AstraZeneca

Evaluating PROTAC Safety from a Functional and Mechanistic Perspective

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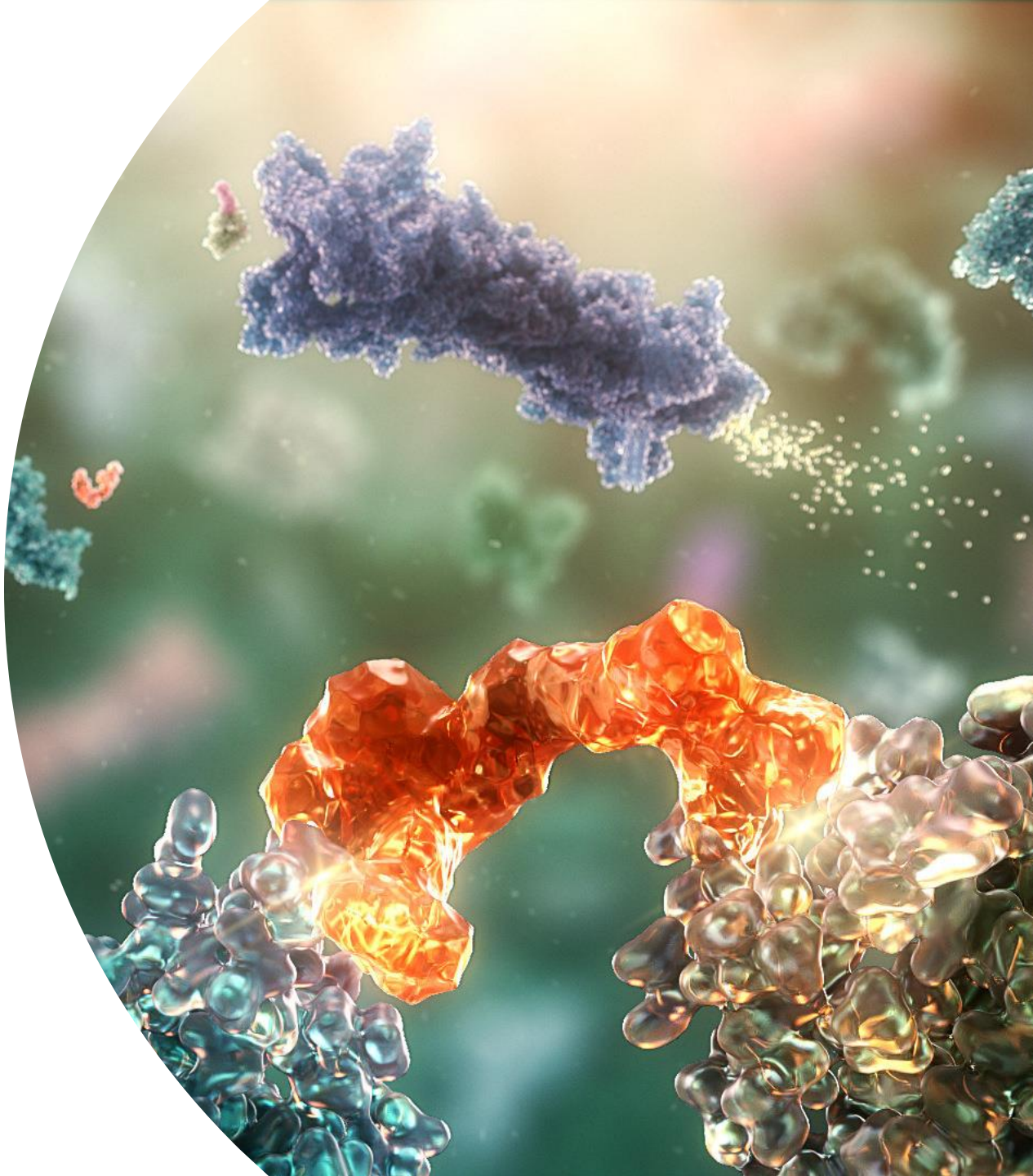
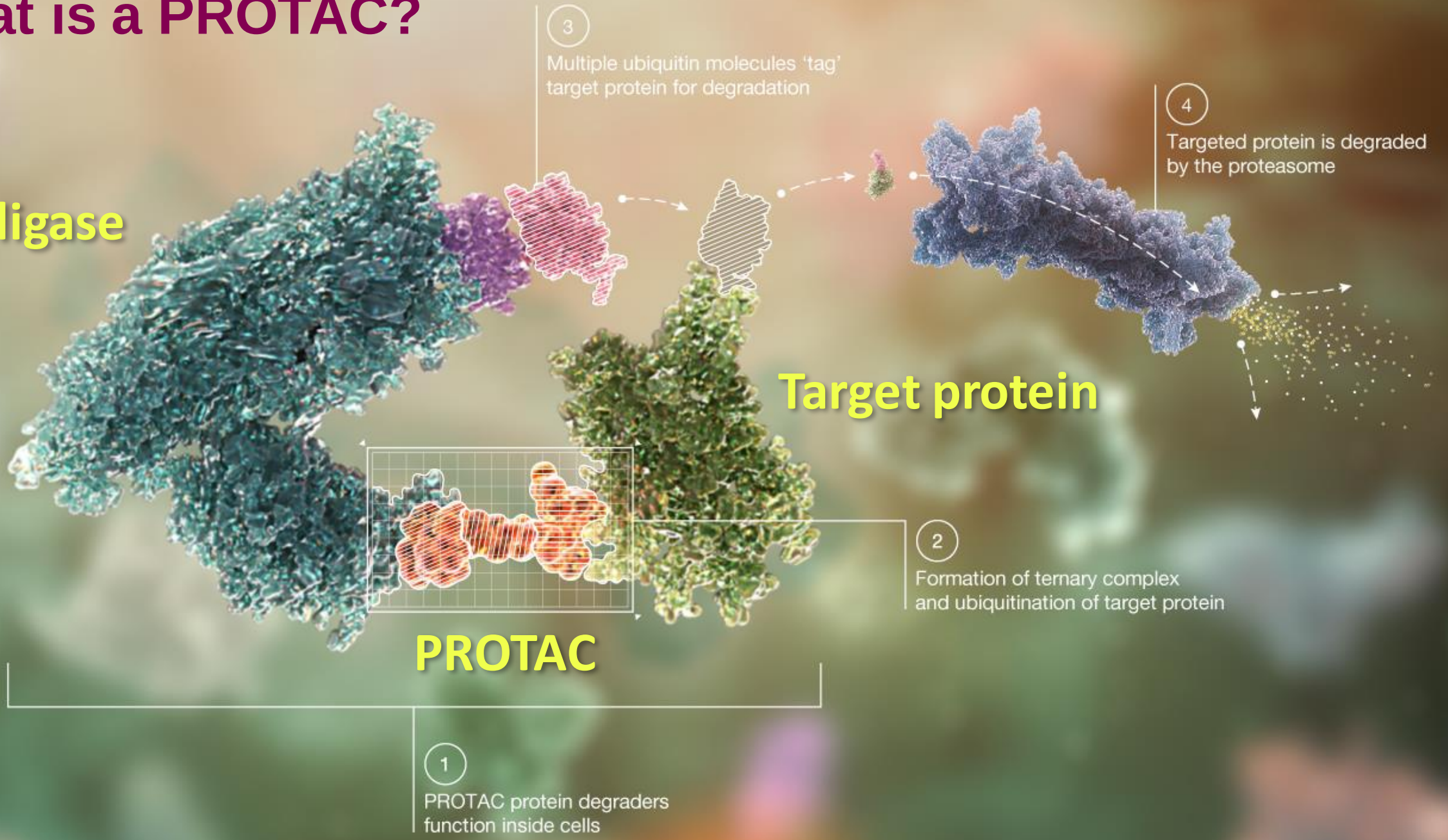
Protein Degradation & Targeting Undruggables Congress EUROPE



What is a PROTAC?



