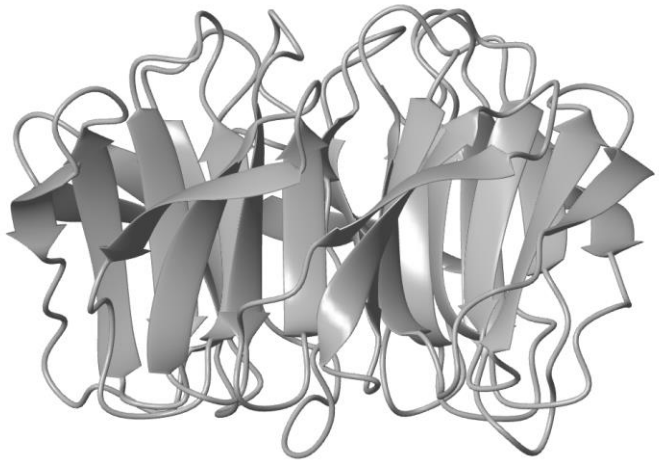
WDR5 INHIBITORS FOR THE TREATMENT OF CANCER

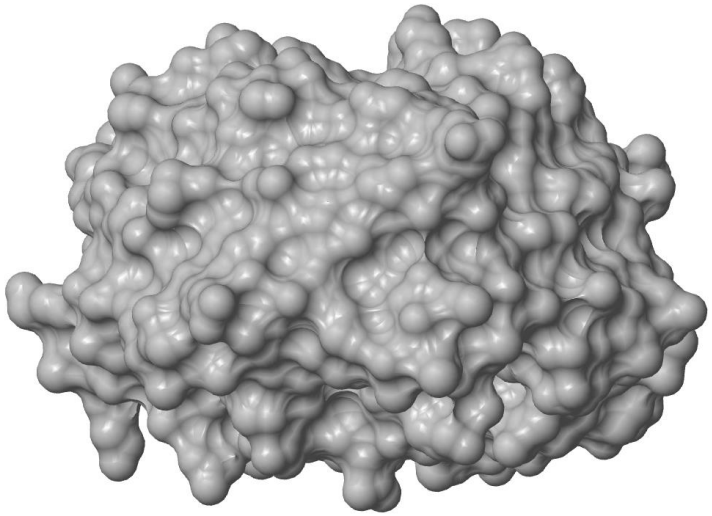
Steve Fesik, Ph.D.

Bill Tansey, Ph.D.

Vanderbilt University School of Medicine

WDR5 is a high-value target in cancer

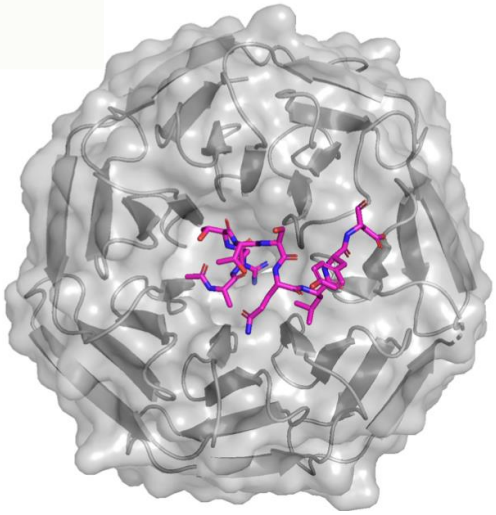


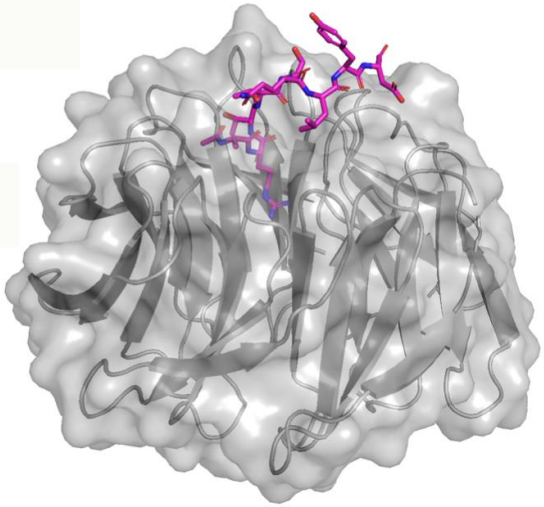


OVER-EXPRESSED IN:
Head/neck squamous cell carcinoma
Gastric cancer
Pancreatic cancer
Lung cancer
Breast cancer
Bladder cancer
Prostate cancer
Leukemia

CRITICAL ROLE IN:
MLLr-driven cancers
ER-driven cancers
C/EBPα-mutant cancers
p53 GOF cancers
MYC-driven cancers

This project targets the “WIN” site of WDR5

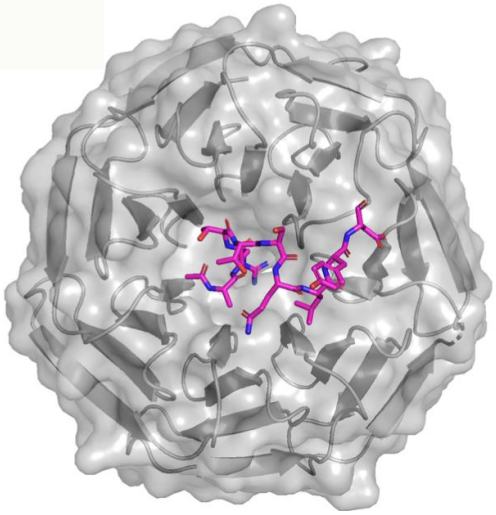


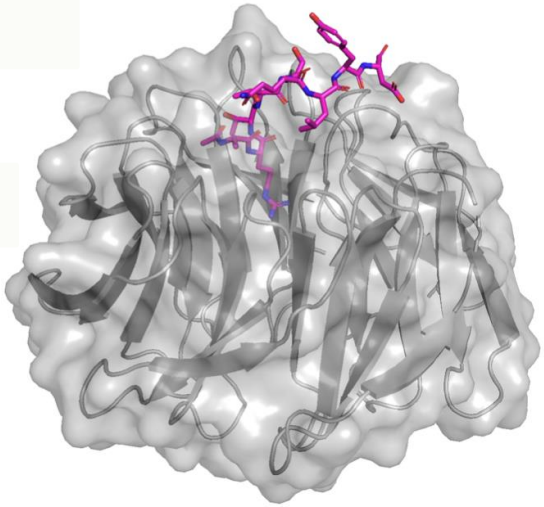


The WIN site is an arginine-binding cavity

```
PDPK1_HUMAN      ----ARITSQLYDAVP
H3_HUMAN         ----ARIKQTARKSTG
KANSL1_HUMAN     TCVAARIRPVLSCKKR
KMT2A_HUMAN      PHGSARAEVHLRKSAF
KMT2D_HUMAN      PTGCARSEPKILTHYK
KMT2C_HUMAN      PTGCARSEPKMSAHVK
KMT2B_HUMAN      PHGAARAEVYLRKCTF
SET1A_HUMAN      QTGSARSEGYYPISKK
SET1B_HUMAN      VTGCARSEGFYTIDKK
KIF2A_HUMAN      VVGSARARPSQFPEQS
```

This project targets the “WIN” site of WDR5





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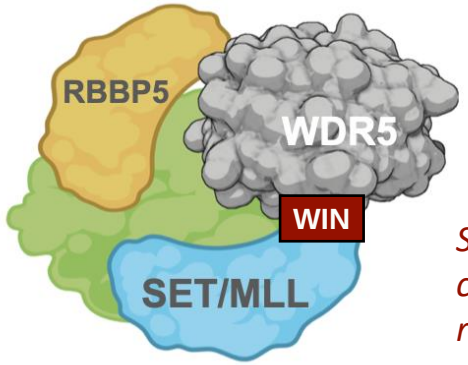
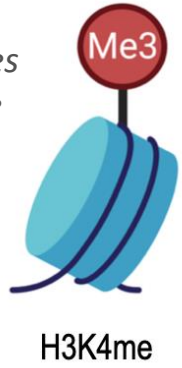
KMT2C_HUMAN      PTGCARSEPKMSAHVK
KMT2B_HUMAN      PHGAARAEVYLRKCTF
SET1A_HUMAN      QTGSARSEGYYPISKK
SET1B_HUMAN      VTGCARSEGFYTIDKK
KIF2A_HUMAN      VVGSARARPSQFPEQS
```

Original premise: WIN site inhibitors reprogram H3K4me in MLLr



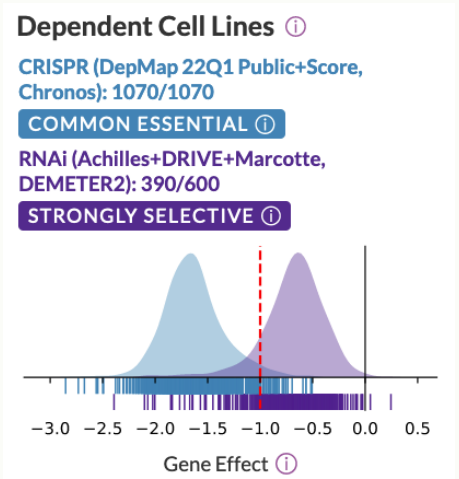
Inhibitors drive changes in ‘oncogenic’ H3K4me

*Selective inhibition of cancer cells with **MLL1**-rearrangements.*

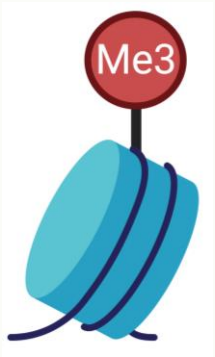


What are the challenges in targeting WDR5?

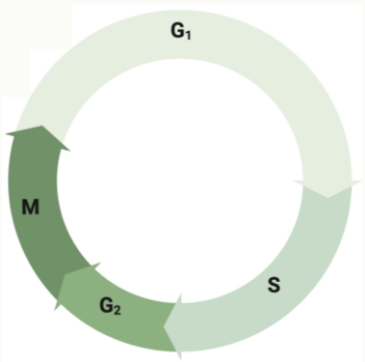
WDR5 is pan-essential



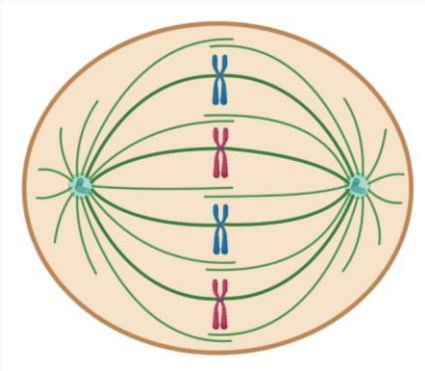
WDR5 is a versatile cellular multi-tasker



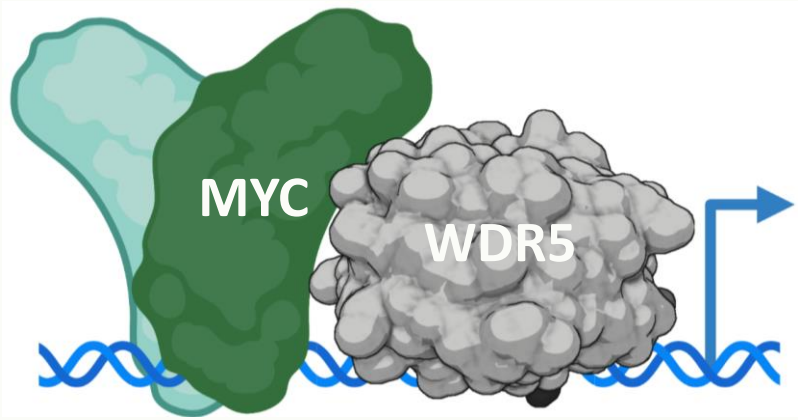
Epigenetics



Bookmarking



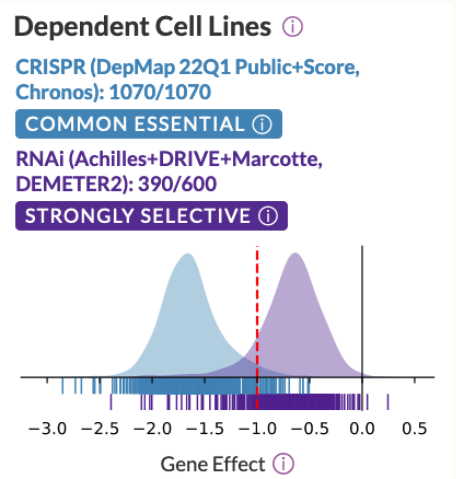
Mitotic spindle



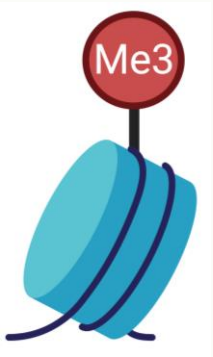
Recruits MYC to chromatin

What are the challenges in targeting WDR5?

