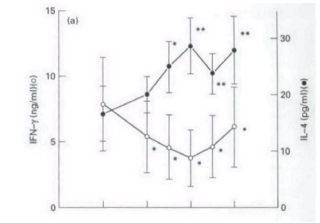
Dr. Frances Kelsey

TNF-α

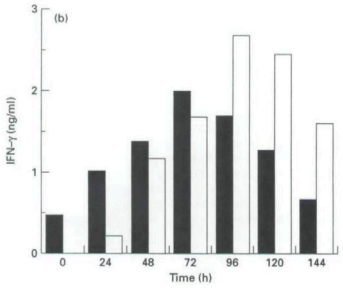


Sampaio et.al., *J.Exp.Med* 1991

IFNγ

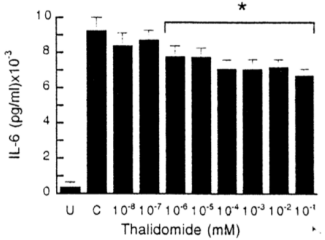


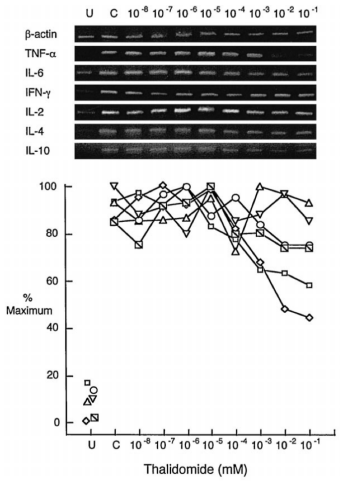
	PHA (72 h)	
	Thalidomide	
	(None)	(1000 ng/ml)
IFN-γ (ng/ml)	7·845	3·738 ($P = 0·024$)
IL-4 (pg/ml)	16·553	28·637 ($P < 0·01$)
IL-5 (pg/ml)	2·718	3·998 ($P < 0·142$)
IL-2 (U/ml)	0·45	0·71 ($P < 0·327$)



McHugh et.al., *Clin. Exp. Imm.* 1995

IL-6





Rowland et.al., *Immunopharm.* 1998

Thalidomide Therapy for Erythema Nodosum Leprosum (*M. Leprae*)

Table 2
Double Blind, Controlled Clinical Trials of Thalidomide in Patients with ENL: Cutaneous Response

Reference	No. of Patients	No. Treatment Courses*	Percent Responding**	
Iyer *et al.*[9] Bull World Health Organization 1971; 45:719	92	204	Thalidomide 75%	Aspirin 25%
Sheskin *et al.*[10] Int J Lep 1969; 37:135	52	173	Thalidomide 66%	Placebo 10%

WARNING: SEVERE, LIFE-THREATENING HUMAN BIRTH DEFECTS

IF THALIDOMIDE IS TAKEN DURING PREGNANCY, IT CAN CAUSE SEVERE BIRTH DEFECTS OR DEATH TO AN UNBORN BABY. THALIDOMIDE SHOULD NEVER BE USED BY WOMEN WHO ARE PREGNANT OR WHO COULD BECOME PREGNANT WHILE TAKING THE DRUG. EVEN A SINGLE DOSE [1 CAPSULE (50 mg)] TAKEN BY A PREGNANT WOMAN DURING HER PREGNANCY CAN CAUSE SEVERE BIRTH DEFECTS.

BECAUSE OF THIS TOXICITY AND IN AN EFFORT TO MAKE THE CHANCE OF FETAL EXPOSURE TO THALOMID AS NEGLIGIBLE AS POSSIBLE, THALOMID IS APPROVED FOR MARKETING ONLY UNDER A SPECIAL RESTRICTED DISTRIBUTION PROGRAM APPROVED BY THE FOOD AND DRUG ADMINISTRATION. THIS PROGRAM IS CALLED THE "SYSTEM FOR THALIDOMIDE EDUCATION AND PRESCRIBING SAFETY (S.T.E.P.S.)".

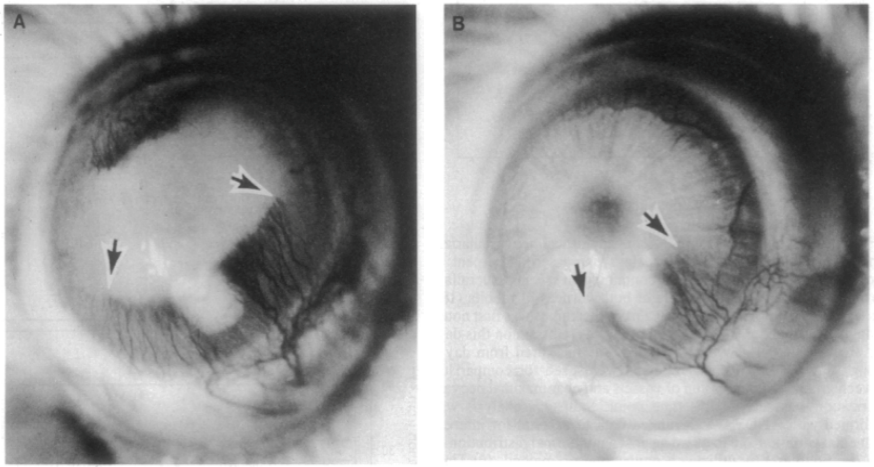
UNDER THIS RESTRICTED DISTRIBUTION PROGRAM, ONLY PRESCRIBERS AND PHARMACISTS REGISTERED WITH THE PROGRAM ARE ALLOWED TO PRESCRIBE AND DISPENSE THE PRODUCT. IN ADDITION, PATIENTS MUST BE ADVISED OF, AGREE TO, AND COMPLY WITH THE REQUIREMENTS OF THE S.T.E.P.S. PROGRAM IN ORDER TO RECEIVE PRODUCT.

PLEASE SEE THE FOLLOWING BOXED WARNINGS CONTAINING SPECIAL INFORMATION FOR PRESCRIBERS, FEMALE PATIENTS, AND MALE PATIENTS ABOUT THIS RESTRICTED DISTRIBUTION PROGRAM.

Thalidomide Package Insert

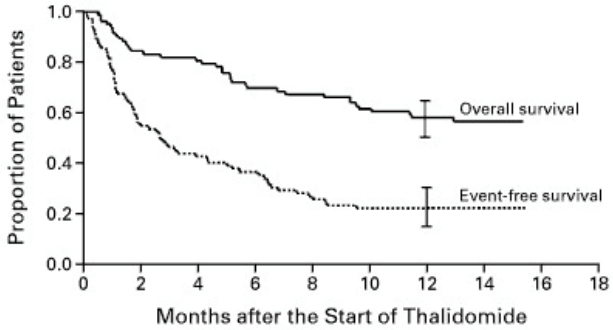
control

+ thalidomide



J. Folkman et al *PNAS* 91:4082- 4085, 1994

Antitumor Activity of Thalidomide in Refractory Multiple Myeloma

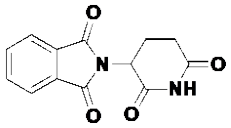


No. at Risk

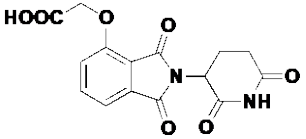
Overall survival	84	78	69	64	58	56	51	34
Event-free survival	84	65	39	32	24	19	18	11

Seema Singhal et.al., *N Engl J Med* 18 November 1999

Thalidomide Target Identification



thalidomide



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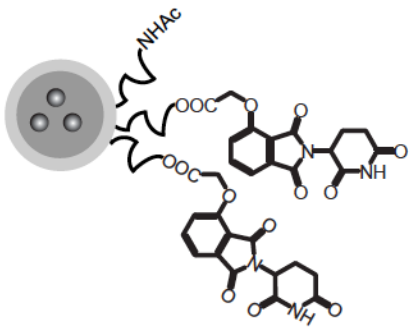
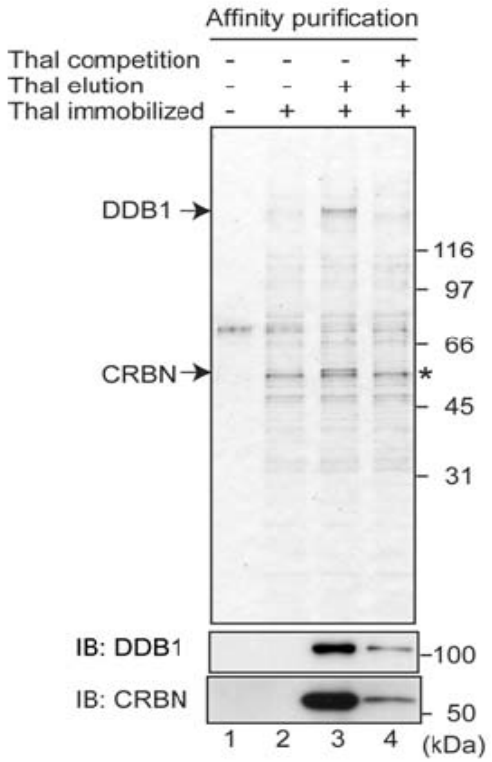
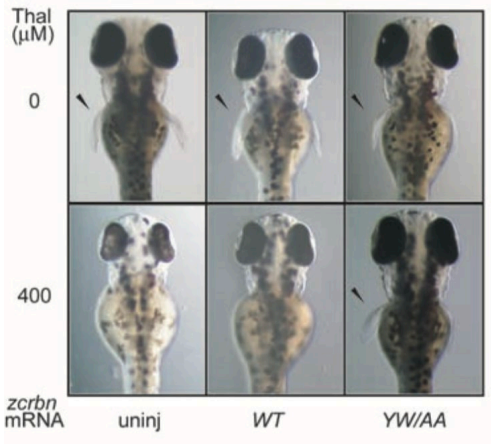
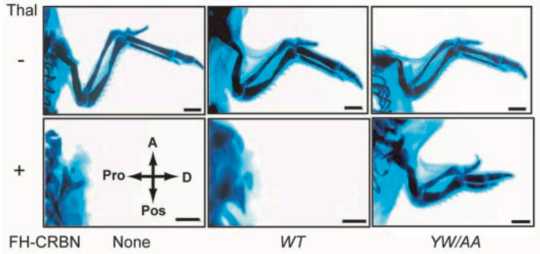


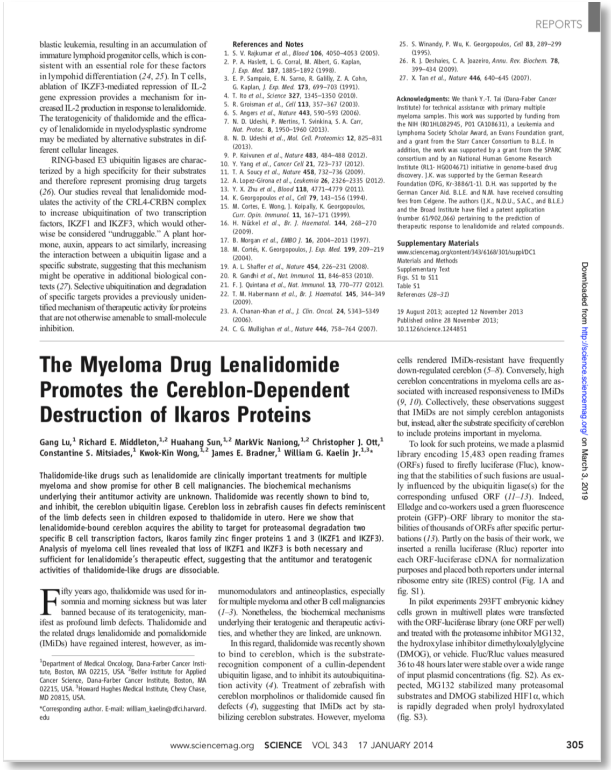






Handa and colleagues, *Science* 2010

Cereblon Substrate Identification – Myeloma



REPORTS

blastic leukemia, resulting in an accumulation of immature lymphoid progenitor cells, which is consistent with an essential role for these factors in lymphoid differentiation (*24*, *25*). In T cells, ablation of IKZF3-mediated repression of IL-2 gene expression provides a mechanism for increased IL-2 production in response to lenalidomide. The teratogenicity of thalidomide and the efficacy of lenalidomide in myelodysplastic syndrome may be mediated by alternative substrates in different cellular lineages.

RING-based E3 ubiquitin ligases are characterized by a high specificity for their substrates and therefore represent promising drug targets (*26*). Our studies reveal that lenalidomide modulates the activity of the CRL4-CRBN complex to increase ubiquitination of two transcription factors, IKZF1 and IKZF3, which would otherwise be considered "undruggable." A plant hormone, auxin, appears to act similarly, increasing the interaction between a ubiquitin ligase and a specific substrate, suggesting that this mechanism might be operative in additional biological contexts (*27*). Selective ubiquitination and degradation of specific targets provides a previously unidentified mechanism of therapeutic activity for proteins that are not otherwise amenable to small-molecule inhibition.

References and Notes

1. S. V. Rajkumar *et al.*, *Blood* **106**, 4050–4053 (2005).
2. P. A. Haslett, L. G. Corral, M. Albert, G. Kaplan, *J. Exp. Med.* **187**, 1885–1892 (1998).
3. E. P. Sampaio, E. N. Sarno, R. Galilly, Z. A. Cohn, G. Kaplan, *J. Exp. Med.* **173**, 699–703 (1991).
4. T. Ito *et al.*, *Science* **327**, 1345–1350 (2010).
5. R. Groisman *et al.*, *Cell* **113**, 357–367 (2003).
6. S. Angers *et al.*, *Nature* **443**, 590–593 (2006).
7. N. D. Udeshi, P. Mertins, T. Svinkina, S. A. Carr, *Nat. Protoc.* **8**, 1950–1960 (2013).
8. N. D. Udeshi *et al.*, *Mol. Cell. Proteomics* **12**, 825–831 (2013).
9. P. Kaivonen *et al.*, *Nature* **483**, 484–488 (2012).
10. Y. Yang *et al.*, *Cancer Cell* **21**, 723–737 (2012).
11. T. A. Soucy *et al.*, *Nature* **458**, 732–736 (2009).
12. A. Lopez-Girona *et al.*, *Leukemia* **26**, 2326–2335 (2012).
13. Y. X. Zhu *et al.*, *Blood* **118**, 4771–4779 (2011).
14. K. Georgopoulos *et al.*, *Cell* **79**, 143–156 (1994).
15. M. Cortes, E. Wong, J. Koipally, K. Georgopoulos, *Curr. Opin. Immunol.* **11**, 167–171 (1999).
16. H. Nikkel *et al.*, *Br. J. Haematol.* **144**, 268–270 (2009).
17. B. Morgan *et al.*, *EMBO J.* **16**, 2004–2013 (1997).
18. M. Cortés, K. Georgopoulos, *J. Exp. Med.* **199**, 209–219 (2004).
19. A. L. Shaffer *et al.*, *Nature* **454**, 226–231 (2008).
20. R. Gandhi *et al.*, *Nat. Immunol.* **11**, 846–853 (2010).
21. F. J. Quintana *et al.*, *Nat. Immunol.* **13**, 770–777 (2012).
22. T. M. Habermann *et al.*, *Br. J. Haematol.* **145**, 344–349 (2009).
23. A. Chanan-Khan *et al.*, *J. Clin. Oncol.* **24**, 5343–5349 (2006).
24. C. G. Mullighan *et al.*, *Nature* **446**, 758–764 (2007).
25. S. Winandy, P. Wu, K. Georgopoulos, *Cell* **83**, 289–299 (1995).
26. R. J. Deshaies, C. A. Joazeiro, *Annu. Rev. Biochem.* **78**, 399–434 (2009).
27. X. Tan *et al.*, *Nature* **446**, 640–645 (2007).