Clinical PROTAC landscape in 2022

Company	Degrader	Target	Indications	E3 ligase	ROA	Highest phase	Clinical trial no. (if applicable)
Arvinas	ARV-110	AR	Prostate cancer	CRBN	Oral	Phase II	NCT03888612
Arvinas/Pfizer	ARV-471	ER	Breast cancer	CRBN	Oral	Phase II	NCT04072952
Accutar Biotech	AC682	ER	Breast cancer	CRBN	Oral	Phase I	NCT05080842
Arvinas	ARV-766	AR	Prostate cancer	Undisclosed	Oral	Phase I	NCT05067140
Bristol Myers Squibb	CC-94676	AR	Prostate cancer	CRBN	Oral	Phase I	NCT04428788
Dialectic Therapeutics	DT2216	BCL-x_L	Liquid and solid tumours	VHL	I.v.	Phase I	NCT04886622
Foghorn Therapeutics	FHD-609	BRD9	Synovial sarcoma	Undisclosed	I.v.	Phase I	NCT04965753
Kymera/Sanofi	KT-474	IRAK4	Autoimmune diseases (e.g., AD, HS, RA)	Undisclosed	Oral	Phase I	NCT04772885
Kymera	KT-413	IRAK4	Diffuse large B cell lymphoma (MYD88-mutant)	CRBN	I.v.	Phase I	
Kymera	KT-333	STAT3	Liquid and solid tumours	Undisclosed	Undisclosed	Phase I	
Nurix Therapeutics	NX-2127	BTK	B cell malignancies	CRBN	Oral	Phase I	NCT04830137
Nurix Therapeutics	NX-5948	BTK	B cell malignancies and autoimmune diseases	CRBN	Oral	Phase I	NCT05131022
C4 Therapeutics	CFT8634	BRD9	Synovial sarcoma	CRBN	Oral	IND-e	
C4 Therapeutics	CFT8919	EGFR-L858R	Non-small-cell lung cancer	CRBN	Oral	IND-e	
Cullgen	CG001419	TRK	Cancer and other indications	CRBN	Oral	IND-e	

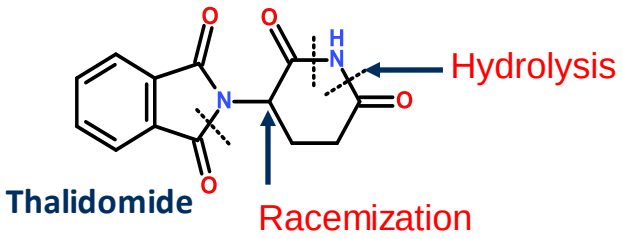
13 Clinical stage PROTACs
(= bifunctional degraders)

Nature Review Drug Discovery, 2021

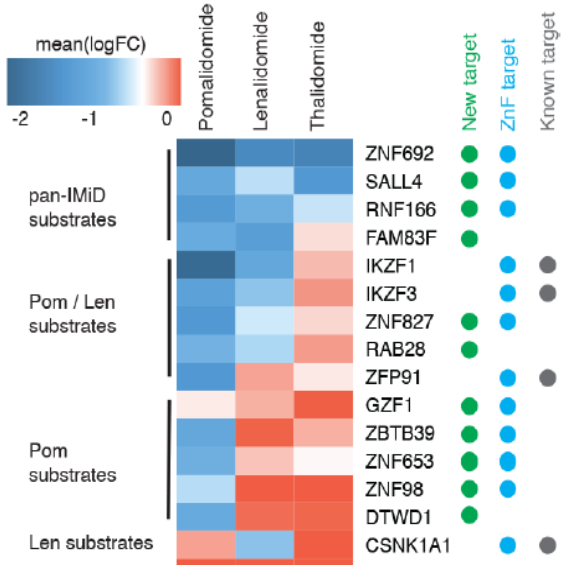
Most PROTAC projects use CRBN as the E3 ligase and many with IMiD warheads

