New Cancer Therapies on the Horizon
New Strategies for therapies of the future

Nov 20th 2020 IUPHAR Meeting
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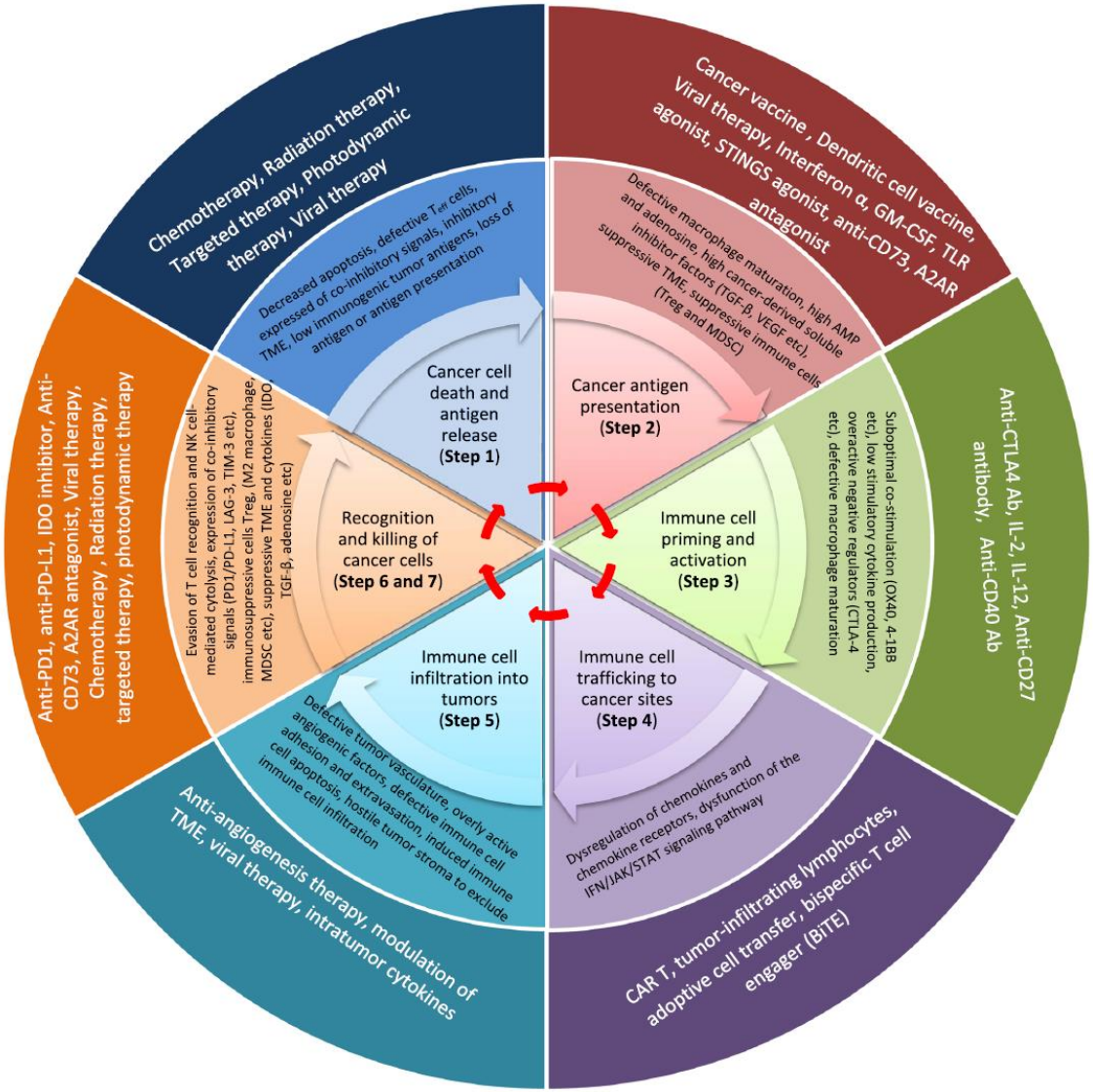
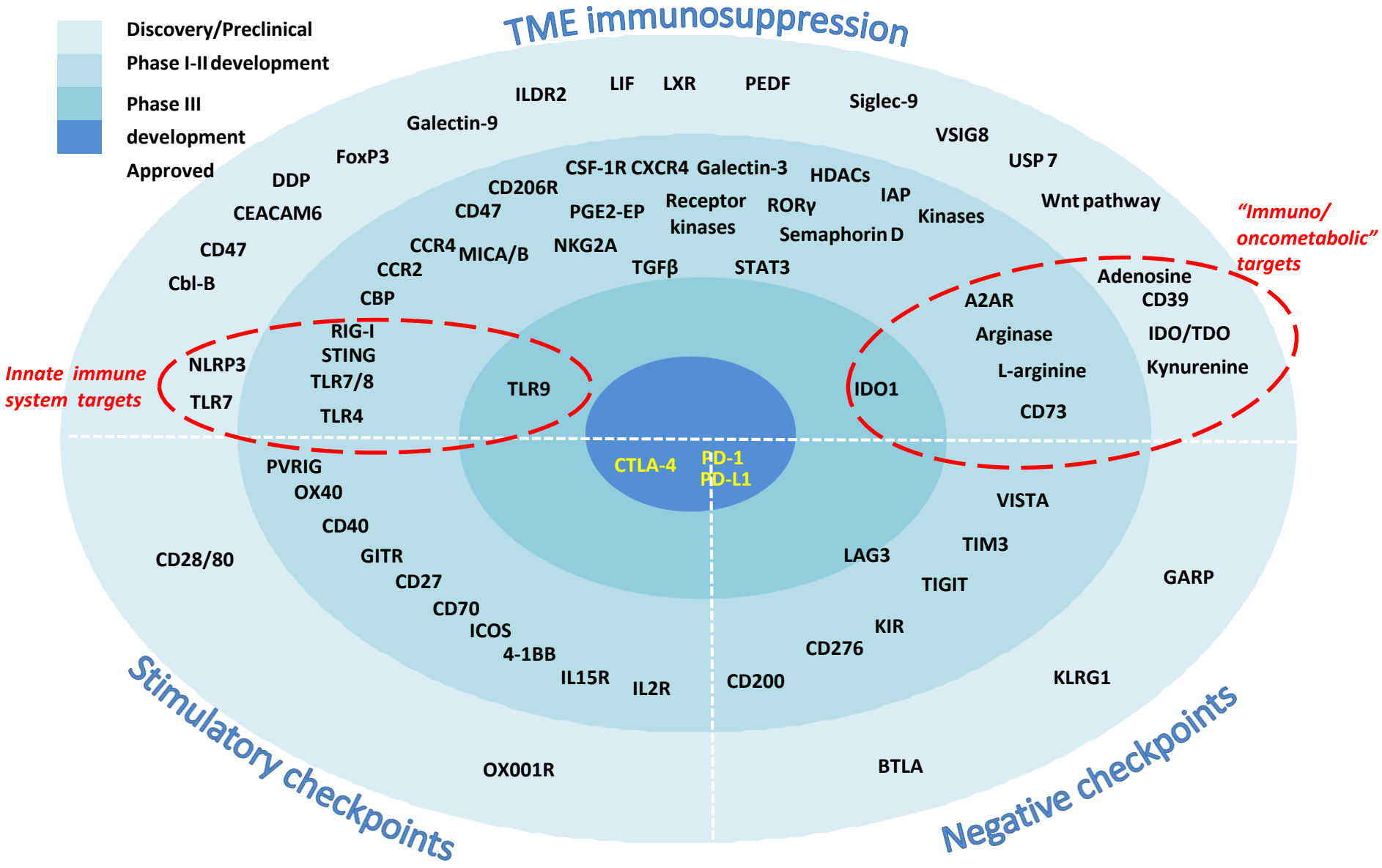
Agenda

- Immuno-oncology approaches
- Epigenetic Targets
- Drugging “undruggable targets”
  - Degraders & MS-ABPP
  - Inhibiting Ras
  - Inhibiting Transcription Factors

The cancer-immunity cycle, immune-resistant mechanisms and strategies for anti-cancer immunotherapy



Next wave IO drug development target landscape



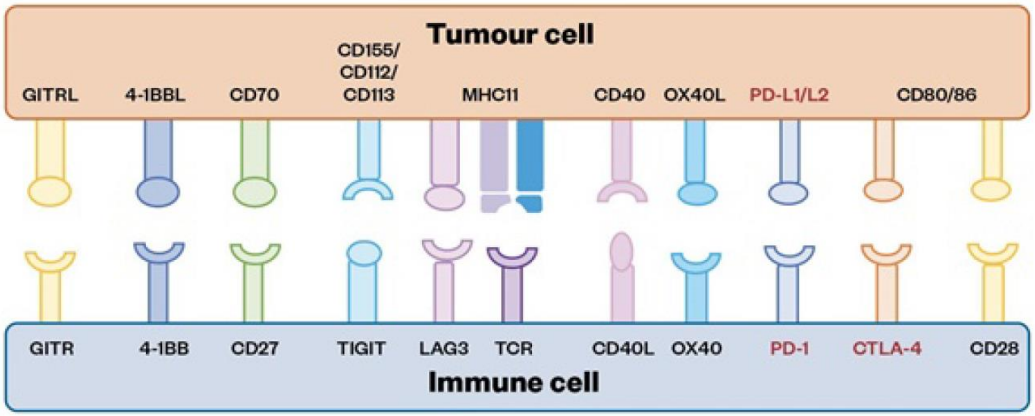
Next wave targets- what's hot?

Commercial development heat map

4-1BB	A2AR	Adenosine	Arginase	Arginine	BTLA	Cbl-b	CBP	CCR2	CCR4
CD28/80	CD200	CD206R	CD27	CD276	CD39	CD40	CD47/ SIRPα	CD70	CD73
CEACAM6	CSF-1R	CXCR4	DPP	EP4	FoxP4	Galectin 3	Galectin 9	GARP	GITR
HDACs	HVEM	IAP	ICOS	IDO/ TDO	IDO1	IL15Rα	IL2R	ILDR2	Kinases
KIR	KLRG1	LAG3	LIF	LXR	NKG2A	NLRP3	OX001R	OX40	PEDF
PVRIG	Receptor kinases	RIG-I pathway	RORγ	Semaphorin	Siglec-9	STAT3	STING	TGFβ	TIGIT
TIM3	TLRs	USP7	VISTA	VSIG8	Wnt pathway				

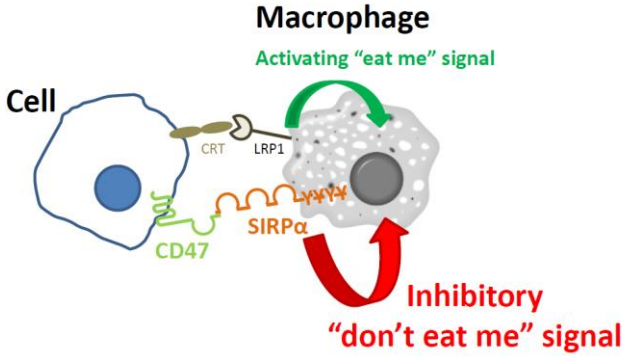
No. identified candidates per target

1	2-3	4-6	7-9	10-12	>12

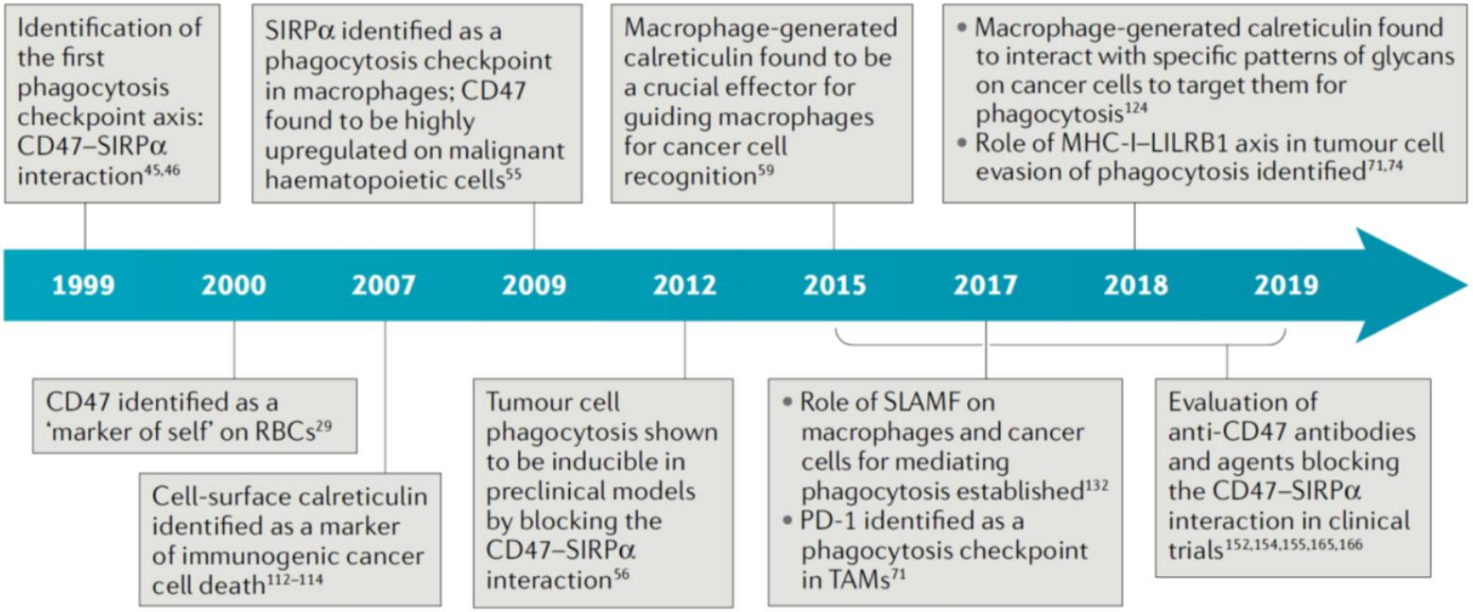


Some of the main ligands and receptors present on the surface of tumor and immune cells that are targets for approved and emerging immuno-oncology therapies

CD47 is an immune checkpoint receptor



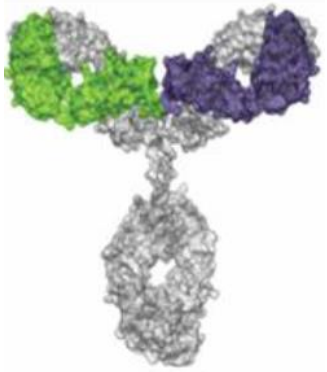
- CD47 is ubiquitously expressed & inhibits phagocytosis and dendritic cell maturation
- CD47 is over expressed by various cancers and poor clinical prognosis correlates with increased levels of CD47 expression
- Blocking the «dont eat me signal» could be beneficial for cancer treatrment

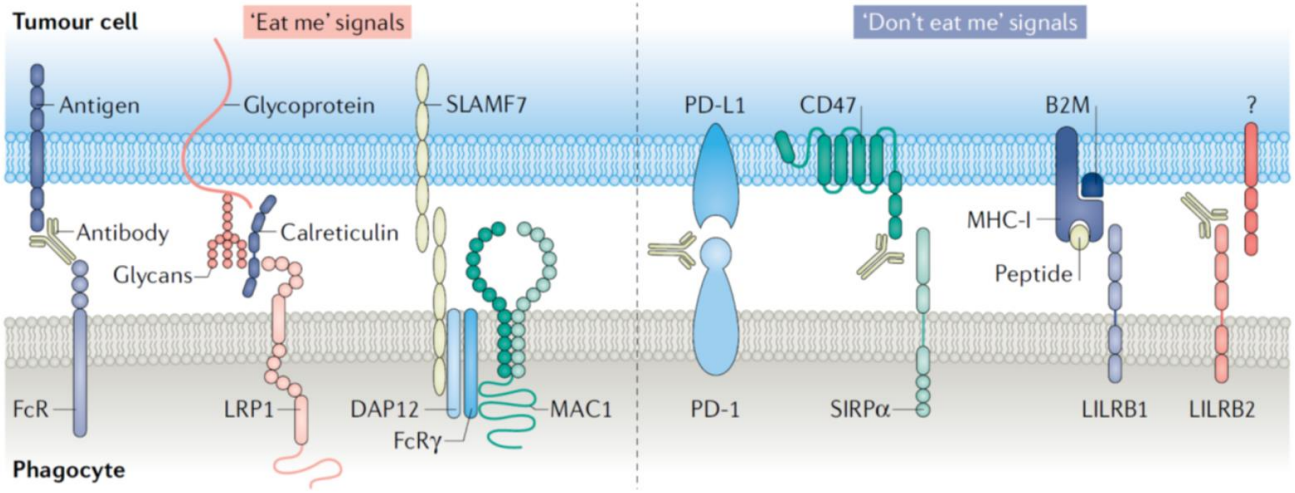


CD47 antagonist

- Blocking of “Don’t Eat-me-signal” activates macrophage and DC-mediated phagocytosis as well as T-cell-mediated killing for antitumor activity
- Antibodies or SIRP1α fusion proteins have been made by Fortyseven Inc., Celgene, Surface Oncology, Trillium, ALX.
- **However, Fc-containing agents (ie Antibodies) have**
  - Significant DLTs like anemia, thrombocytopenia and leukopenia
  - Impact on normal tissues, such as liver, lung and brain – caused by high level of macrophages, expression of CD47 coupled with FcR activation
- Need for either bispecific ABs (CD47 with tumor antigen (TA) like CD19/Mesothelin etc.) or small molecules (Aurigen et al.)