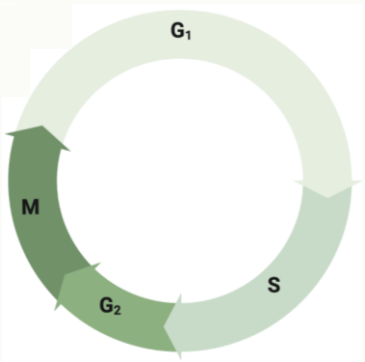
WDR5 is pan-essential



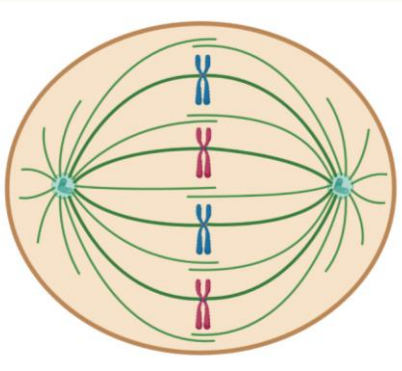
WDR5 is a versatile cellular multi-tasker



Epigenetics

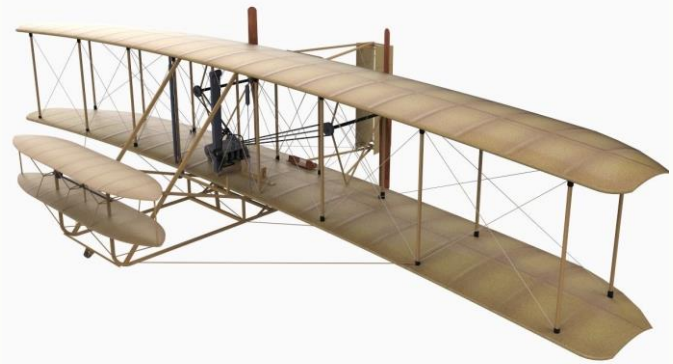


Bookmarking

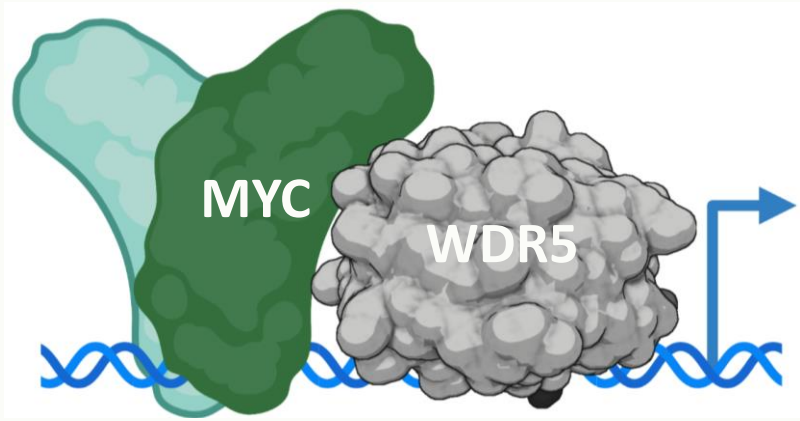


Mitotic spindle

We didn't know as much about WDR5 as we thought



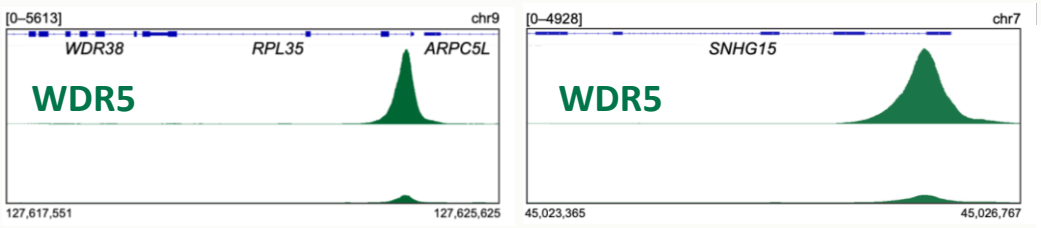
"Build the plane while flying it"

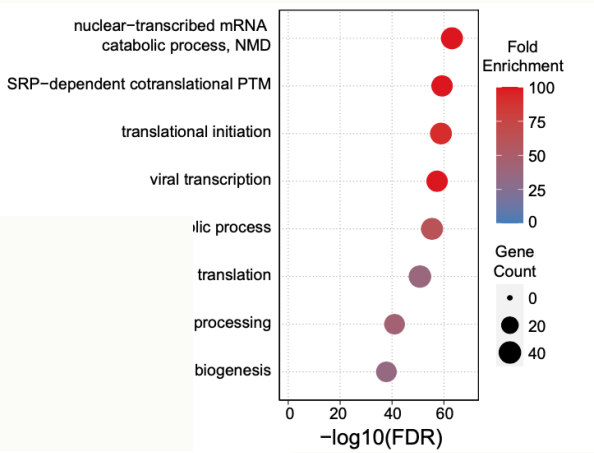


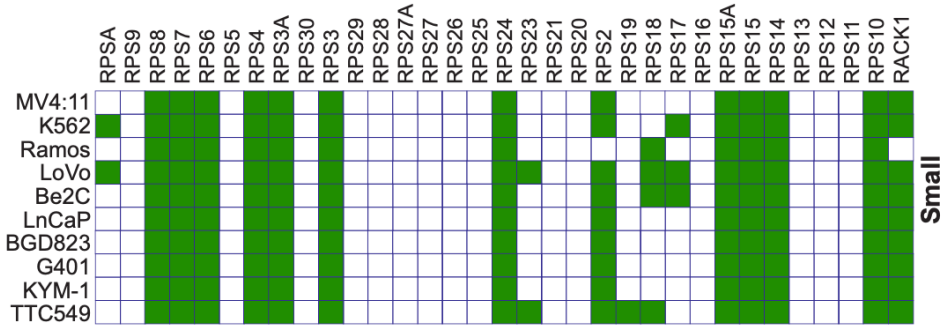
Recruits MYC to chromatin

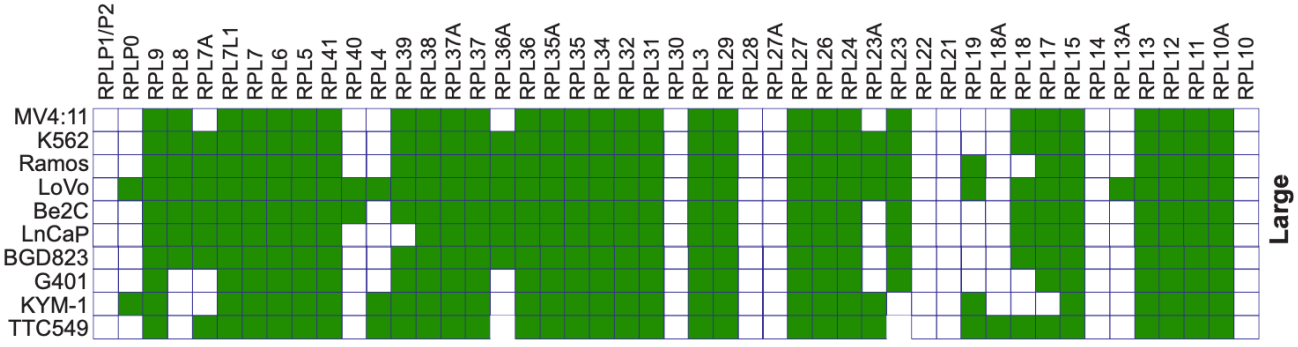
WDR5 is a conserved regulator of protein synthesis genes

WDR5 binds to and regulates a highly predictable set of genes linked to protein synthesis

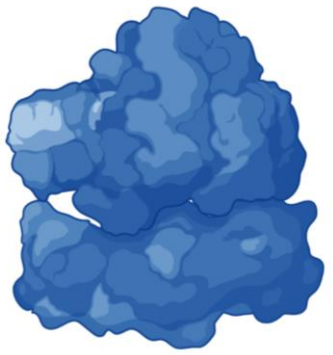








WDR5 target gene non-target



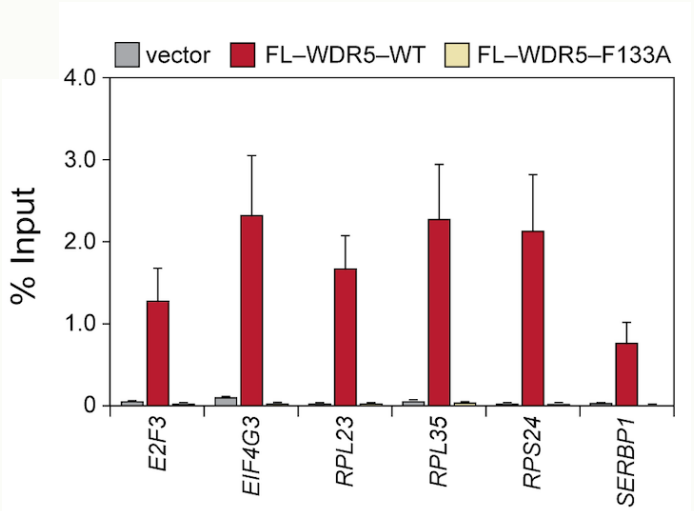
ribosomal subunits

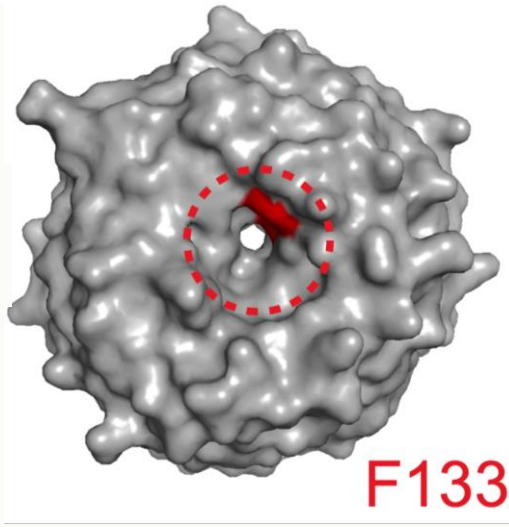
Developing PD biomarkers based on RPG pattern

Foshage et al., 2019; Aho et al., 2019; Florian et al., 2022

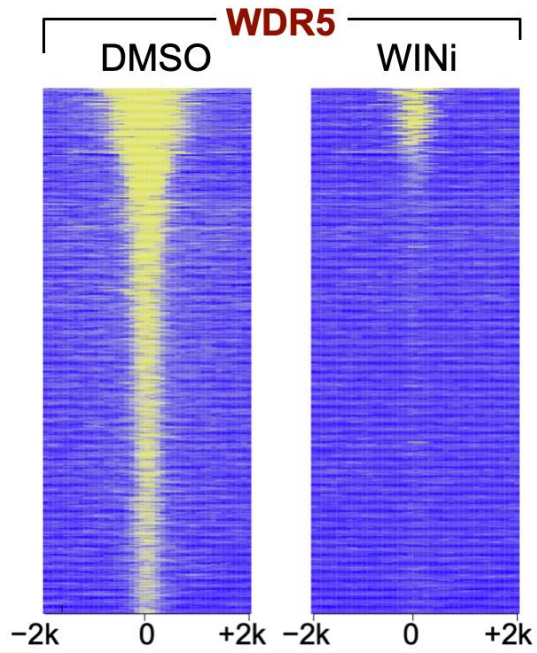
The WIN site tethers WDR5 to chromatin

Genetic disruption of the WIN site



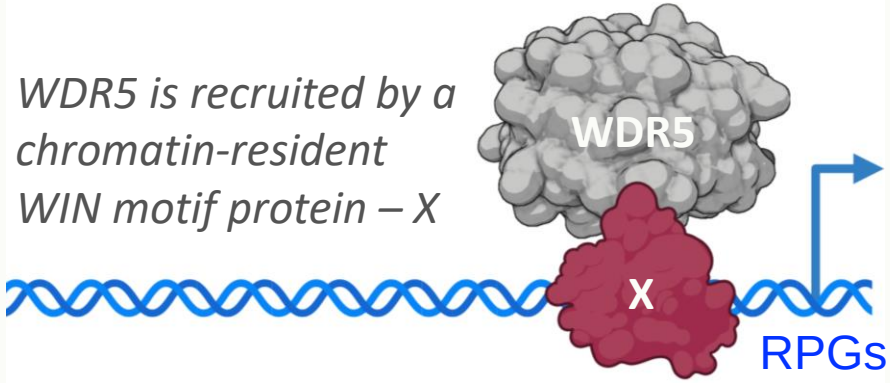


WIN site inhibitors globally evict WDR5 from chromatin



- Rapid
- Comprehensive
- Universal

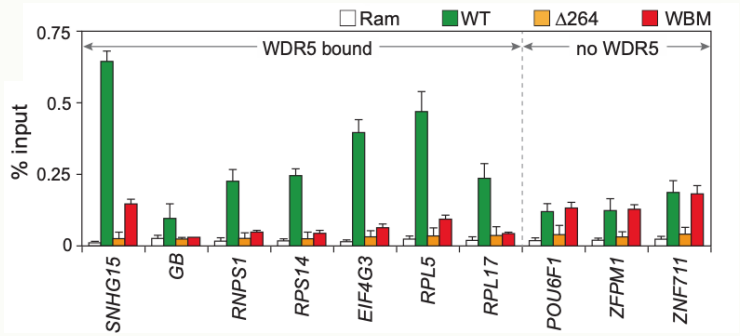