Safety concerns with IMiD-based PROTACs

- IMiDs can show instability
  - Undergo racemization and hydrolysis



- IMiDs induce degradation of numerous proteins



Donovan et al eLife 2018

- IMiDs are associated with a range of clinical toxicities

	Thalidomide	Lenalidomide	Pomalidomide
Reprotoxicity	Teratogen	Teratogen	Teratogen
Hemetoxicity	**Neutropenia**: 15-25% (reversible)	**Neutropenia** and thrombocytopenia, grade 3-4: 80% (patients with del 5q MDS; reversible)	**Neutropenia**: 45%; thrombocytopenia: 21%; anemia: 17% (reversible)
Neurotoxicity	**Peripheral neuropathy** (Mild: 85%; Severe: 3-5%)	**Peripheral neuropathy** (29%)	**Peripheral neuropathy** (9%, no grade 3/4 reported)
Thromboembolic complications (low incidence/high impact)	**DVT/PE** (mono: 1-3%; with dex: 10-12%)	**DVT/PE** (7.4%/3.7% with dex)	**DVT/PE** (grade 3/4: mono: 3%; with dex: 4%)

DVT: deep vein thrombosis; PE: pulmonary embolism

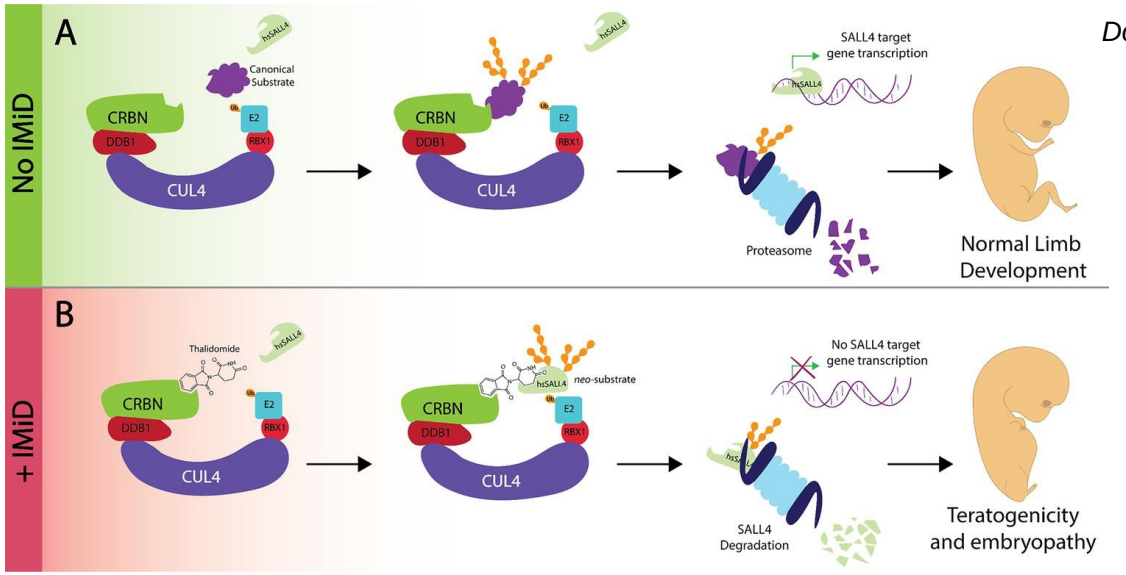
Conclusion

IMiD-PROTACs may carry the IMiD safety risks of degradation of numerous proteins and clinical toxicities.