CD47 TA





Don’t eat me signals expressed by tumor cells and avoid phagocytoses by macrophages

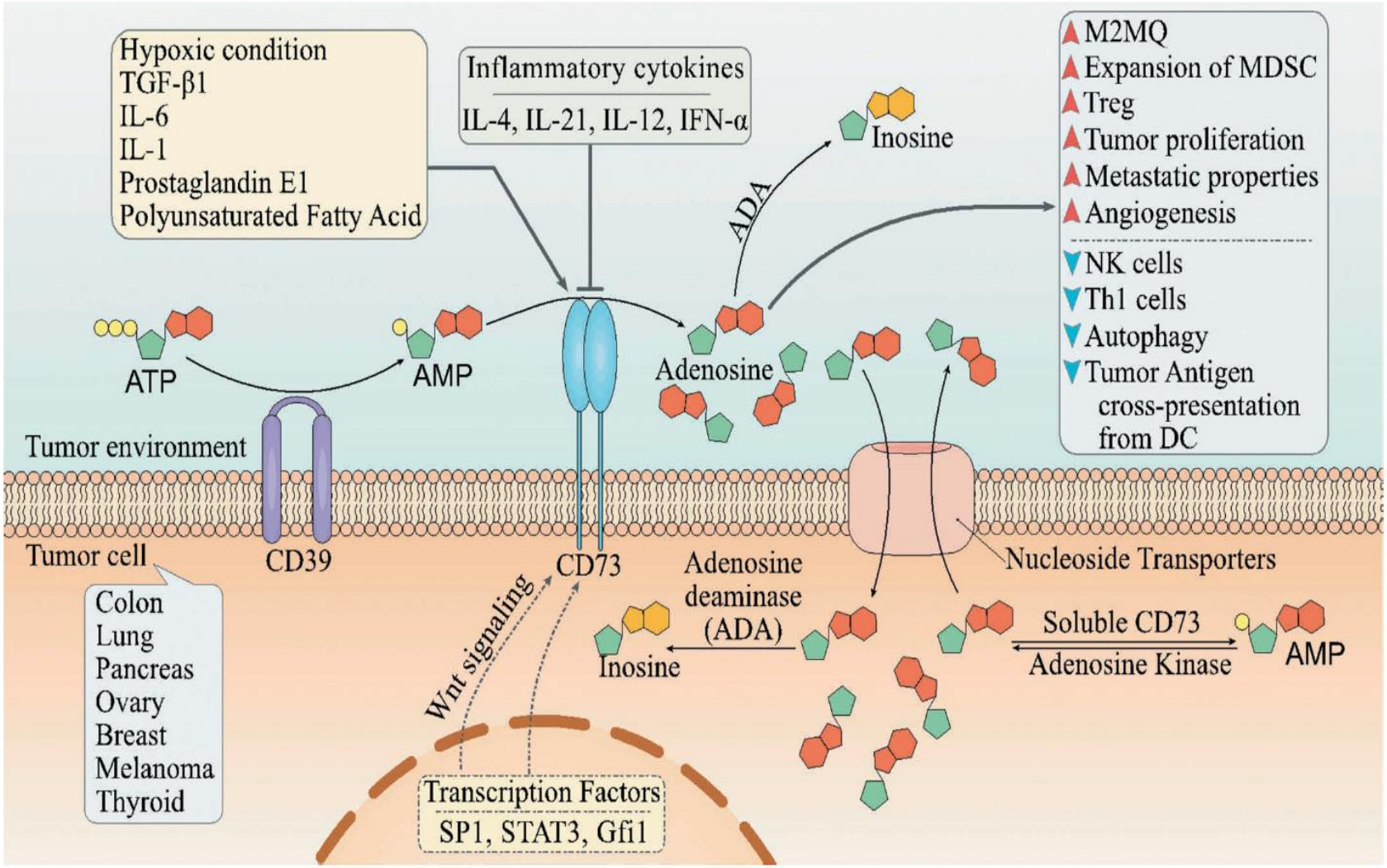
Developing Anti-CD47: mostly Abs, except Aurigen

Name	Company	Composition	Target	Fc	Stage	Clinical Start
Hu5F9	Forty Seven	Human monoclonal antibody	CD47	IgG4	Phase 2	Aug. 2014
CC90002	Inhibrix/Celgene	Human monoclonal antibody	CD47	IgG4	Phase 1	Mar. 2015
SRF-231	Surface Oncology	Human monoclonal antibody	CD47	IgG4	Phase 1	Mar. 2018
TTI-621/622	Trillium Therapeutics	Fusion protein, human SIRPa w/ active human Fc	CD47	IgG4/1	Phase 1	Jan 2016 (May 2018)
ALX-148	Alexo	High affinity IgG fusion protein w/ inactive Fc domain	CD47	Inactive Fc	Phase 1	Feb 2017
OSE-172	OSE Therapeutics (Boehringer Ingelheim)	Human monoclonal antibody	SIRPa	-	Preclinical	-
NI-1701 (TG-1801)	Novimmune	Human IgG bispecific	CD47/ CD19	IgG1	Preclinical	-
NI-1801	Novimmune	Human IgG bispecific	CD47/ Mesothelin	IgG1	Preclinical	-
AUR-104/105	Aurigene	Small molecule inhibitor	CD47	-	Preclinical	-
Undisclosed	Biocad	Human monoclonal antibody	CD47	-	Preclinical	-
Undisclosed	Synthon	Monoclonal antibody	CD47	-	Preclinical	-

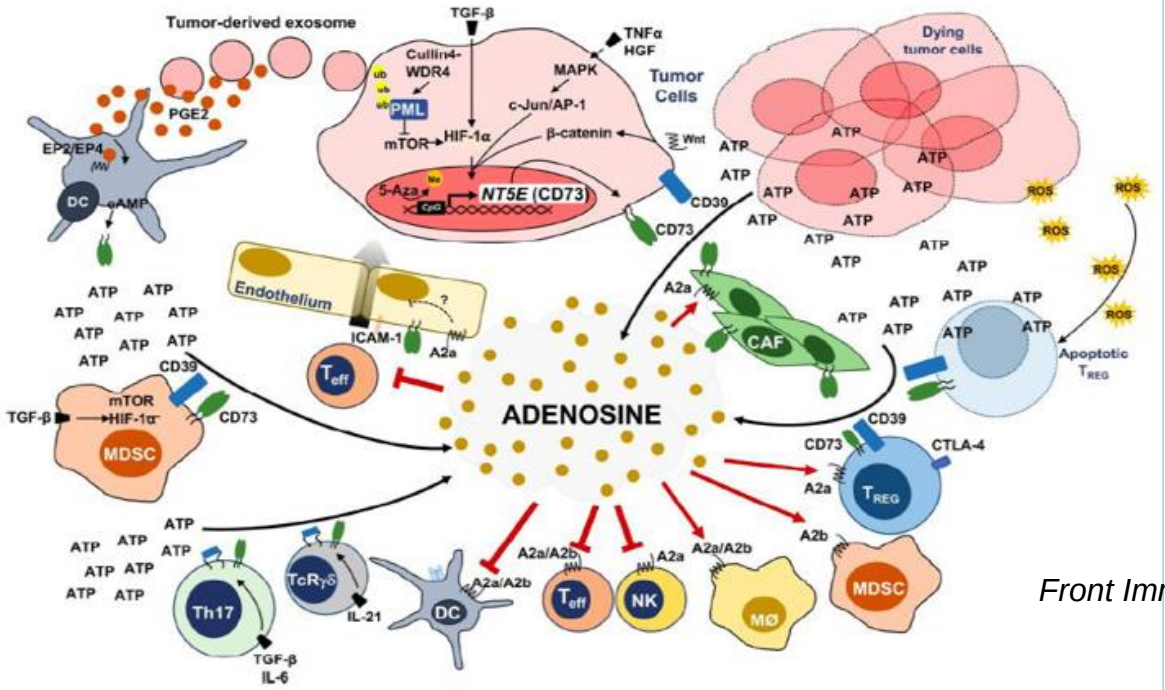
ClinicalTrials.gov identifier[a]	Phase	Intervention	Trial design	Estimated enrolment (*n*)	Cancer type	Primary end points
Monotherapy trials						
NCT03763149	I	Anti-CD47 antibody (IBI188)	Dose escalation	42	Advanced malignancies and lymphoma	Safety and tolerability
NCT03717103	I	Anti-CD47 antibody (IBI188) alone (Ia) or in combination with rituximab (Ib)	Dose escalation	92	Advanced malignancies	Safety and tolerability
NCT02678338	I	Anti-CD47 antibody (Hu5F9-G4)	Dose escalation	20	Haematological malignancies	Tolerability
NCT02216409	I	Anti-CD47 antibody (Hu5F9-G4)	Dose escalation	88	Solid tumours	Safety and tolerability
NCT03834948	I	Anti-CD47 antibody (AO-176)	Dose escalation, dose expansion	90	Advanced solid tumours	Safety and tolerability
NCT03013218	I	High-affinity SIRPα fusion protein (ALX148)	Dose escalation	142	Advanced solid tumours and lymphoma	Dose-limiting toxicity
NCT03512340	I/Ib	Anti-CD47 antibody (SRF231)	Dose escalation, dose expansion	148	Advanced solid and haematological cancers	Safety and tolerability
Combination trials						
NCT02367196	I	Anti-CD47 antibody (CC-90002) alone or in combination with rituximab	Dose escalation	110	Advanced solid and haematological malignancies	Tolerability and safety
NCT02663518	I	SIRPαFc (TTI-621) alone or in combination with rituximab or nivolumab	Dose escalation	260	Relapsed/refractory haematological and solid malignancies	Safety and tolerability
NCT02890368	I	SIRPαFc (TTI-621) alone or in combination with an anti-PD-1/PD-L1 agent, pegylated IFNα2a, T-VEC or radiation	Non-randomized, parallel assignment	240	Solid tumours and mycosis fungoides	Optimal delivery regimen
NCT03248479	Ib	Anti-CD47 antibody (Hu5F9-G4) alone or in combination with azacitidine	Non-randomized	96	AML and MDS	Safety and tolerability
NCT02953509	Ib/II	Anti-CD47 antibody (Hu5F9-G4) in combination with rituximab	Single-arm, non-randomized	72	Refractory/relapsed non-Hodgkin lymphoma	Safety, tolerability and efficacy
NCT02953782	I/II	Anti-CD47 antibody (Hu5F9-G4) in combination with cetuximab	Single-arm, non-randomized	112	Solid tumours and advanced CRC	Safety, tolerability and efficacy

CD73 and A2AR as IO targets

Blocking Adenosine production, transport and signaling



Immunosuppressive tumor microenvironment due to adenosine



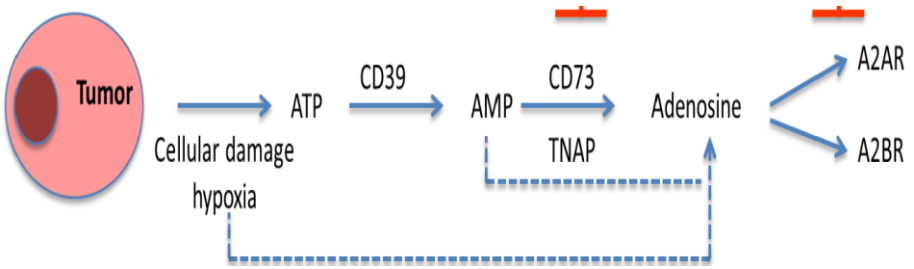
Front Immunol. 2019; 10: 453.

Limitations of single agent CD73 inhibition:

- Adenosine from direct release from tumor cells
- Adenosine generation from tissue non-specific alkaline phosphatase (TNAP)

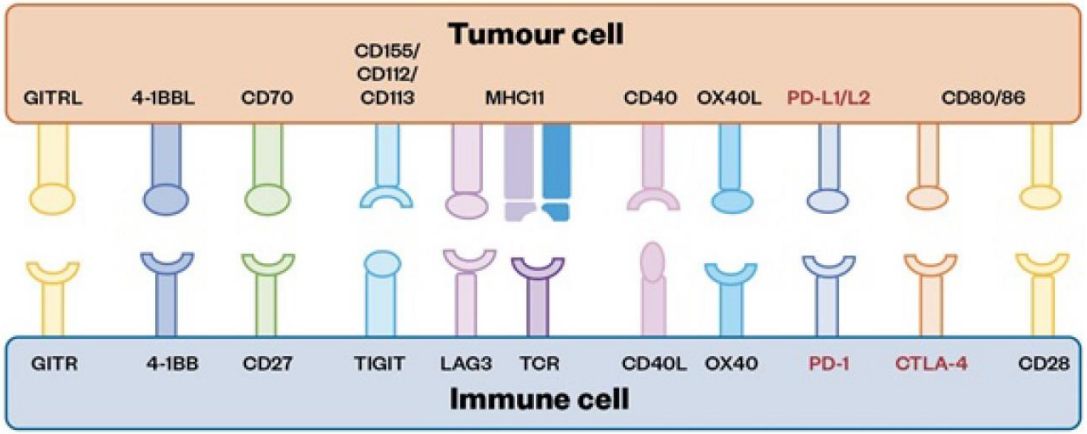
Limitations of single agent A2AR inhibition:

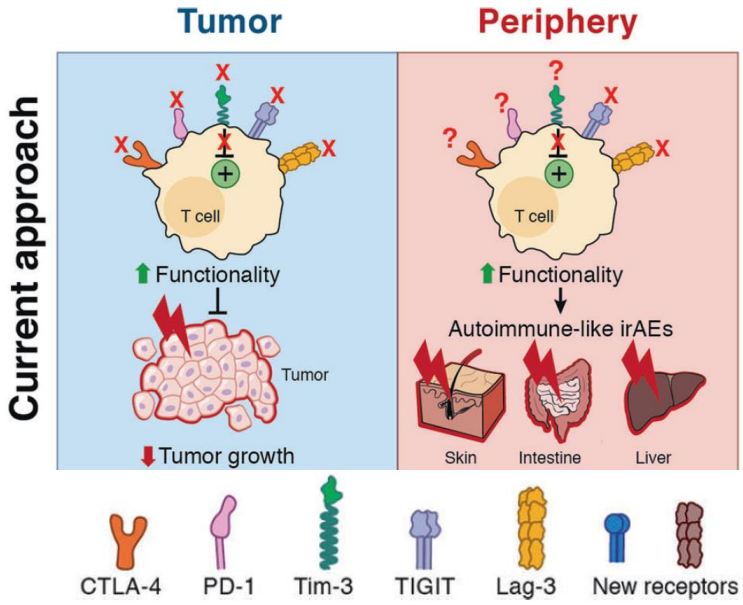
- increased CD73 expression upon A2AR inhibition
- compensatory activity of other adenosine receptors such as A2BR upon A2AR inhibition

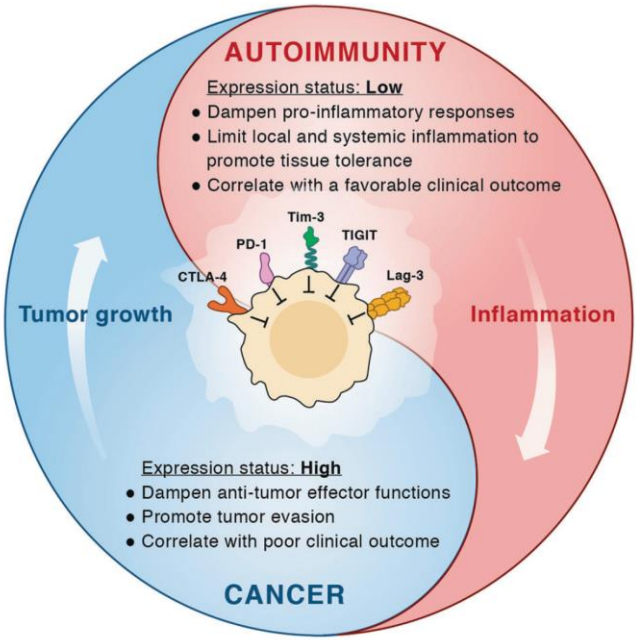


TIM-3 and TIGIT and LAG-3

- T cells express multiple cell surface immune check points like TIM-3, TIGIT and LAG-3
- Blockage is beneficial for activating T-cells but can cause auto immunity (or irAEs)







TIGIT/PD-L1: Rationale

- TIGIT expressed on NK and T cells inhibits the immune function by binding to PVR (CD155)
- TIGIT on tumor cells is an independent checkpoint pathway contributing to intrinsic resistance to anti-PD1.
- Co-blockade of TIGIT and PD-1/PD-L1 elicits synergistic activity in vivo
- Therefore, TIGIT combination opens all indications approved for PD-1/PD-L1
- Genentech (completed Ph-2 in NSCLC), Merck and Merck (Ph-2 in NSCLC), Arcus (completed Ph-2), BMS, Celgene, Astellas , Seattle Genetics, Iteos Therapeutics (Ph-1 studies in solid tumors ongoing)

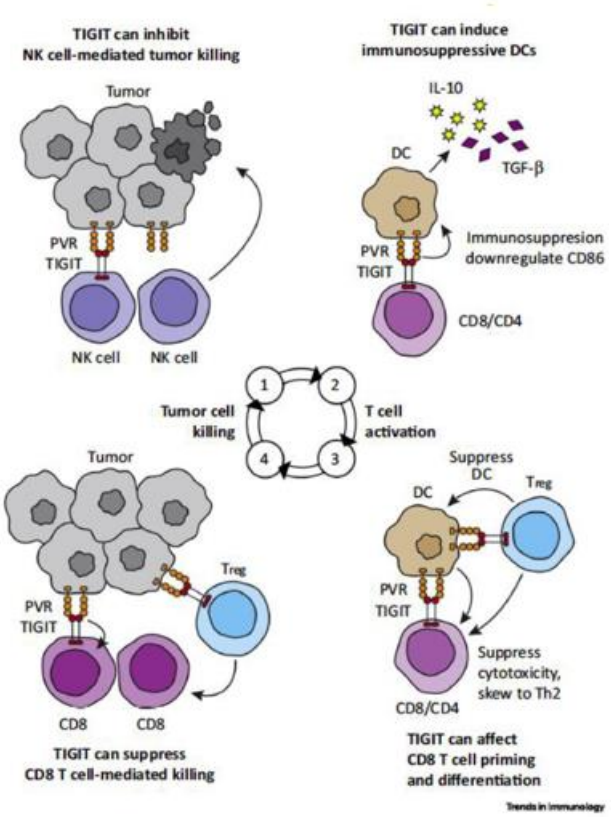


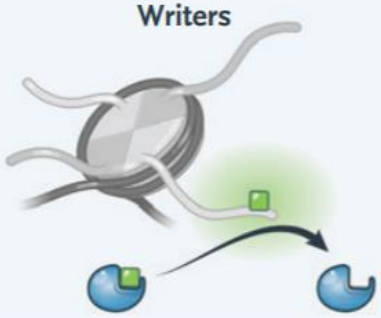
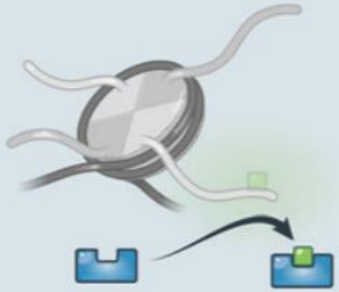
Table 2 Clinical trials of TIM-3 inhibitors

Year	Drug	Phase	Company	Type	Objective	ClinicalTrial.gov identifier
2015	MBG453	I	Novartis Pharmaceuticals (Basel, Switzerland)	Anti-TIM-3	MBG453 given alone or combined with PDR001 in adult patients with advanced malignancies	NCT02608268
2016	TSR-022	I	Tesaro, Inc. (Waltham, MA, USA)	Anti-TIM-3	Dose escalation and cohort expansion study of TSR-022 in advanced solid tumors	NCT02817633
2017	LY3321367	I	Eli Lilly and Company (Indianapolis, IN, USA)	Anti-TIM-3	LY3321367 alone or combined with an anti-PD-L1 antibody in advanced relapsed/refractory solid tumors	NCT03099109
2017	MBG453	I	Novartis Pharmaceuticals	Anti-TIM-3	PDR001 and/or MBG453 in combination with decitabine in AML or high-risk MDS	NCT03066648

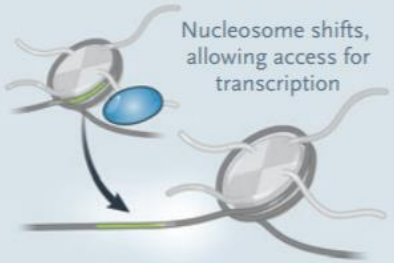
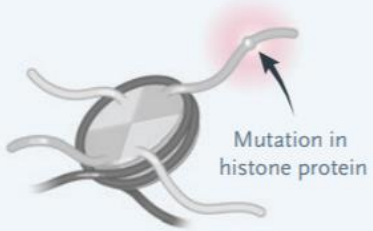
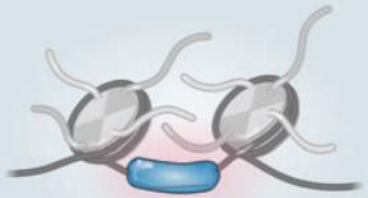
Significant rationales for

TIGIT or TIM-3 or LAG-3 single agents and/or
TIGIT or TIM-3 or LAG-3 combo with PD-L1

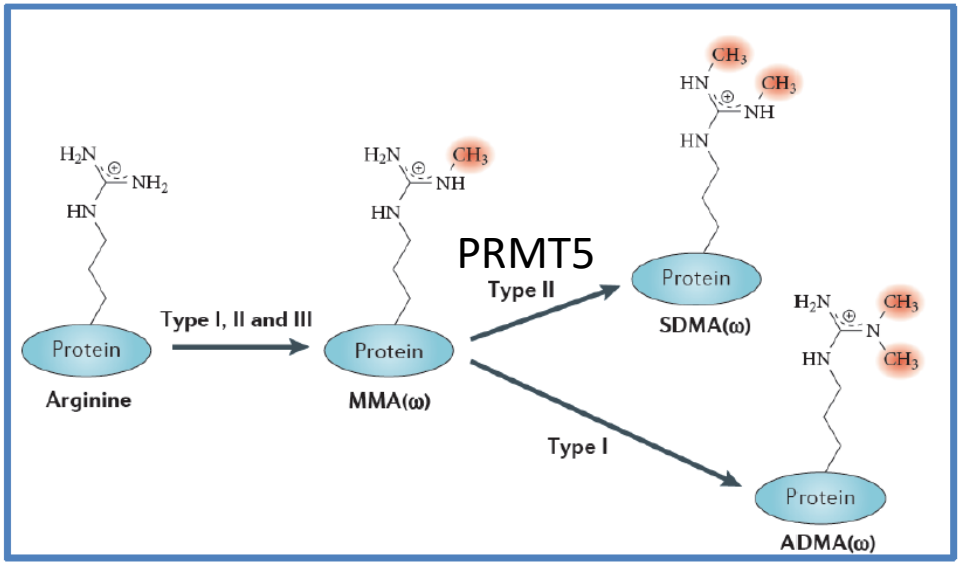
Epigenetic targets
Writers, Erasers, Readers