WDR5 is recruited by a chromatin-resident WIN motif protein – X

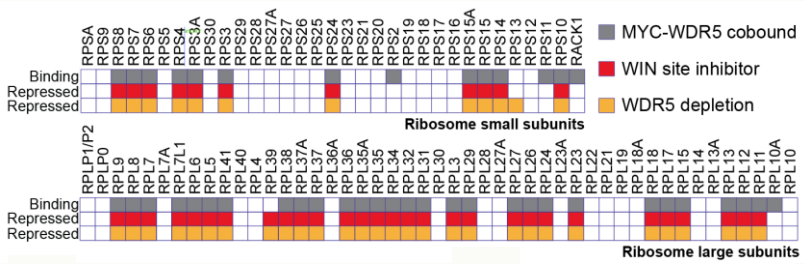


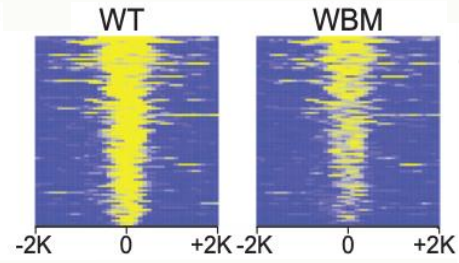
Foshage et al., 2019; Aho et al., 2019; Florian et al., 2022

The function of WDR5 at RPGs is to recruit MYC

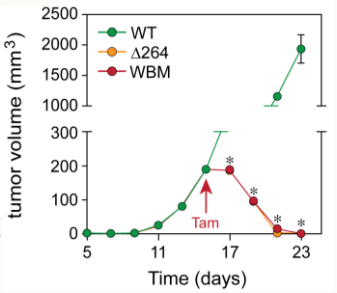
Genetic disruption of the MYC–WDR5 interaction





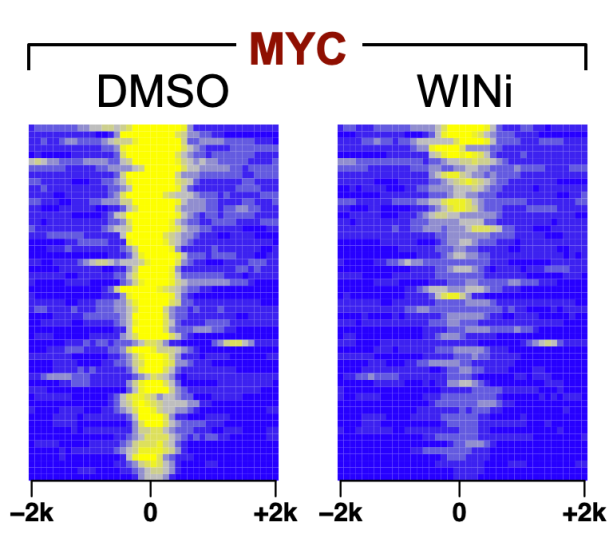


Myc binding reduced at ~90 sites



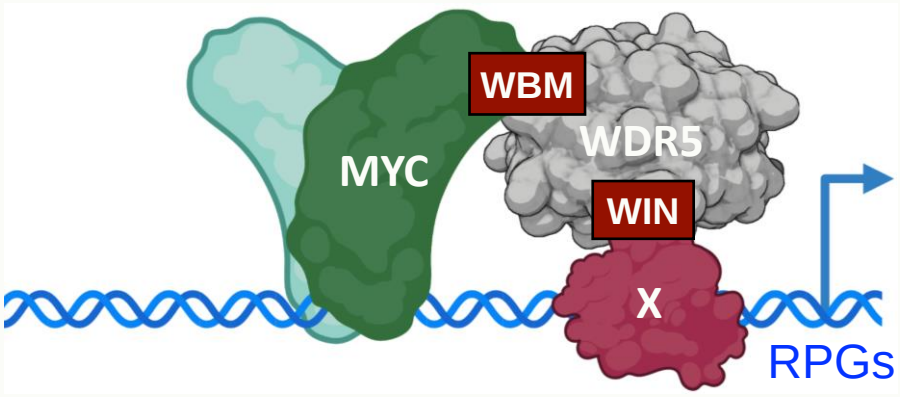
Tumor regression

WIN site inhibitors evict MYC from chromatin at WDR5-targets



- Rapid
- Selective
- Universal

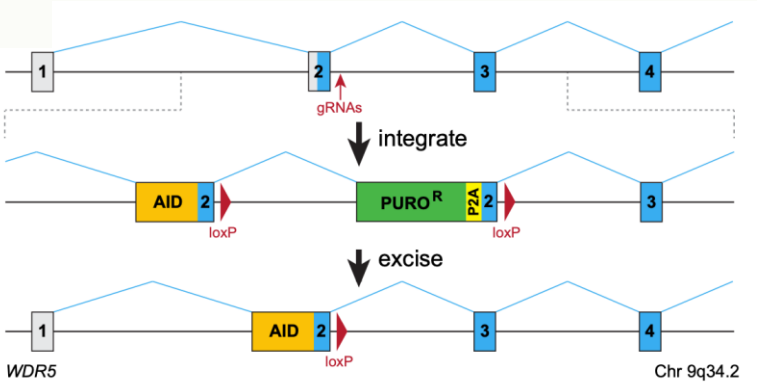
MYC is recruited to protein synthesis genes via WDR5

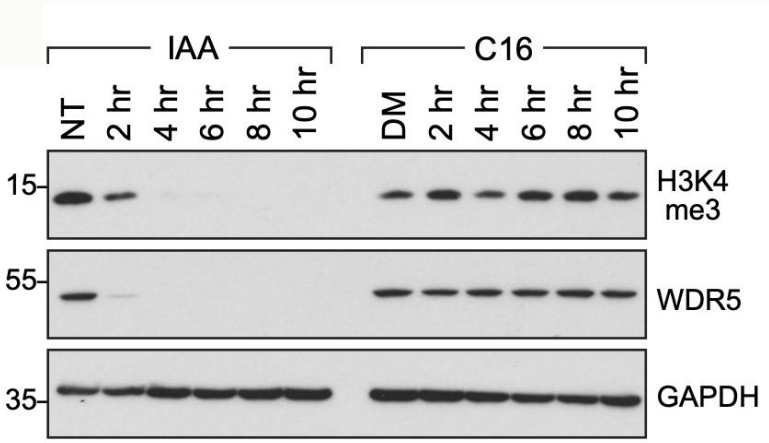


Thomas et al., 2019

WIN site inhibitors disrupt a subset of WDR5 function

A system to compare degradation of WDR5 with WINi

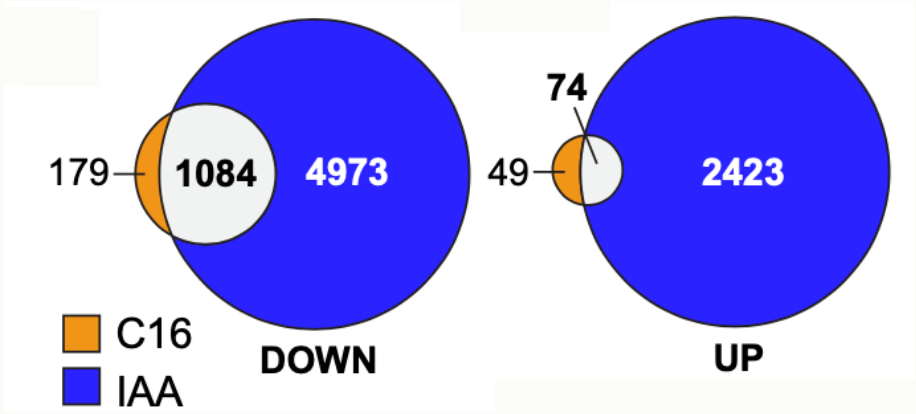


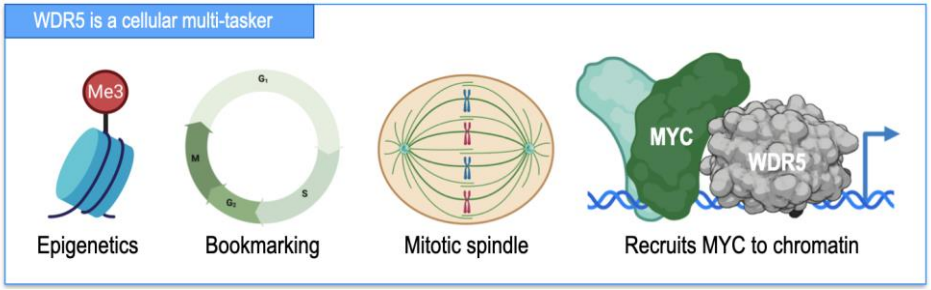


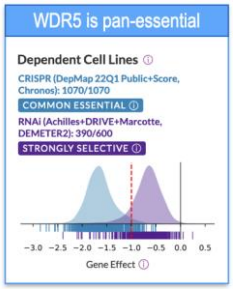
Acute WDR5 depletion *500 nM C16*

WIN site inhibitors disable only part of WDR5 function

Transcriptomic changes (direct and indirect)





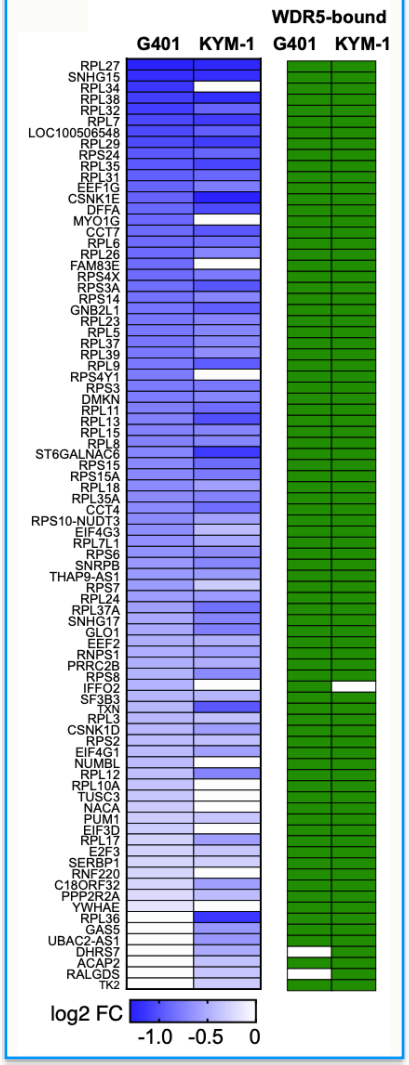


Although WDR5 is pan-essential, the WIN site is not. WDR5 does not need to be tethered to chromatin in all cells (therapeutic window).