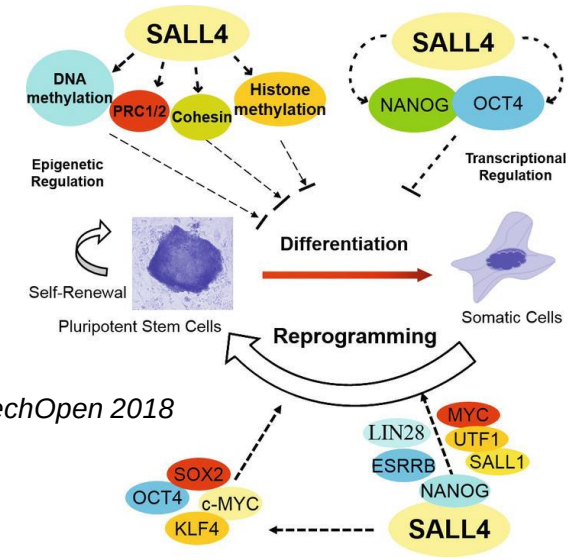
IMiD reprotox linked to SALL4 degradation

- SALL4 is a zinc finger DNA-binding protein that has been well characterized in development and in embryonic stem cell (ESC) maintenance

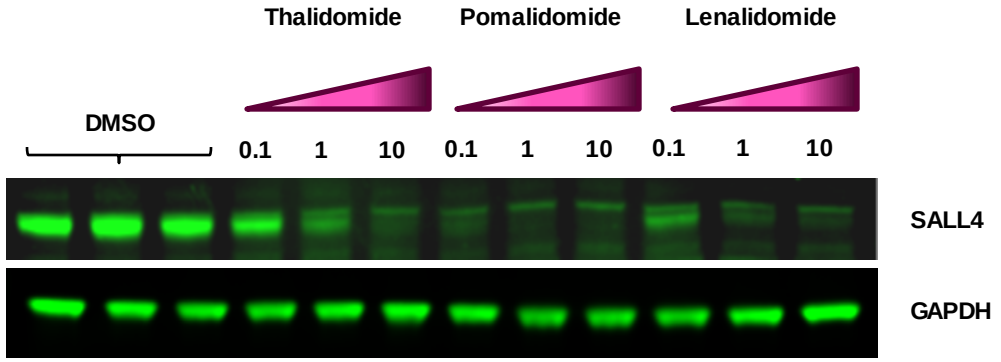
- SALL4 is recruited to CRBN by IMiDs inducing its degradation and has been linked to the reprotoxicity seen with these drugs