Category	Epigenetic Regulators	Function	FDA-Approved Drug
Writers 	**DNMT1, 3A, and 3B**	Methylates cytosines on DNA, and mutation can lead to aberrant methylation	Azacitidine, decitabine
	EZH2	Methylates histone H3K27	Tazemetostat
	DOT1L	Methylates histone H3K79	
	KMT2A–D, SETD2, NSD1	Methylate histone lysines	
	EP300, CREBBP	Acetylate histone lysines	
Erasers 	**TET2**	Is the first step in cytosine demethylation; is inhibited by 2-hydroxyglutarate (2-HG)	Azacitidine, decitabine
	IDH1, IDH2	Mutated protein produces 2-HG from isocitrate that inhibits TET2 and lysine demethylases	Ivosidenib, enasidenib
	HDAC1–3, 8 HDAC6	Deacetylase removes acetyl groups from histone lysines	Vorinostat, belinostat, panobinostat, romidepsin
	KDM1A, **KDM6A (UTX)**	Demethylates histone lysines	
Readers 	BRD4	Bromodomain proteins read acetyl groups on histone lysines	
	CBX family, CHD1	Chromodomain proteins read methyl groups	

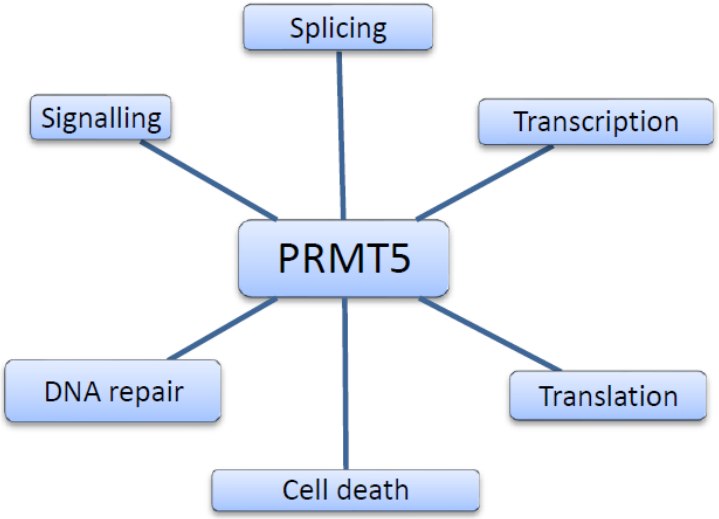
Epigenetic targets
Movers, Shapers, Insulators

Category	Epigenetic Regulators	Function	FDA-Approved Drug
Movers 	**ARID1A, ARID1B, ARID2** SMARCA2, **SMARCA4, SMARCB1,** CHD1	Proteins in the chromatin remodeling complex use ATP to move nucleosomes away from DNA; loss-of-function mutations common in cancer	
Shapers 	**HIST1H1B, HIST1H1C, HIST1H3B, H3F3A, H3F3B**	Structural histone proteins acquire mutations that can be oncogenic	
Insulators 	**CTCF, STAG2, RAD21,** CHD8	Normal binding to CTCF sites on DNA defines and protects gene neighborhoods from inappropriate expression	

PRMT5:
Protein Arginine Methyltransferase 5



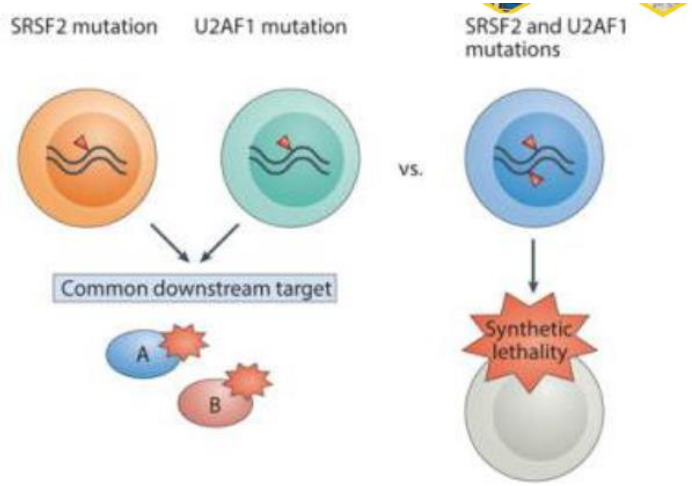
Nature Reviews Cancer 2013, 13, 37–50

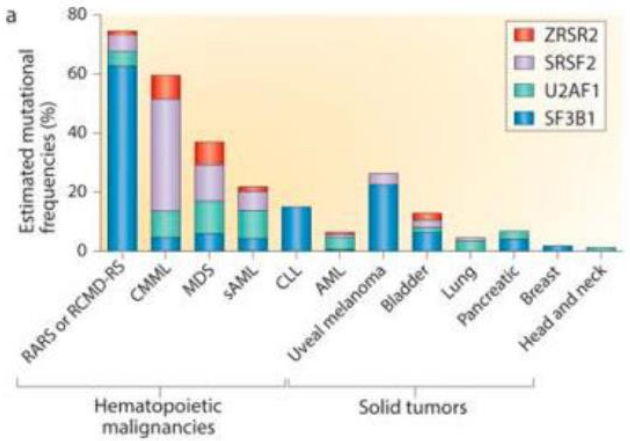


- PRMT5 catalyzes the formation of symmetric dimethyl arginine (SDMA) in many cellular proteins
- SDMA regulates cancer relevant proteins and pathways
- PRMT5 is key regulator of cellular splicing and its overexpression signifies poor prognosis

Potential Therapeutic Application of PRMT5i Synthetic Lethality in Spliceosome Mutant Cancers

- Splicing of mRNA precursors is a key step in regulating expression of many genes
- Spliceosome proteins are rendered functional by post-translational methylation by PRMT5
- High frequency of hotspot mutations in the spliceosome proteins SF3B1, SRSF2, and/or U2AF1 have been reported in several cancers (35-40% of MDS, 5-18% in CLL, 5-25% in AML, 14-29% in uveal melanoma)
- Functional redundancy exists among members of the spliceosome complex
- PRMT5 inhibition potentiates synthetic lethality in spliceosome mutant cancers
- Approach is currently being evaluated in clinical trials

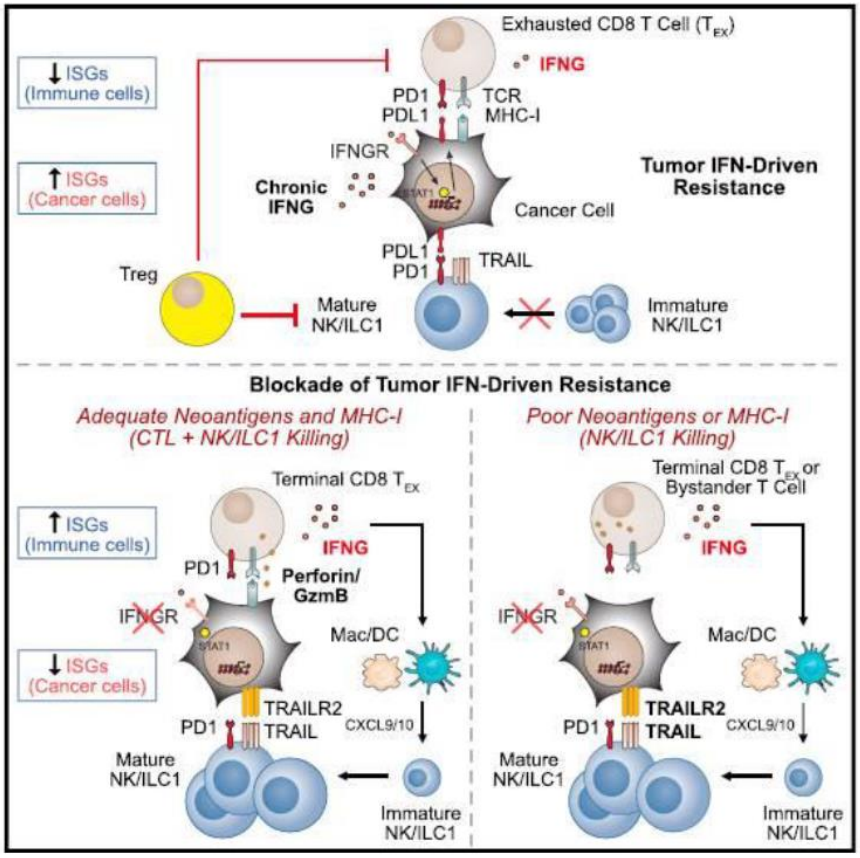




Nat Rev Cancer 2016 17; 413-430; Nat Struct Mol Biol 2019; 26, 999–1012

Potential Therapeutic Application of PRMT5i
Turning cold tumors to hot

- Epigenetic changes are required for the expression of ISG genes in tumor cells leading to tumor IFN-driven resistance
- PRMT5i is reported to inhibit expression of resistant genes in tumor cells
- Conversely, PRMT5i allows uninterrupted IFNG signaling in immune cells
- PRMT5i would phenocopy tumor IFNGR ablation while allowing IFNG-driven favorable immune response.
- Expected to enhance anti-tumor efficacy of anti-PD1 antibody. Combination is currently being evaluated in clinical trials



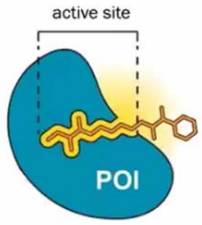
Cell Reports 2020, 30, 1935–1950; Cell 2016, 167, 1540-1554; Cell 2019, 178, 933–948

PRMT5i: Competitive Landscape

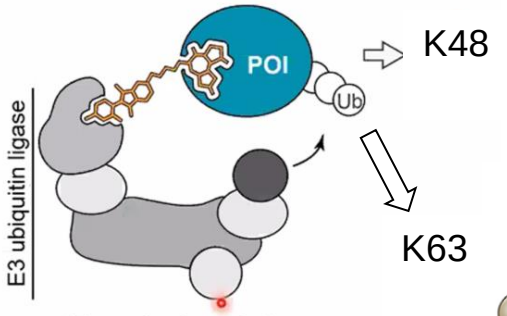
Drug/Company name	Type	Highest Dev. Status
GSK-3326595/ GSK and Epizyme Inc	Substrate competitive	Phase 1/2 Clinical
JNJ-64619178/Janssen Research & Development LLC	SAM competitive	Phase 1 Clinical
PF-06939999/Pfizer Inc.	SAM competitive	Phase 1 Clinical
PRT-811/Prelude Therapeutics Inc.	Unknown	Phase 1 Clinical
PRT-543/Prelude Therapeutics Inc.	Unknown	Phase 1 Clinical
Cancer Therapeutics CRC/Merck MSD	-	Discovery
CT-300/Celleron Therapeutics Ltd	-	Discovery
Bayer Pharma	-	Discovery
Argonaut Therapeutics Ltd	-	Discovery

- GSK has initiated Phase 2 trials in MDS and AML; Combination with 5-azaciditine and Pembrolizumab

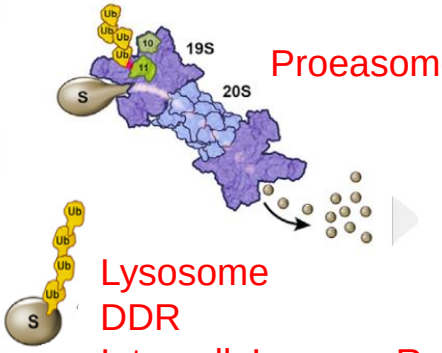
Novel paradigm in drug development using the UPS



Traditional inhibitors



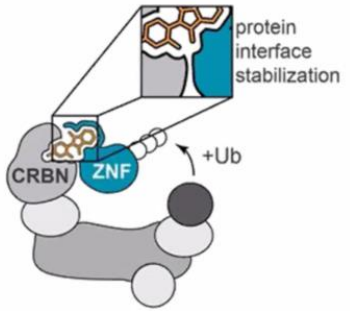
Targeted protein degradation



Proeasome

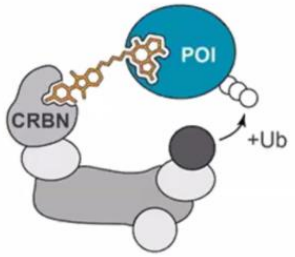
Lysosome
DDR
Intracell. Immune Response

Molecular Glues



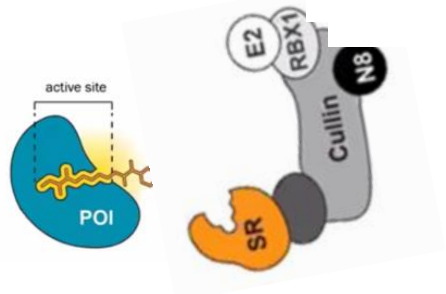
Modulate E3 surface to recognize neo-substrate

PROTACs



Tethering to enforce proximity to E3

Destabilizer



Destabilize target to make it E3 degradable

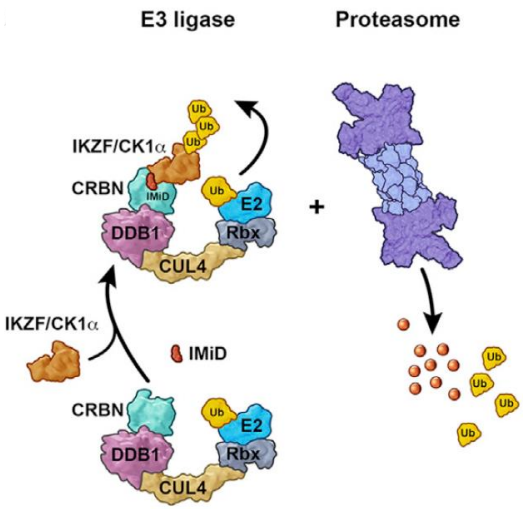
Type of molecule	monovalent	heterobifunctional
Design principles	Non-rational	Modular: ligand^{E3}-linker-ligand^{POI}
Examples	IMiDs (clinical reality)	ARV-110, ARV-471 (in clinical trials)

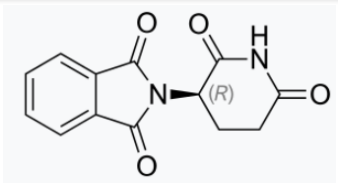
Pros and Cons

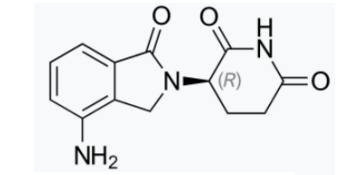
PROTAC	Molecular Glue	Destabilizers
Bi-functional molecules bind to E3 ligase and a single protein simultaneously, causing proximity induced degradation	**Bind to E3 ligases and degrade recruited protein(s) via a ternary complex**	**Monovalent molecules that bind to a protein and cause destabilization and degradation**
Amenable to any protein with a known binder. Optimization of linkers, ligands and couplings very well suited for combinatorial probing. **Path to ID'ing degraders & optimizing efficacy, differentiation and validation is well defined**	Smaller and more drug-like molecules e.g. IMIDs and indusulam	Small and drug like – derived from recognizable inhibitor templates, often with very small (single atom) changes
Wide variety of targets already demonstrated to be amenable for PROTAC degradation	Not target-biased; Potential to target 'undruggable' proteins	Target biased: bromodomains, nuclear receptors and kinases all shown to be capable of being degraded
Molecules tend to be larger and less drug-like; Beyond Ro5 with implications for achieving good PK/ADME/tox	No rational way to design glues, and no way to predict which protein(s) they will degrade Pure screening and empiricism; difficult to predict pleotropy	No rational way – yet – to design destabilizers. Examples so far found serendipitously during SAR campaigns for inhibitors
Requires a binding site on the protein	Validation of therapeutic potential & safety of novel degraded proteins can be a massive effort	Factors controlling degradation efficiency and selectivity are not well understood

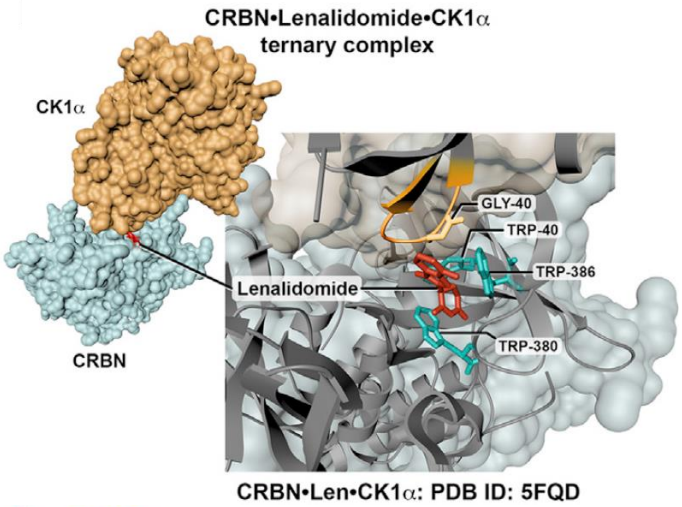
Glue-degrader

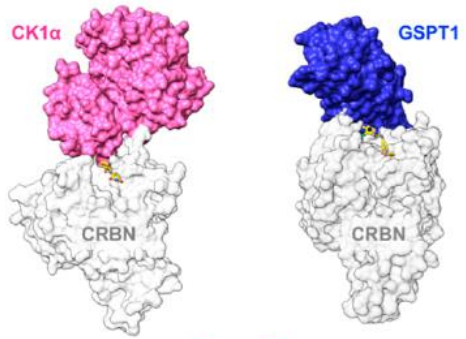
IMiD-Induced CRBN-Dep. Degradation of Neosubstrates

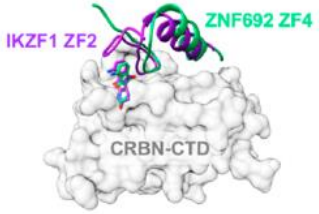


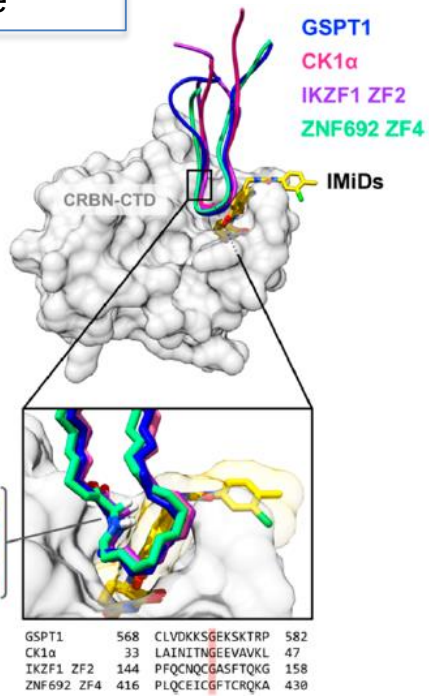










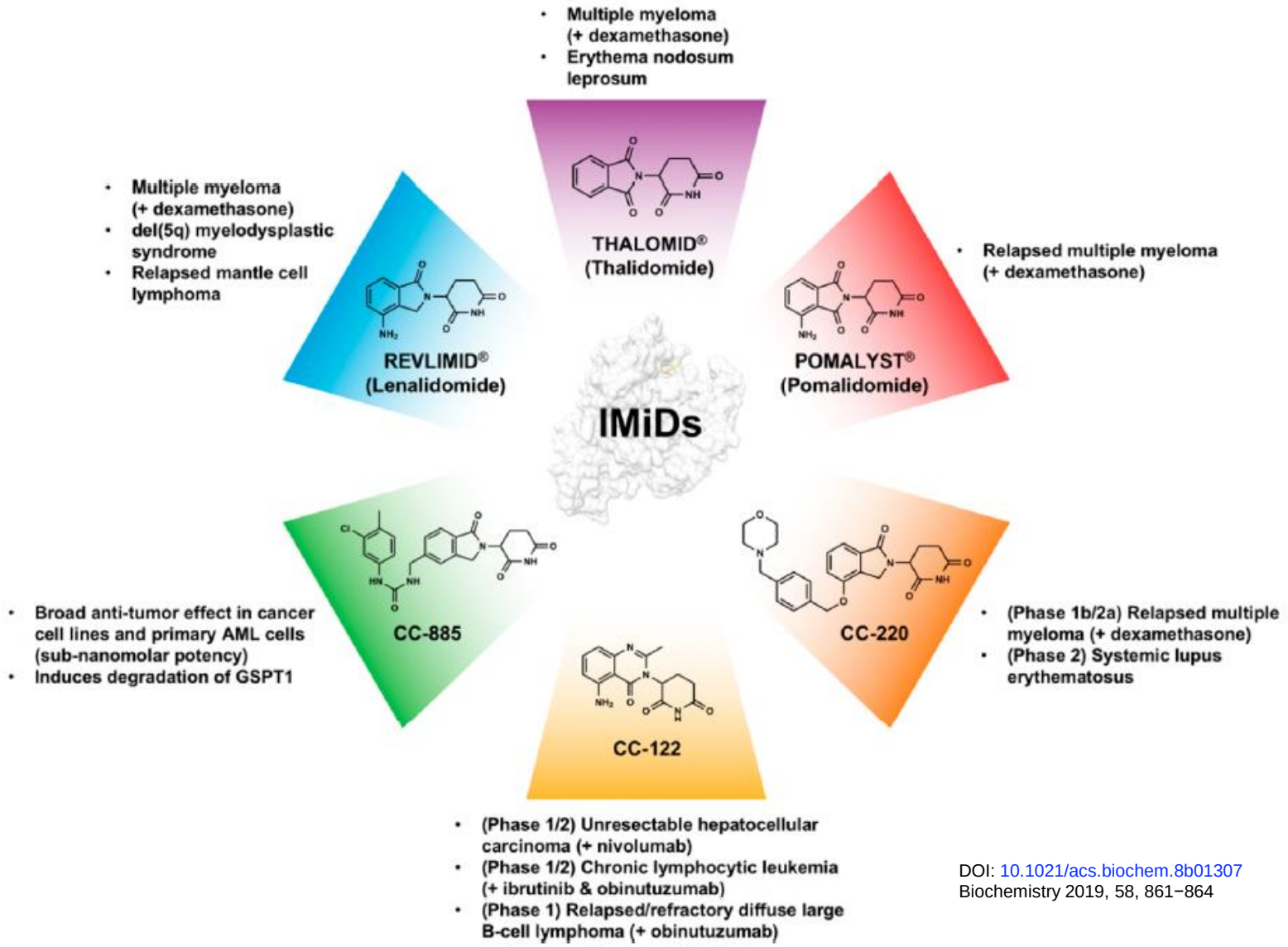


ps://doi.org/10.1016/j.molcel.2020.01.010

DOI: 10.1021/acs.biochem.8b01307
Biochemistry 2019, 58, 861−864

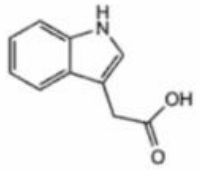
Glue degrader

Structures and clinic for FDA-approved Imids



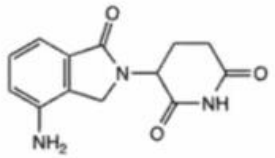
DOI: 10.1021/acs.biochem.8b01307
Biochemistry 2019, 58, 861−864