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Supplementary Materials

www.sciencemag.org/content/343/6168/301/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S11
Table S1
References (*28*–*32*)

19 August 2013; accepted 12 November 2013
Published online 28 November 2013;
10.1126/science.1244851

The Myeloma Drug Lenalidomide Promotes the Cereblon-Dependent Destruction of Ikaros Proteins

Gang Lu,[1] Richard E. Middleton,[1,2] Huahang Sun,[1,2] MarkVic Naniong,[1,2] Christopher J. Ott,[1] Constantine S. Mitsiades,[1] Kwok-Kin Wong,[1,2] James E. Bradner,[1] William G. Kaelin Jr.[1,3,*]

Thalidomide-like drugs such as lenalidomide are clinically important treatments for multiple myeloma and show promise for other B cell malignancies. The biochemical mechanisms underlying their antitumor activity are unknown. Thalidomide was recently shown to bind to, and inhibit, the cereblon ubiquitin ligase. Cereblon loss in zebrafish causes fin defects reminiscent of the limb defects seen in children exposed to thalidomide in utero. Here we show that lenalidomide-bound cereblon acquires the ability to target for proteasomal degradation two specific B cell transcription factors, Ikaros family zinc finger proteins 1 and 3 (IKZF1 and IKZF3). Analysis of myeloma cell lines revealed that loss of IKZF1 and IKZF3 is both necessary and sufficient for lenalidomide's therapeutic effect, suggesting that the antitumor and teratogenic activities of thalidomide-like drugs are dissociable.

Fifty years ago, thalidomide was used for insomnia and morning sickness but was later banned because of its teratogenicity, manifest as profound limb defects. Thalidomide and the related drugs lenalidomide and pomalidomide (IMiDs) have regained interest, however, as immunomodulators and antineoplastics, especially for multiple myeloma and other B cell malignancies (*1*–*3*). Nonetheless, the biochemical mechanisms underlying their teratogenic and therapeutic activities, and whether they are linked, are unknown.

In this regard, thalidomide was recently shown to bind to cereblon, which is the substrate-recognition component of a cullin-dependent ubiquitin ligase, and to inhibit its autoubiquitination activity (*4*). Treatment of zebrafish with cereblon morpholinos or thalidomide caused fin defects (*4*), suggesting that IMiDs act by stabilizing cereblon substrates. However, myeloma cells rendered IMiDs-resistant have frequently down-regulated cereblon (*5*–*8*). Conversely, high cereblon concentrations in myeloma cells are associated with increased responsiveness to IMiDs (*9*, *10*). Collectively, these observations suggest that IMiDs are not simply cereblon antagonists but, instead, alter the substrate specificity of cereblon to include proteins important in myeloma.

To look for such proteins, we made a plasmid library encoding 15,483 open reading frames (ORFs) fused to firefly luciferase (Fluc), knowing that the stabilities of such fusions are usually influenced by the ubiquitin ligase(s) for the corresponding unfused ORF (*11*–*13*). Indeed, Elledge and co-workers used a green fluorescence protein (GFP)–ORF library to monitor the stabilities of thousands of ORFs after specific perturbations (*13*). Partly on the basis of their work, we inserted a renilla luciferase (Rluc) reporter into each ORF-luciferase cDNA for normalization purposes and placed both reporters under internal ribosome entry site (IRES) control (Fig. 1A and fig. S1).

In pilot experiments 293FT embryonic kidney cells grown in multiwell plates were transfected with the ORF-luciferase library (one ORF per well) and treated with the proteasome inhibitor MG132, the hydroxylase inhibitor dimethyloxalylglycine (DMOG), or vehicle. Fluc/Rluc values measured 36 to 48 hours later were stable over a wide range of input plasmid concentrations (fig. S2). As expected, MG132 stabilized many proteasomal substrates and DMOG stabilized HIF1α, which is rapidly degraded when prolyl hydroxylated (fig. S3).

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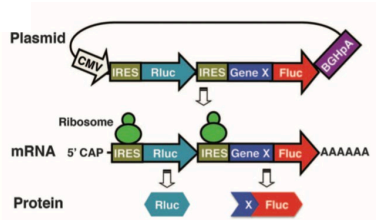
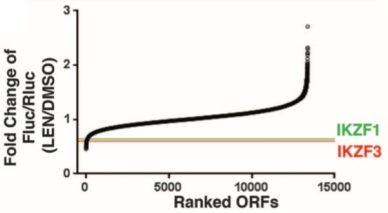
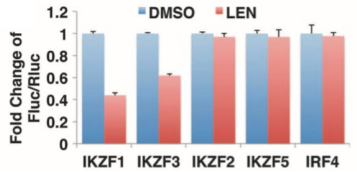
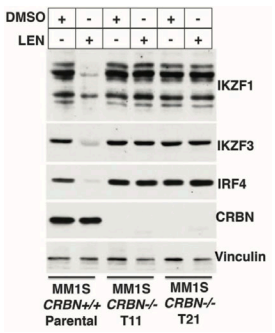
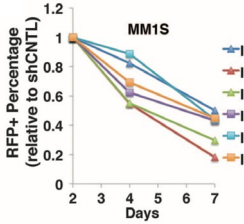
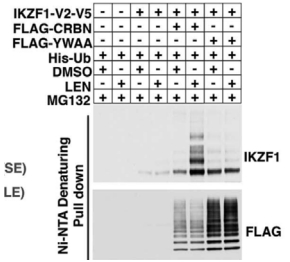


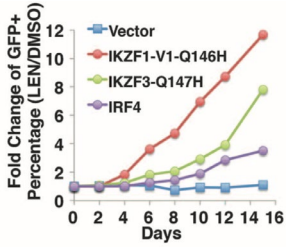






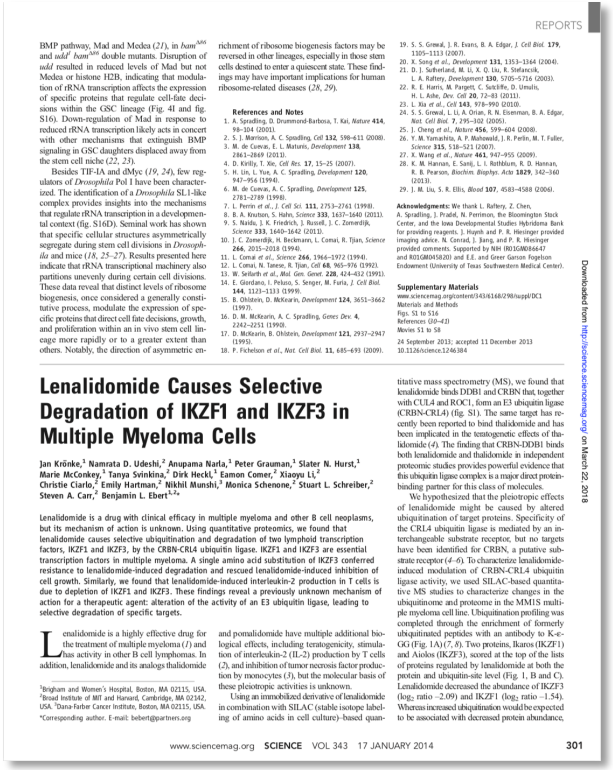






With B. Kaelin, *Science* 2014

Cereblon Substrate Identification – Myeloma



REPORTS

BMP pathway, Mad and Medea (*21*), in *bam*$^{\Delta 86}$ and *add*^{1} *bam*$^{\Delta 86}$ double mutants. Disruption of *add*^{1} resulted in reduced levels of Mad but not Medea or histone H2B, indicating that modulation of rRNA transcription affects the expression of specific proteins that regulate cell-fate decisions within the GSC lineage (Fig. 4I and fig. S16). Down-regulation of Mad in response to reduced rRNA transcription likely acts in concert with other mechanisms that extinguish BMP signaling in GSC daughters displaced away from the stem cell niche (*22*, *23*).

Besides TIF-IA and dMyc (*19*, *24*), few regulators of *Drosophila* Pol I have been characterized. The identification of a *Drosophila* SL1-like complex provides insights into the mechanisms that regulate rRNA transcription in a developmental context (fig. S16D). Seminal work has shown that specific cellular structures asymmetrically segregate during stem cell divisions in *Drosophila* and mice (*18*, *25*–*27*). Results presented here indicate that rRNA transcriptional machinery also partitions unevenly during certain cell divisions. These data reveal that distinct levels of ribosome biogenesis, once considered a generally constitutive process, modulate the expression of specific proteins that direct cell fate decisions, growth, and proliferation within an in vivo stem cell lineage more rapidly or to a greater extent than others. Notably, the direction of asymmetric enrichment of ribosome biogenesis factors may be reversed in other lineages, especially in those stem cells destined to enter a quiescent state. These findings may have important implications for human ribosome-related diseases (*28*, *29*).

References and Notes

1. A. Spradling, D. Drummond-Barbosa, T. Kai, *Nature* **414**, 98–104 (2001).
2. S. J. Morrison, A. C. Spradling, *Cell* **132**, 598–611 (2008).
3. M. de Cuevas, E. L. Matunis, *Development* **138**, 2861–2869 (2011).
4. D. Kirilly, T. Xie, *Cell Res.* **17**, 15–25 (2007).
5. H. Lin, L. Yue, A. C. Spradling, *Development* **120**, 947–956 (1994).
6. M. de Cuevas, A. C. Spradling, *Development* **125**, 2781–2789 (1998).
7. L. Perrin *et al.*, *J. Cell Sci.* **111**, 2753–2761 (1998).
8. B. A. Knutson, S. Hahn, *Science* **333**, 1637–1640 (2011).
9. S. Naidu, J. K. Friedrich, J. Russell, J. C. Zomerdijk, *Science* **333**, 1640–1642 (2011).
10. J. C. Zomerdijk, H. Beckmann, L. Comai, R. Tjian, *Science* **266**, 2015–2018 (1994).
11. L. Comai *et al.*, *Science* **266**, 1966–1972 (1994).
12. L. Comai, N. Tanese, R. Tjian, *Cell* **68**, 965–976 (1992).
13. W. Seiberth *et al.*, *Mol. Gen. Genet.* **228**, 424–432 (1991).
14. E. Giordano, J. Peluso, S. Senger, M. Furia, *J. Cell Biol.* **144**, 1123–1133 (1999).
15. B. Ohstein, D. McKearin, *Development* **124**, 3651–3662 (1997).
16. D. M. McKearin, A. C. Spradling, *Genes Dev.* **4**, 2242–2251 (1990).
17. D. McKearin, B. Ohstein, *Development* **121**, 2937–2947 (1995).
18. P. Fichelson *et al.*, *Nat. Cell Biol.* **11**, 685–693 (2009).
19. S. S. Grewal, J. R. Evans, B. A. Edgar, *J. Cell Biol.* **179**, 1305–1313 (2007).
20. X. Song *et al.*, *Development* **131**, 1353–1364 (2004).
21. D. J. Sutherland, M. Li, X. Q. Liu, R. Stefanyck, L. A. Raftery, *Development* **130**, 5705–5716 (2003).
22. R. E. Harris, M. Pargett, C. Sutcliffe, D. Umulis, H. L. Ashe, *Dev. Cell* **20**, 72–83 (2011).
23. L. Xia *et al.*, *Cell* **143**, 978–990 (2010).
24. S. S. Grewal, L. Li, A. Orian, R. N. Eisenman, B. A. Edgar, *Nat. Cell Biol.* **7**, 295–302 (2005).
25. J. Cheng *et al.*, *Nature* **456**, 599–604 (2008).
26. Y. M. Yamashita, A. P. Mahowald, J. R. Perlin, M. T. Fuller, *Science* **315**, 518–521 (2007).
27. X. Wang *et al.*, *Nature* **461**, 947–955 (2009).
28. K. M. Hannan, E. Sanij, L. I. Rothblum, R. D. Hannan, R. B. Pearson, *Biochim. Biophys. Acta* **1829**, 342–360 (2013).
29. J. M. Liu, S. R. Ellis, *Blood* **107**, 4583–4588 (2006).

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Supplementary Materials

www.sciencemag.org/content/343/6168/298/suppl/DC1
Materials and Methods
Figs. S1 to S16
References (*10*–*42*)
Movies S1 to S8

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Lenalidomide Causes Selective Degradation of IKZF1 and IKZF3 in Multiple Myeloma Cells

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Lenalidomide is a drug with clinical efficacy in multiple myeloma and other B cell neoplasms, but its mechanism of action is unknown. Using quantitative proteomics, we found that lenalidomide causes selective ubiquitination and degradation of two lymphoid transcription factors, IKZF1 and IKZF3, by the CRBN-CRL4 ubiquitin ligase. IKZF1 and IKZF3 are essential transcription factors in multiple myeloma. A single amino acid substitution of IKZF3 conferred resistance to lenalidomide-induced degradation and rescued lenalidomide-induced inhibition of cell growth. Similarly, we found that lenalidomide-induced interleukin-2 production in T cells is due to depletion of IKZF1 and IKZF3. These findings reveal a previously unknown mechanism of action for a therapeutic agent: alteration of the activity of an E3 ubiquitin ligase, leading to selective degradation of specific targets.

Lenalidomide is a highly effective drug for the treatment of multiple myeloma (*1*) and has activity in other B cell lymphomas. In addition, lenalidomide and its analogs thalidomide and pomalidomide have multiple additional biological effects, including teratogenicity, stimulation of interleukin-2 (IL-2) production by T cells (*2*), and inhibition of tumor necrosis factor production by monocytes (*3*), but the molecular basis of these pleiotropic activities is unknown.

Using an immobilized derivative of lenalidomide in combination with SILAC (stable isotope labeling of amino acids in cell culture)–based quantitative mass spectrometry (MS), we found that lenalidomide binds DDB1 and CRBN that, together with CUL4 and ROC1, form an E3 ubiquitin ligase (CRBN-CRL4) (fig. S1). The same target has recently been reported to bind thalidomide and has been implicated in the teratogenetic effects of thalidomide (*4*). The finding that CRBN-DDB1 binds both lenalidomide and thalidomide in independent proteomic studies provides powerful evidence that this ubiquitin ligase complex is a major direct protein-binding partner for this class of molecules.

We hypothesized that the pleiotropic effects of lenalidomide might be caused by altered ubiquitination of target proteins. Specificity of the CRL4 ubiquitin ligase is mediated by an interchangeable substrate receptor, but no targets have been identified for CRBN, a putative substrate receptor (*4*–*6*). To characterize lenalidomide-induced modulation of CRBN-CRL4 ubiquitin ligase activity, we used SILAC-based quantitative MS studies to characterize changes in the ubiquitinome and proteome in the MM1S multiple myeloma cell line. Ubiquitination profiling was completed through the enrichment of formerly ubiquitinated peptides with an antibody to K-ε-GG (Fig. 1A) (*7*, *8*). Two proteins, Ikaros (IKZF1) and Aiolos (IKZF3), scored at the top of the lists of proteins regulated by lenalidomide at both the protein and ubiquitin-site level (Fig. 1, B and C). Lenalidomide decreased the abundance of IKZF3 ($\log_2$ ratio −2.09) and IKZF1 ($\log_2$ ratio −1.54). Whereas increased ubiquitination would be expected to be associated with decreased protein abundance,