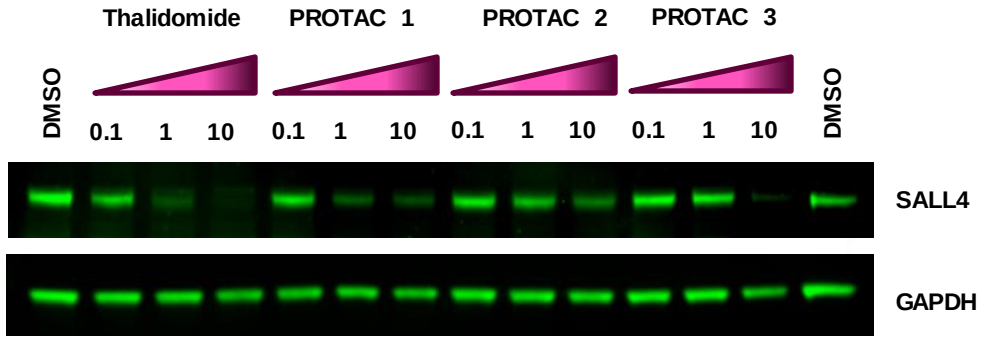
Donovan et al eLife 2018



Yang, IntechOpen 2018



- PROTAC based on IMiDs are able to degrade SALL4

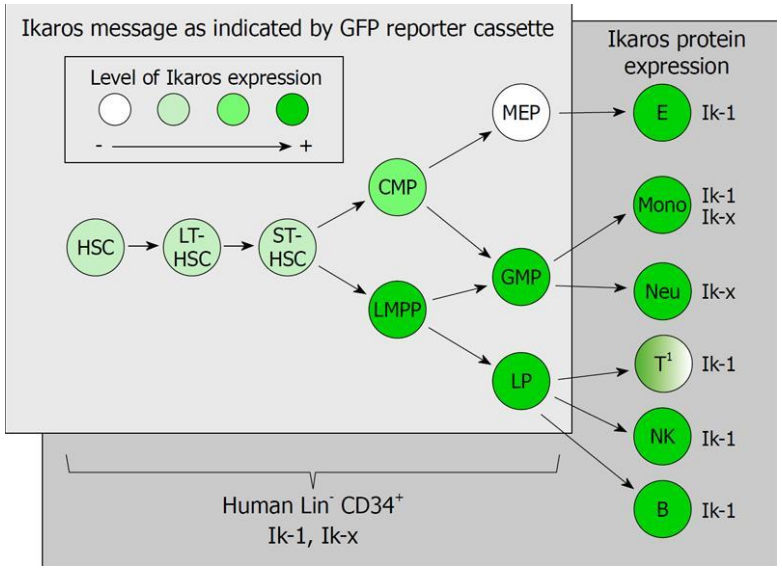


Conclusion

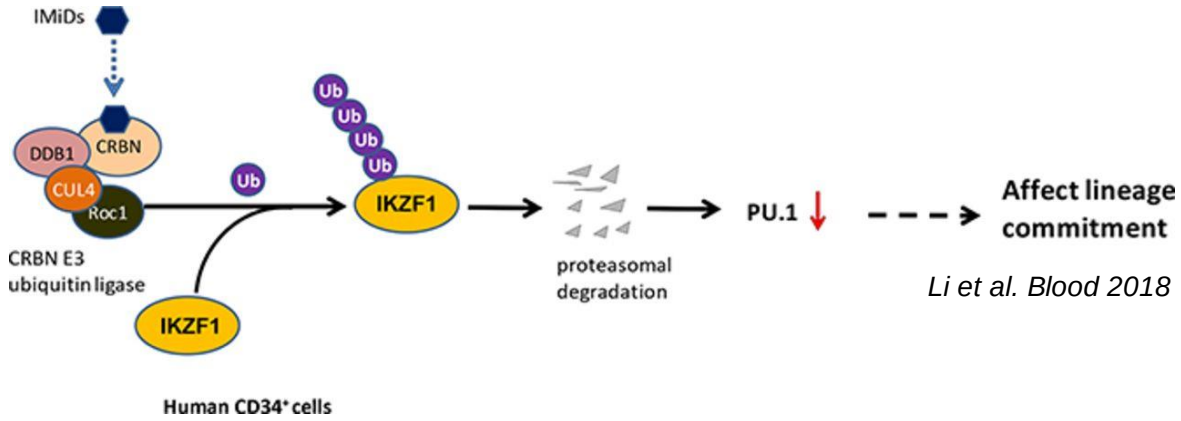
SALL4 could be a good biomarker for PROTAC reprotoxicity risk prediction

IMiD-induced neutropenia linked to cell maturation arrest

- IKAROS (IKZF1) is a zinc finger DNA-binding protein that displays crucial functions in the hematopoietic system
- IKRAOS is recruited to CRBN by IMiDs, inducing its degradation and has been linked to neutropenia seen in clinic
- Literature suggests IMiDs arrest myeloblast differentiation via IKAROS degradation and downstream PU.1 downregulation