Glue degrader
Expanding on Glue degrader



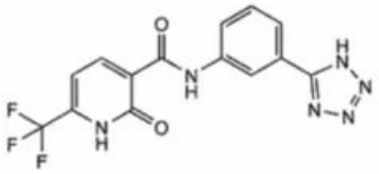
Auxin

TIR1



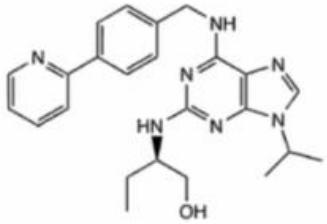
Lenalidomide

CRBN



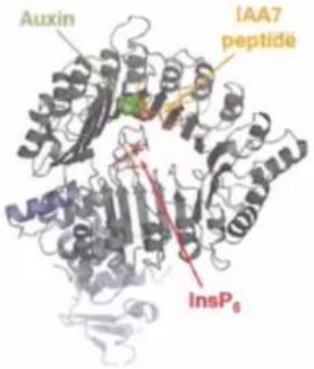
NRX-1933

B-TrCP

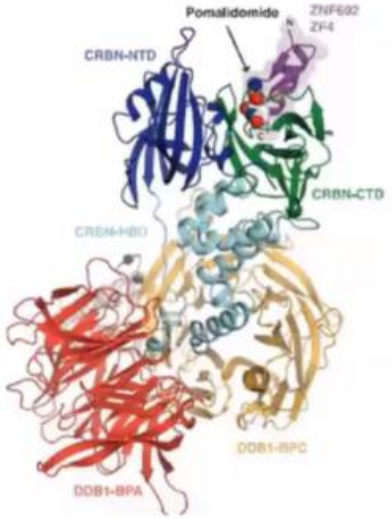


(R)-CR-8

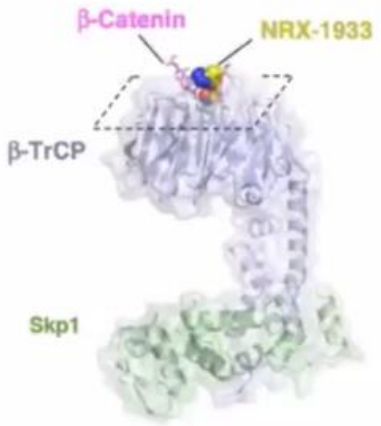
DDB1



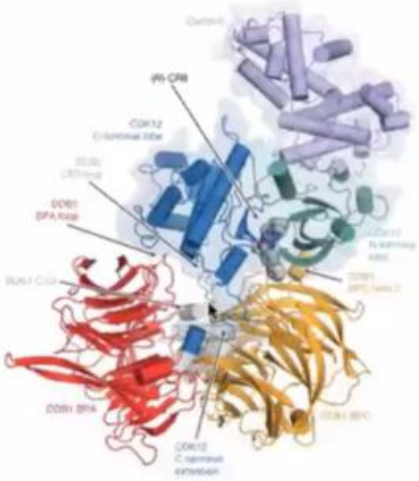
Tan et al., 2007, *Nature*



Sievers et al., 2018, *Science*



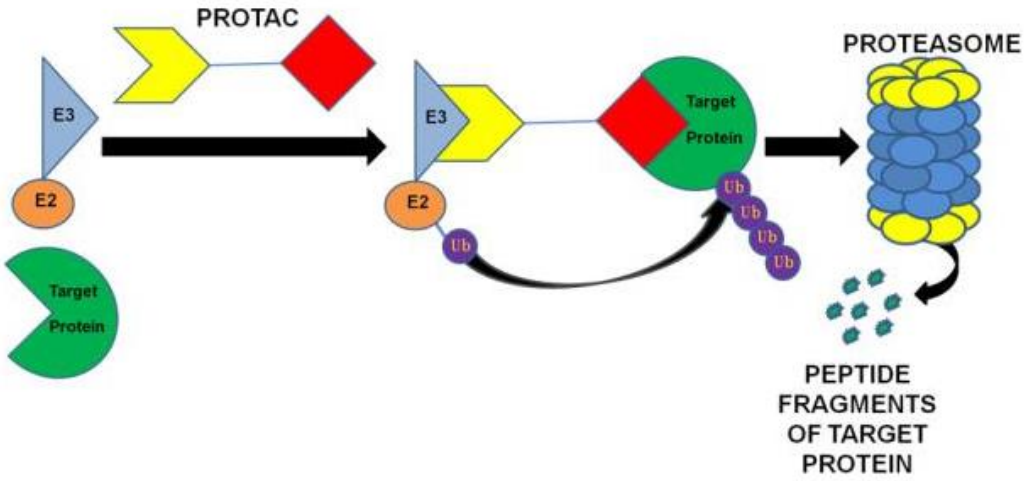
Simonetta et al., 2019, *Nat. Comm.*

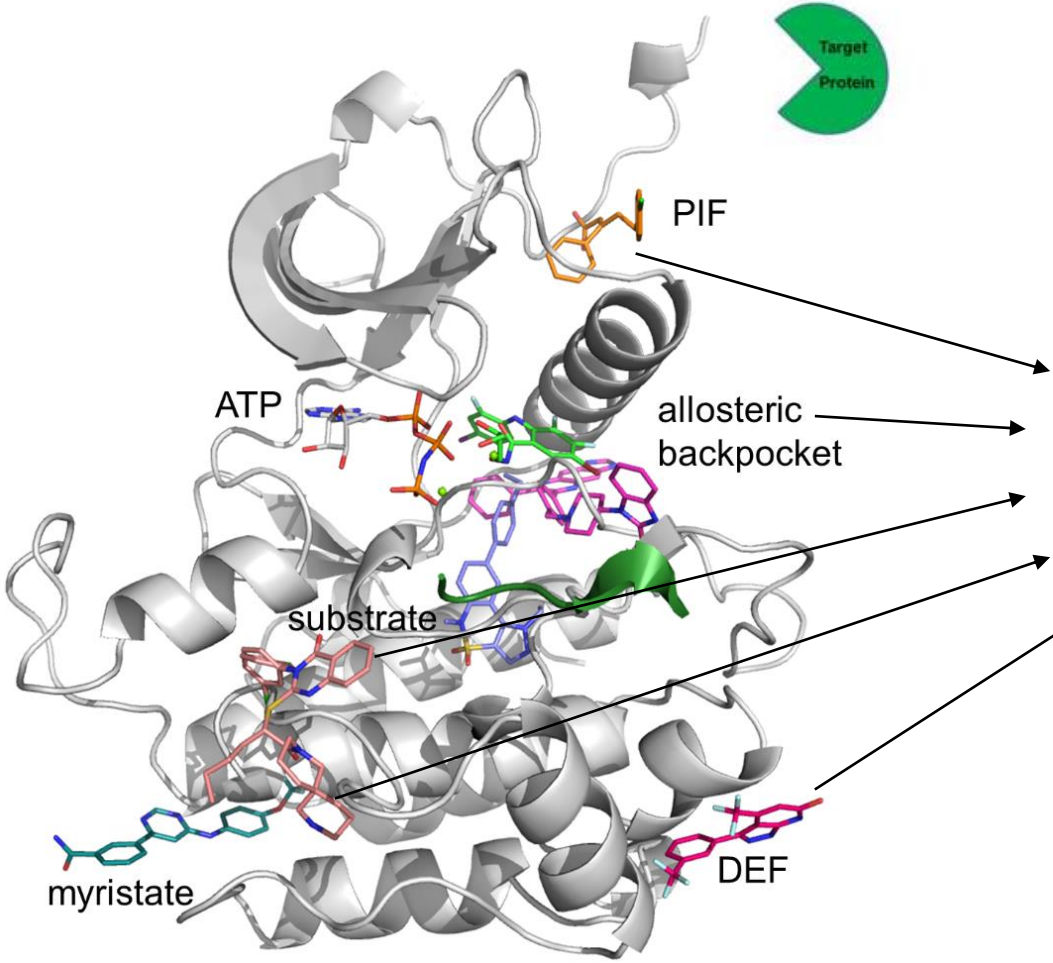


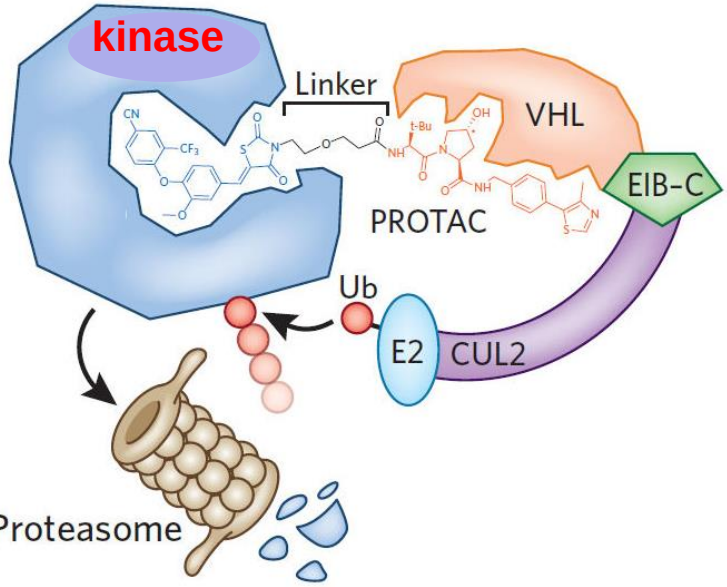
Słabicki et al., 2019, *Nature.*

Bifunctional degrader approach for protein kinases or other POIs

Destroy the kinase target rather than inhibiting it

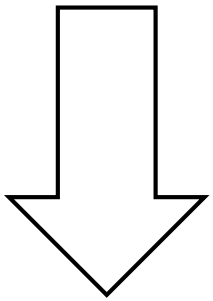






Target(s)	Ligase(s) (References)
ABC50	CRBN (Cie slak et al., 2019)
ALK	CRBN (Powell et al., 2018; Zhang et al., 2018)
AR	MDM2 (Schneekloth et al., 2008)
AURKA/B	CRBN (Huang et al., 2018)
BCL2	CRBN (Wang et al., 2019)
BCL6	CRBN (McCoull et al., 2018)
BCR-ABL	IAP; CRBN; VHL (Demizu et al., 2016; Lai et al., 2016)
BRAF	CRBN (Chen et al., 2019a)
BRD2, BRD3, BRD4	CRBN; VHL (Lu et al., 2015; Winter et al., 2015; Zengerle et al., 2015)
BRD7, BRD9	VHL (Zoppi et al., 2019)
BTK	CRBN (Buhimschi et al., 2018; Sun et al., 2018; Zorba et al., 2018)
c-ABL	CRBN; VHL (Lai et al., 2016)
CDK4/6	CRBN (Jiang et al., 2019; Zhao and Burgess, 2019a)
CDK9	CRBN (Olson et al., 2018; Robb et al., 2017)
cIAP1	IAP (Itoh et al., 2012)
CRABP I/II	IAP (Itoh et al., 2012; Itoh et al., 2010)
CRBN	VHL; CRBN (Steinebach et al., 2019; Steinebach et al., 2018)
EGFR	VHL (Burslem et al., 2018a)
ER	IAP (Demizu et al., 2012; Itoh et al., 2011)
ERK1/2	CRBN (Lebraud et al., 2016)
ERRa	VHL (Bondeson et al., 2015)
FAK	VHL (Cromm et al., 2018)
FKBP12	CRBN (Winter et al., 2015)
FLT3	VHL; CRBN (Burslem et al., 2018b; Huang et al., 2018)
GSPT1	CRBN (Matyskiela et al., 2016)
HCV	NS3/4A CRBN (de Wispelaere et al., 2019)
HDAC6	CRBN (Yang et al., 2018)
HER2	VHL (Burslem et al., 2018a)
IRAK4	VHL (Nunes et al., 2019)
ITK	CRBN (Huang et al., 2018)
Mcl1	CRBN (Wang et al., 2019)
MDM2	CRBN (Li et al., 2019b)
p38a/delta	VHL; CRBN (Bondeson et al., 2018; Smith et al., 2019)
PARP1	MDM2 (Zhao et al., 2019)
PCAF/GCN5	CRBN (Bassi et al., 2018)
PIRIN	CRBN (Chessum et al., 2018)
PTK2/B	VHL; CRBN (Popow et al., 2019)
RAR	IAP (Itoh et al., 2011)
RIPK2	VHL (Bondeson et al., 2015)
Rpn13	CRBN (Song et al., 2019)
SGK3	VHL (Tovell et al., 2019b)
sirtuin-2	CRBN (Schiedel et al., 2018)
SMAD3	VHL (Wang et al., 2016)
SMARCA2/4	VHL (Farnaby et al., 2019)
TACC3	IAP (Ohoka et al., 2014)
tau	CRBN (Silva et al., 2019)
TBK1	VHL (Crew et al., 2018)
TEC	CRBN; VHL; IAP (Zorba et al., 2018; Huang et al., 2018)
TRIM24	VHL (Gechijian et al., 2018)
TrkC	CRBN (Zhao and Burgess, 2019b)
ULK1	CRBN (Huang et al., 2018)
VHL	VHL (Maniaci et al., 2017)

List of present targets for non-peptidic PROTACs



Two thus far in clinical trials

https://doi.org/10.1016/j.molcel.2020.01.010

ARVINAS

		Programs [Target]	Discovery	Lead Optimization	IND Enabling	Phase 1	Arvinas Owned
Oncology	Metastatic Castration-resistant Prostrate Cancer	ARV-110 [Androgen Receptor]					✓
		Next Generation Degrader [Androgen Receptor]					✓
		AR Variant Degrader [AR-V7]					✓
	Locally Advanced or Metastatic ER+ / HER2- Breast Cancer	ARV-471 [Estrogen Receptor]					✓
	Additional Oncology Indications	e.g., CRC, NSCLC [Various Undisclosed]					✓
Neurology	Tauopathies	e.g., PSP[2] [Tau]					✓
	Synucleinopathies	e.g., MSA[3], Parkinson's [α-synuclein]					✓
	Additional Neurology Indications	Various [Undisclosed]					✓

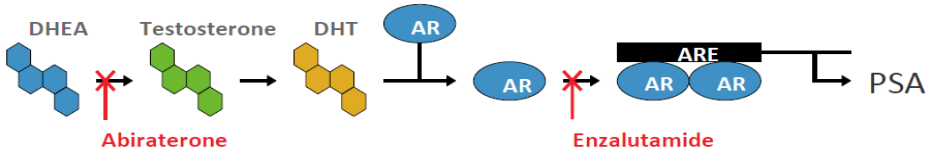
ARV-110

Androgen Receptor (AR) Activity Drives Prostate Cancer

- Current agents work by decreasing androgen levels (abiraterone) or blocking androgen binding to AR (enzalutamide)
- **15-25%** of patients never respond to abiraterone or enzalutamide (**intrinsic resistance**)
- **Resistance mechanisms** to abiraterone and enzalutamide include:
  - **AR gene amplification** (40-60% of patients)
  - **AR gene enhancer amplification** (>70% of patients)
  - **AR point mutations** (~15% of patients)
  - **Intra-tumoral androgen production**

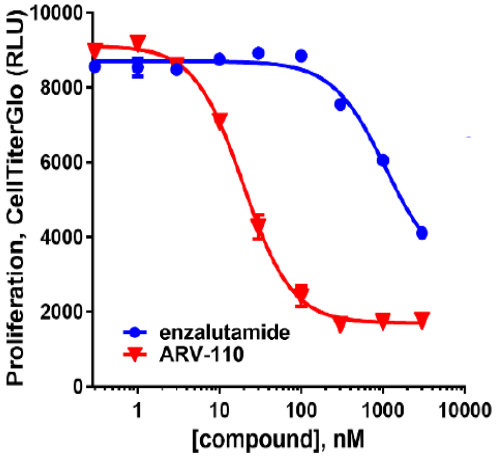
PROTAC® Degrader ARV-110

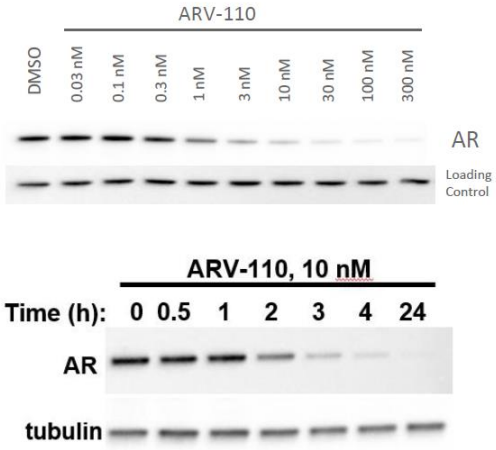
- Highly selective degrader of AR; DC_{50} = 1 nM
- In preclinical models, overcomes resistance mechanisms to enzalutamide and abiraterone
- Not brain penetrant
- First-in-class AR degrader being tested in men with metastatic castration-resistant prostate cancer who have progressed on standards of care (enzalutamide, abiraterone)
- **Phase 1 clinical trial initiated 1Q19**
- **Received FDA "Fast Track" designation in May 2019**



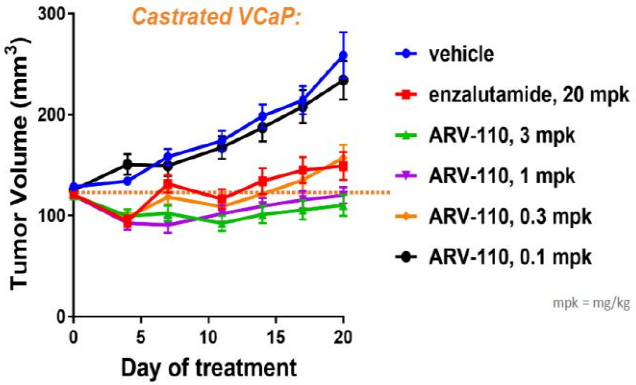
ARV-110 degraded 95% to 98% of AR in multiple cell lines typically used in prostate cancer research, including VCaP cells