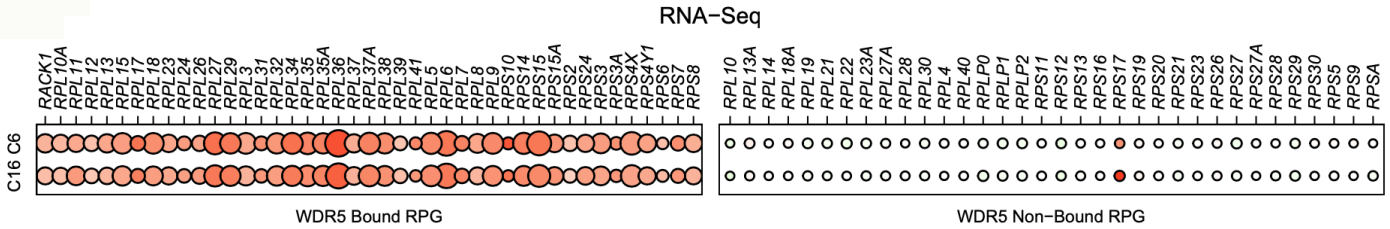
Siladi et al., 2022

WIN site inhibitors decrease PSG expression

Transcription (4 hr)



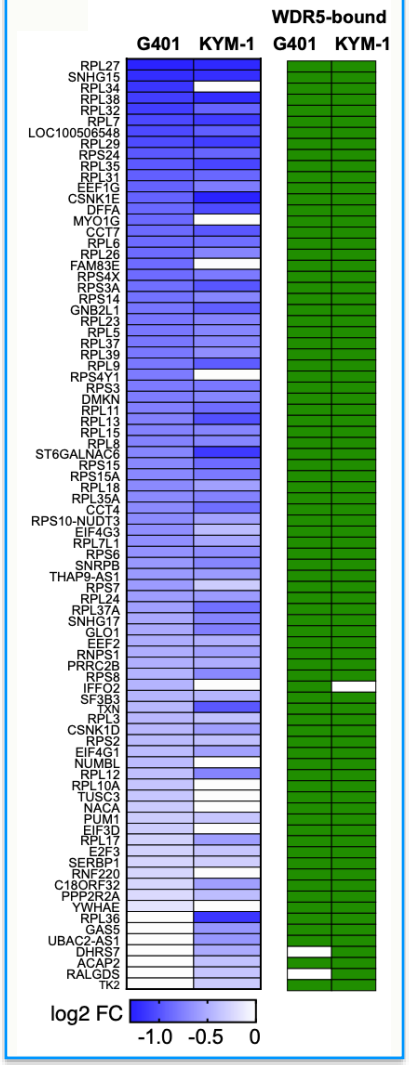
Steady-state transcripts (2 days)



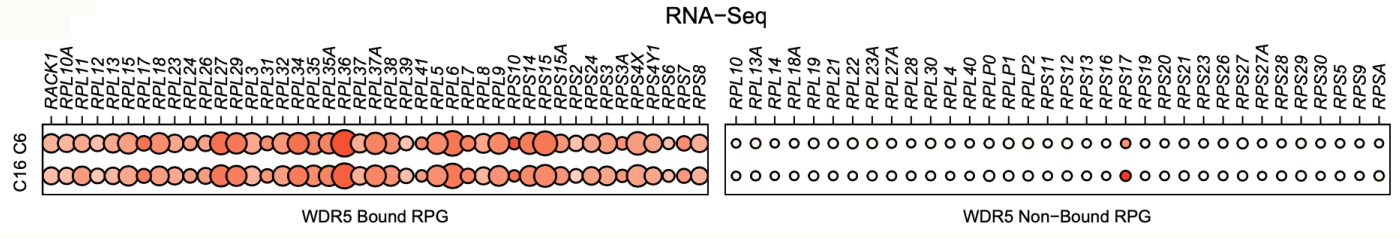
Aho et al., 2019; Florian et al., 2022; unpublished

WIN site inhibitors decrease ribosome inventory

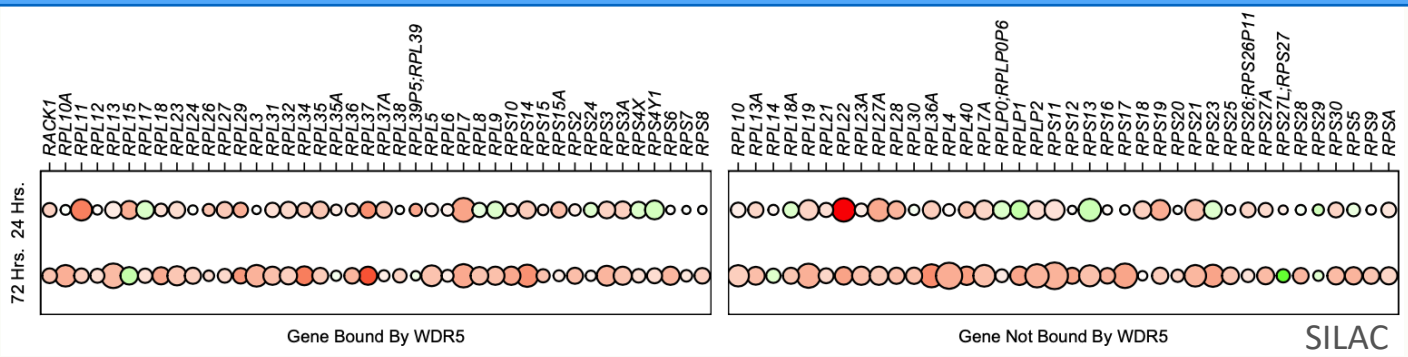
Transcription (4 hr)

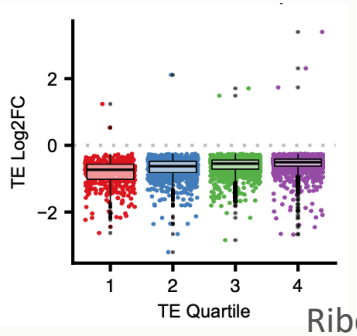


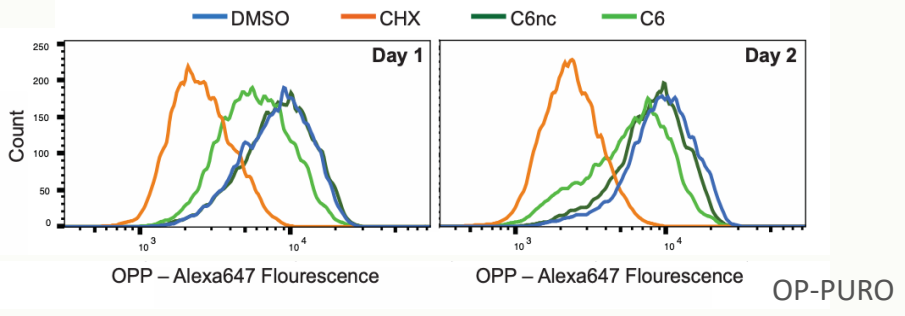
Steady-state transcripts (2 days)



Proteomic changes show decreased ribosomes and general translational choke

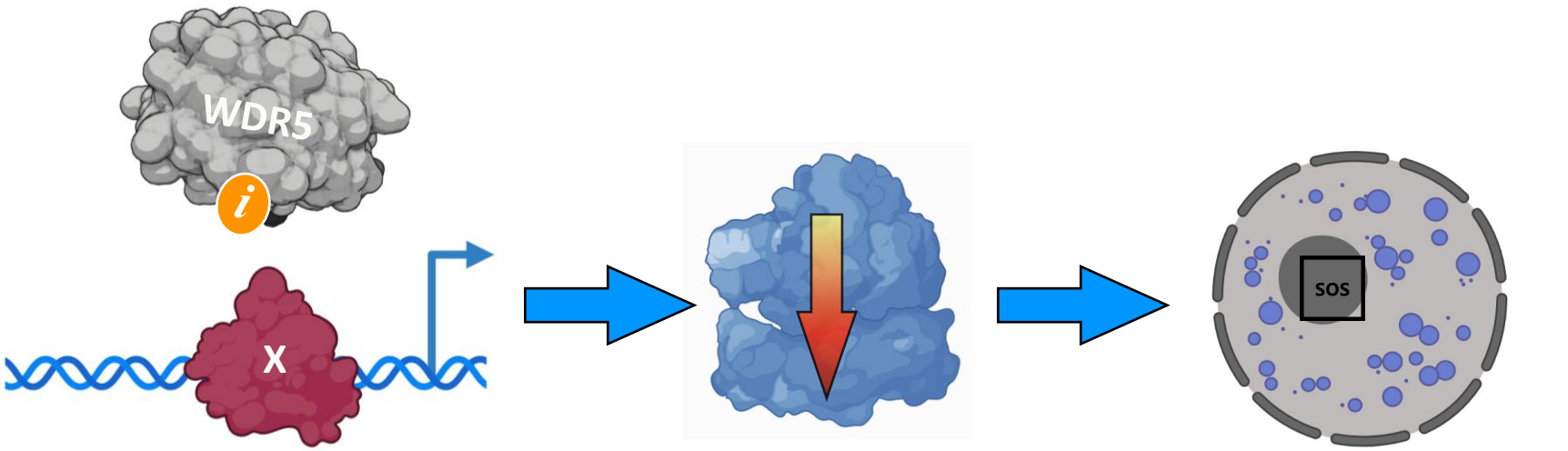






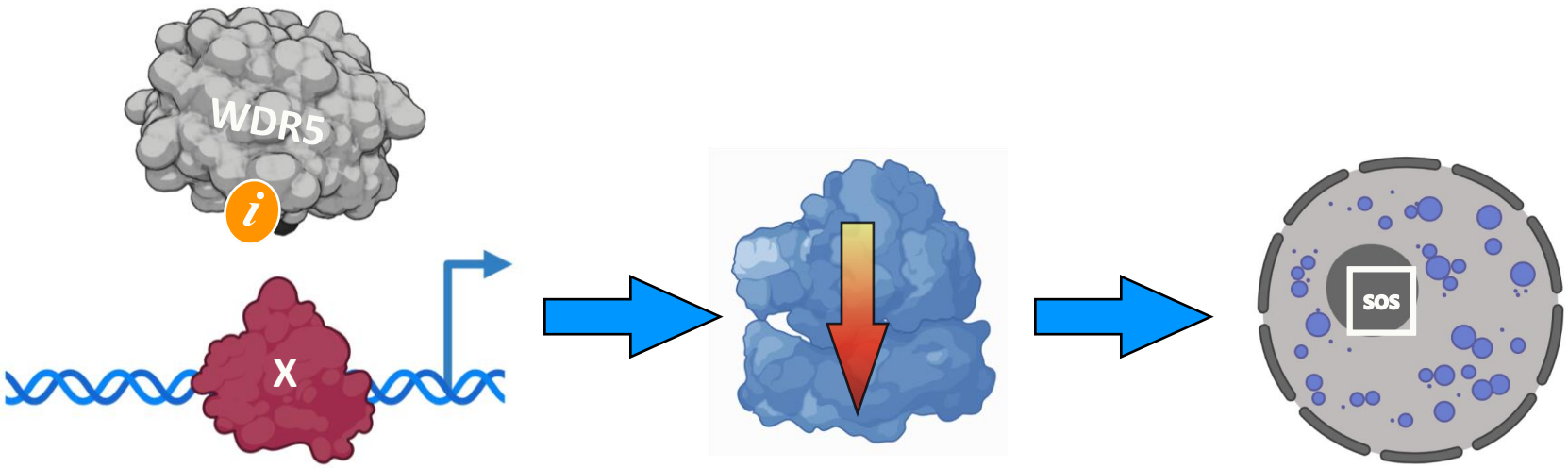
Aho et al., 2019; Florian et al., 2022; unpublished

Mechanism of action of WIN site inhibitors



Mechanism of action of WIN site inhibitors

VULNERABILITY



Chromatin displacement at RPGs

↓ Ribosome inventory

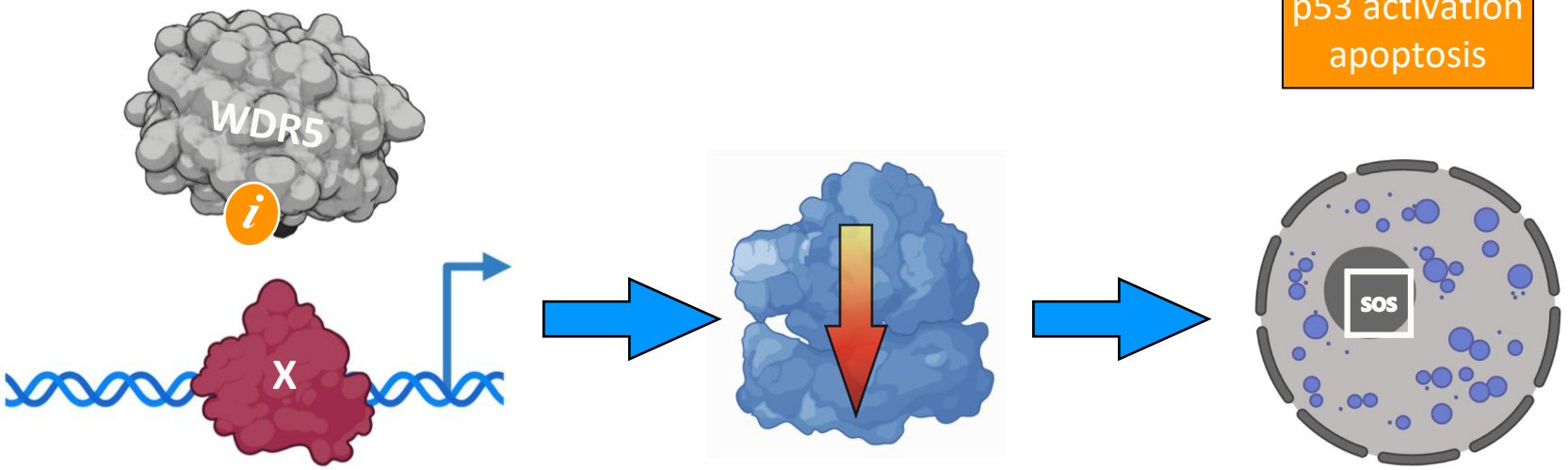
Nucleolar stress response

MYC
Oncogenes (e.g., MLLr)

Mechanism of action of WIN site inhibitors

VULNERABILITY

RESPONSE MECHANISM



p53 activation
apoptosis

Chromatin displacement at RPGs

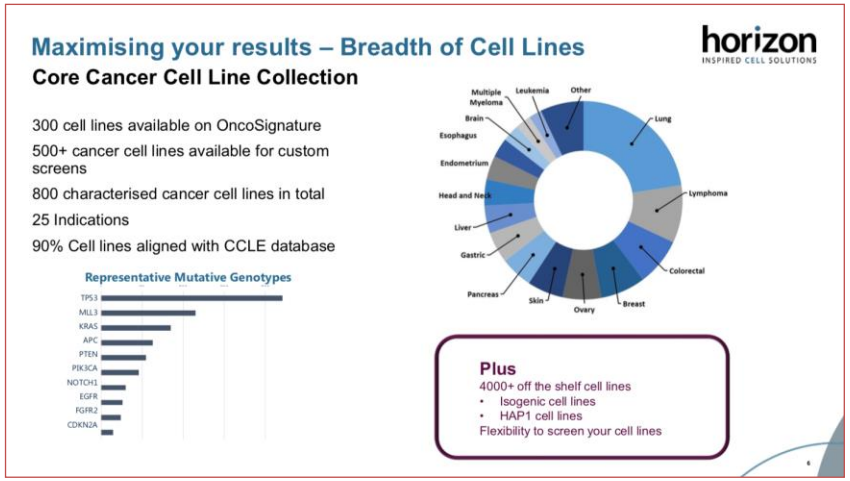
↓ Ribosome inventory

Nucleolar stress response

MYC
Oncogenes (e.g., MLLr)

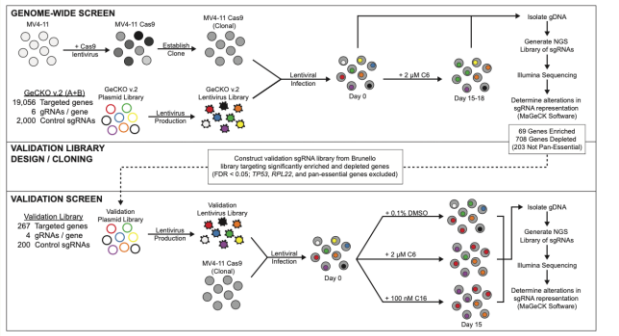
p53 independent
response

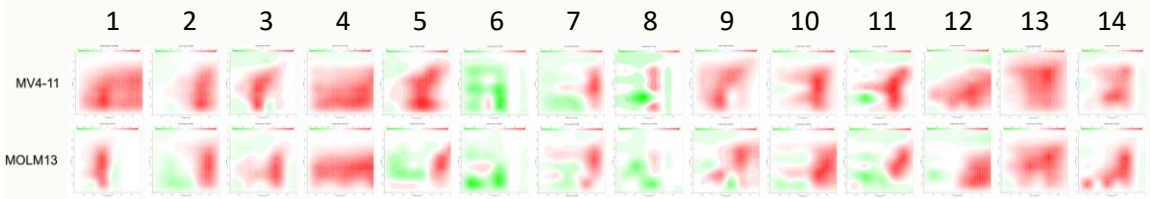
Clinical indications



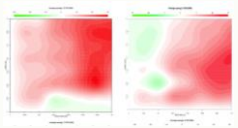
- **Blood-borne cancers strongly represented**
- **MLLr cancers strongly represented**
- **DLBCL strongly represented**
- **WT p53 not required**

Genome-wide CRISPR screens informs synergy choices





CRISPR screens performed in MLLr and solid cancer cell lines

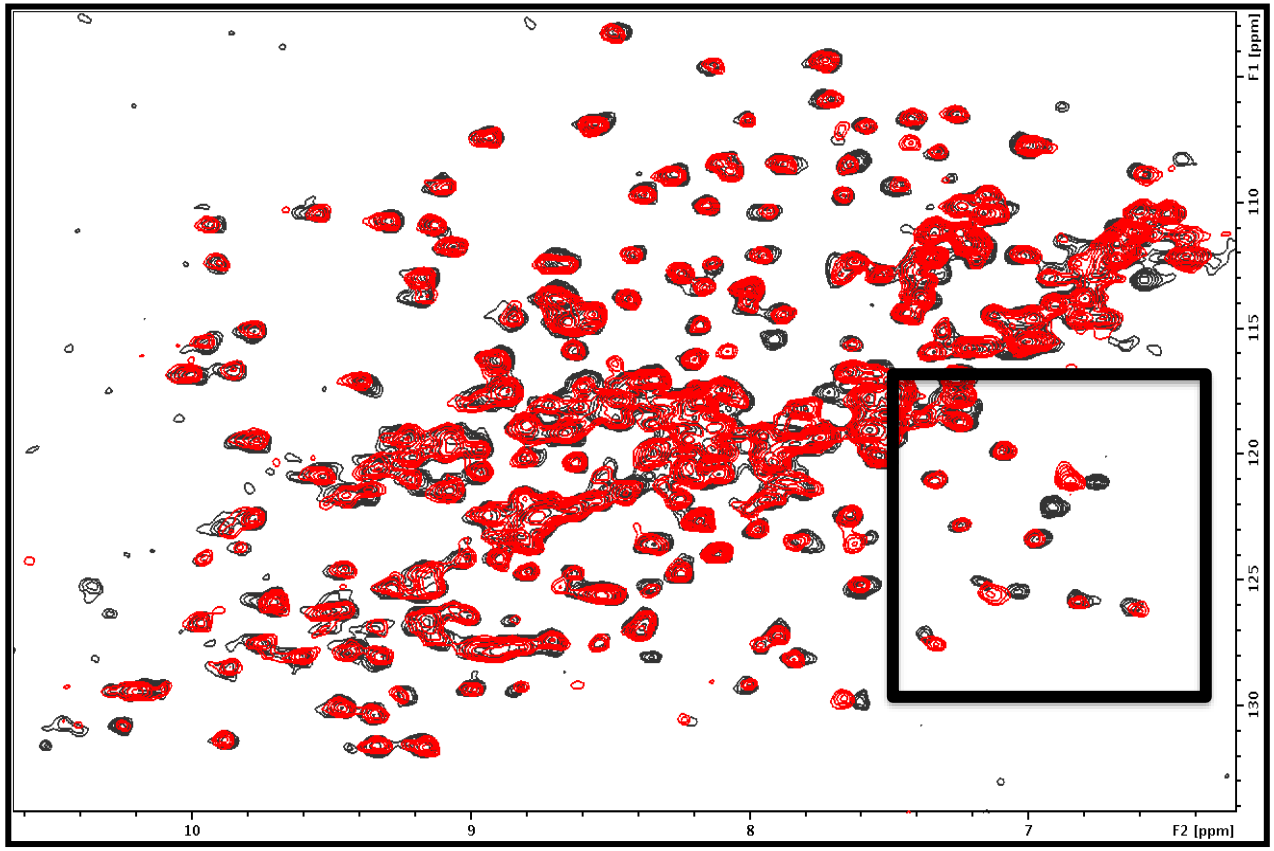


Summary of the biology of WIN site inhibitors

- WIN site inhibitors evict WDR5 and MYC from chromatin at RGPs