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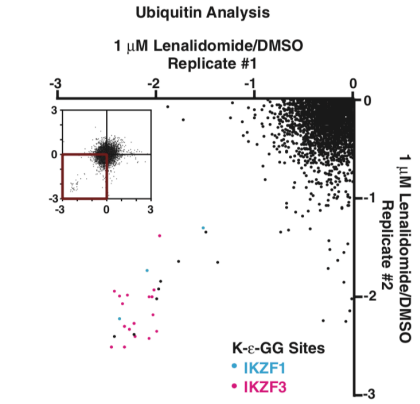
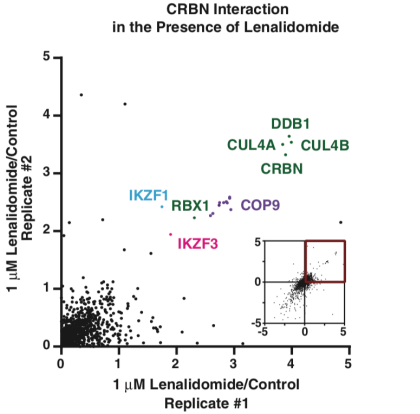
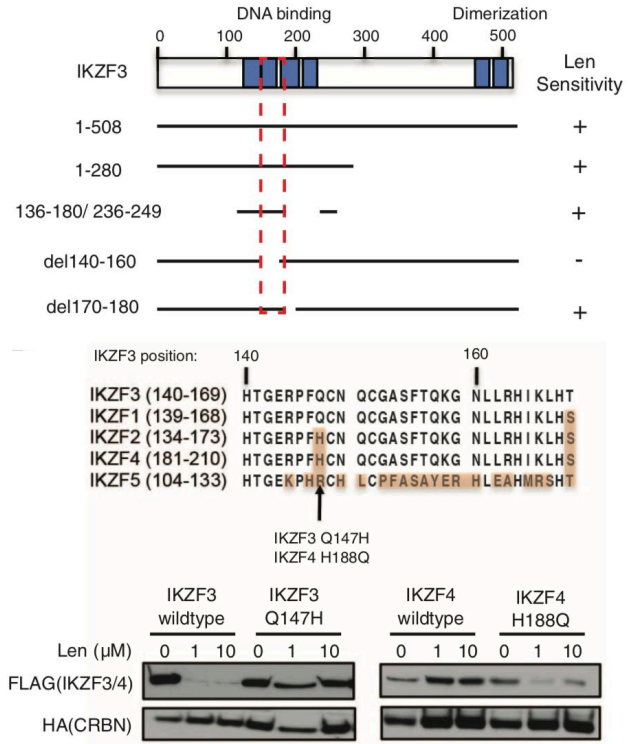
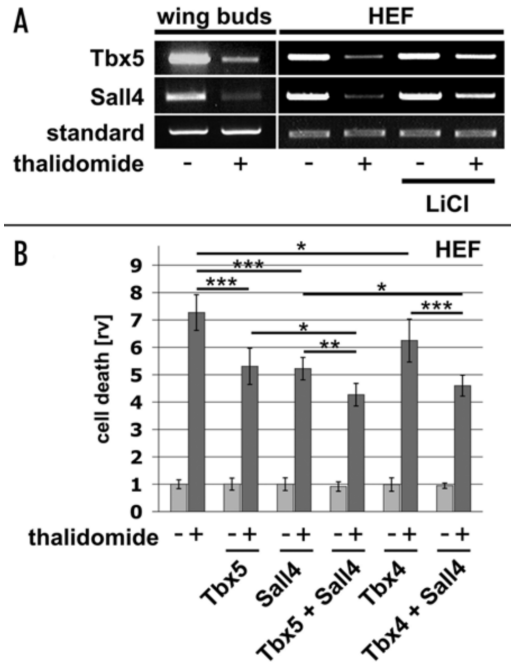




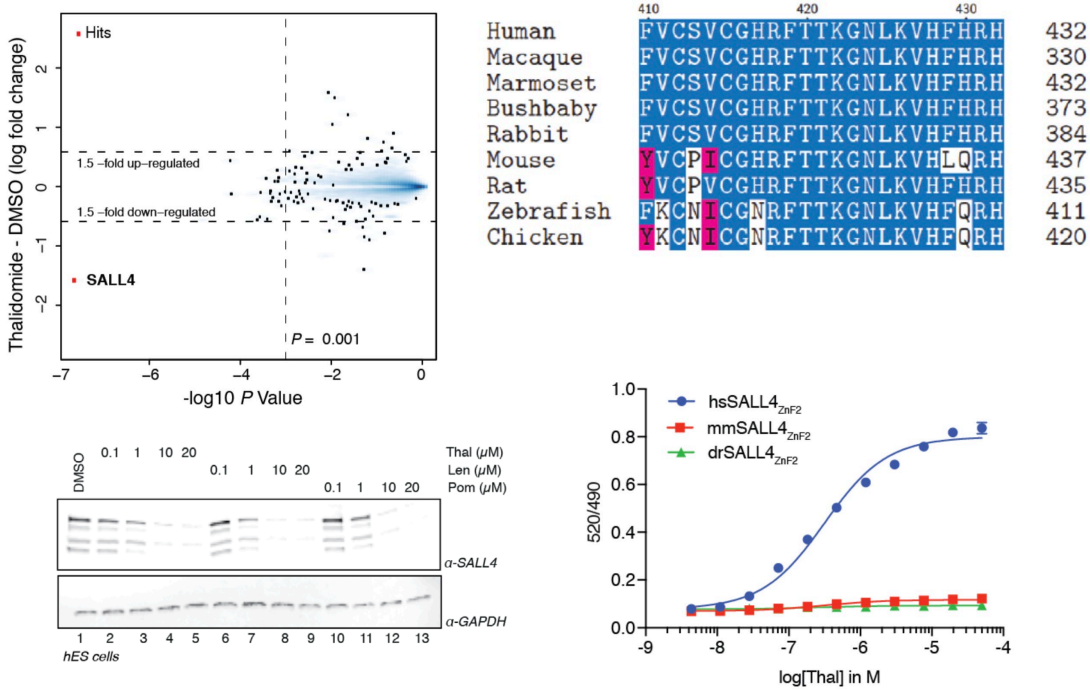


B. Ebert, *Science* 2014

Cereblon Substrate Identification – Phocomelia



Knobloch and Ruther, *Cell Cycle* 2008

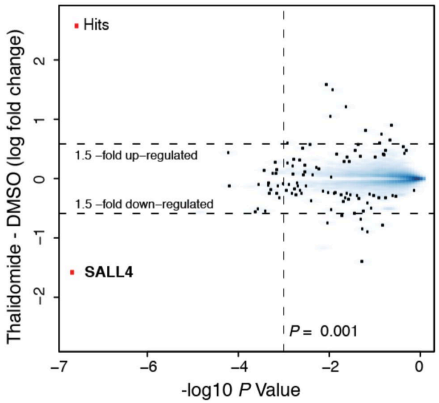


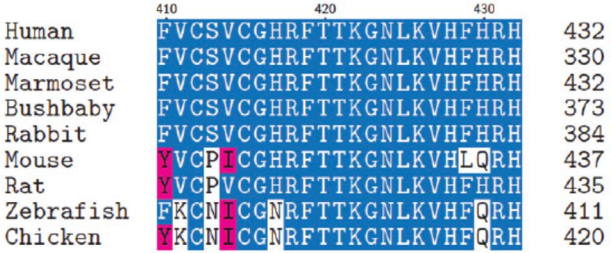
E. Fischer, *eLife* 2018

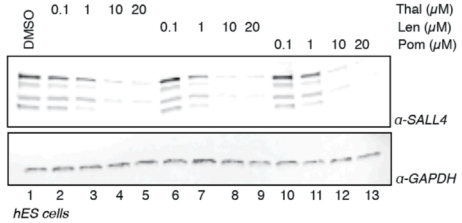
Cereblon Substrate Identification – Phocomelia

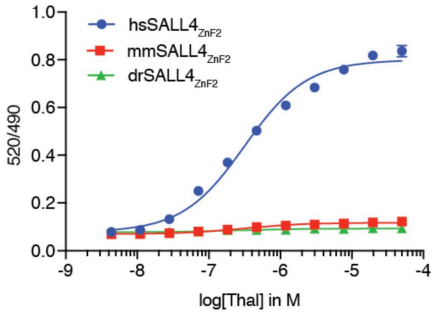
Table 1. Common phenotypes in thalidomide syndrome, Duane Radial Ray syndrome, and Holt-Oram syndrome

	Thalidomide Syndrome	Duane Radial Ray Syndrome	Holt-Oram Syndrome
Upper limbs			
	Thumbs Radius Humerus Ulna Fingers	Thumbs Radius Humerus Ulna Fingers	Thumbs Radius Humerus Ulna Fingers
Lower limbs			
	Mostly normal lower limbs Talipes dislocation Hip dislocation Shortening of long bones	Mostly normal lower limbs Talipes dislocation	
Ears			
	Absence or abnormal pinnae Deafness Microtia	Abnormal pinnae Deafness	
Eyes			
	Colobomata Microphthalmos Abduction of the eye Duane anomaly	Colobomata Microphthalmos Abduction of the eye Duane anomaly	
Stature			
	Short stature	Postnatal growth retardation	
Heart			
	Ventricular septal defects Atrial septal defects Pulmonary stenosis	Ventricular septal defects Atrial septal defects	Ventricular septal defects Atrial septal defects

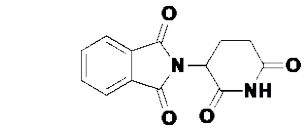




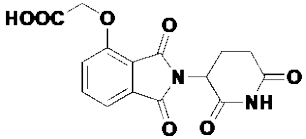




E. Fischer, *eLife* 2018



thalidomide



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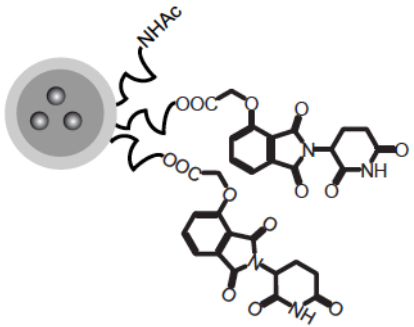
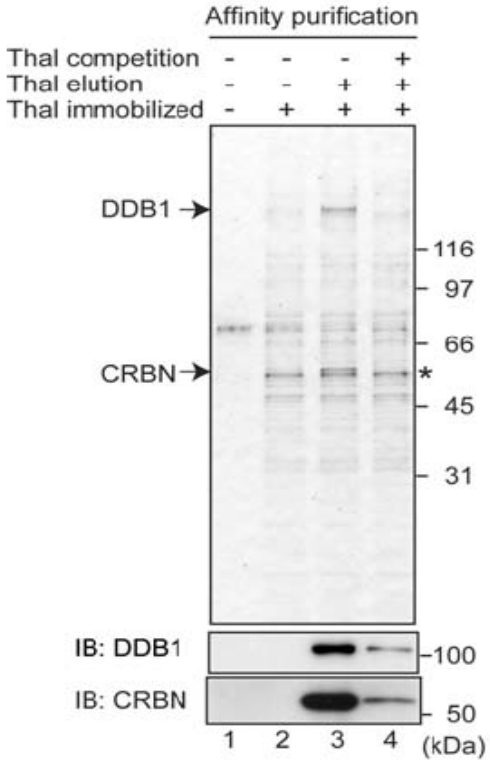
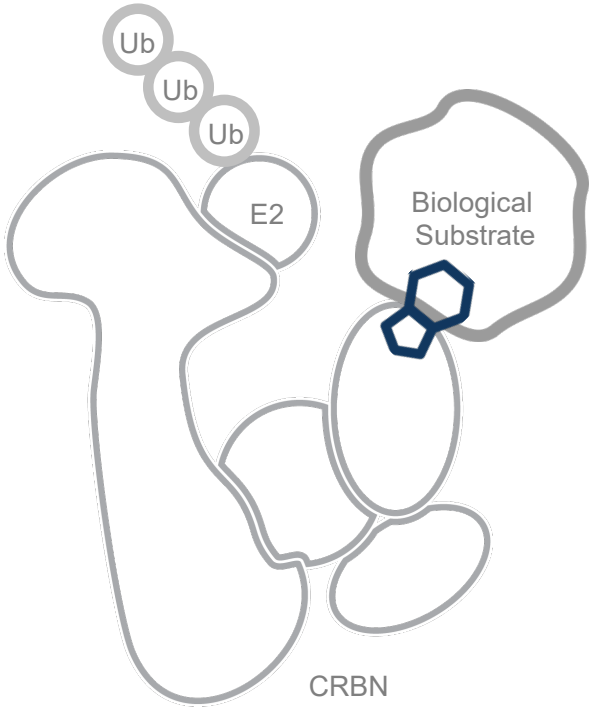
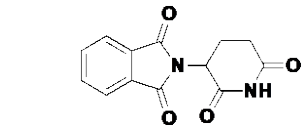
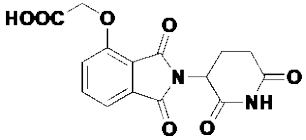


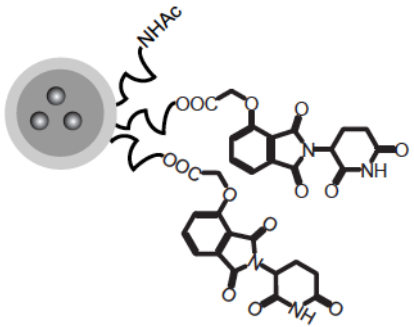


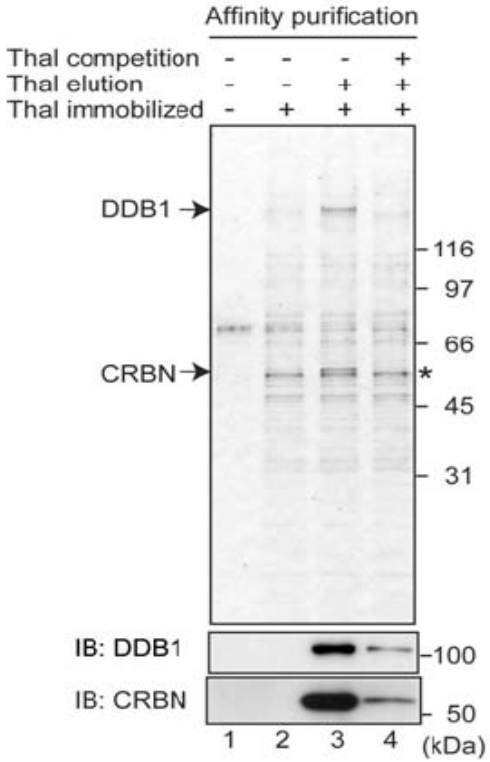


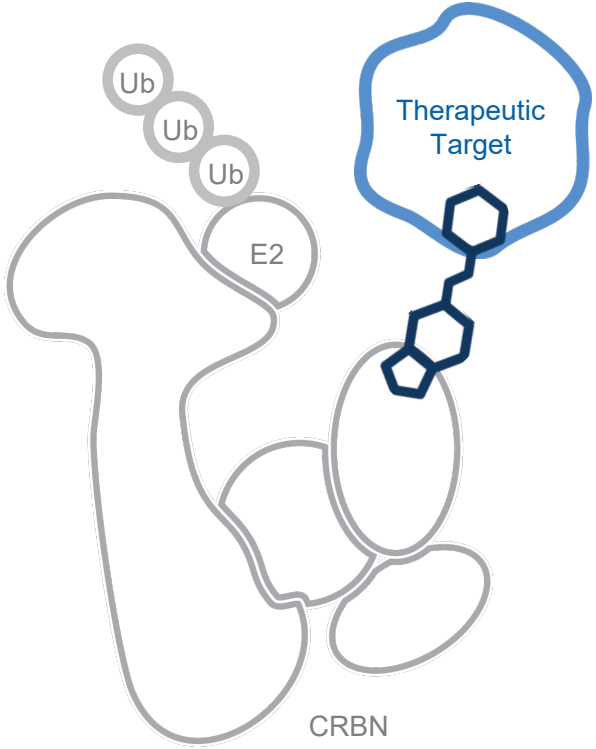
Handa and colleagues, *Science* 2010











Handa and colleagues, *Science* 2010

rationale for targeted protein degradation

targeting entire protein vs. single domain

the addressable domain may not be active

pairs well with shRNA and CRISPR validation

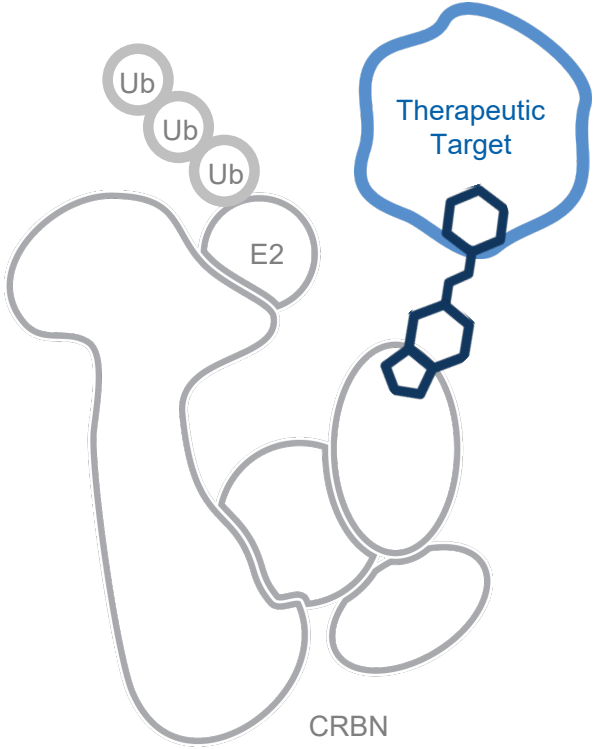
improved Emax

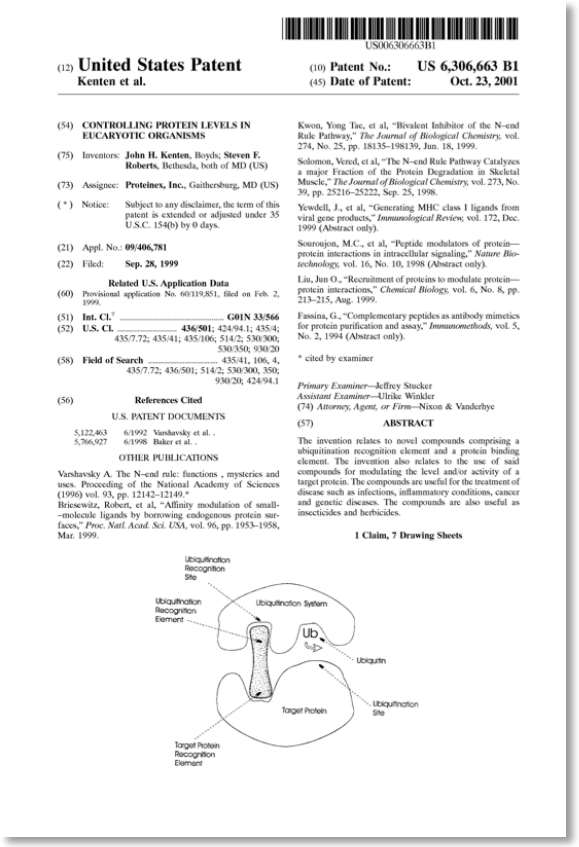
kinetic advantage: prolonged duration of effect

catalytic turnover feasible

overcome mechanisms of resistance

a new path to the waterfall





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(12) **United States Patent**
Kenten et al.