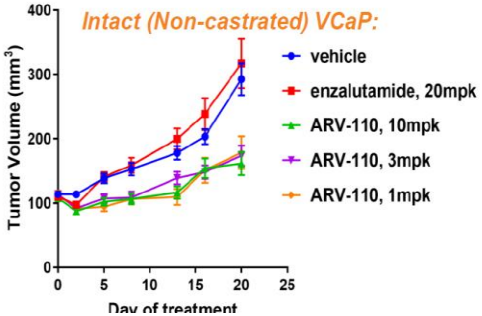
- DC_{50} in VCaP = 1 nM , Dmax@4 hrs
- 60x more potent than enzalutamide

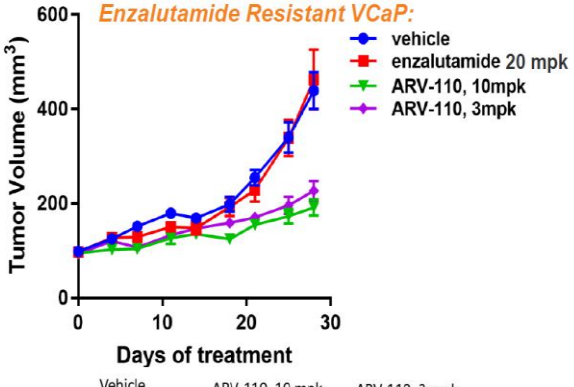


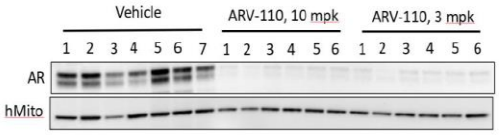


ARV-110 Inhibits AR-Dependent Tumor Growth in Xenograft Models with Oral, Daily Dosing



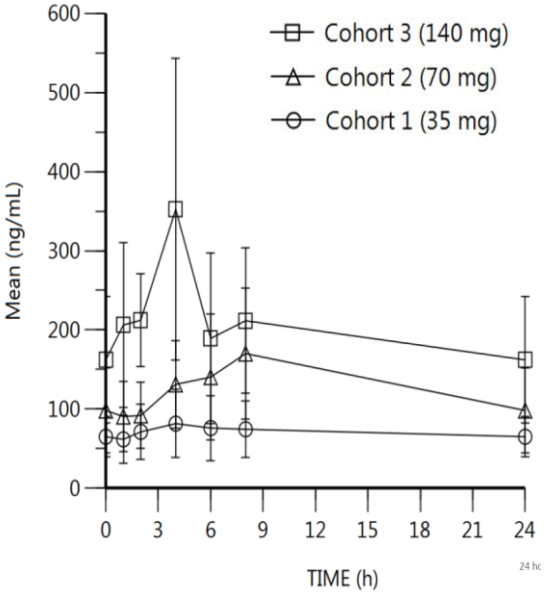






Dose po, qd	Mean AUC_{0-24} ng*hr/ml†	Mean C_{max} ng/ml‡
1 mpk	3628	224
3 mpk	8106	507

ARV-110 Ph-1 Day 15 Pharmacokinetics



- 10 patients with mCRPC treated across three dose levels
- At doses up to 280 mg with an acceptable safety profile
- PK dose-proportional increase in exposure
- PSA and RECIST responses and PD/molecular marker planned in 1st half 2020 at major medical conference

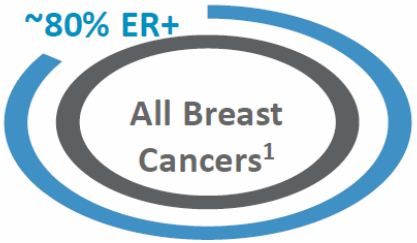
ARV-471: ER Degrader for Patients with Locally Advanced or Metastatic Breast Cancer

Breast cancer is the second most common cancer in women[1]

- ~268,000 women are expected to be diagnosed with invasive breast cancer in the US in 2019[1]
- Metastatic breast cancer accounts for ~6% of newly diagnosed cases[2]
- 80% of newly diagnosed breast cancers are estrogen receptor (ER) positive[3]
- Fulvestrant has validated the relevance of ER degradation in breast cancer
- After 6 months of fulvestrant treatment, up to 50% of ER baseline levels remain[4]

PROTAC® Degrader ARV-471

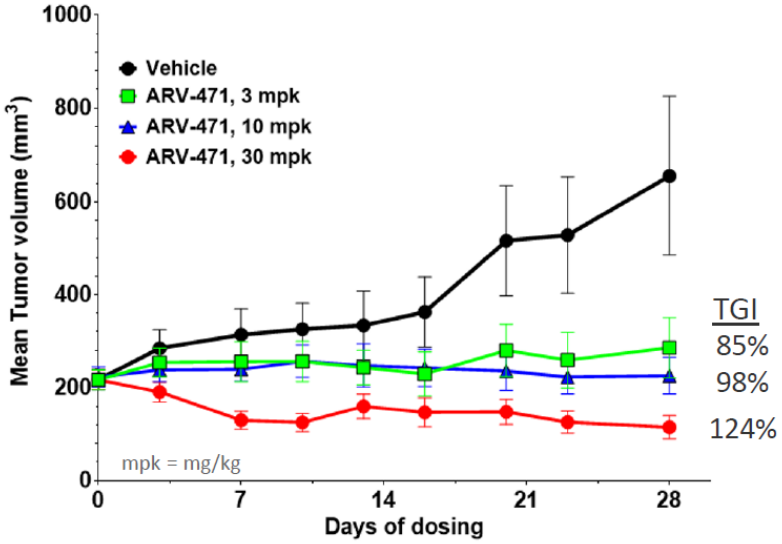
- ARV-471 is a potent degrader (DC_{50} = 1.8 nM) of the estrogen receptor, which is in development for the treatment of patients with ER+ locally advanced or metastatic breast cancer
- **Phase 1 clinical trial initiated 3Q2019**
- After Phase 1 dose escalation, a Phase 1b trial in combination with CDK4/6 inhibitor is planned



1 American Cancer Society;

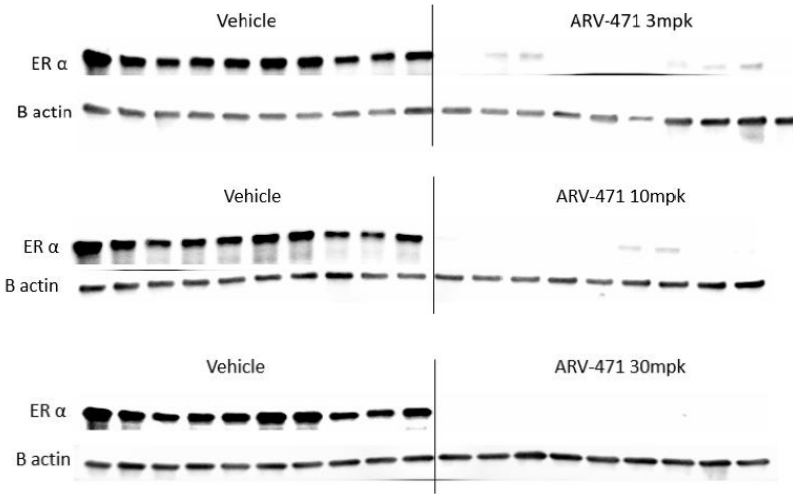
3 National Cancer Institute, Hormone Therapy for Breast Cancer;

Orally Dosed ARV-471 Shrinks Tumors and Robustly Degrades ER in MCF7 Xenografts



Dose po, qd	Mean AUC_{0-24} ng*hr/ml	Mean C_{max} ng/ml
3 mpk	658	84
10 mpk	2538	312
30 mpk[a]	5717	962

WESTERN BLOT PD (18 hours post last dose)	% ER REDUCTION
3 mpk	95
10 mpk	97
30 mpk	94



ARV-471: Phase 1 Study: FIH 08-2019

Design:

- "3 + 3" dose escalation; starting dose = 30 mg orally, once daily (po, qd) with food
- Dose increases dependent on toxicities: range 25% (if 1 DLT in 6 pts) to 100% (≤Grade 1 Adverse Events)

Key Entry Criteria:

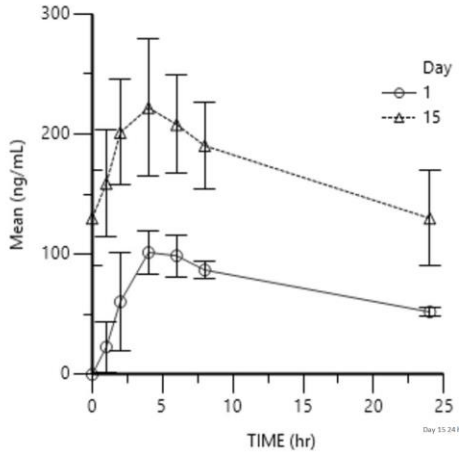
- ER+/HER2- advanced breast cancer
- At least two prior endocrine therapies in any setting, and a CDK4/6 inhibitor
- Up to three prior cytotoxic chemotherapy regimens

Key Objectives:

- Maximum Tolerated Dose/ Recommended Phase 2 Dose/Safety
- Pharmacokinetics
- Anti-tumor activity (RECIST, CBR)
- Biomarkers

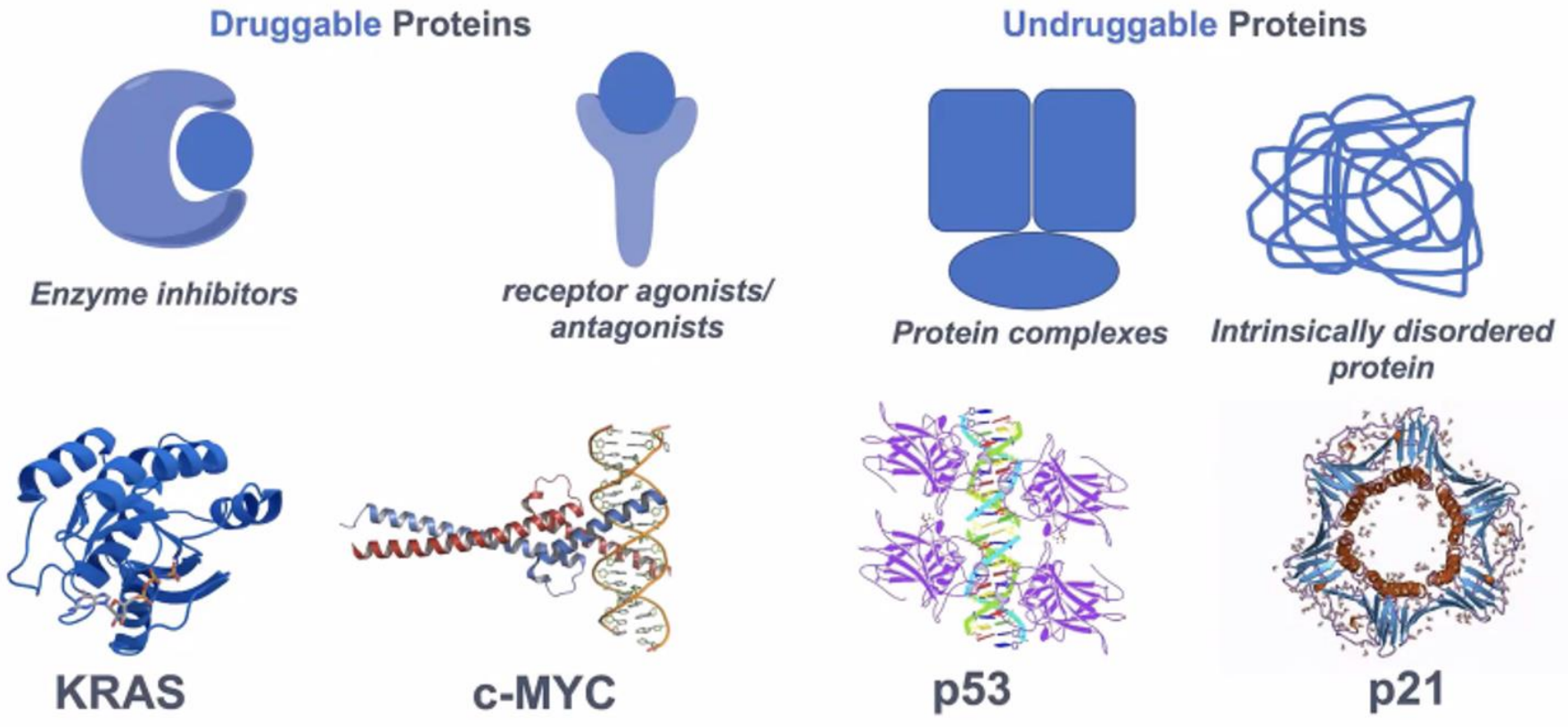
Biomarkers:

- ER gene (ESR1) mutational status in ctDNA and/or tumor tissue
- ER, Progesterone Receptor and Ki-67 levels in pre- and post-treatment tumor biopsies in patients with accessible tumor tissue



Accumulation occurs between Day 1 and Day 15 (30 mg)

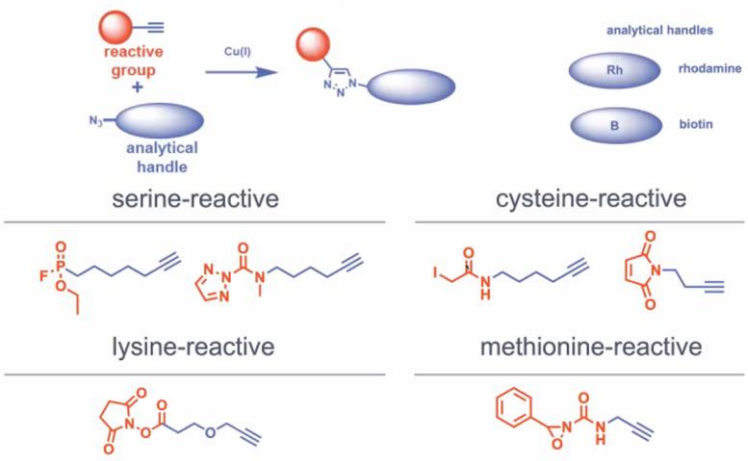
The undruggable proteome



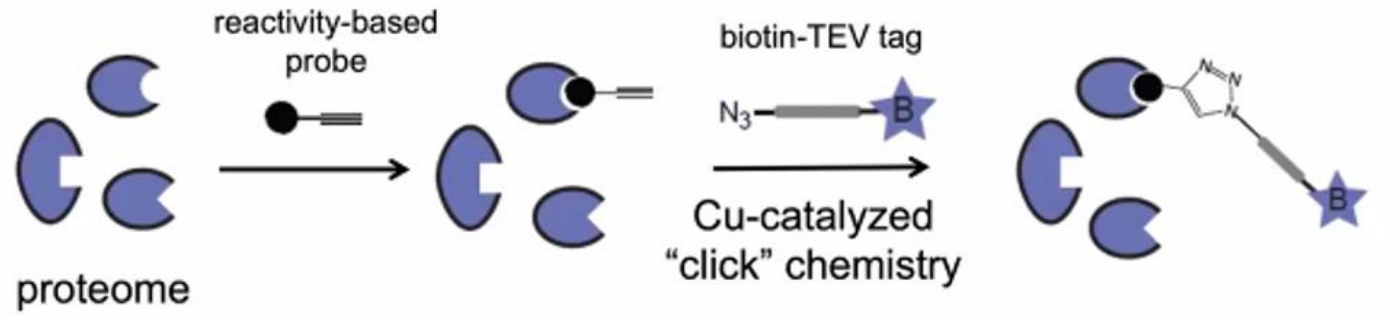
Over 90 % of proteins do not have known pocket that small molecule can bind

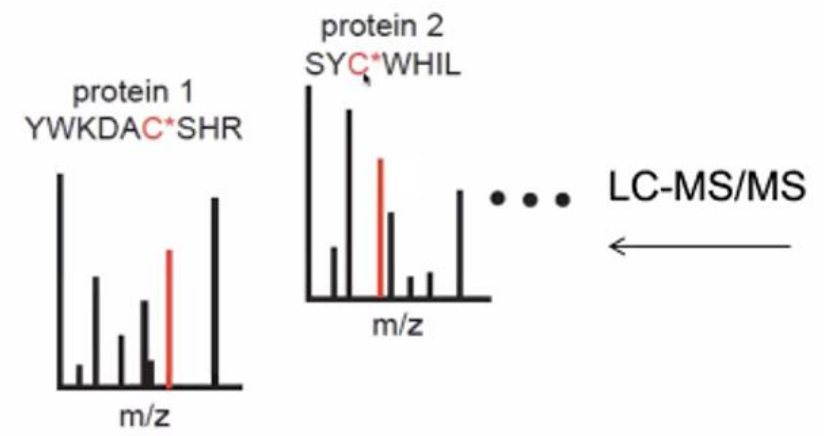
Identify target binding pockets «druggable hotspots» across the entire proteome

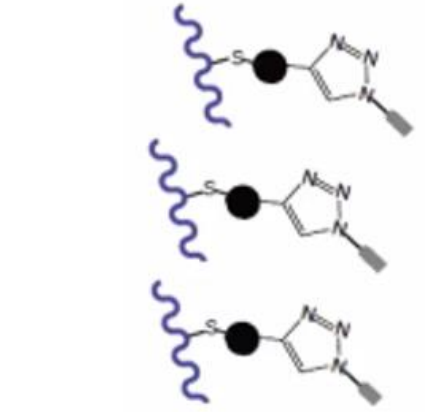
MS-Activity Based Protein Profiling



Reactivity based probes for mapping ligandable «druggable hotspots» across the entire proteome

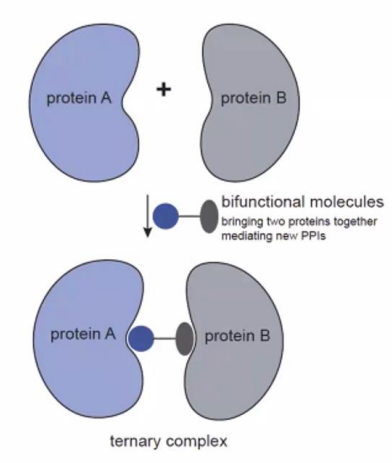






1. Avidin enrichment
2. Tryptic digest
3. TEV digest

TPD
Inventing induced proximity druggable mechanims



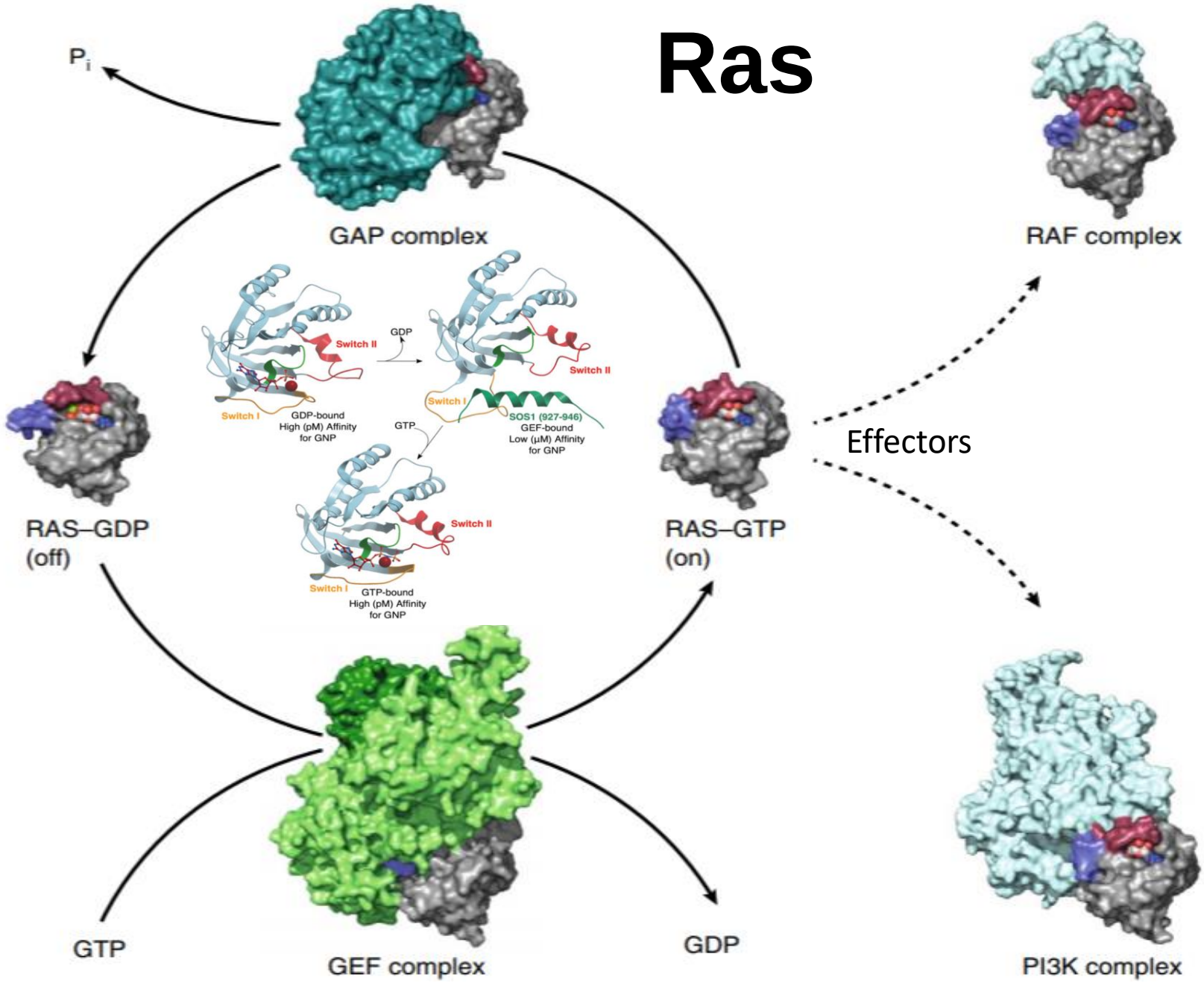
- Dub targeting Chimeras (DubTACs)
- Phospatase targeting Chimeras (PhosphaTACs)
- Acetyltransferase targeting Chimeras (AATACs)
- Deacetylase targeting Chimeras (DATACs)
- Conformation targeting Chimeras (DubTACs)
- Etc…