- WIN site inhibitors are selective loss of function agents
- WIN site inhibitors induce a translational choke
- WIN site inhibitors act via p53-dependent and independent ways
- Expect single agent activity in blood-borne cancers
- Expanded/improved activity via drug synergy (solid cancers)

Discovery of WDR5-MLL Inhibitors

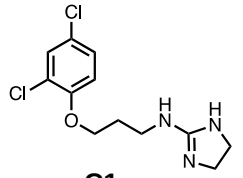
Initial Fragment Screen



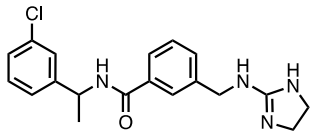
- **~13,824 Fragments screened:**
  48 primary hits
  K_d ~60 – 1000 µM

Enhance Binding Affinity by Fragment Growing

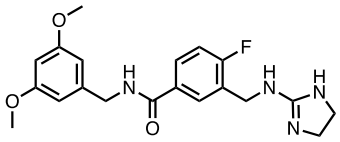
Gen 1



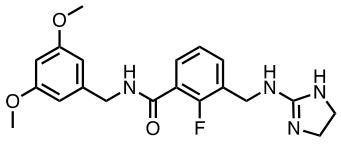
C1
K_d = 66,200 nM



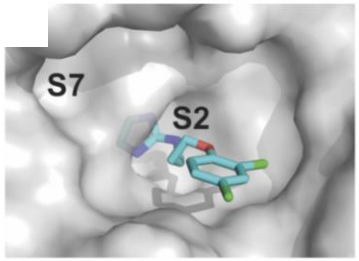
C2
K_i = 22 nM

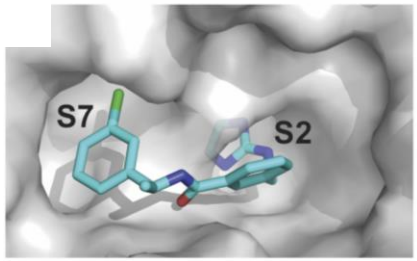


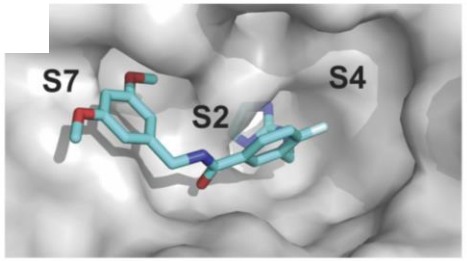
C3
K_i = 1.3 nM



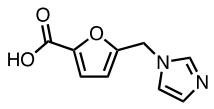
C3nc
K_i = 35 nM



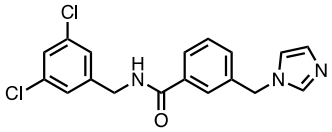




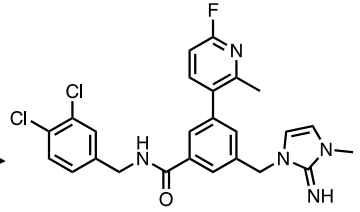
Gen 2



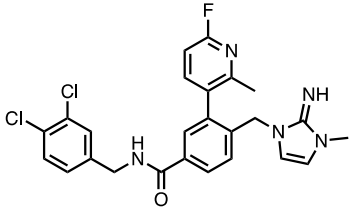
C4
K_d = 150,000 nM



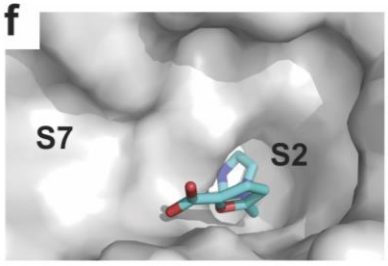
C5
K_i = 680 nM

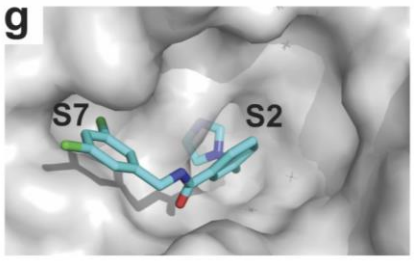


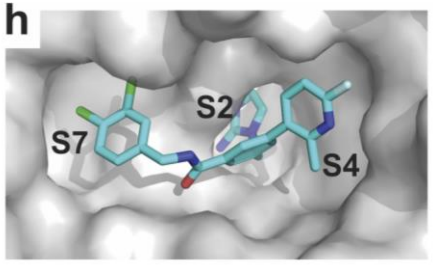
C6
K_i = 0.12 nM



C6nc
K_d > 330 nM

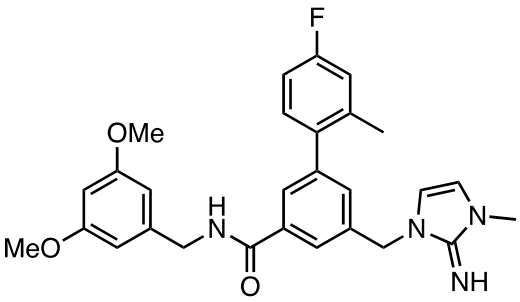






Aho, E. R. et. al. *Cell Reports* **2019**, *26*, 2916-2928.

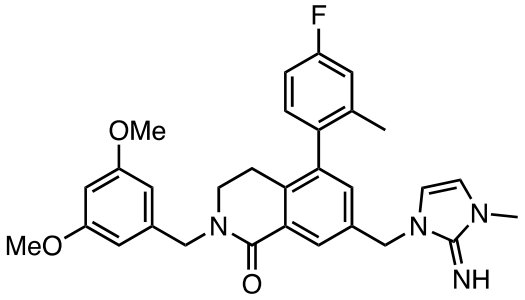
Structure Based Core Modification



VU2318

WDR5 K_i =	49 pM
5d GI_{50}, MV4:11 =	470 nM
Molm13 =	482 nM
K562 =	15000 nM
K562 / MV4:11 =	32

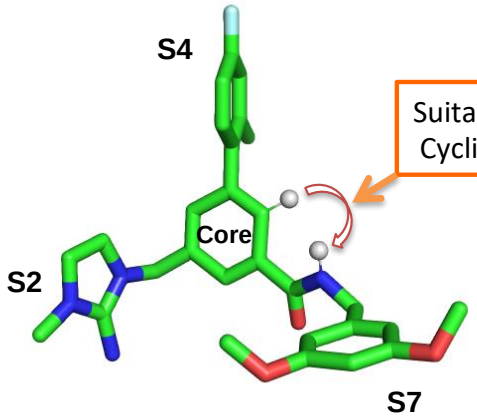
↓ 1 H-bond donor
↓ 2 Rotatable bonds



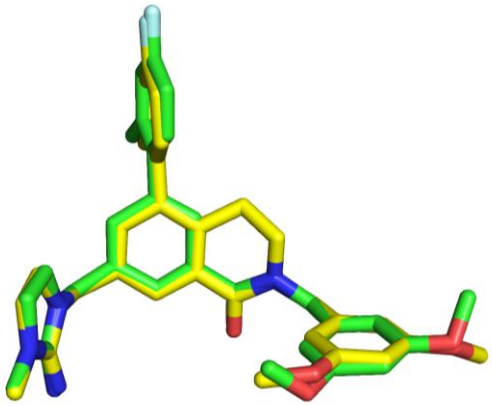
VU7584

WDR5 K_i =	< 20 pM
5d GI_{50}, MV4:11 =	38 nM
Molm13 =	78 nM
K562 =	8000 nM
K562 / MV4:11 =	210

WDR5 binding conformation of VU2318



Overlay of VU2318 & VU7584



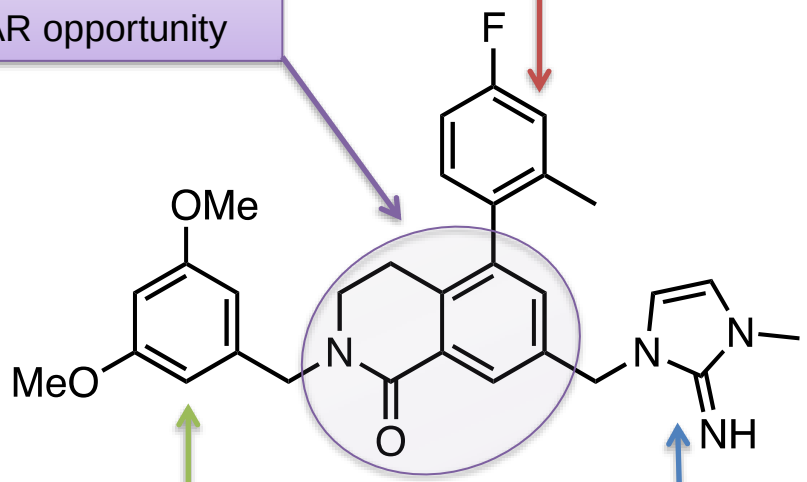
Tian, J. et.al. *J. Med. Chem*. **2020**, *63* (2), 656-675.