(10) **Patent No.: US 6,306,663 B1**
(45) **Date of Patent: Oct. 23, 2001**

(54) **CONTROLLING PROTEIN LEVELS IN EUCARYOTIC ORGANISMS**

(75) Inventors: **John H. Kenten**, Boyds; **Steven F. Roberts**, Bethesda, both of MD (US)

(73) Assignee: **Proteinex, Inc.**, Gaithersburg, MD (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/406,781**

(22) Filed: **Sep. 28, 1999**

Related U.S. Application Data

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(51) **Int. Cl.**[7] **G01N 33/566**

(52) **U.S. Cl.** **436/501**; 424/94.1; 435/4; 435/7.72; 435/41; 435/106; 514/2; 530/300; 530/350; 930/20

(58) **Field of Search** 435/41, 106, 4, 435/7.72; 436/501; 514/2; 530/300, 350; 930/20; 424/94.1

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,122,463 6/1992 Varshavsky et al. .
5,766,927 6/1998 Baker et al. .

OTHER PUBLICATIONS

Varshavsky A. The N–end rule: functions , mysteries and uses. Proceeding of the National Academy of Sciences (1996) vol. 93, pp. 12142–12149.*

Briesewitz, Robert, et al, "Affinity modulation of small–molecule ligands by borrowing endogenous protein surfaces," *Proc. Natl. Acad. Sci. USA*, vol. 96, pp. 1953–1958, Mar. 1999.

Kwon, Yong Tae, et al, "Bivalent Inhibitor of the N–end Rule Pathway," *The Journal of Biological Chemistry*, vol. 274, No. 25, pp. 18135–198139, Jun. 18, 1999.

Solomon, Vered, et al, "The N–end Rule Pathway Catalyzes a major Fraction of the Protein Degradation in Skeletal Muscle," *The Journal of Biological Chemistry*, vol. 273, No. 39, pp. 25216–25222, Sep. 25, 1998.

Yewdell, J., et al, "Generating MHC class I ligands from viral gene products," *Immunological Review*, vol. 172, Dec. 1999 (Abstract only).

Souroujon, M.C., et al, "Peptide modulators of protein—protein interactions in intracellular signaling," *Nature Biotechnology*, vol. 16, No. 10, 1998 (Abstract only).

Liu, Jun O., "Recruitment of proteins to modulate protein—protein interactions," *Chemical Biology*, vol. 6, No. 8, pp. 213–215, Aug. 1999.

Fassina, G., "Complementary peptides as antibody mimetics for protein purification and assay," *Immunomethods*, vol. 5, No. 2, 1994 (Abstract only).

* cited by examiner

Primary Examiner—Jeffrey Stucker
Assistant Examiner—Ulrike Winkler
(74) *Attorney, Agent, or Firm*—Nixon & Vanderhye

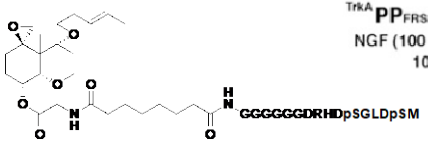
(57) **ABSTRACT**

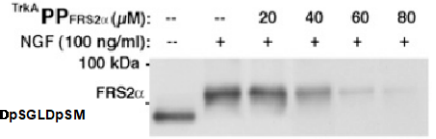
The invention relates to novel compounds comprising a ubiquitination recognition element and a protein binding element. The invention also relates to the use of said compounds for modulating the level and/or activity of a target protein. The compounds are useful for the treatment of disease such as infections, inflammatory conditions, cancer and genetic diseases. The compounds are also useful as insecticides and herbicides.

1 Claim, 7 Drawing Sheets

PROTACS

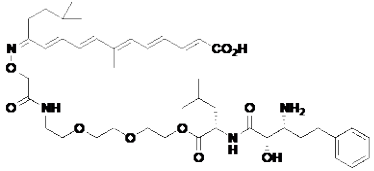
PROTAC-1; Crews & Deshaies, *PNAS* 2001
TrkA-PPFRS2α; Crews, *PNAS* 2012

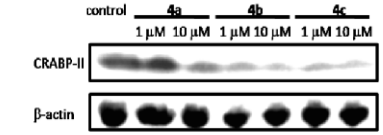




SNIPER System

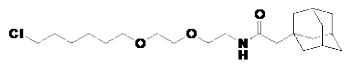
Cpd 4; Naito, *JACS* 2010
SNIPER(ER); Naito, *Cancer Science* 2013

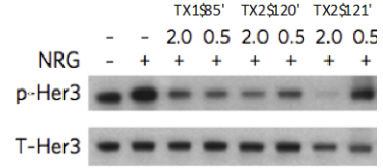




Hydrophobic Tagging

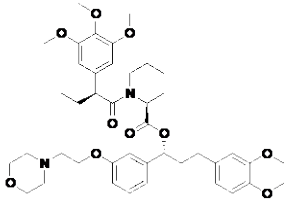
HyT13; Crews, *NCB* 2011
TX2-121; Gray and Crews, *NCB* 2014





Shield System

Shield-1; Wandless, *Cell* 2006
F. Stegmeier, *Nature Biotech* 2015



US 6,306,663 (Filed 1999)
NIBR

NOVARTIS

technological challenges

engineered approaches predominated

peptide synthesis challenging

limited biochemical characterization

absent mechanistic controls

low intracellular permeability

poor potency

poorly reproducible

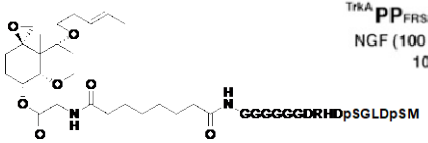
inactive *in vivo*

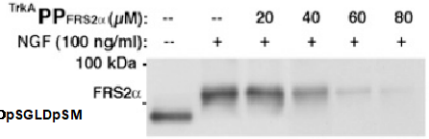
not extensible to diverse targets

PROTACS

PROTAC-1; Crews & Deshaies, *PNAS* 2001

TrkA-PPFRS2α; Crews, *PNAS* 2012





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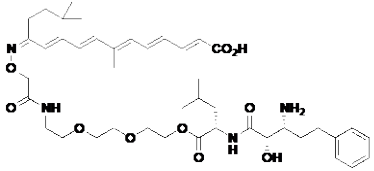
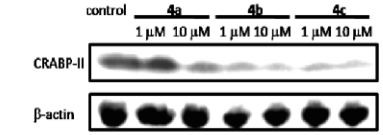
Cpd 4; Naito, *JACS* 2010

SNIPER(ER); Naito, *Cancer Science* 2013





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HyT13; Crews, *NCB* 2011

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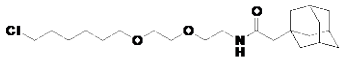
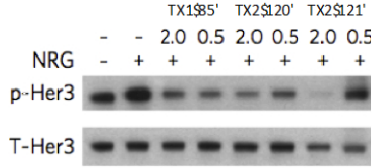
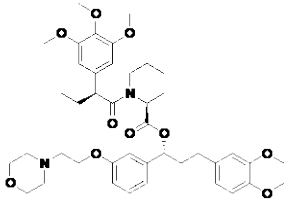




Shield System

Shield-1; Wandless, *Cell* 2006

F. Stegmeier, *Nature Biotech* 2015



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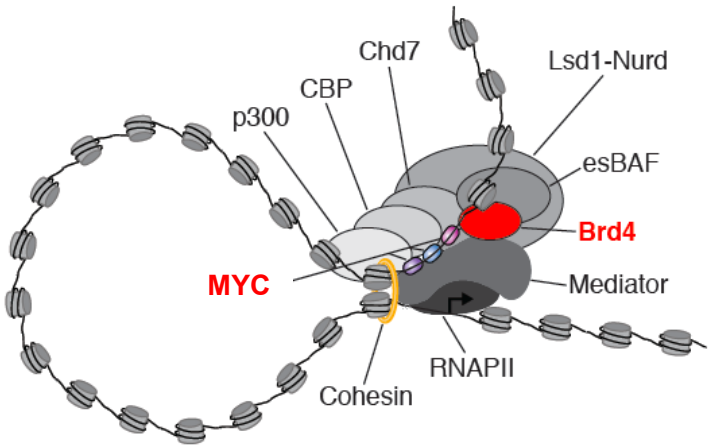
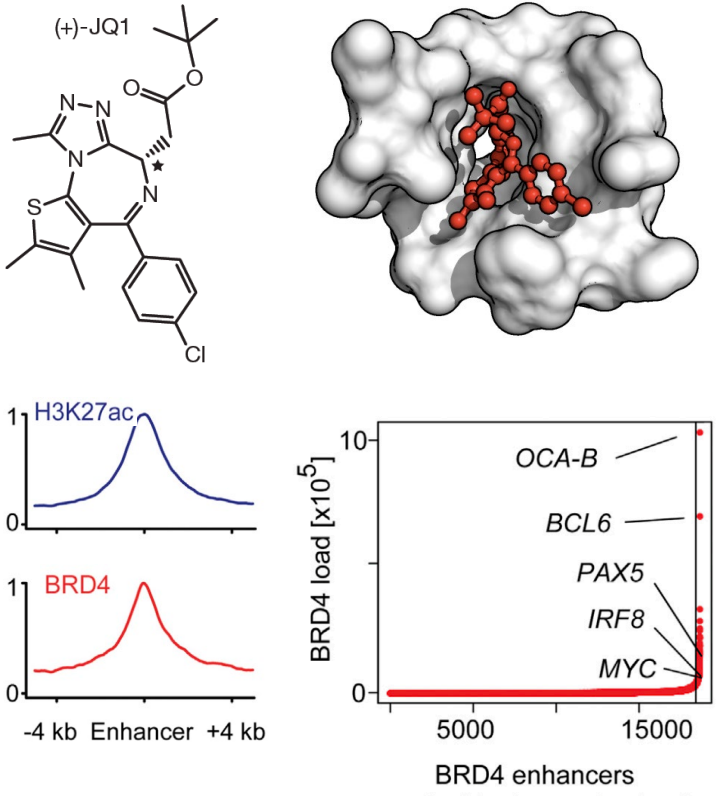
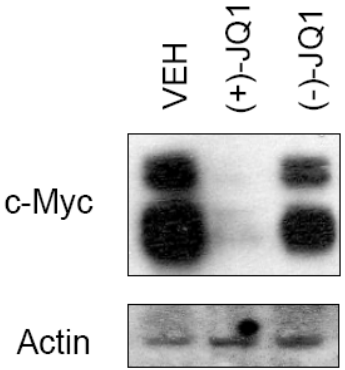
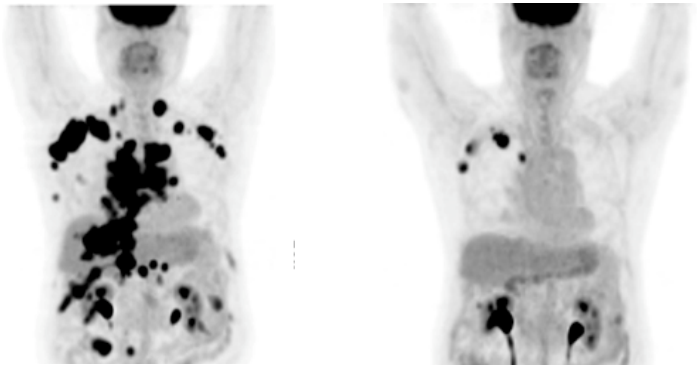




Bradner Lab, *Nature* (2010), *Cell* (2011), *Cancer Cell* (2013)
With R. Young (2013)

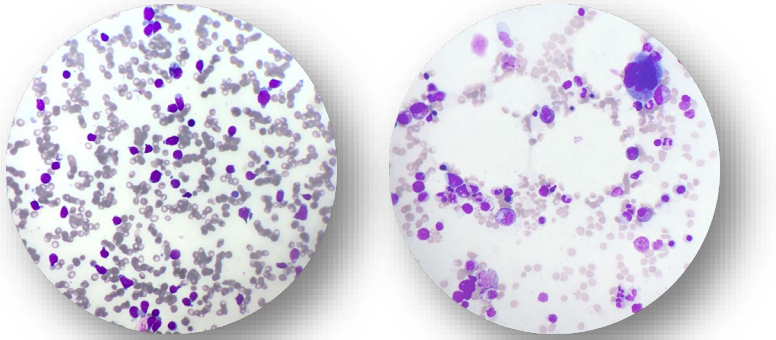


With C. Mitsiades *Cell* (2011)



Baseline RG-6146

With Vakoc and Lowe *Nature* (2011)



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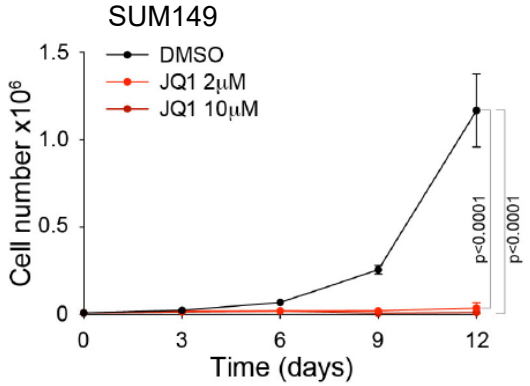
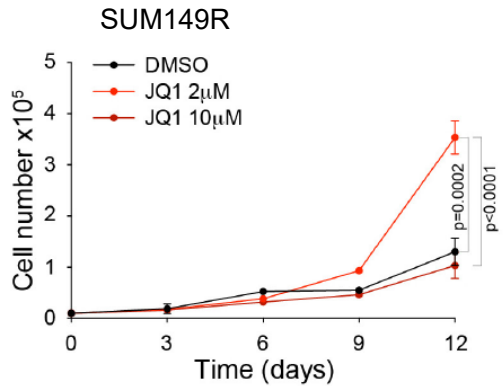
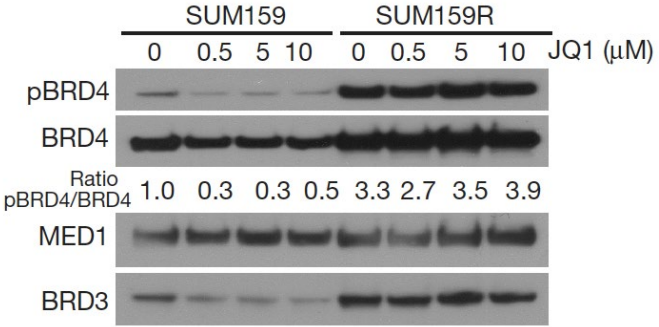
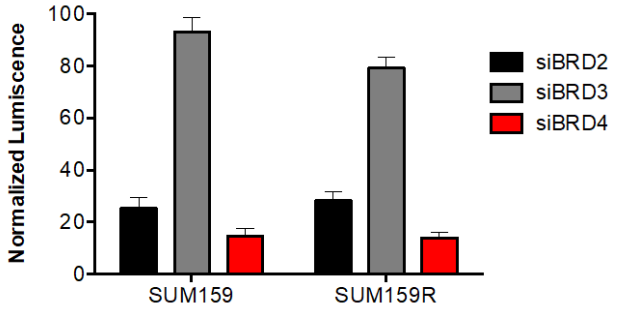
Tensha (Roche) | Armand, Deangelo, Roboz, Shapiro