Table 2. Summary of the Advantages and Disadvantages of Described Methods of Targeted Protein Degradation

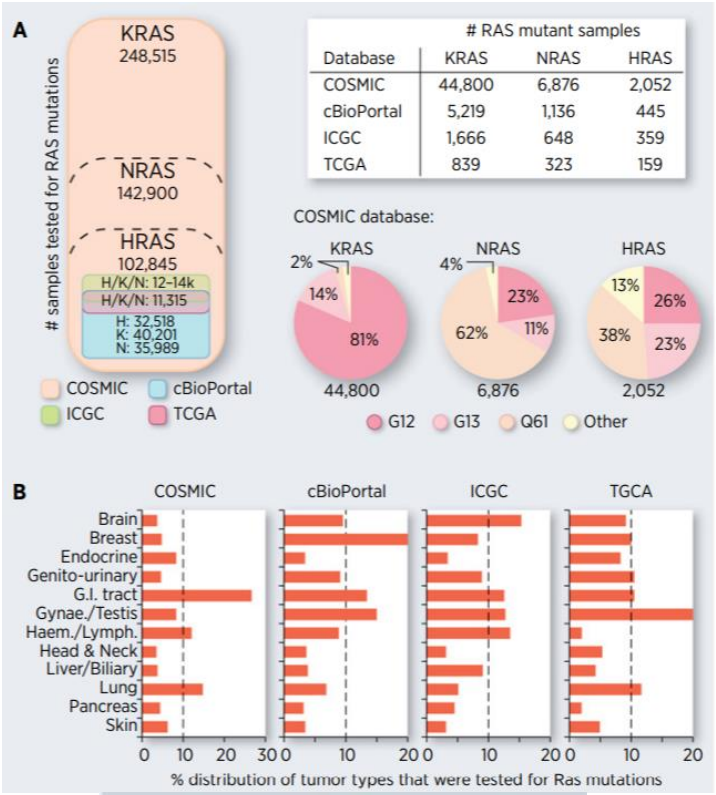
Method	Advantages	Disadvantages
PROTACs	Catalytic; reversible; endogenous wild-type and mutant targets; paralog-selective; *in vivo* applications.	Lengthy SAR analyses of linker and target ligands; high MW and not "rule-of-5" compliant.
Molecular glues	Catalytic; reversible; endogenous wild-type and mutant targets; paralog-selective; *in vivo* efficacy in humans with some BBB-permeable.	Challenging to identify/synthesize prospectively; specificity issues.
Trim-Away	Endogenous targets.	Limited by endogenous levels of Trim21, Ab access to cytosolic but not nuclear targets; Ab specificity to target.
CMA	No dependency on ubiquitination machinery for targeting to lysosome.	Ectopic expression of engineered constructs; non-catalytic; only cytosolic targets.
AID	Reversible; versatile.	Ectopic expression of F-box receptor; 7 kDa target modification; no *in vivo* application; leaky degradation in absence of auxin.
dTAG	Catalytic; reversible; *in vivo* applications.	12 kDa target modification.
IKZF3 degron	Catalytic; reversible; *in vivo* applications including CNS; rodent IKZF3 not targeted.	3 kDa target modification best at C terminus; not all targets degraded.
SMASh	Reversible.	Only for newly synthesized protein.
HaloTag-HyT; HaloPROTACs	Tagged ORFs and PROTAC commercially available; *in vivo* applications.	Covalent 33 kDa target modification; non-catalytic.
Nanobodies: deGradFP and AID nanobody	Suitable for GFP-tagged ORFs available commercially.	Target modification; substrate receptor engineering.

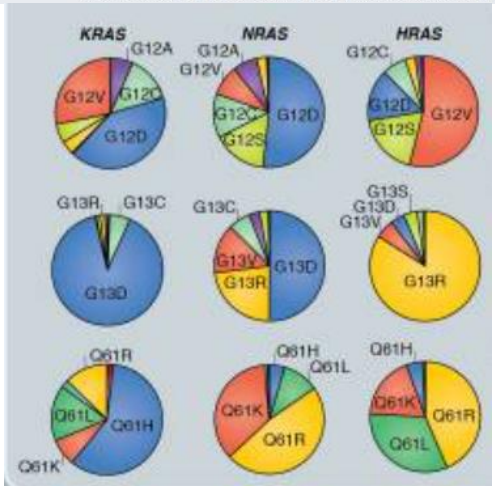
The broad applicability of emerging modalities such as AUTACs, ENDTACs/LYTACs, and RIBOTACs briefly mentioned in this Review remains to be determined and hence is not summarized here. SAR, structure-activity relationship; SMs, small molecules; BBB, blood-brain barrier; CNS, central nervous system; ORFs, open reading frames.

Ras

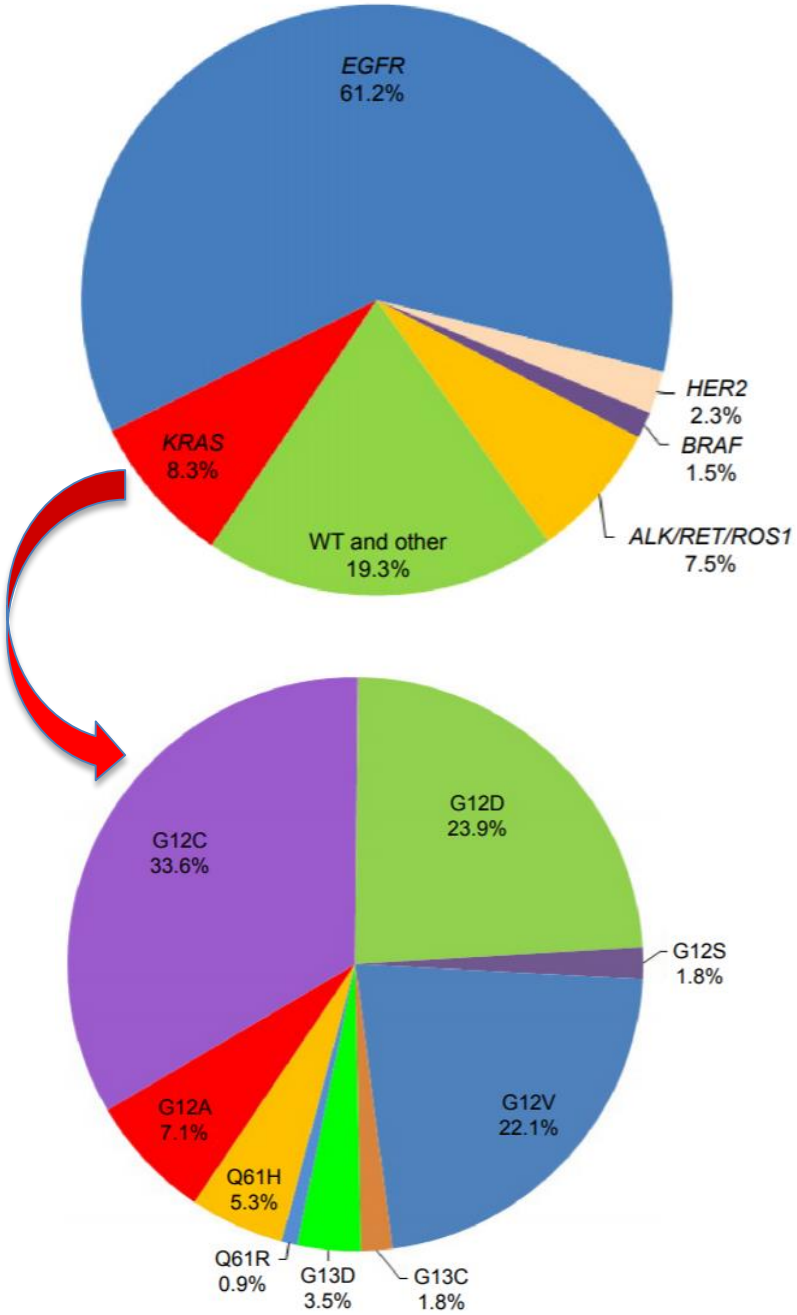

P_i
GAP complex
RAF complex
Switch II
GDP
Switch I
GDP-bound
High (pM) Affinity
for GNP
Switch II
Switch I
SOS1 (927-946)
GEF-bound
Low (μM) Affinity
for GNP
GTP
Switch II
Switch I
GTP-bound
High (pM) Affinity
for GNP
RAS–GDP
(off)
RAS–GTP
(on)
Effectors
GTP
GEF complex
GDP
PI3K complex

Ras

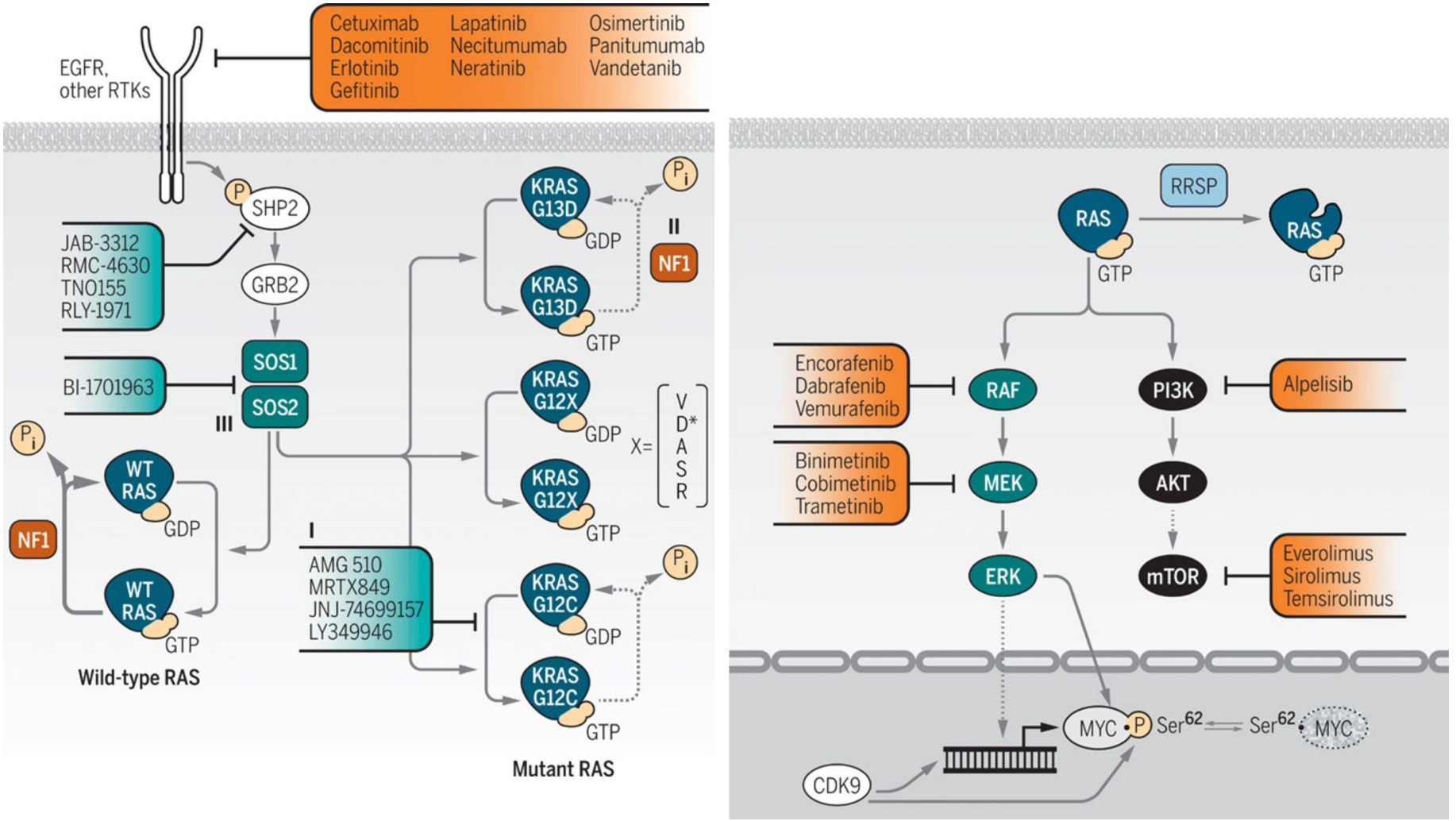




LUNG Carcinomas



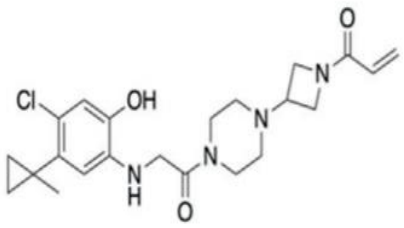
Ras vulnerabilities for anti-Ras strategies



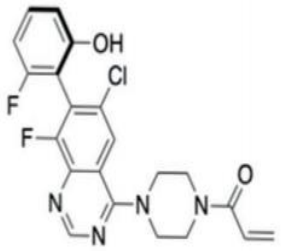
Clint A. Stalnecker, and Channing J. Der Sci. Signal. 2020;13:eaay6013

Science Signaling
AAAS

K-Ras inhibitors targeting the Cys12



ARS-853 (**9**)
IC_{50} 1.6μM
KRas G12C



ARS-1620 (**10**)
IC_{50} 120 nM
KRas G12C

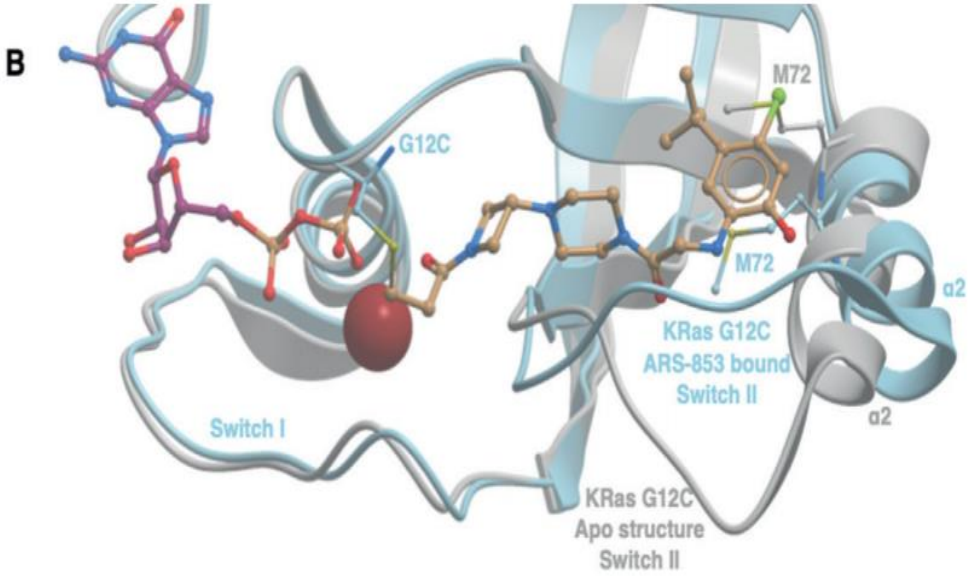
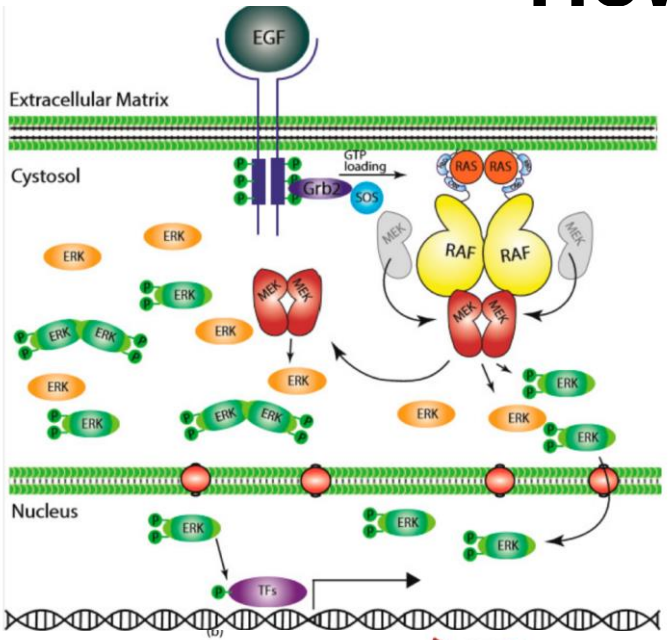


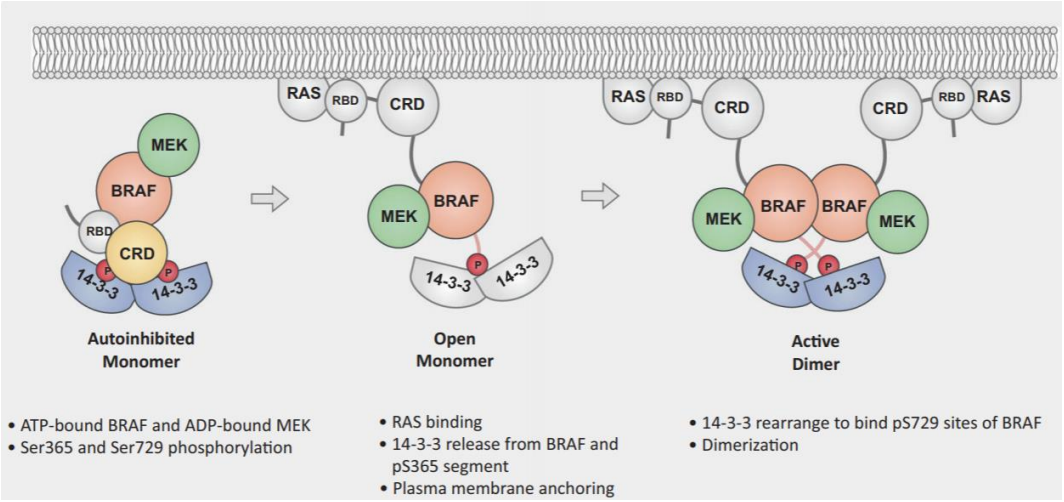
Table 1 | Companies with RAS inhibitor in clinical development

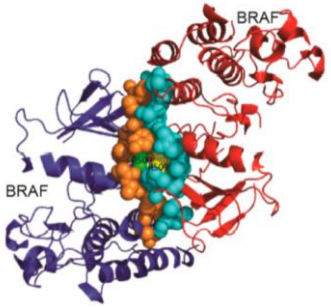
Developer	Molecule	Description	Clinical status
Amgen, Carmot Therapeutics	AMG-510	$KRAS^{G12C}$ inhibitor	Phase 1/2
Mirati Therapeutics	MRTX849	$KRAS^{G12C}$ inhibitor	Phase 1/2
Johnson & Johnson, Wellspring Biosciences	JNJ-74699157 (formerly ARS-3248)	$KRAS^{G12C}$ inhibitor	Phase 1
Eli Lilly	LY3499446	$KRAS^{G12C}$ inhibitor	Phase 1/2
Moderna, Merck	V941 (mRNA-5671)	Lipid-nanoparticle-formulated mRNA-based vaccine targeting $KRAS^{G12D}$, $KRAS^{G12V}$, $KRAS^{G13D}$ and $KRAS^{G12C}$	Phase 1
Revolution Medicines	NA	Inhibitors of $KRAS^{G12C}$, $KRAS^{G13C}$, $KRAS^{G12D}$ and $NRAS^{G12C}$	Preclinical
Mirati Therapeutics	NA	$KRAS^{G12D}$ inhibitor	Preclinical

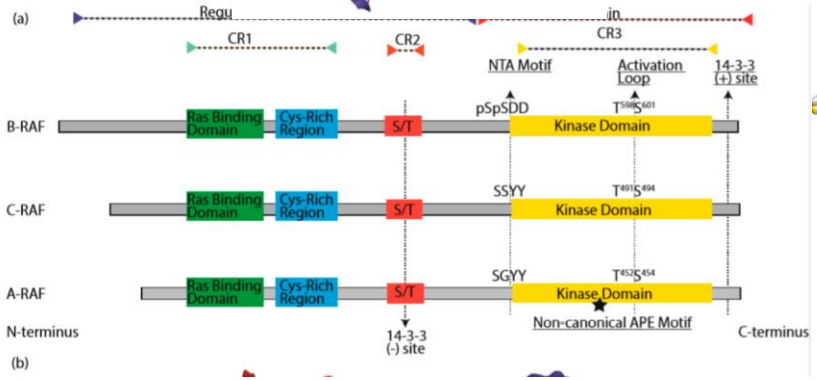
NA, not available. Sources: ClinicalTrials.gov, company websites, Cancer.gov.