Pharmacophore Based SAR Strategy

Objectives : Enhance on target potency, PK properties (oral %F, IV CL), and safety profiles (i.e. hERG liability, CYP inhibition)

Core:
Identified 3 viable bicyclic cores that offer different properties & SAR opportunity

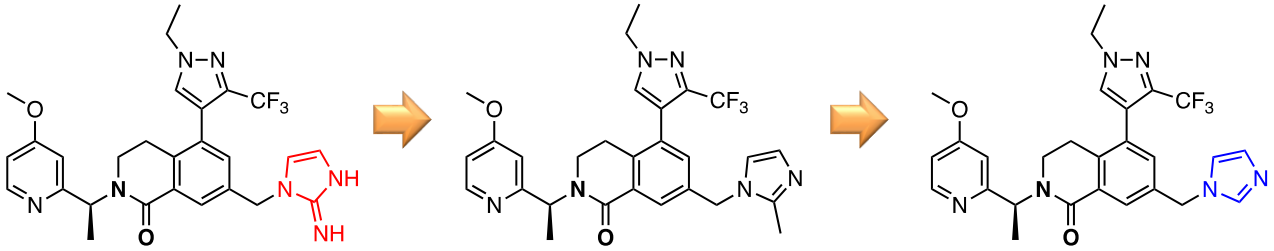
S4 unit:
Properly substituted 5- or 6-membered heterocycles



S7 unit:
Substituted mono- or bicyclic heterocycles

S2 unit:
Basic or neutral warhead (most critical for potency, PK, and safety)

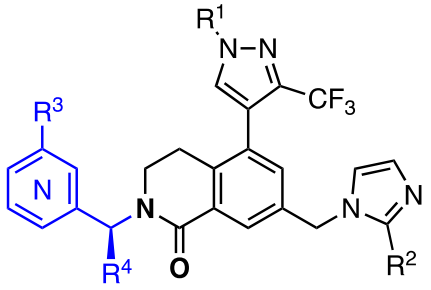
Build-in Oral Bioavailability through S2 Modification



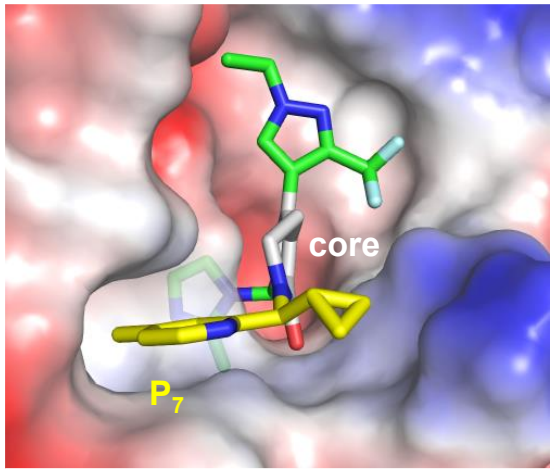
VU ID	VU831417	VU848313	VU848427
WDR5 K_i (pM)	<20	<20	<20
GI_{50} MV4-11 (nM)	20	6.9	13
GI_{50} K526 (nM)	4200	2500	2800
Mouse PK (IV @ 3 mg/kg, PO @ 10 mg/kg)			
IV CL (mL/min/kg)	**25**	**118**	**76**
PO %F	**0**	**28**	**73**
PO AUC_{inf} (h*ng/mL)	**0**	397	**2013**

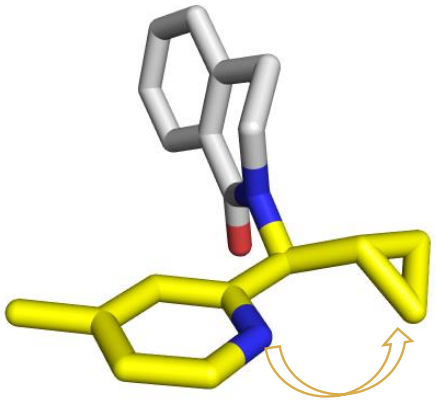
- Matching pair with different S2 subunits
- Choice of S2 subunit is critical for oral bioavailability
- IV CL of S2 need to be optimize
- SAR is focused on new S2 sub-series

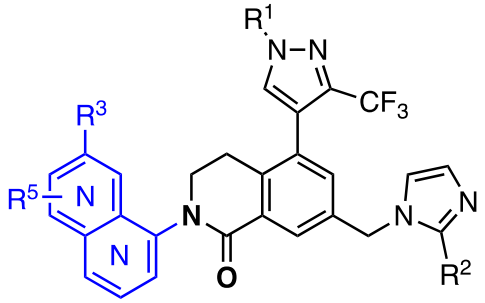
Structure-Based Design of Bicyclic Heteroaryl P7 Units



Optimal P7
R^4 = small Alkyl
S-enantiomer is preferred.





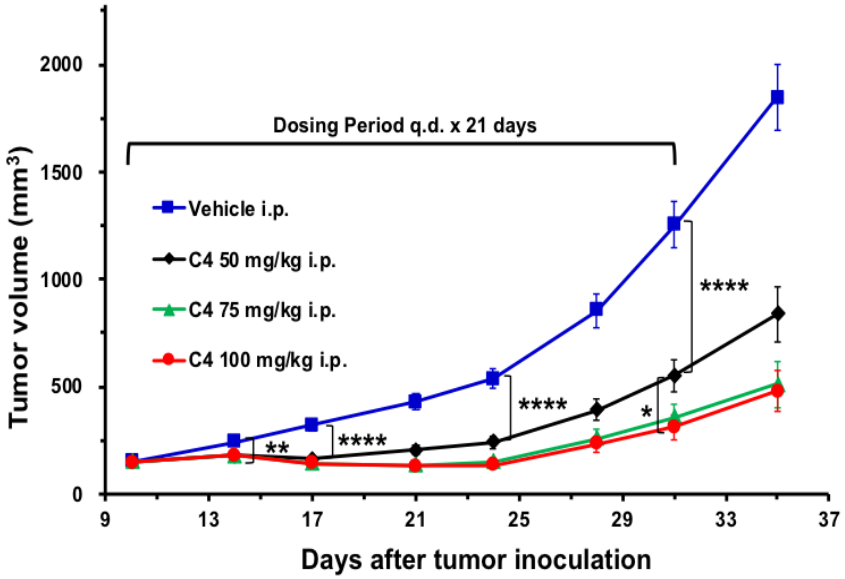


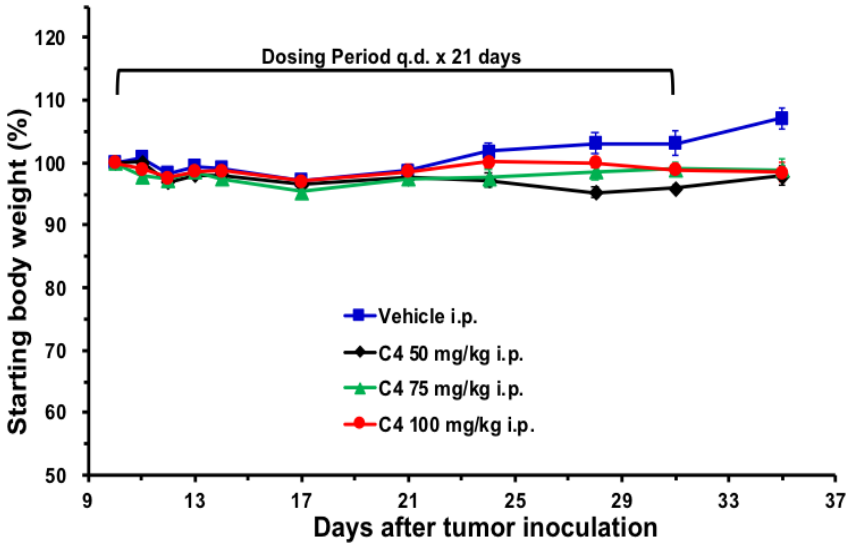
Challenges:
- Limited SAR expandability
- Limited accessibility of P7 amines
- Lacking efficient conversion synthesis

Benefits:
- Enhanced potency & PK
- High SAR expandability
- High accessibility of bicyclic heteroaryl P7 units
- Efficient conversion synthesis

In Vivo Tumor Growth Inhibition Was Achieved by IP Dosing

VU0849716 : Dosed at 50, 75, 100 mg/kg by IP QD x 21 days

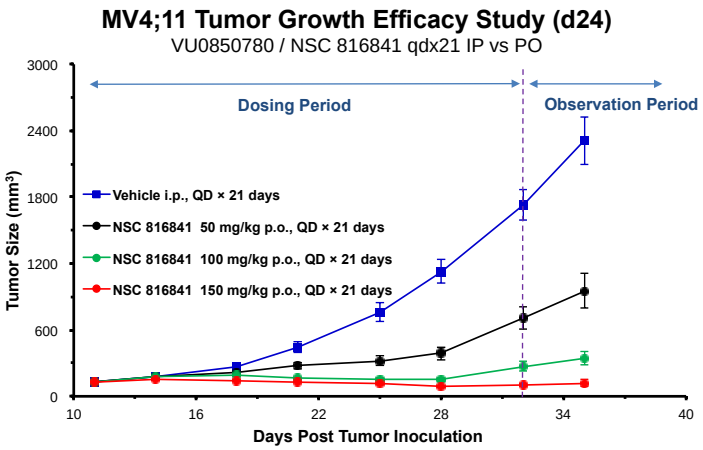


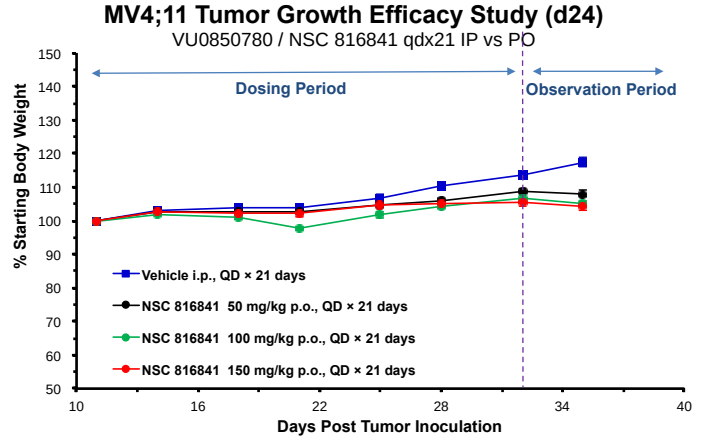


In Vivo Tumor Growth Inhibition Was Achieved by PO Dosing

VU0850780

Dosed at 50, 100, 150 mg/kg by PO QD x 21 days

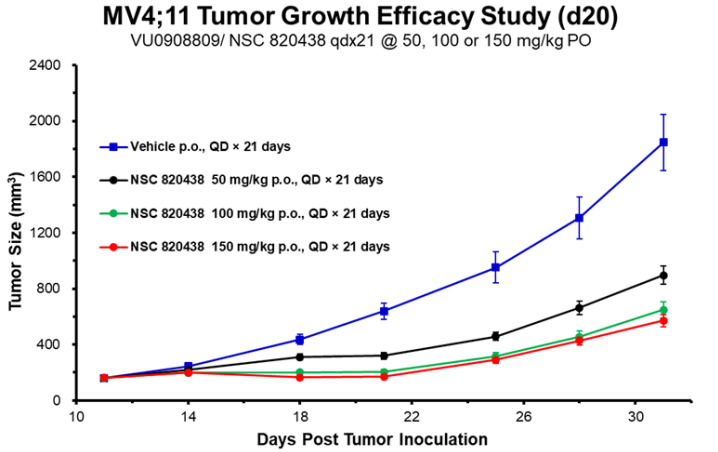


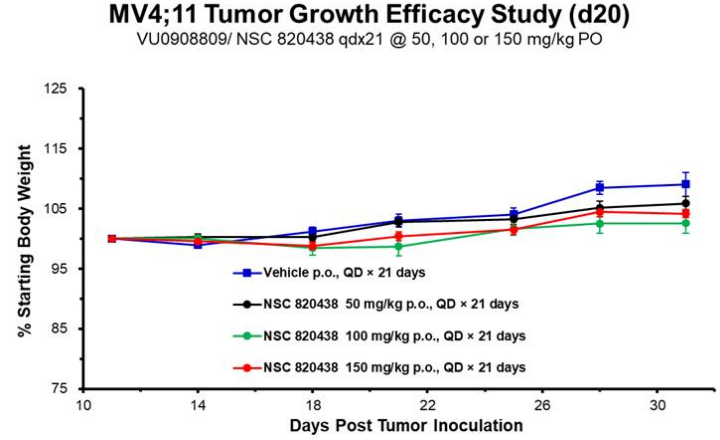


- Exhibited robust dose-dependent tumor growth inhibition by oral dosing
- Advancement was stopped due to high CL in rat

VU0908809

Dosed at 50, 100, 150 mg/kg by PO QD x 21 days





- Exhibited robust tumor growth inhibition at 100 mg/kg
- Exhibit superior PK's in all species
- Lower aqueous solubility
- Selected as the 1st IND candidate

Current IND Candidate: VU0914813

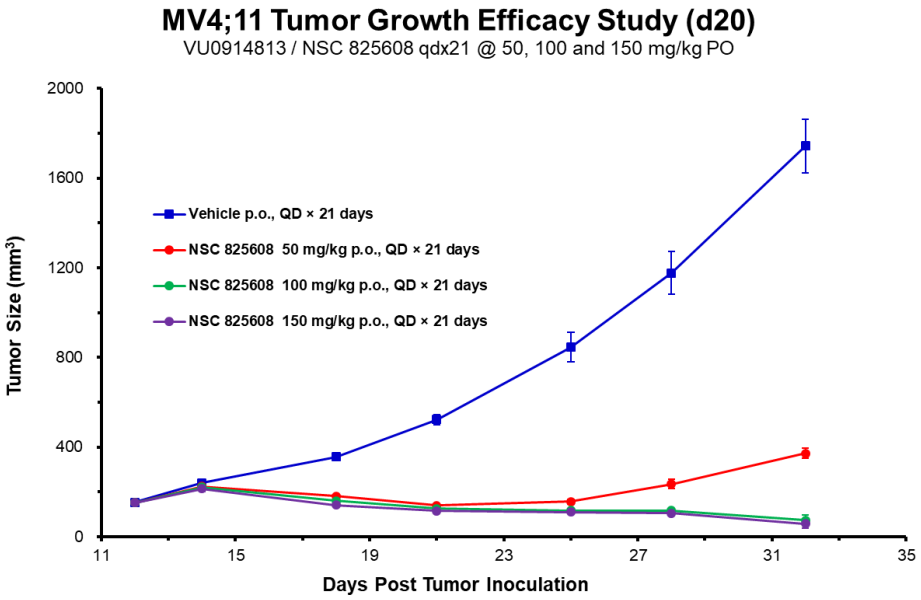
VU ID	VU0914813
NSC ID	825608
MW	561.57
tPSA	85.5