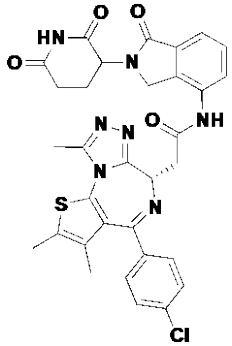






Bradner and Polyak Labs, *Nature* 2016

an all-chemical solution for targeted protein degradation



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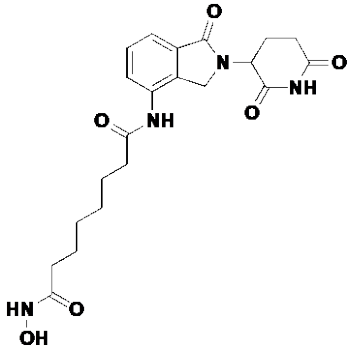

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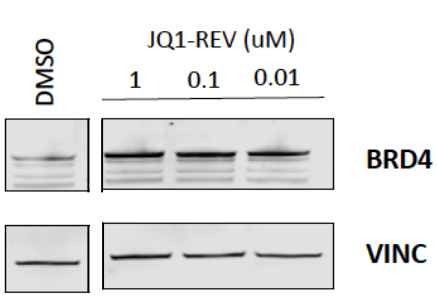

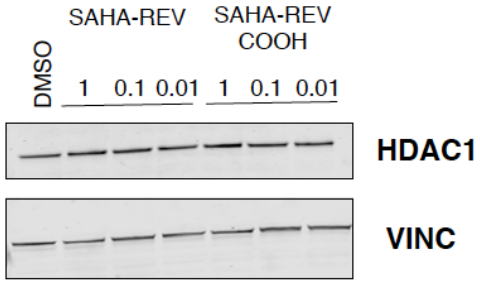

Bradner Lab (Unpublished)

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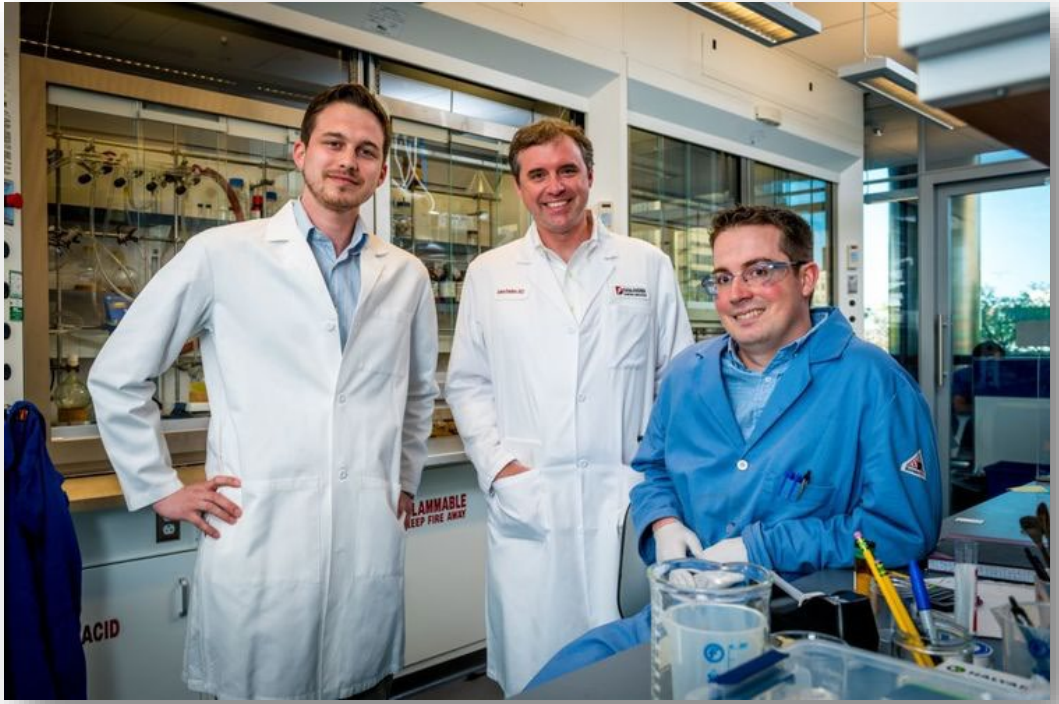
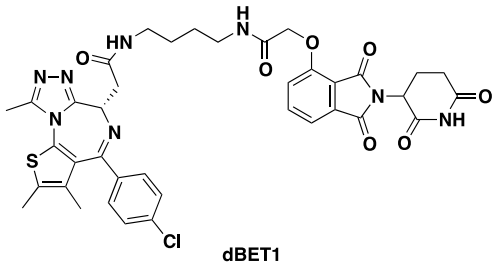
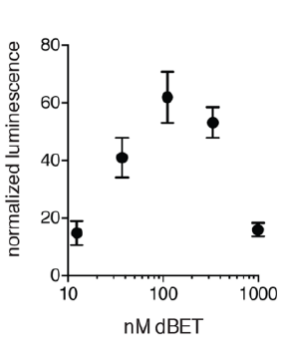

LAMMABLE
EEP FIRE AWAY
ACID

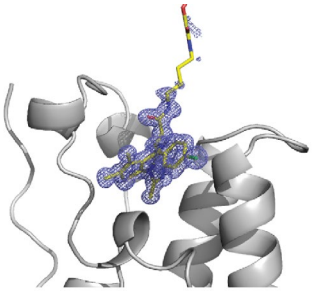
technological challenges

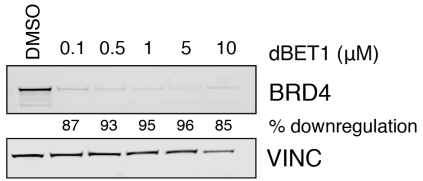
- ~~engineered approaches predominated~~
- ~~peptide synthesis challenging~~
- ~~limited biochemical characterization~~
- ~~absent mechanistic controls~~
- ~~low intracellular permeability~~
- ~~poor potency~~
- ~~poorly reproducible~~
- ~~inactive *in vivo*~~

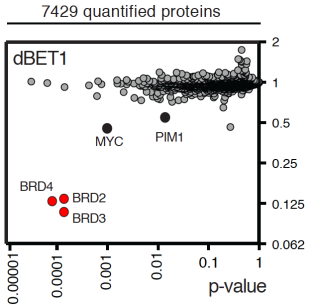
not extensible to diverse targets

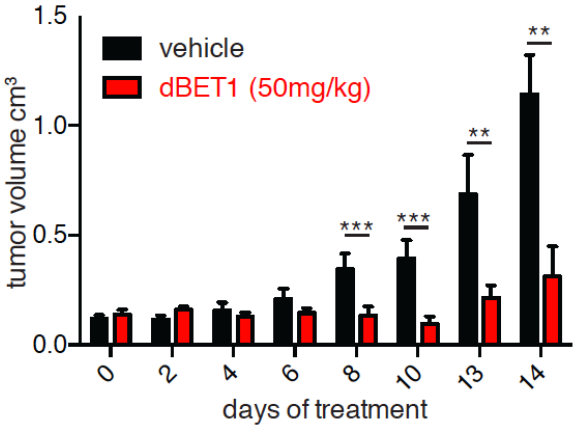




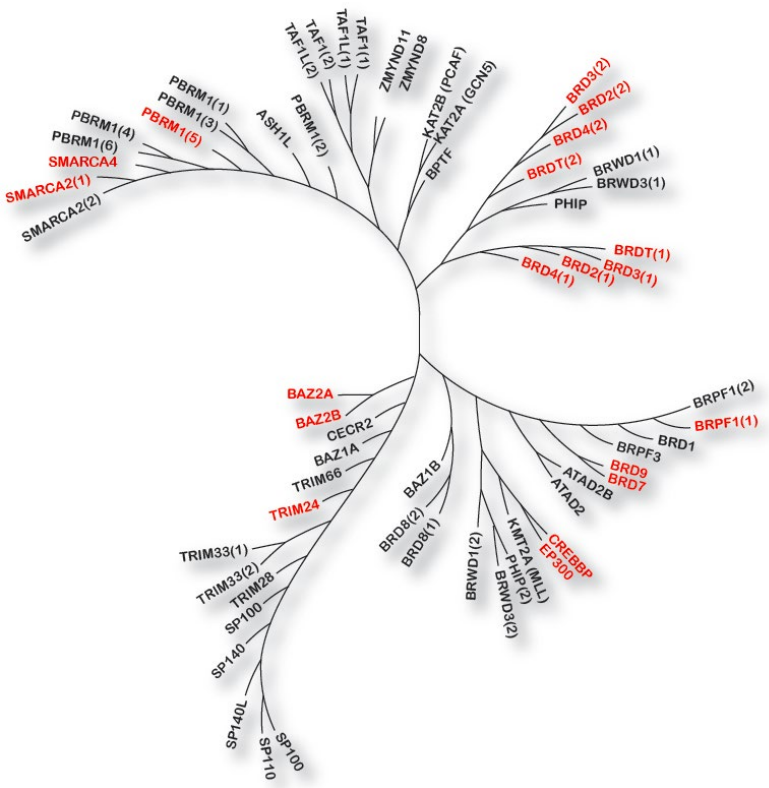


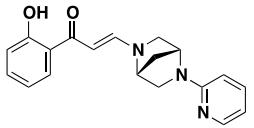




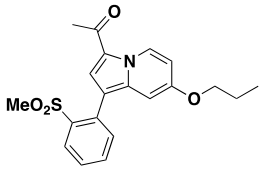


Winter et.al., *Science* 2015





PFI-3 I Pfizer
SMARCA2, SMARCA4, PB1



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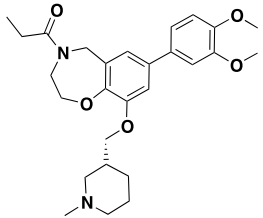
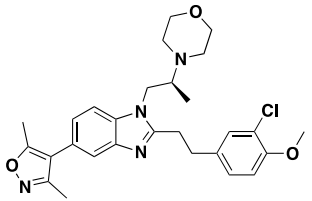
BAZ2A, BAZ2B



I-CBP-112 I GSK
CBP, EP300



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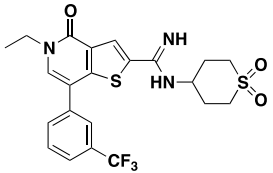

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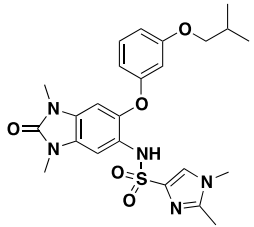

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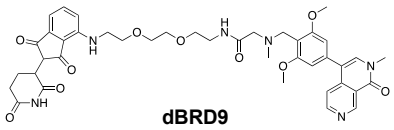

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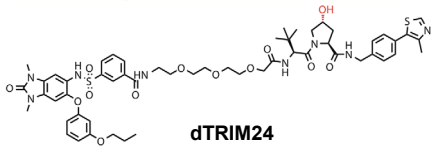

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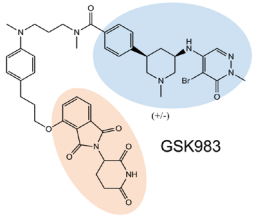

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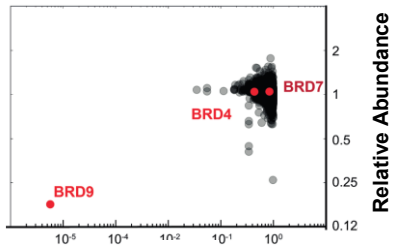
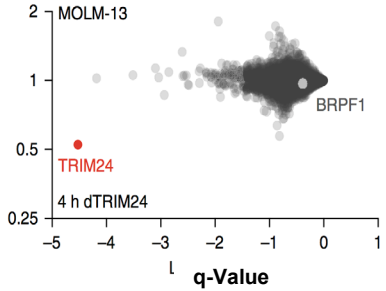
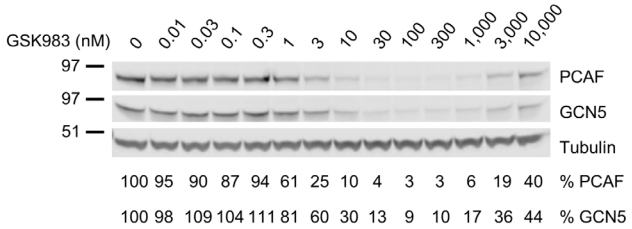
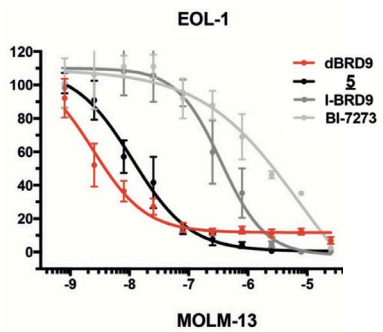
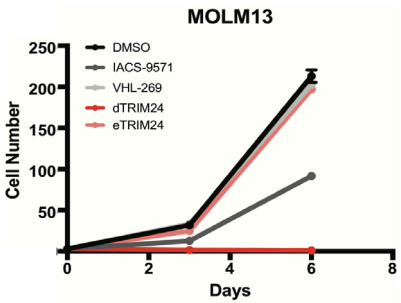
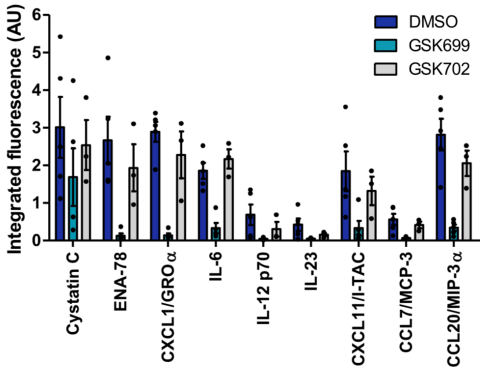












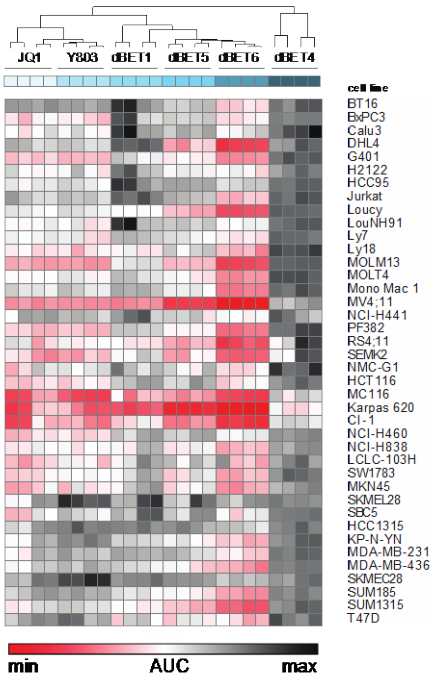
Bradner Lab, *Angewandte Chemie* 2017

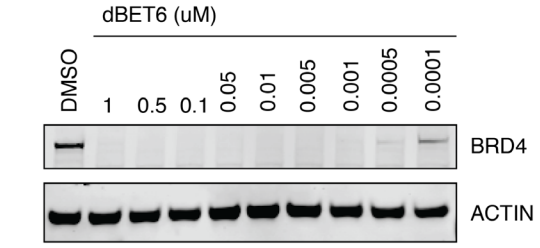
Bradner Lab, *Nature Chemical Biology* 2018