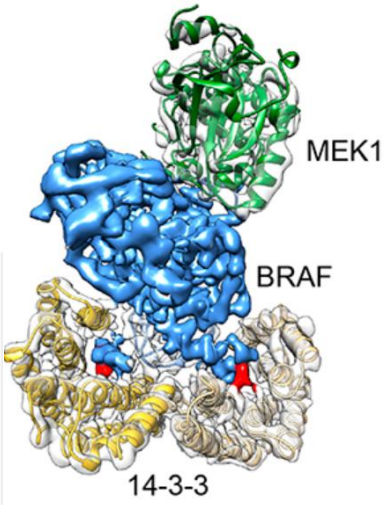
How Ras talks to Raf



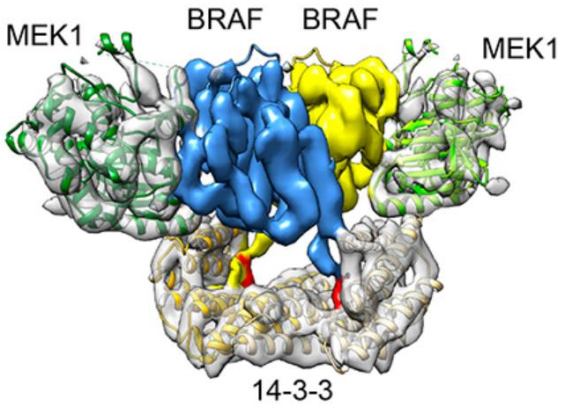






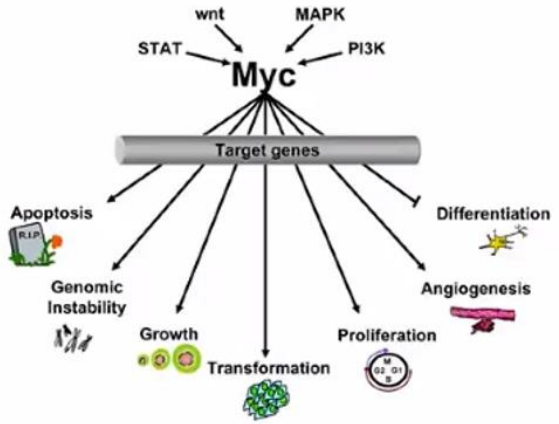


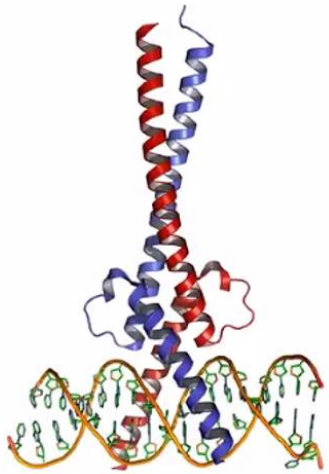
"OFF-state"

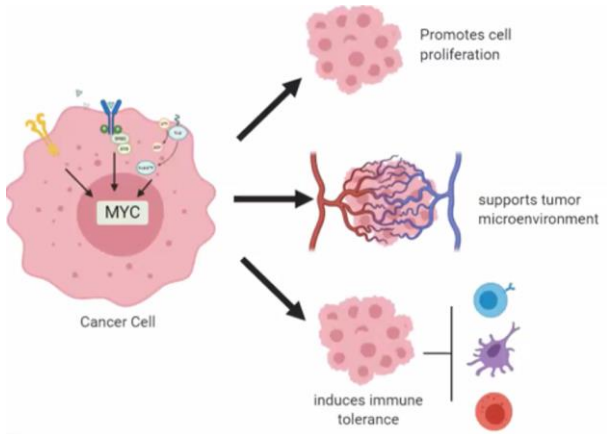


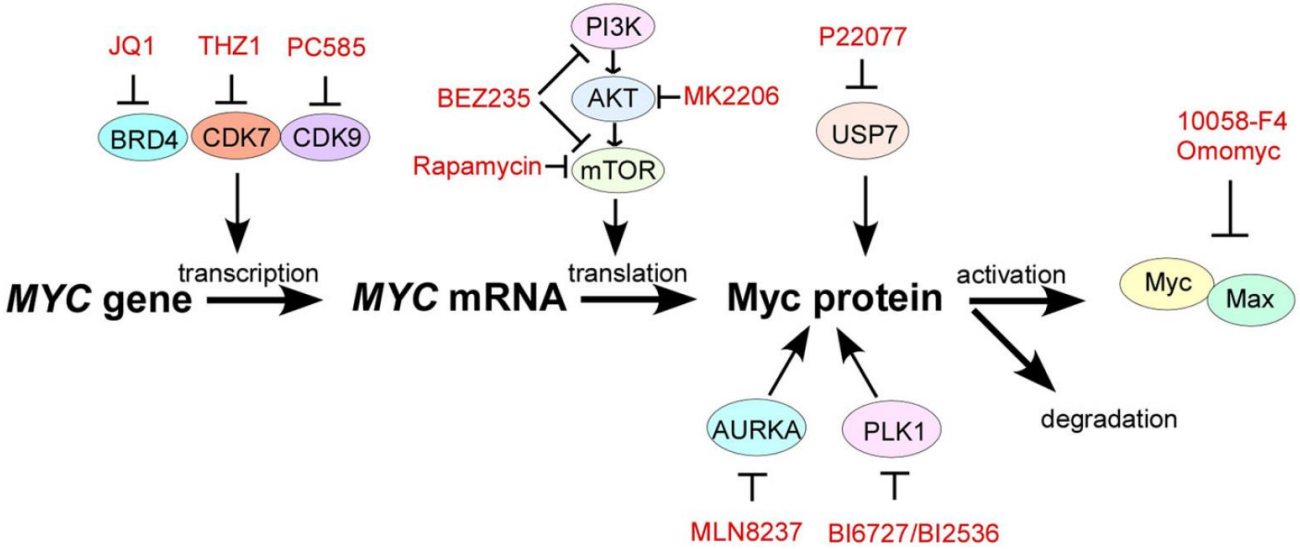
"On-state"

Targeting Myc in Cancer



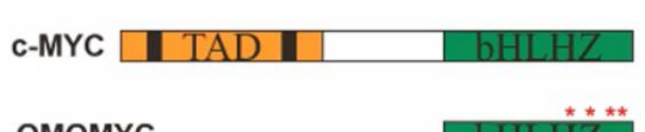


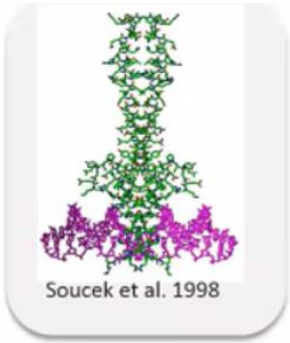


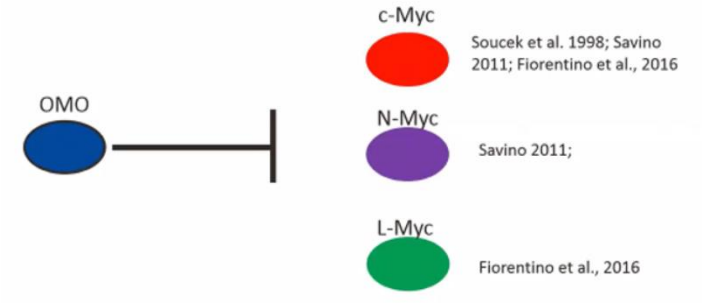


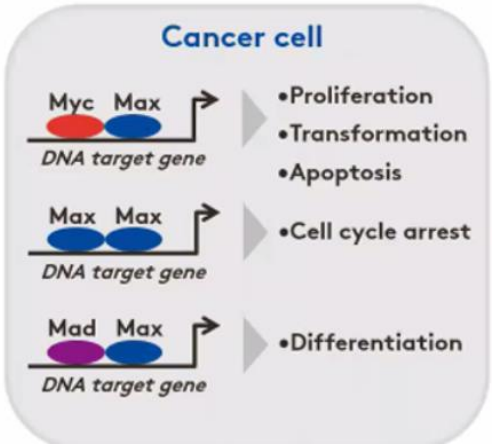
Targeting Myc transcription: BRD4, CDK7, CDK9, p300/CBP-BRD
Trageting Myc mRNA translation: mTOR, CPEBP
Targeting Myc stability: USP28, USP36, AURKA, PLK1, Omomyc, GTI19077

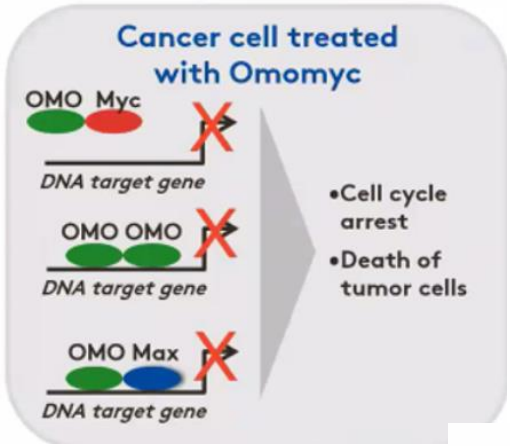
Design of OMOMYC

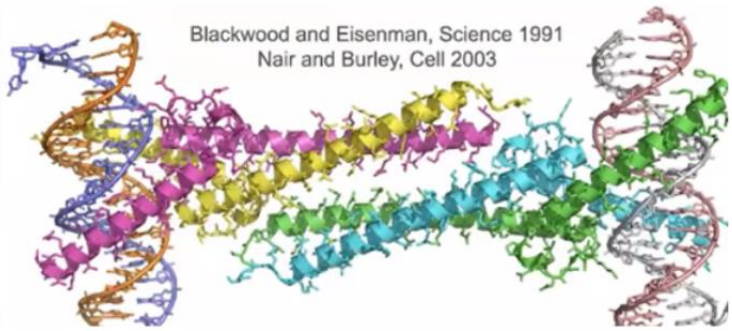


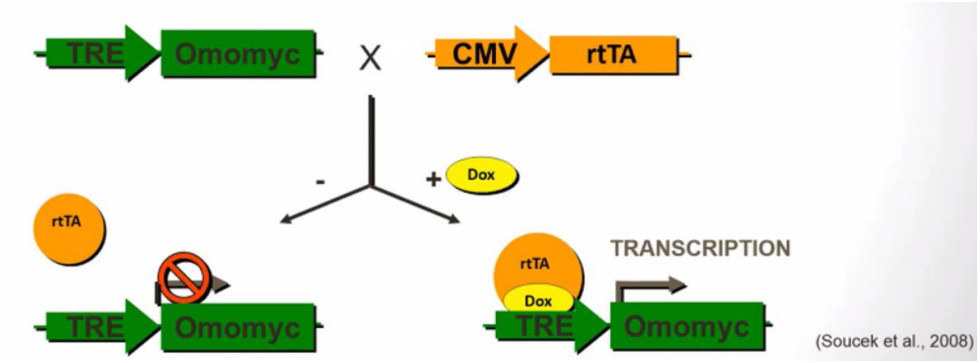


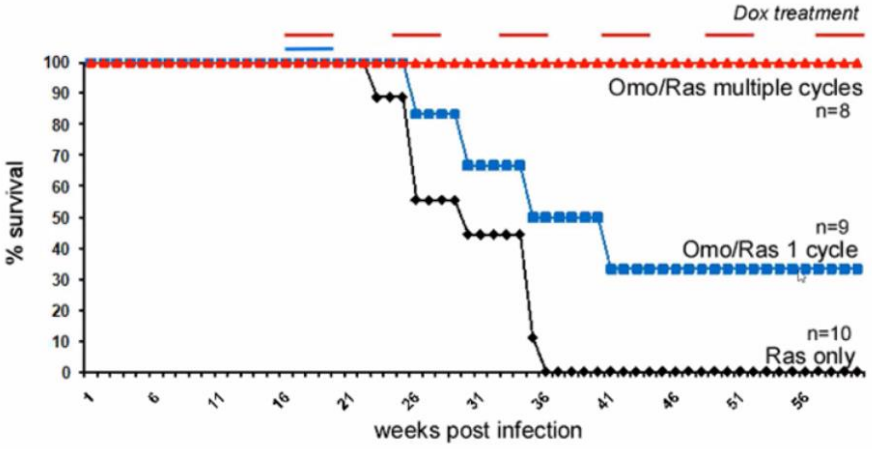






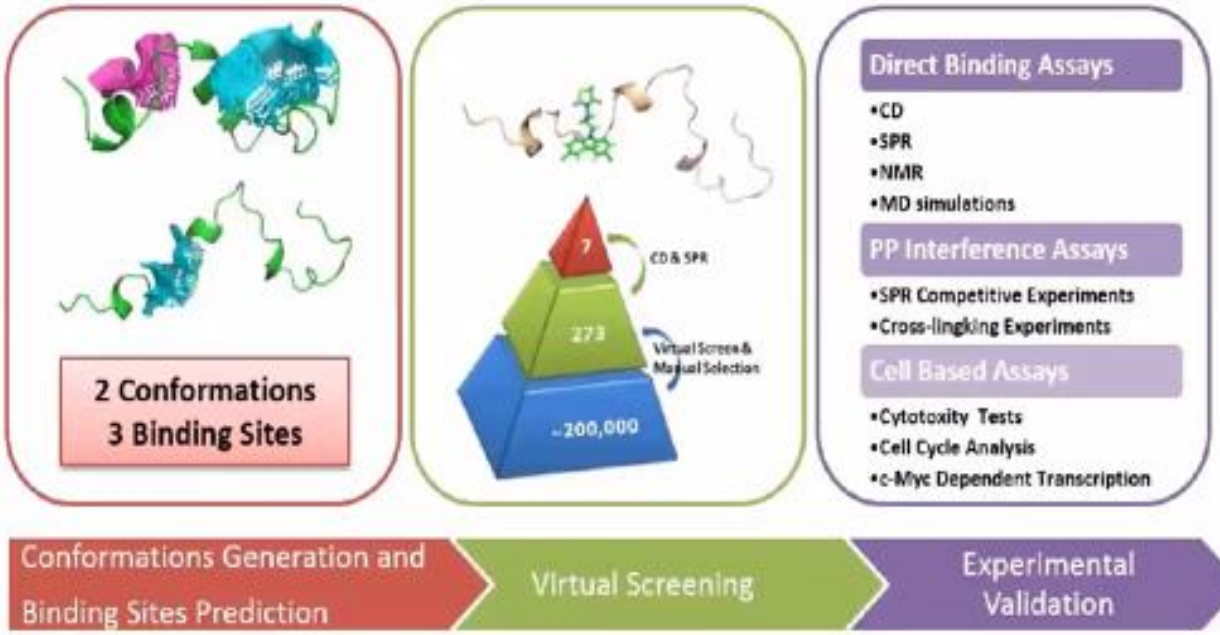






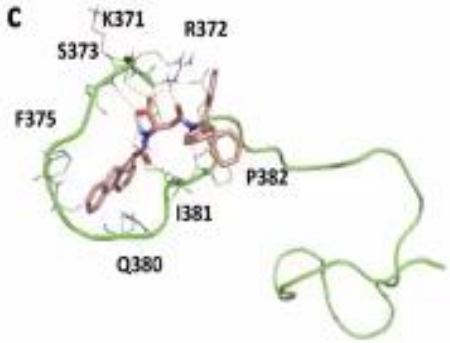
Discovery of Hit compound (A0) that directly targets the intrinsically disordered protein c-Myc

A



B

A0 binding site in c-Myc (aa370-409)



PKUMDL -A0

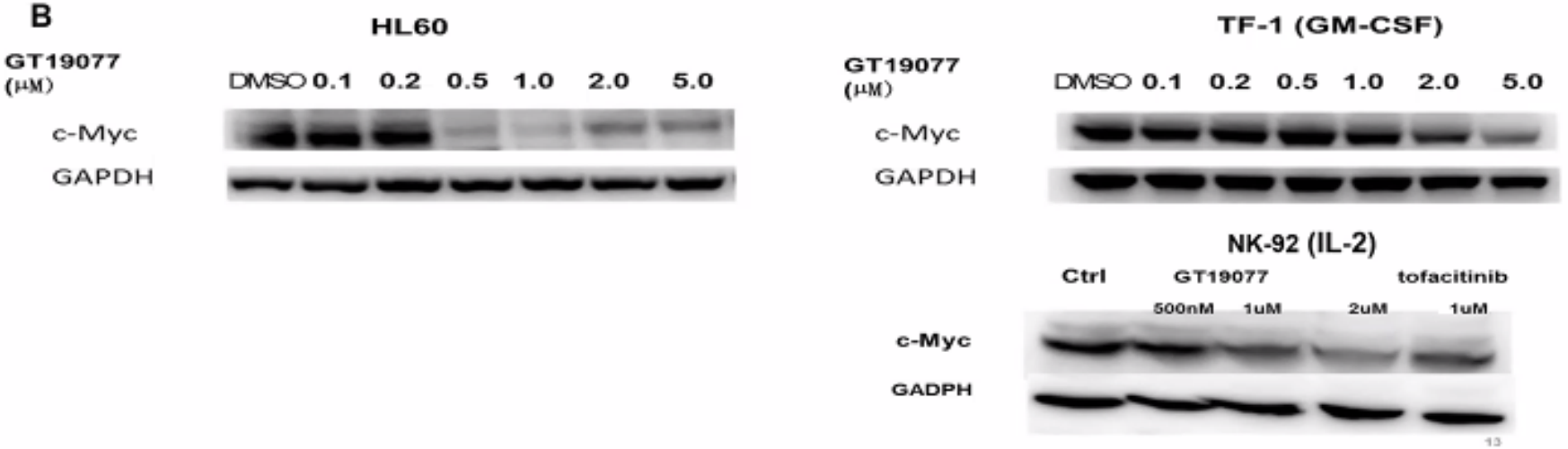
- A0 Hit compound identification (A)
- Predicted A0 binding sites in c-Myc (B)

GT19077 selectively degrades c-Myc protein and inhibits cell proliferation in HL-60 cells

A

Compounds	C-Myc degradation (ELISA)		Cell proliferation (CTG)	
	HL-60	TF-1 (GM-CSF)	HL-60	TF-1 (GM-CSF)
GT19077 IC50(μM)	0.39	2.30	0.338±0.01	1.65±0.055

B



- GT19077 selectively degraded c-Myc in HL-60 cells with much less activity in GM-CSF stimulated TF-1 or IL-2 stimulated NK-92 cells (erythroblast and natural killer cells, respectively)
- GT19077 also selectively inhibited proliferation of HL-60 cells with less potency in GM-CSF stimuled TF-1 cells