CLogP	2.35
pH 6.8 Kinetic Solubility (µM)	**62**
WB (Mouse) % PB	**98.7**
WDR5 K_i (pM)	**<20**
LE	>0.36
GI_{50} MV4-11 (nM)	**10**
GI_{50} Molm-13 (nM)	**18**
GI_{50} K562 (nM)	2038
K562 Max % inh.	70
hERG IC_{50} (µM)	**7.5**
CYP3A4 IC_{50} (µM) Midazolam / Testosterone	0.26 / 1.8

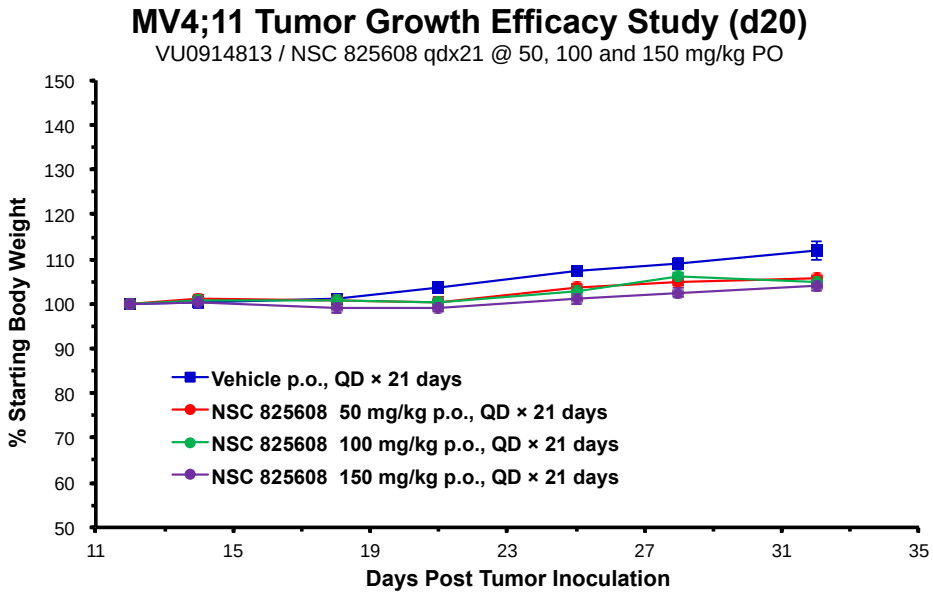
IV PK		Mouse	Rat	Dog	Cyno
PK parameters	Unit	3 mg/kg	3 mg/kg	0.5 mg/kg	0.5 mg/kg
Cl_obs	mL/min/kg	**21**	**36**	**5.1**	**34**
Q%		23	43	17	77
AUC_{last}	h*ng/mL	2396	1381	1754	255
AUC_{last}/D	h*mg/mL	799	460	3508	510
V_{ss}_obs	L/kg	0.8	2.5	0.6	3.3

PO PK		Mouse	Rat	Dog	Cyno
PK parameters	Unit	50 mg/kg	50 mg/kg	5 mg/kg	15 mg/kg
$T_{1/2}$	h	3.8	2.1	2.0	2.4
T_{max}	h	1.7	2.0	1.0	3.3
C_{max}	ng/mL	14033	5623	6805	1563
AUC_{last}	h*ng/mL	75894	23896	23247	7199
MRT_{Inf}_obs	h	6.0	2.7	2.7	4.3
AUC_{last}/D	h*mg/mL	1518	478	4649	480
F	%	**190**	**104**	**126**	**93**

Exhibit: best overall potency, off-target liability potential, and PK profiles
linear PK profile in rodents up to 400 mg/kg by PO-dosing as a suspension

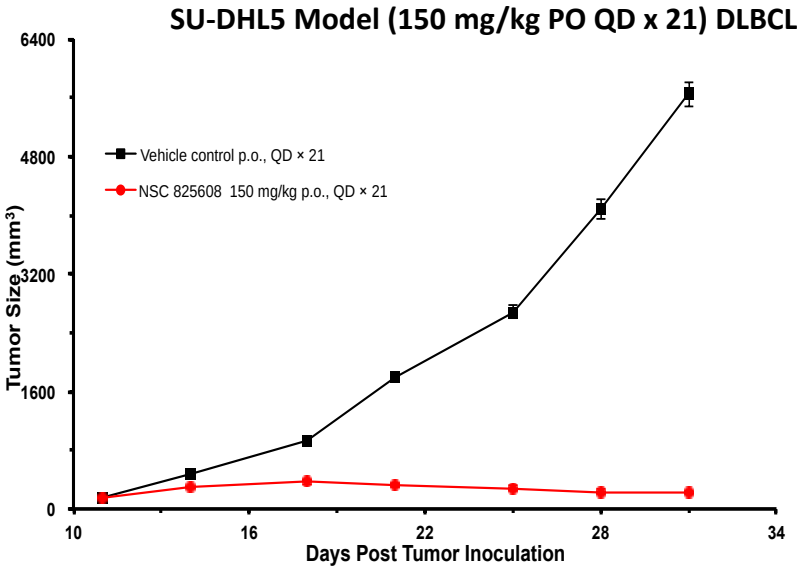
VU0914813 Exhibits Superior *In Vivo* Efficacy

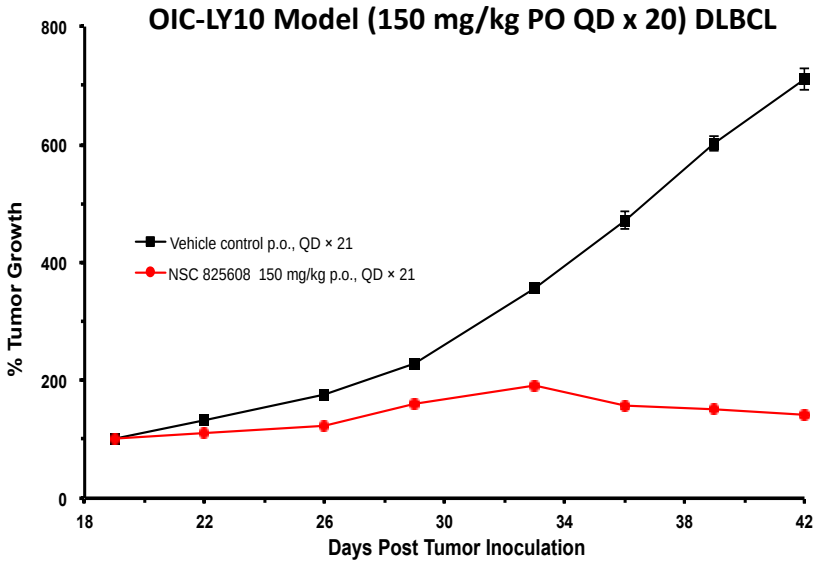


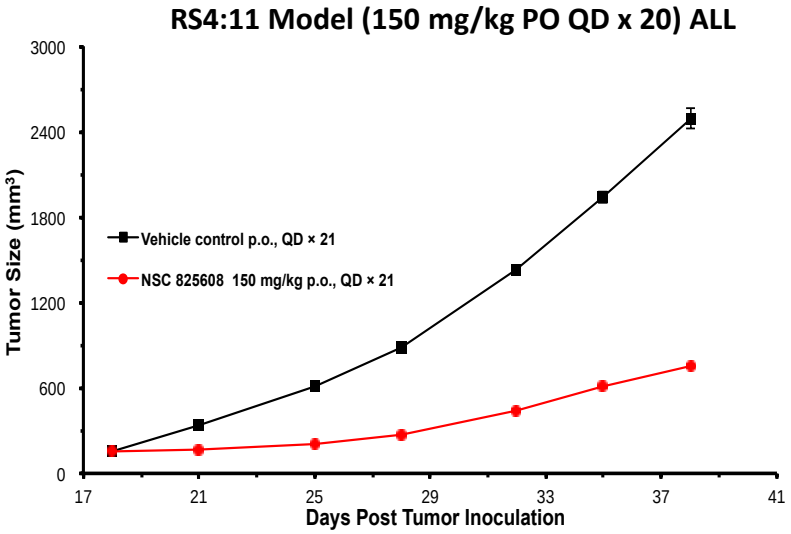


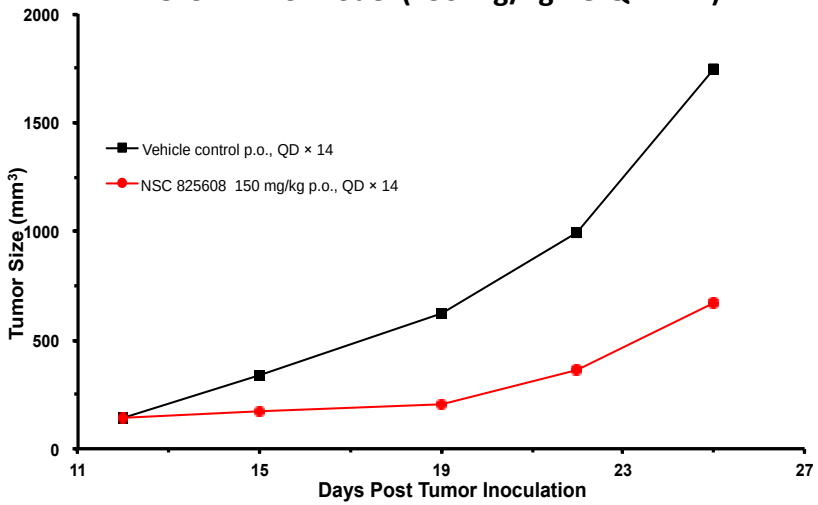
- VU914813 exhibited dose-dependent tumor growth inhibition by oral dosing
- Maximum efficacy was obtained at 100 mg/kg dose
- All doses were tolerated without signs of clinical abnormalities

VU0914813 Is Efficacious in Other Heme Models







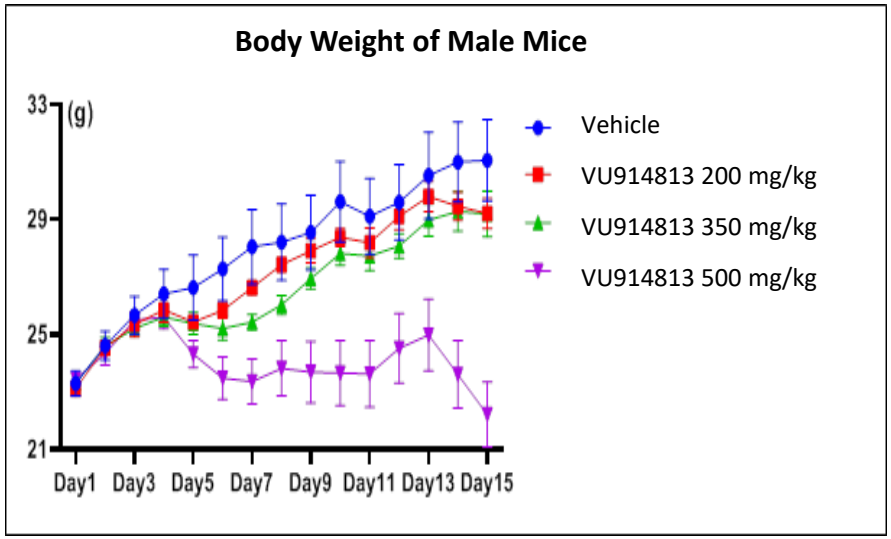


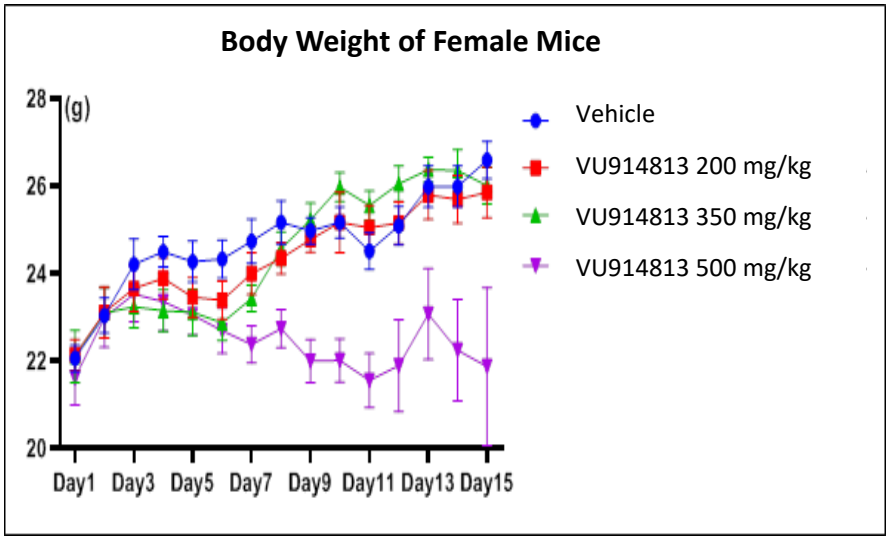
VU0914813 Exhibits >3-Fold Therapeutic Window in Mice

14-Day Tolerability Study in CD-1 Mice

Dose: 200, 350, and 500 mg/kg QD by PO dosing

Measure: Body weight, clinical signs, plasma PK (D1, D14), Tissue exposure (D14), necropsy, hematology, blood chemistry





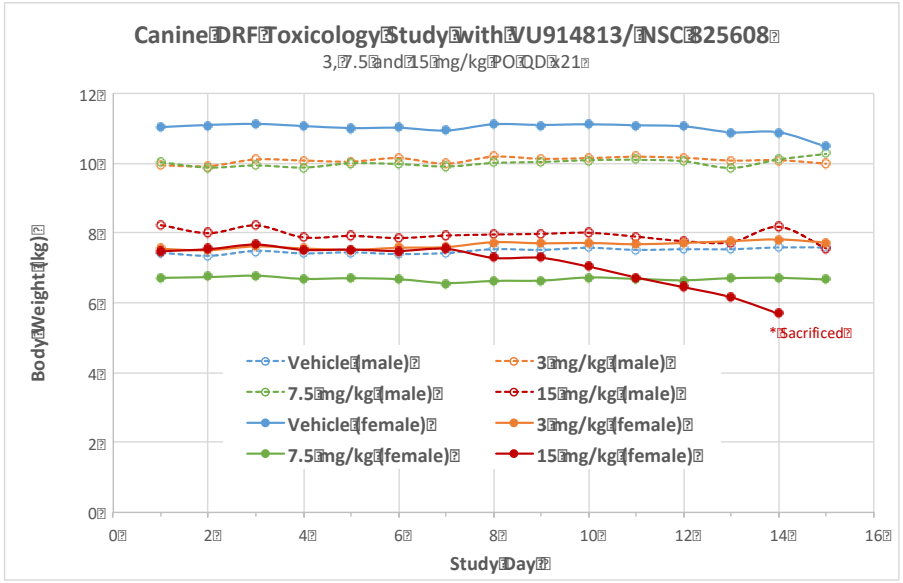
- Fully efficacious dose in mice is 100 mg/kg
- No clinical signs observed in the 200 and 350 mg/kg dose groups
- Lethality (1 out 6 mice D13) and clinical signs at 500 mg/kg
- MTD is 350 mg/kg (3.5-fold therapeutic window)

Determining MTD of VU0914813 in Rats

- **Dosed at 100, 200 and 300 mg/kg PO in SD rats**
- **At 100 & 200 mg/kg PO**
  - Body weights above starting levels
  - No limiting adverse clinical signs
  - Dose-dependent reduction in white blood cells, red blood cells and platelets
- **At 300 mg/kg PO**
  - Reduction in ovary and uterus weights in female rats