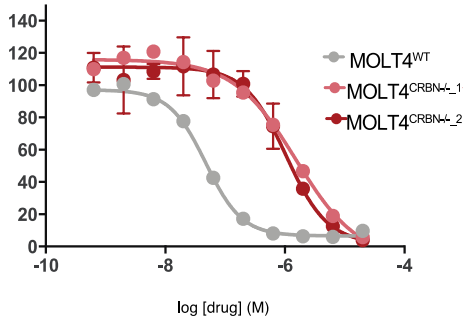
Bassi and colleagues, *ACS Chemical Biology* 2018

emerging themes in targeted protein degradation

Target Potency Reveals Catalytic Activity

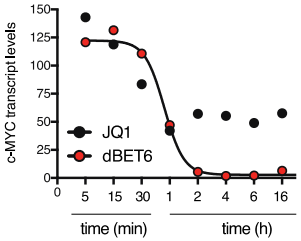


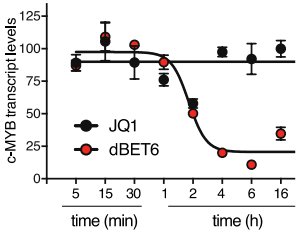


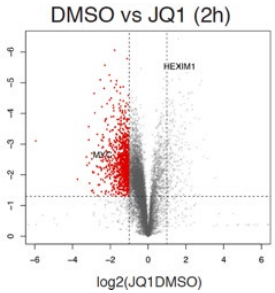


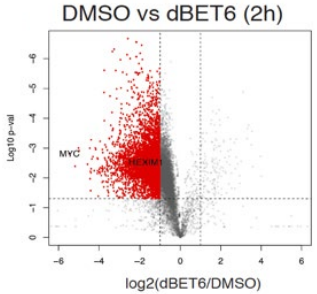
Bradner Lab, Molecular Cell 2017

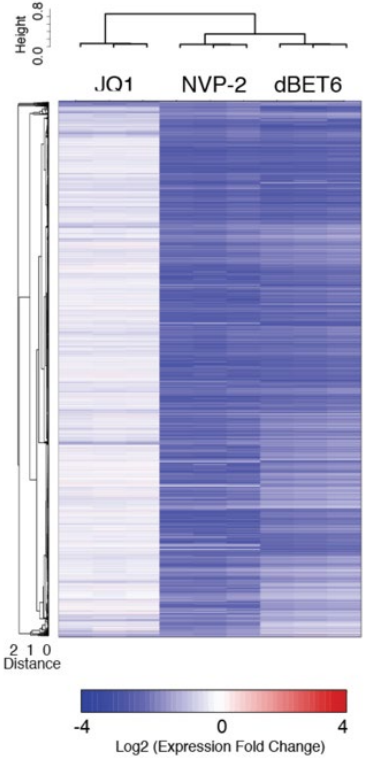
Target Inhibition ≠ Target Degradation

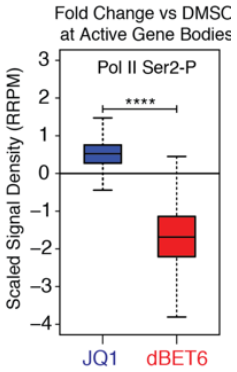






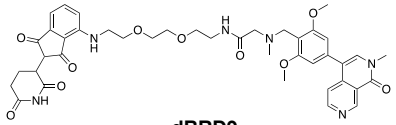






Bradner Lab, Molecular Cell 2017

Ligand Specificity ≠ Degrader Selectivity



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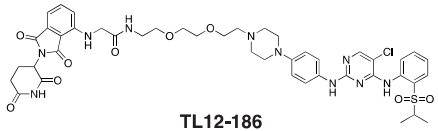

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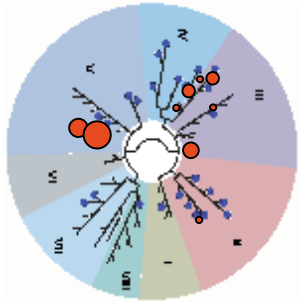
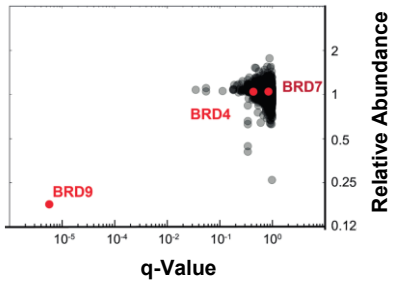
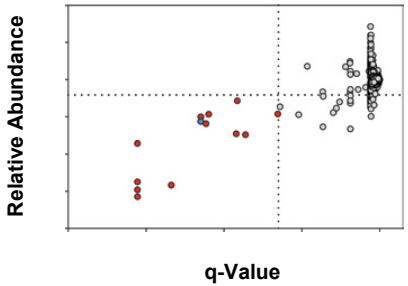


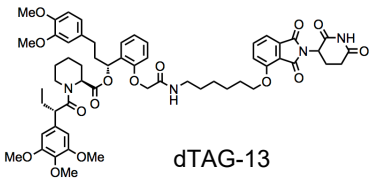


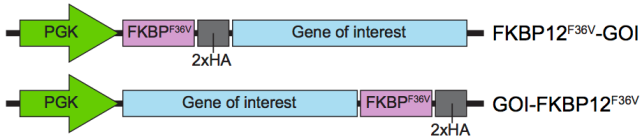


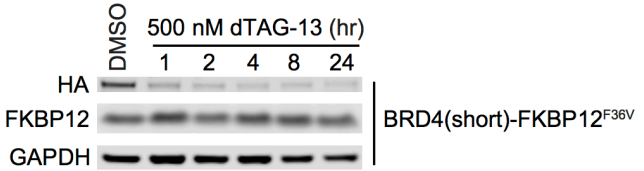
Bradner Lab, *Angewandte Chemie* 2017

With N. Gray, *Cell Chemical Biology* 2017

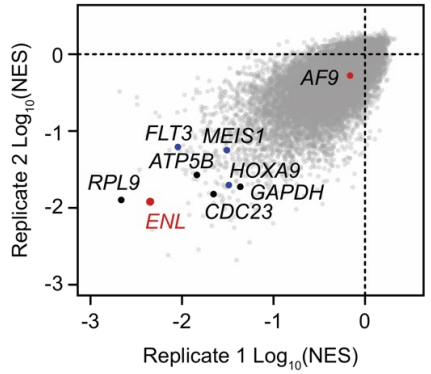
Useful in Target Validation

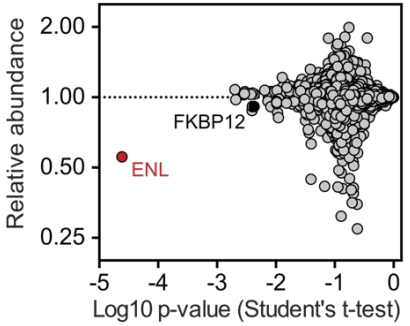


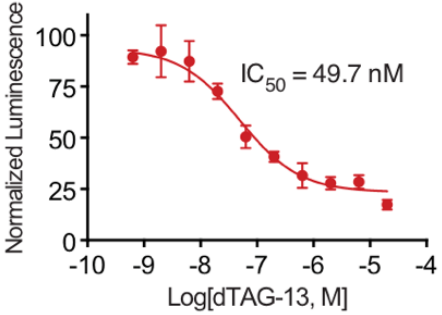


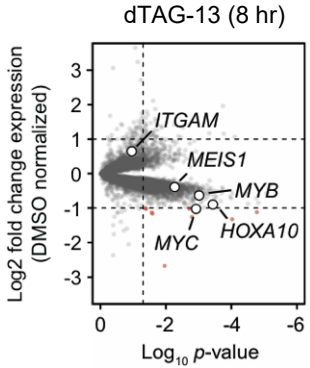


Bradner & Gray Labs, *Nature Chemical Biology* 2018



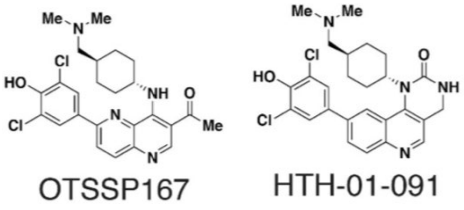


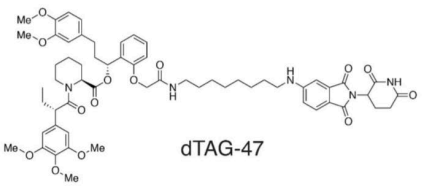


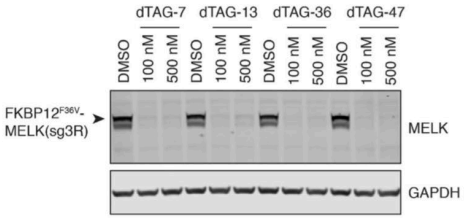


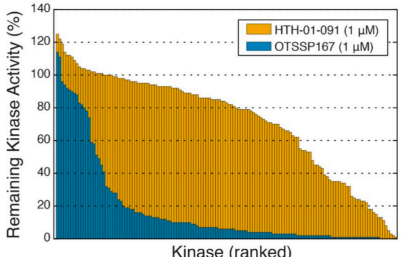
Bradner Lab, *Nature* 2017

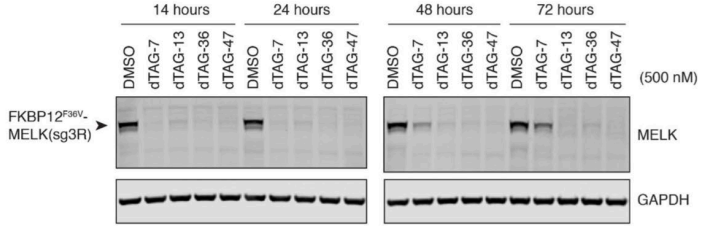
Useful in Target In-Validation

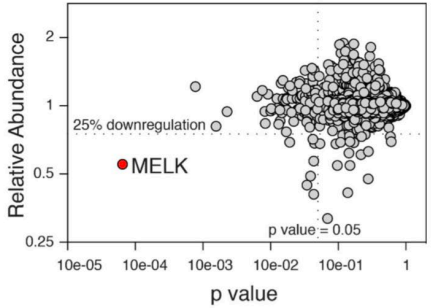


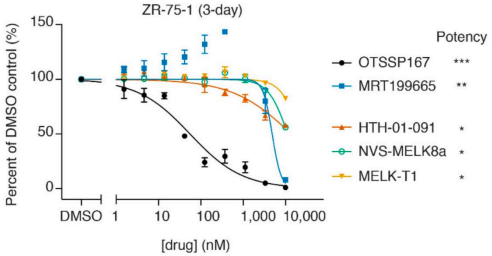


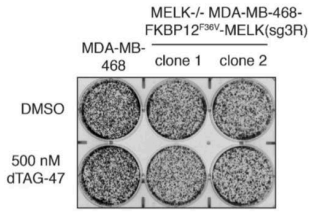


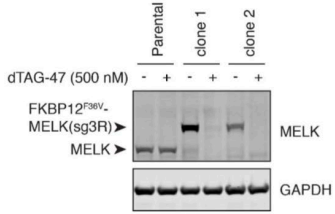






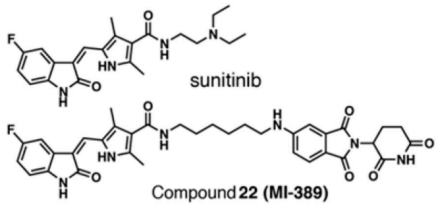


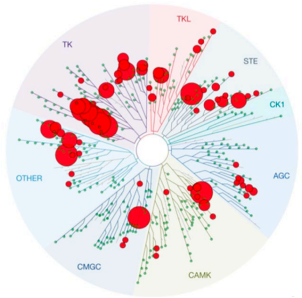


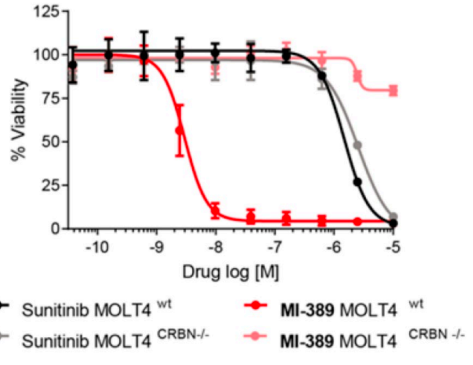


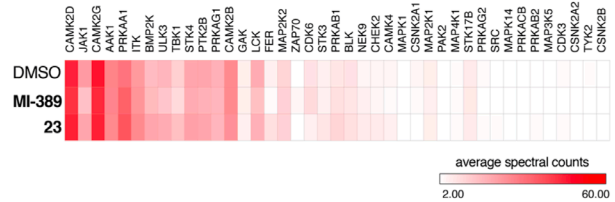
With Nathanael Gray, *eLife* 2017

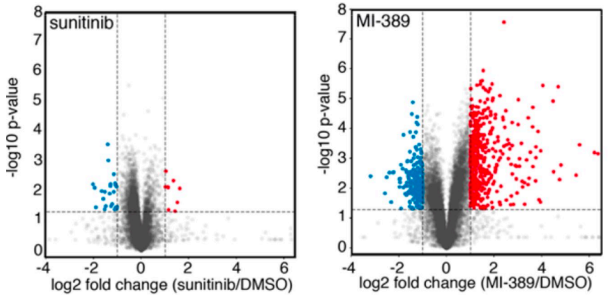
Useful in Target Identification

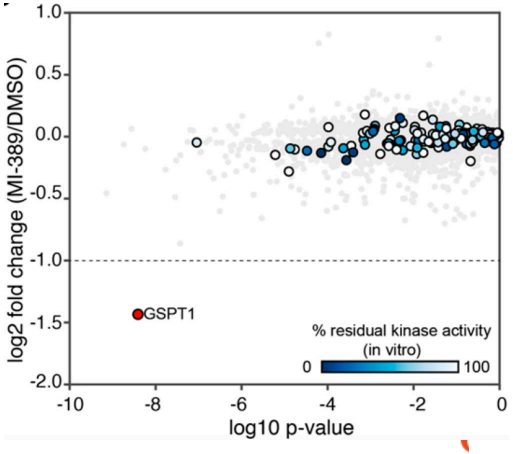






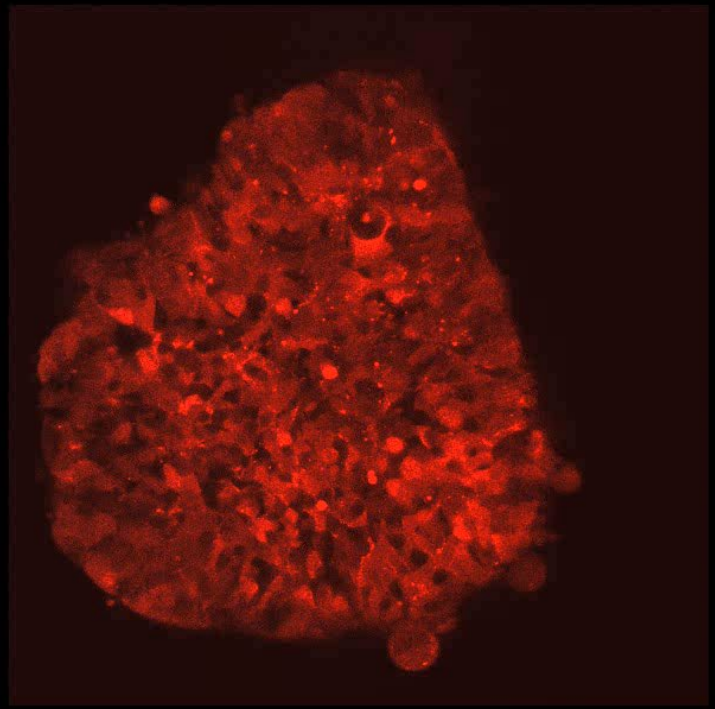


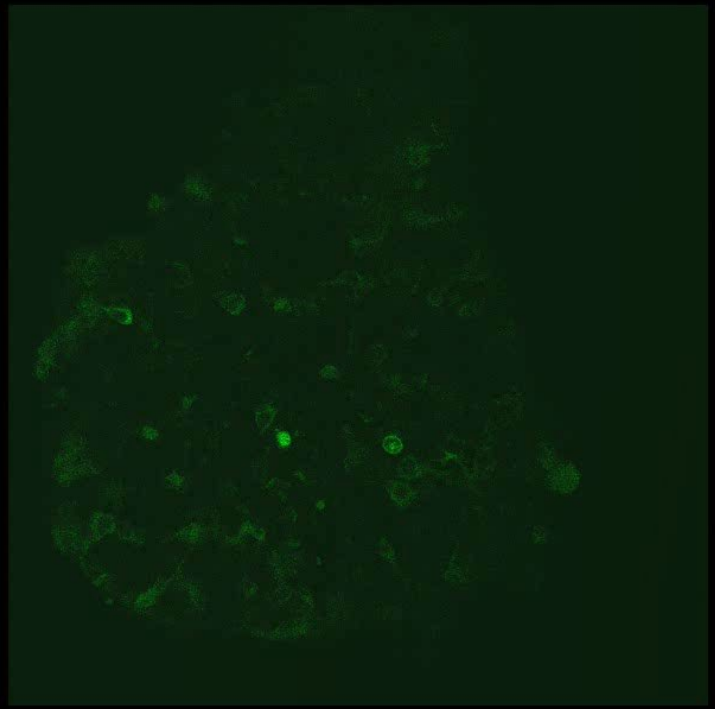




With G. Winter, *ACS Chemical Biology* 2018

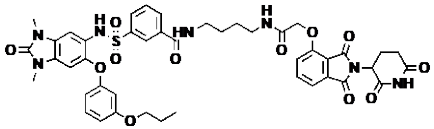
Useful for Fast Biology





Ligases Have Preferences

dTRIM4



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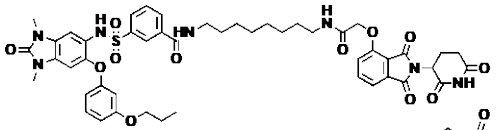