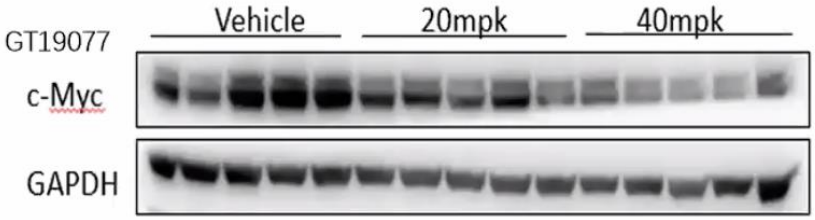
In vivo target engagement of GT19077 with c-Myc in HL-60 and Ramos xenograft tumors (SC)

GT19077 in vivo target inhibition

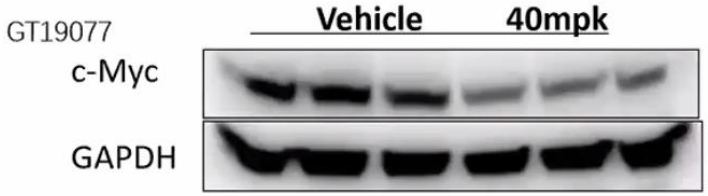
Plasma exposure

GT19077 Doses	Plasma concentration (ng/ml) 0.5 hr post last Rx (3 day Rx)
10mpk	1012±333.75
20mpk	1996±501.48
40mpk	3182±474.10

c-Myc degradation in HL60 tumors



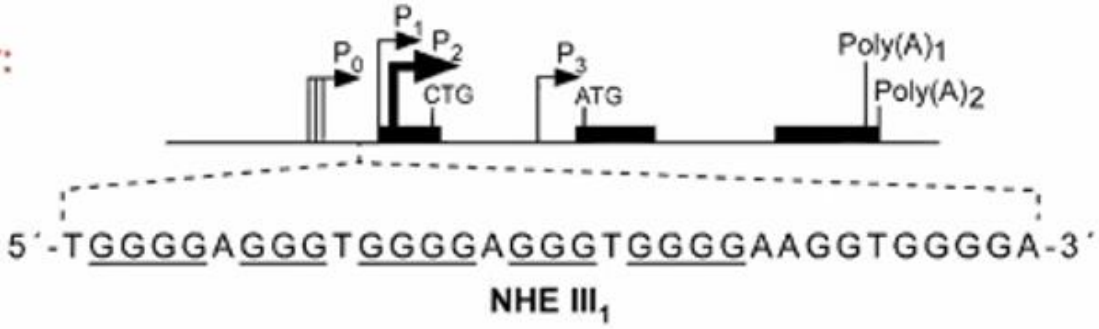
c-Myc degradation Ramos tumors



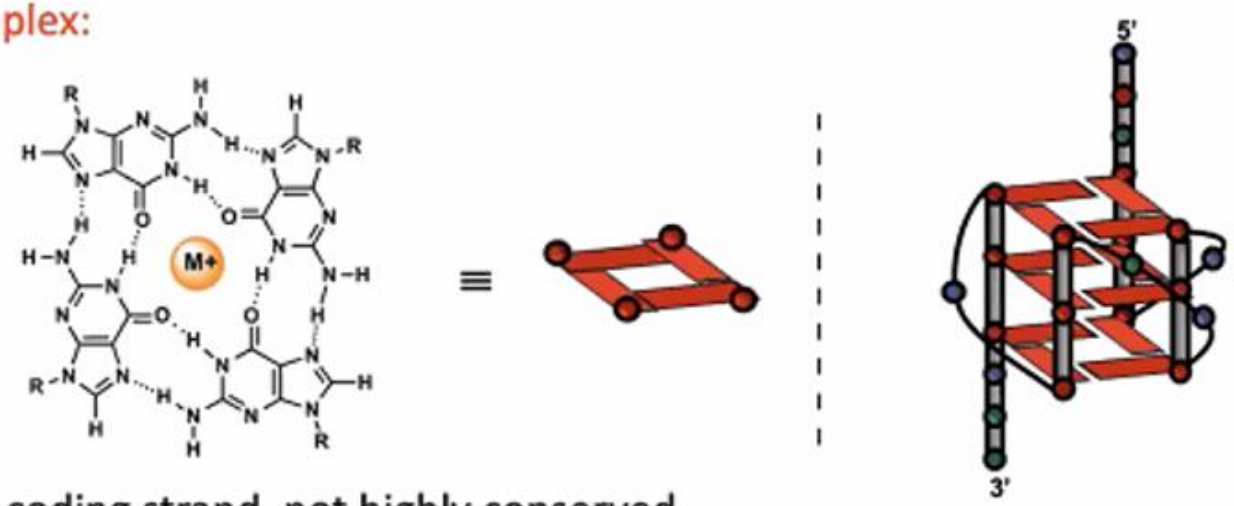
- GT19077 demonstrates PK-dependent c-Myc degradation in HL60 and Ramos xenograft tumors

Targeting Myc Expression Through G-Quadruplexes

MYC Promoter:



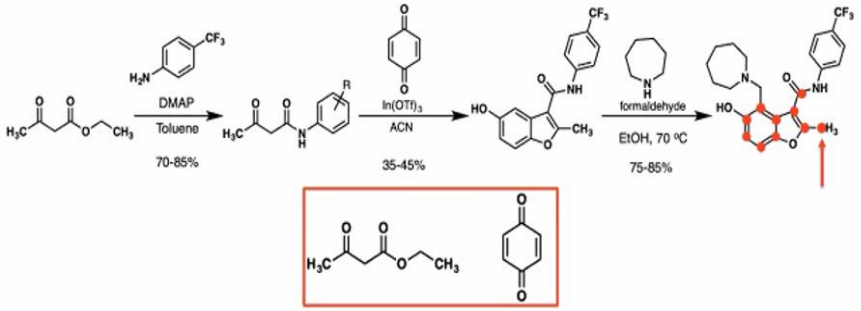
G-Quadruplex:



- Not on coding strand, not highly conserved
- Many quadruplex ligands are known
- It is difficult to identify molecules that ***selectively*** bind to quadruplexes of interest

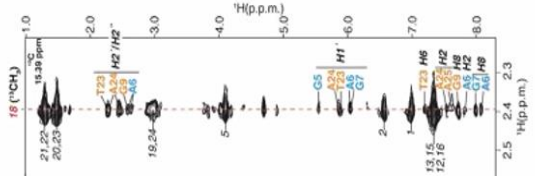
Balasubramanian, Burrows, Hurley, Neidle, Yang, and others

Preparation of an Isotopically labeled DC34 Facilitates an NMR Structure



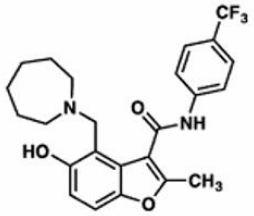
^{13}C-enriched starting materials

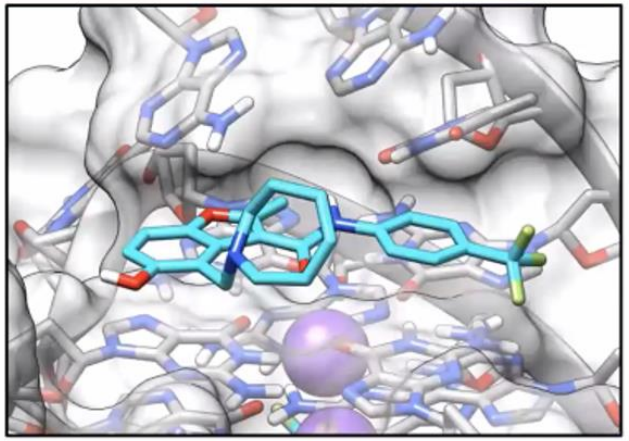
Isotopically labeled probe enables straightforward residue assignment (Half Filtered NOESY) → additional restraints



With Kylie Walters (SBL)

NMR Structure: *MYC* G-quadruplex

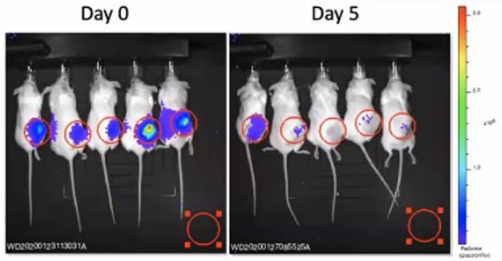


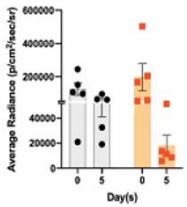


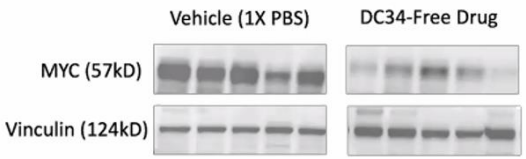
Detail of 3' binding site

- Tails move to accommodate binding (2 sites)
- Ligand: 3-dimensional conformation
- Affinity: hydrogen bonding, cation-π, F-bonding

Evaluation of DC-34 in a Mouse Tumor Xenograft Model





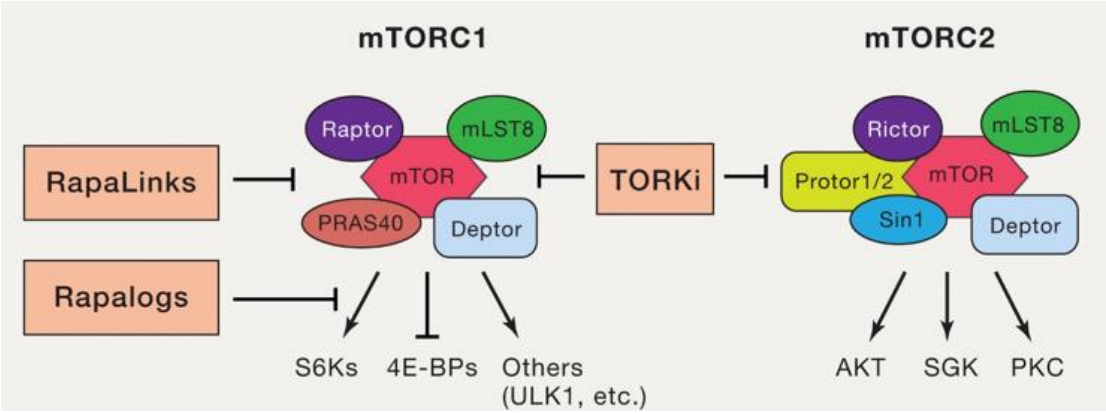


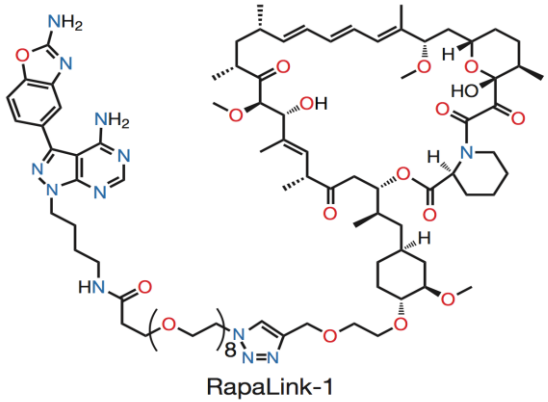
Plasma:
C_{max} = 270 nM, 30 min
$T_{1/2}$ = 24 hours

Tumor:
100-225 pmol/L

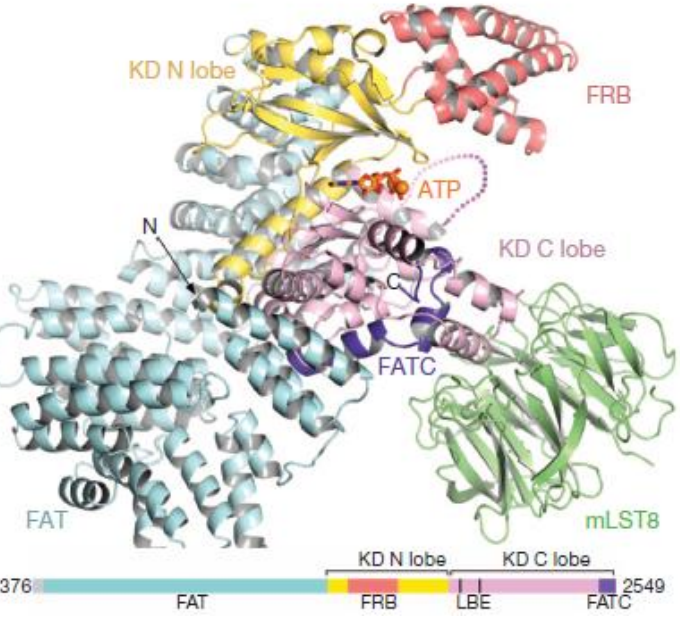
Mock Lab: Wendy DuBois, Snehal Gaikwad, Tyler Peat
Gonzalez Lab: Dax Patel, Kris Krausz, Tom Veleonosi

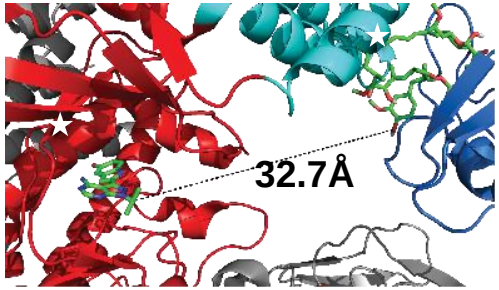
Rapalogs vs TORKi vs Rapalink



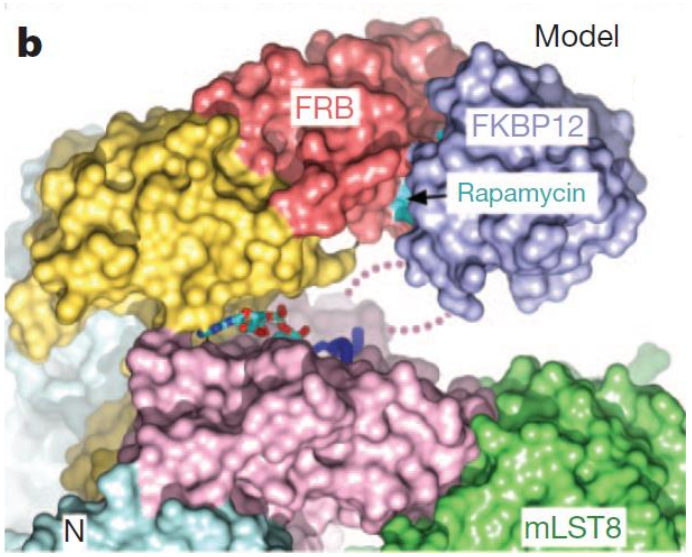


MW = 1784

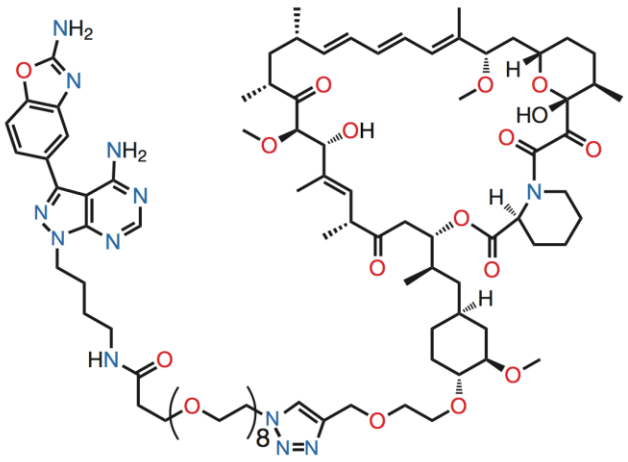




Yang et al. 2013, Nature 47: 217



RapaLink-1 is a non-traditional drug-like molecule with exceptional in vivo efficacy



RapaLink-1

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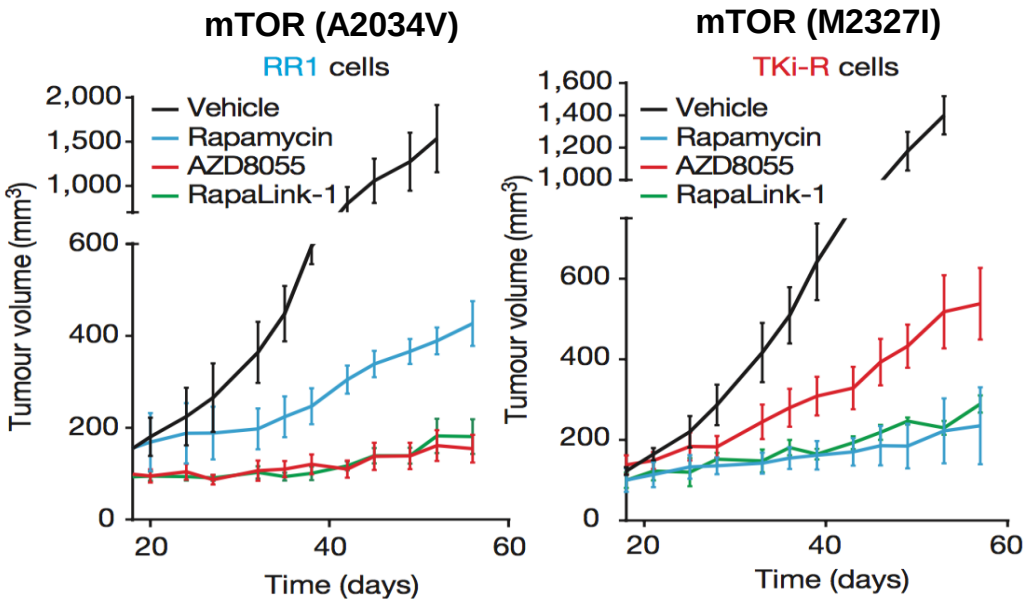

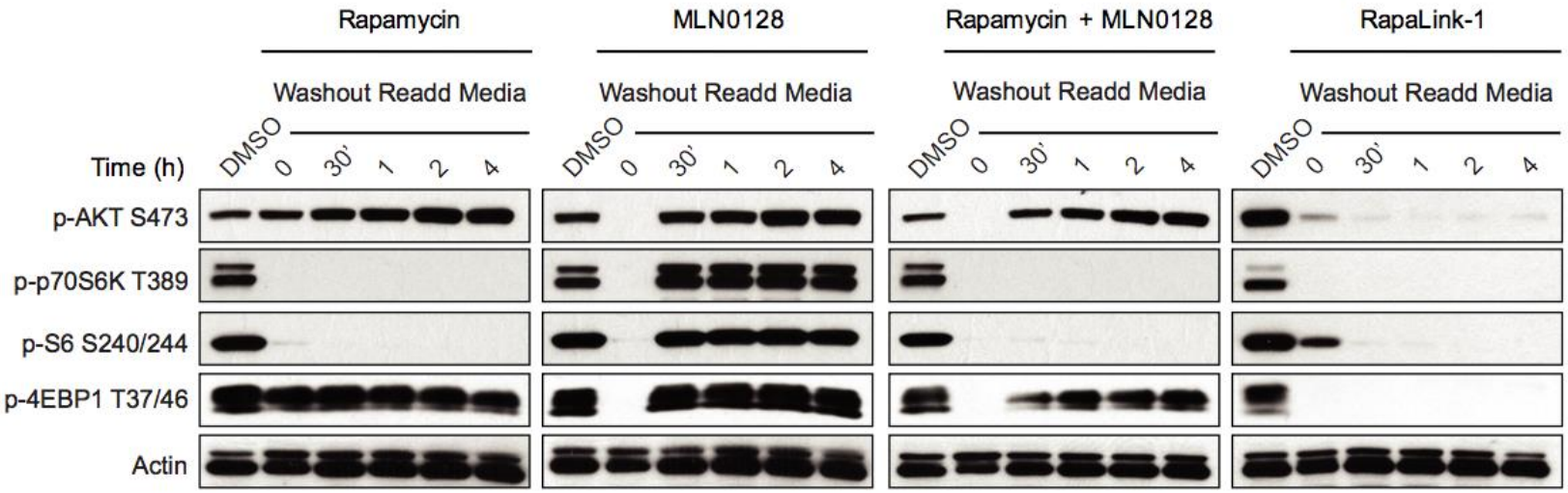

4 hr. Drug Treatment Followed by Drug Washout

MCF7 cells

Rodrik-Outmezguine, V. S. et al. *Nature* 534, 272–276 (2016).