  - Intestinal bleeding found in 2 male rats
  - 2 out of 3 male rats died on D14
- **Determined MTD to be 200 mg/kg in rats**

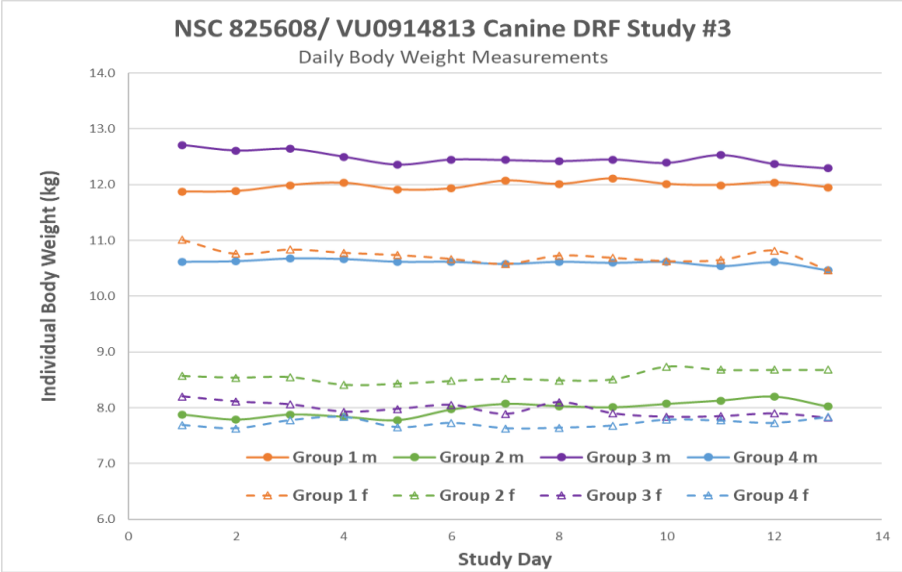
Dog DRF Studies of VU0914813

Initial Study : Dosed at 3, 7.5 and 15 mg/kg QD x 14



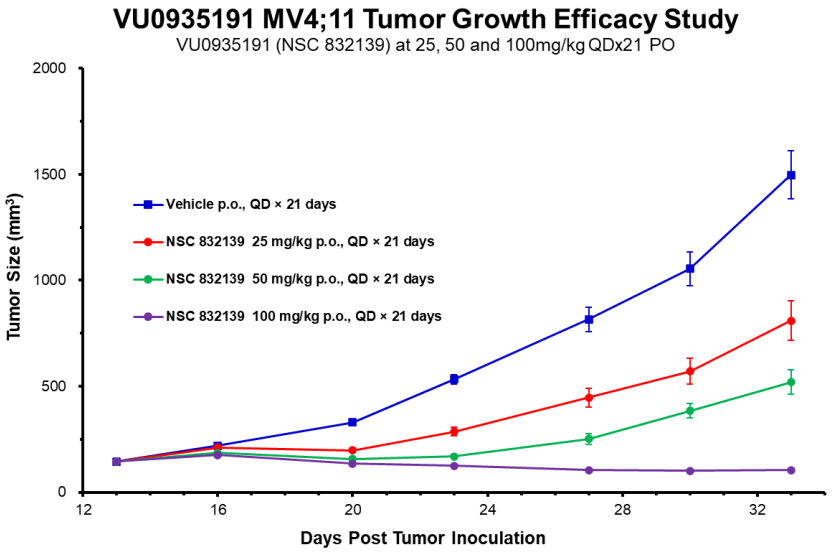
- No significant BW change for all surviving animals
- No limiting clinical signs at 3 and 7.5 mg/kg
- Dose dependent reductions in white blood cells Female dog at the 15mg/kg dose had reduced thymus size, signs of GI toxicity, and sacrificed on day 14
- 7.5 < MTD < 15 mg/kg
- Significant increase in exposure observed at 15 mg/kg at D7 (female) & D14 (male)
- **Toxic effects at 15 mg/kg likely due to compound accumulation**

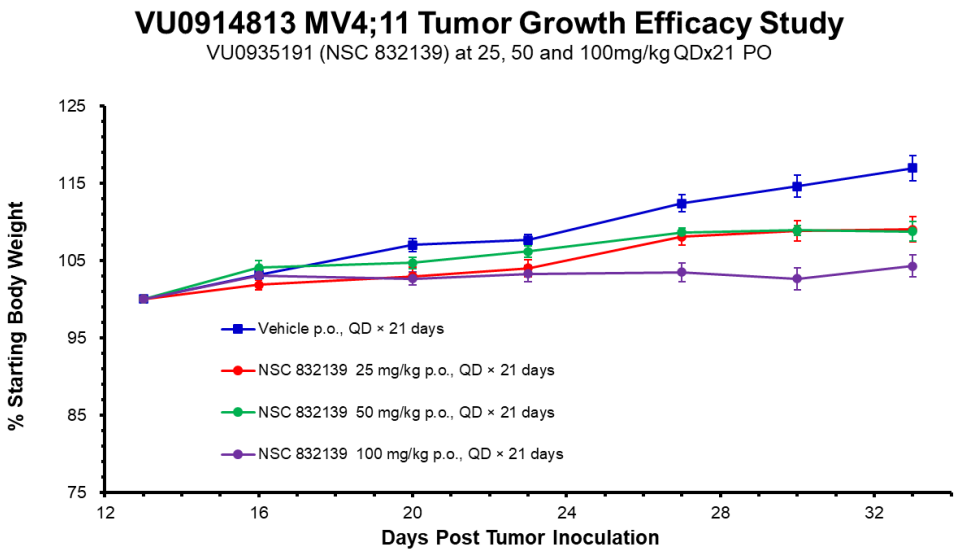
New Study : Dosed at 15 mg/kg QDX4/3 off X2, 30 mg/kg QDX4/3 off X2, 30 mg/kg Q2D



- No significant BW change for all animals
- No limiting clinical signs at all doses
- No significant finding in hematology
- Reduced thymus size observed in all female dogs
- No evidence of compound accumulation at all doses
- Tested doses < MTD

VU0935191: Another Lead Under Profiling





- Exhibit comparable *in vitro* and *in vivo* efficacy to VU0914813
- Similar PK profile to VU0914813 in mice
- Exhibit higher IV CL than 0914813 in dog
- Lower potential for compound accumulation by QD dosing in dog
- Dog DRF study is scheduled in early July
- Final selection will be made between VU0914813 and VU0935191

Summary of WDR5-MLL Program Progress

Medicinal Chemistry

- Filed 8 patents > 2800 New compounds synthesized

Structural Biology

- 85 X-ray co-crystal structures solved

Cell Biology / Assay Development

- Binding assays (FITC-MLL peptide): FPA, TR-FRET, SPR
- Proliferation assays: sensitive (MV4:11 & Molm13) and insensitive (K562) cell lines
- On-mechanism activity assays *(CETSA, HMT inhibition, Caspase 3/7 Glo, SM-biotin IP)*
- External broad panel cellular activity screen (MGH, Horizon)

Cell Biology / MOA

- WDR5 inhibitors do NOT act like previously thought
- Displace WDR5 from chromatin and block selective RPGs
- How to develop WDR5 inhibitors

DMPK & Animal Efficacy Model

- >600 compounds tested in eADME Screen
- PK studies conducted: > 120 Compounds in CD-1 mouse (NCI); >40 compounds in rat
- *In vivo* efficacy studies conducted:
  - Mouse efficacy studies : MV4:11, JeKo-1, SU-DHL-6, OCI-LY-10, DOHH-2, Ramos, Molm13, CHP-134 SQ xenograft ,MV4:11, Nalm-6 disseminated xenograft

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