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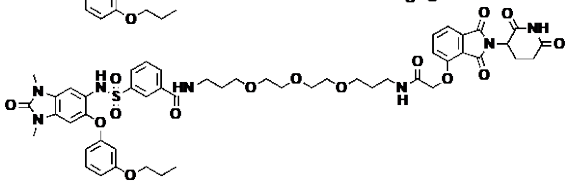
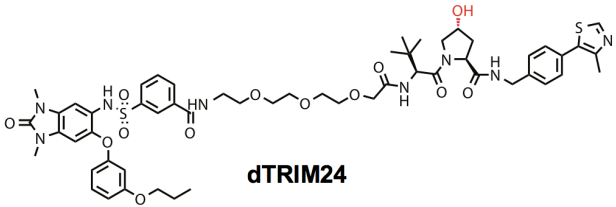
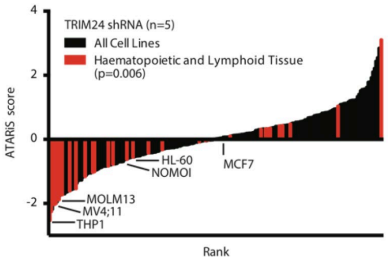
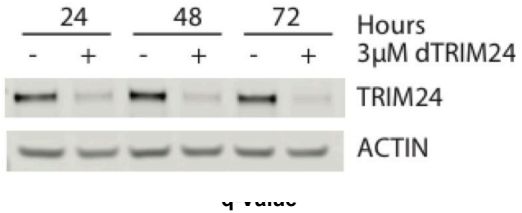
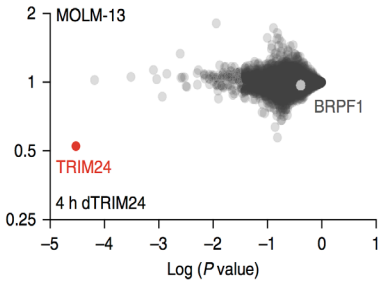
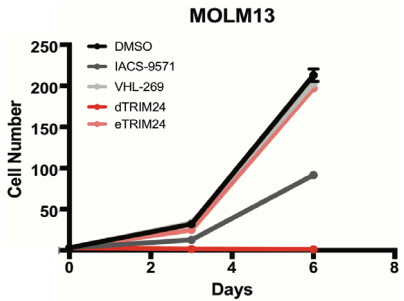


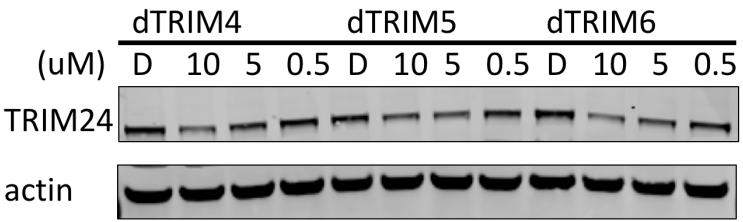






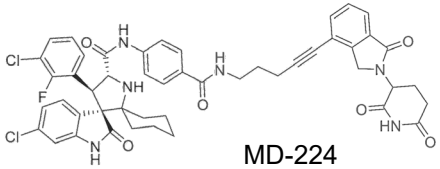


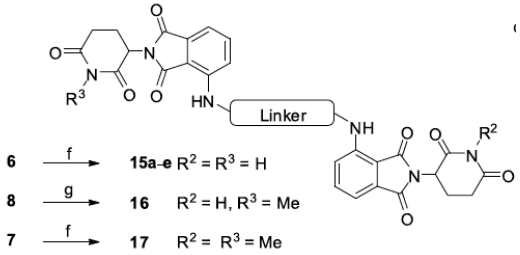


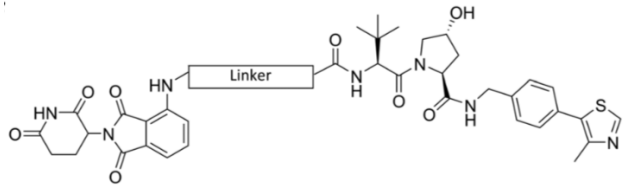


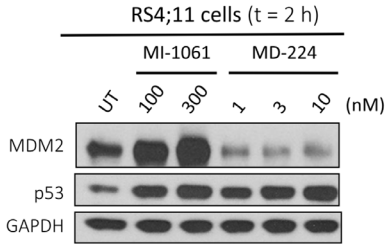
Bradner Lab, *Nature Chemical Biology* 2018

Ligases Degrade Ligases

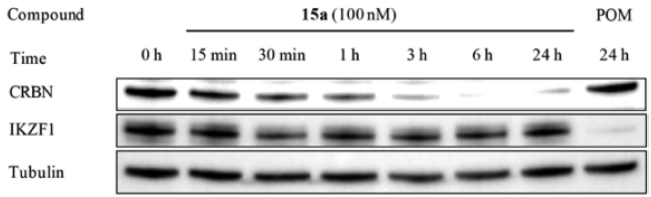




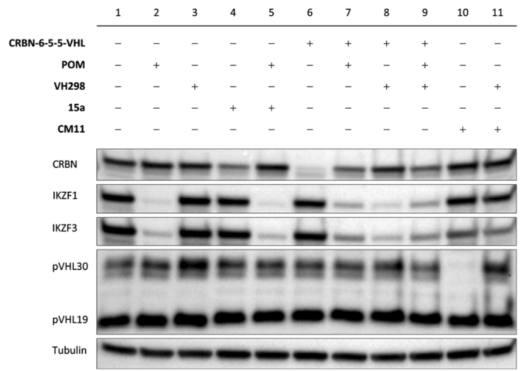




Shaomeng Wang, *J.Med.Chem* 2018

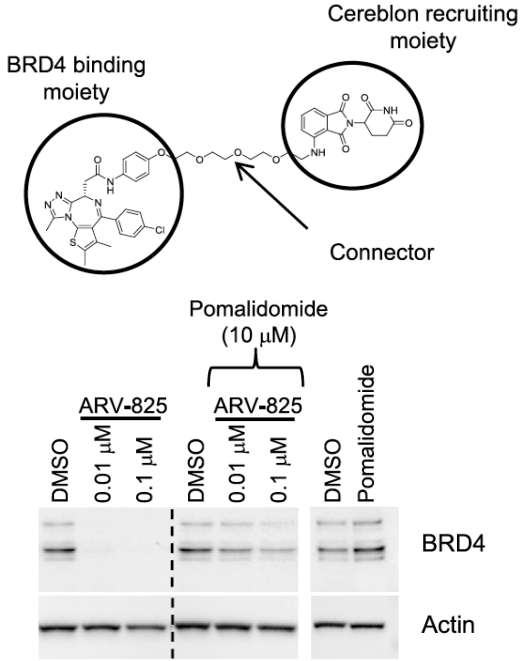


Jan Krönke, *ACS ChemBio* 2018

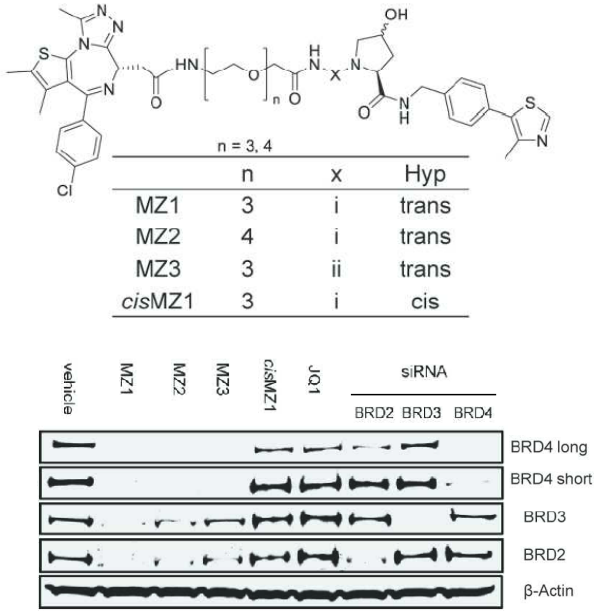


Kronke & Gutschow, *Chem Comm* 2019

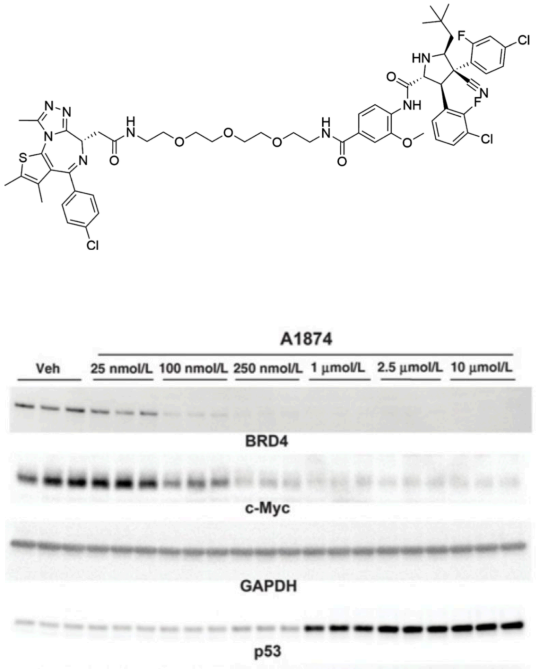
Reproducible & Extensible



Arvinas, *Chemistry & Biology* 2015

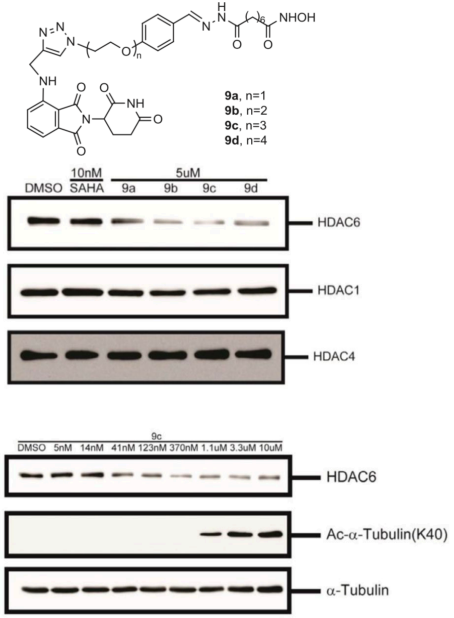


Alessio Ciulli, *Chemistry & Biology* 2015

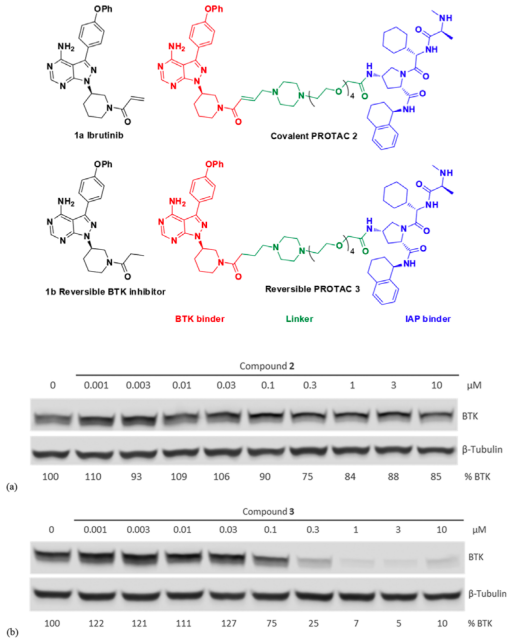


Crews, *Cancer Research* 2018

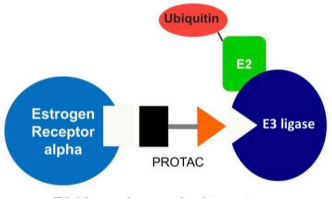
Reproducible & Extensible



W. Tang ert.al., *BMCL* 2018



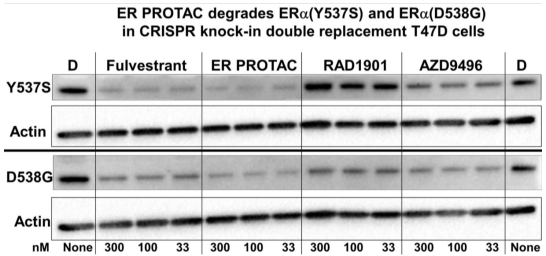
Tinworth ert.al., *ACS Chem. Bio.* 2019



PK summary of ERα PROTAC

Species	% F	AUC/Dose (μM•hr)/(mg/kg)
Mouse	42	0.8 ± 0.3
Dog	57	4.0 ± 1.3
Rat	26	0.6 ± 0.3

PO, 3 mpk



Arvinas, SABCS Poster 2017
[arvinas.com/wp-content/uploads/2017/12/2017-SABCS-poster.pdf]

Deshaies & Crews
PNAS | July 17, 2001 | vol 98 | no. 15

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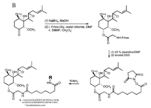
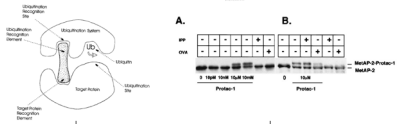


Kenten & Roberts
PROTACS patent



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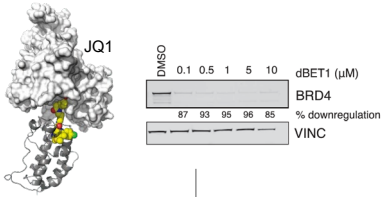

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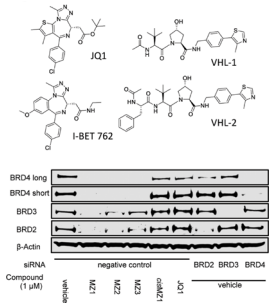

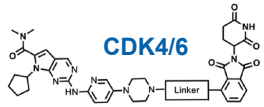

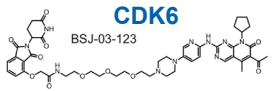

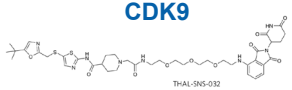

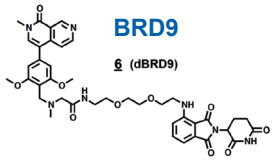

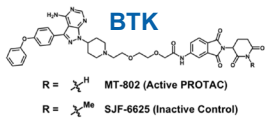

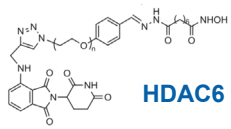

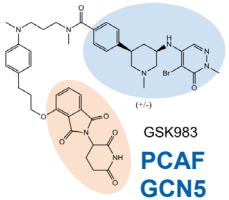

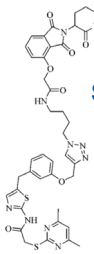

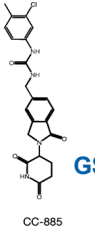

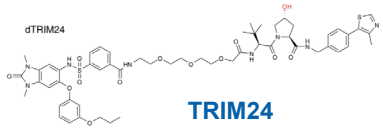

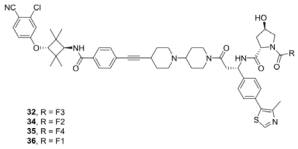

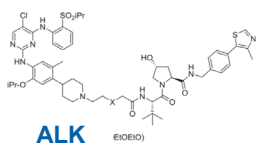

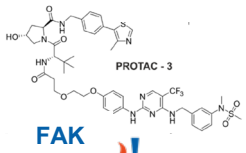

NIBR

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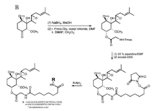
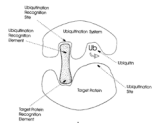
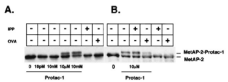


Kenten & Roberts
PROTACS patent





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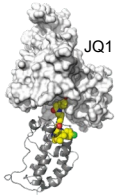

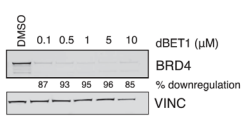

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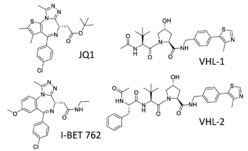

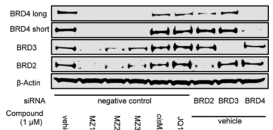

PubMed Citations
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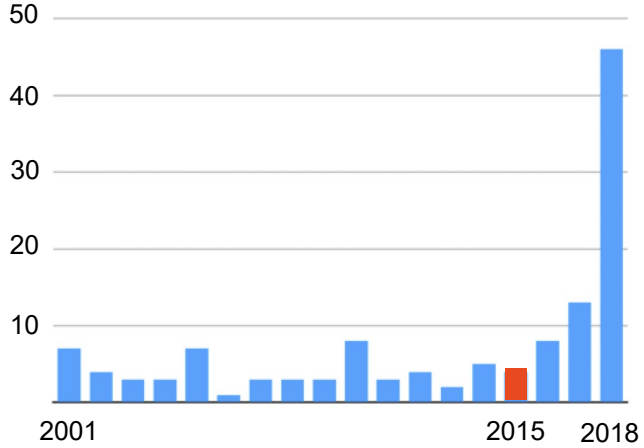



NOVARTIS

ARVINAS

nurix

KYMERA

C4

Genentech

Celgene

Captor
Therapeutics

Roche

MERCK

Bristol-Myers Squibb

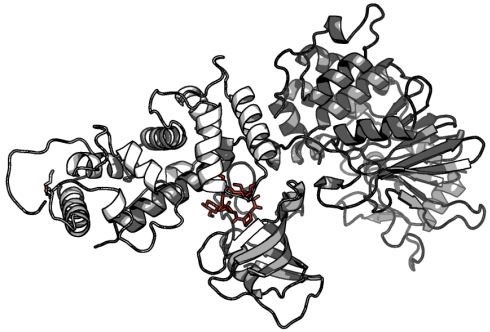
BioTheryX

Calico

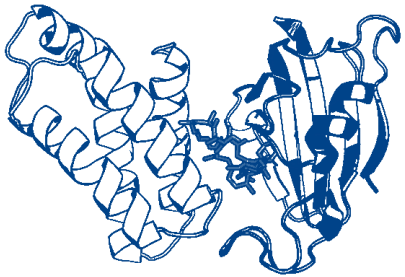
Pfizer

gsk

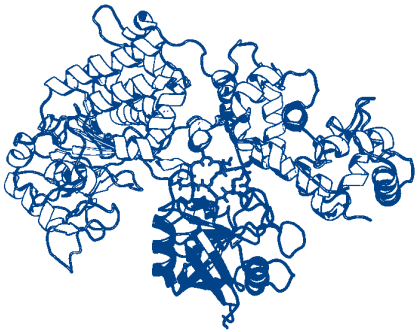
MISSION
Therapeutics



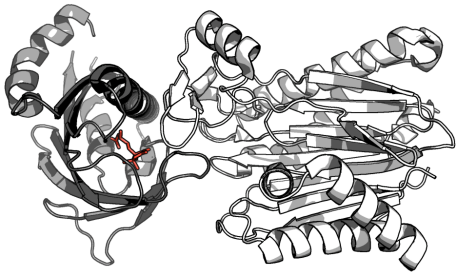
FKBP12:FK506:Calcineurin
Kissinger, *Nature* 1995
Griffith, *Cell* 1995



FRB:Rapamycin:FKBP12
Schreiber & Clardy, *Science* 1996

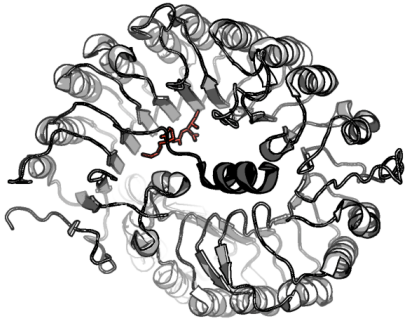


Cyclophilin:Cyclosporine A:Calcineurin
Ke , *PNAS* 2002



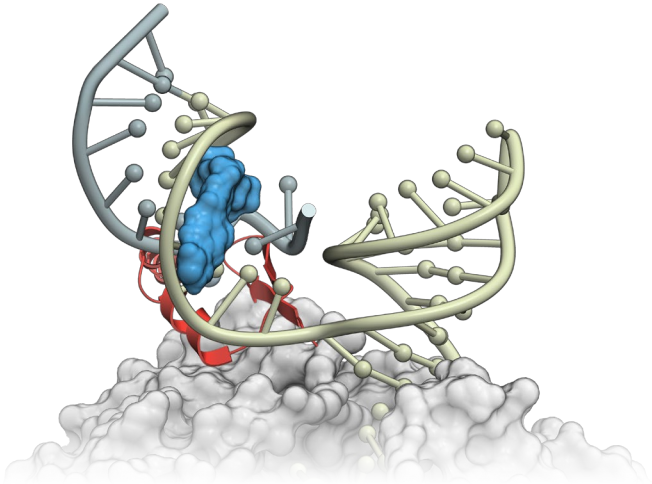
PYL:Abscisic Acid:ABI1
Tanokura, *Nature* 2009

TIR1:Auxin:IAA7
Zheng, *Nature* 2007



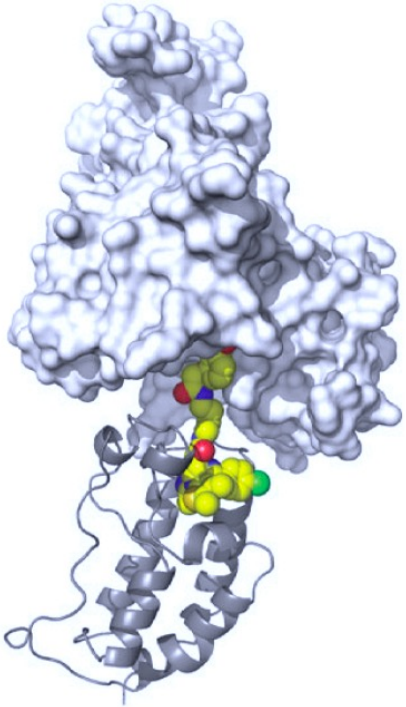
COI1:Jasmonate:JAZ1
Zheng, *Nature* 2010

Molecular Glues



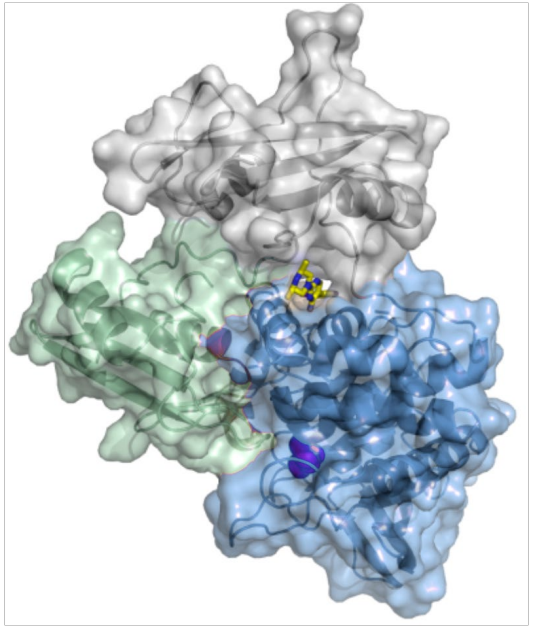
Protein:RNA

LMI070



Protein:Protein

dBET1



Conformational Glues

SHP099


6000
6
340
8
90
8.9

~~Undruggable targets~~ will be drugged

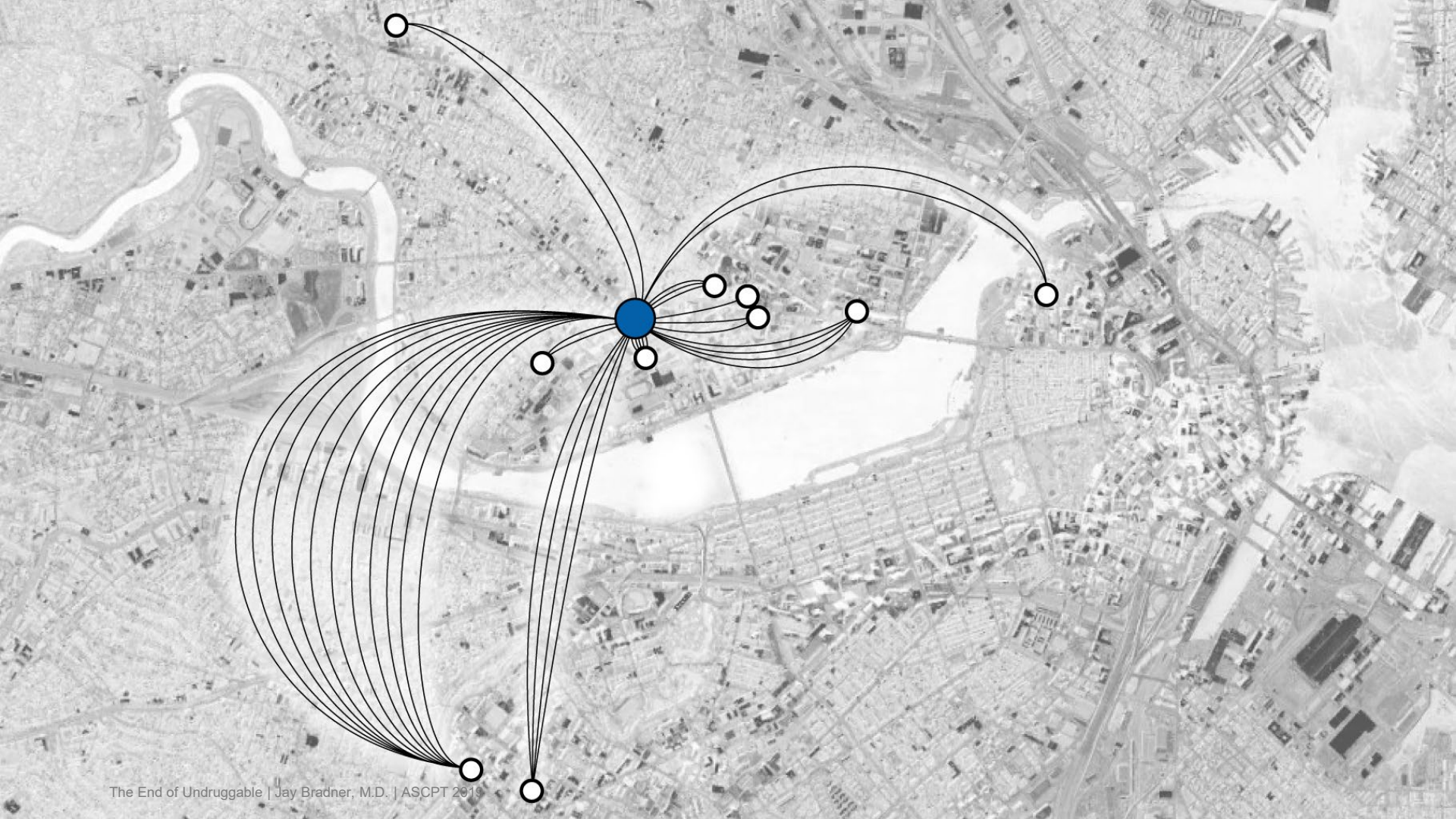
Elusive pathophysiology will be explained

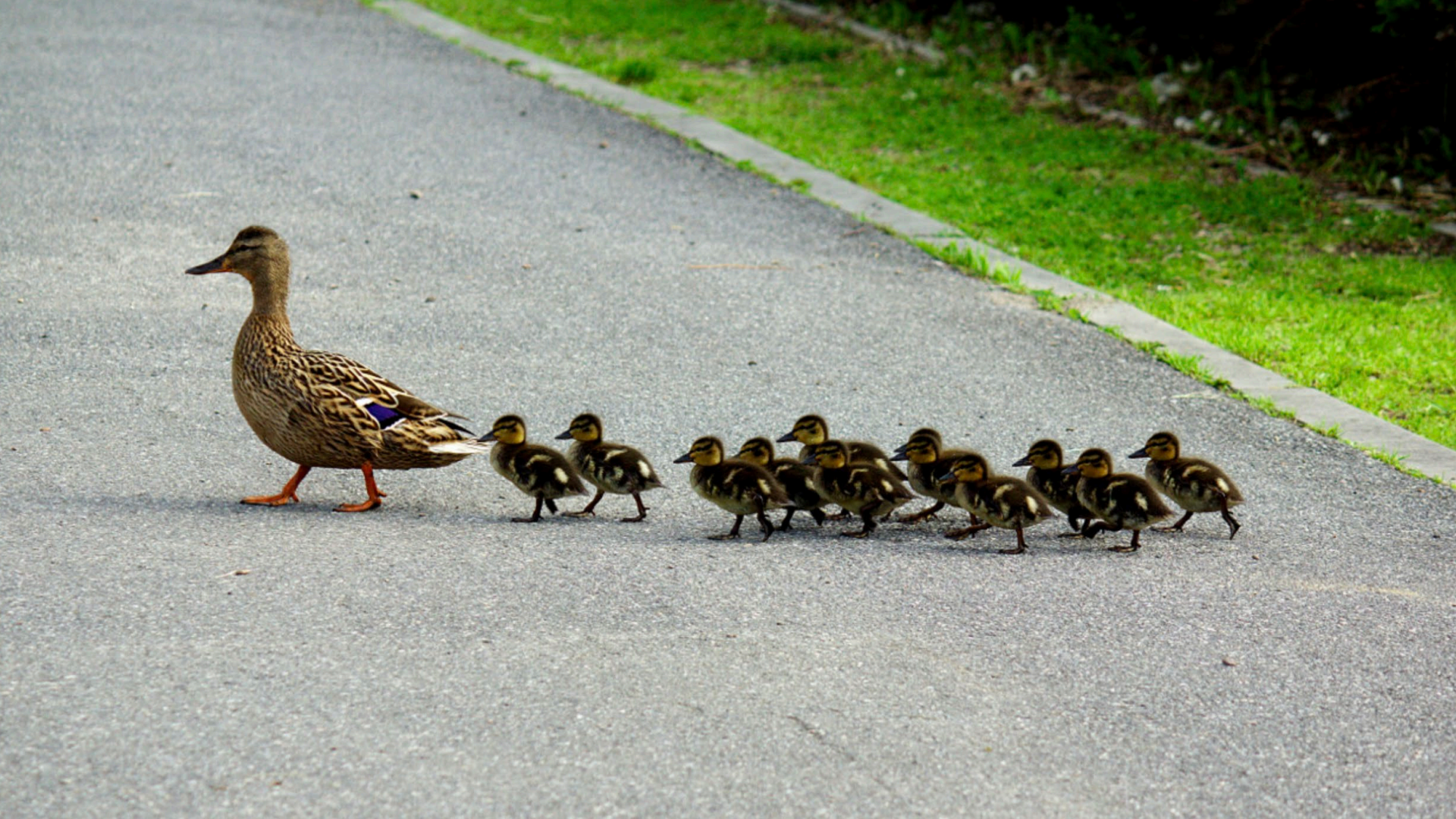
Sciences and medicine will converge

Disease will be treated pre-emptively

Humanity will be quantified

Patients will drive healthcare





Reimagining Medicine