Francis et al. JBC 2011



Li et al. Blood 2018

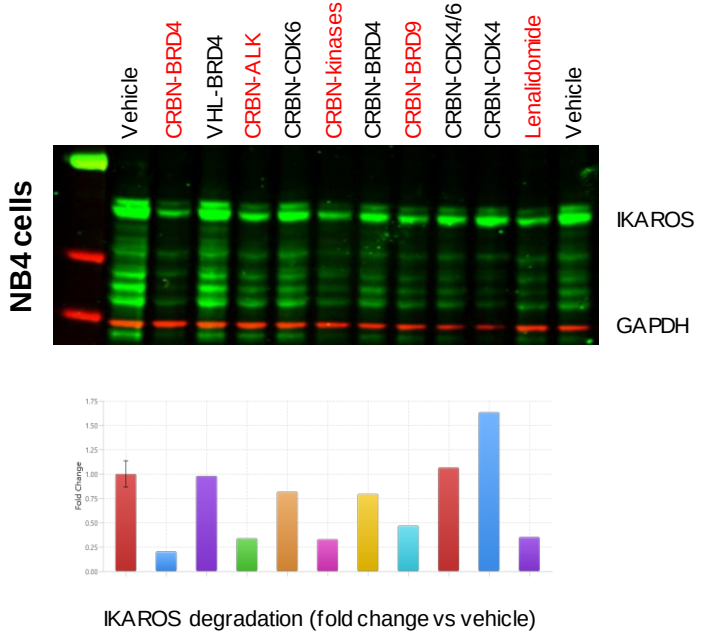
Conclusion

IKAROS could be a good biomarker for PROTAC bone-marrow toxicity risk prediction – need IVIV translation study for validation

Evidence of IKAROS degradation by PROTAC

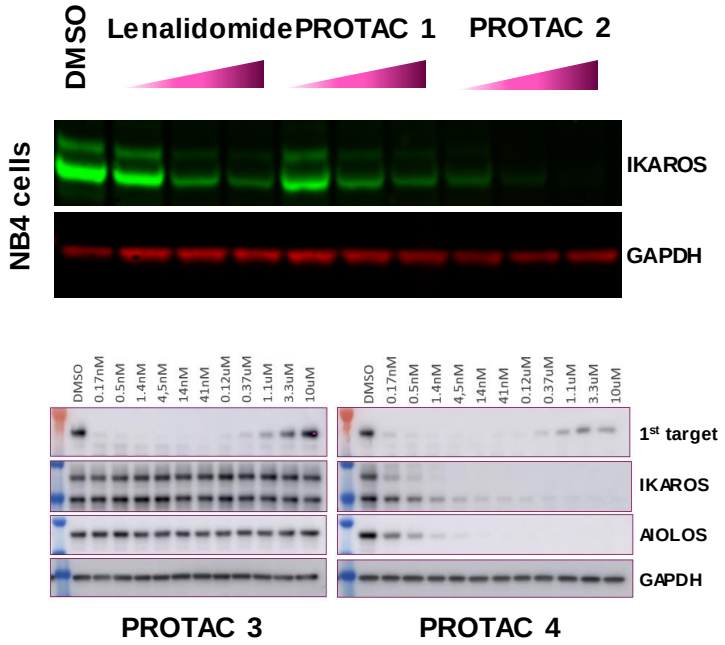
- Most of CRBN-PROTAC tested do not degrade IKAROS
- But some do and can be very potent

Published CRBN-PROTAC can degrade IKAROS

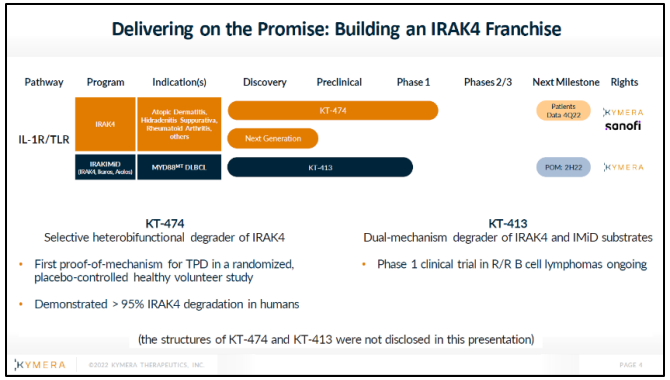


IKAROS degradation (fold change vs vehicle)

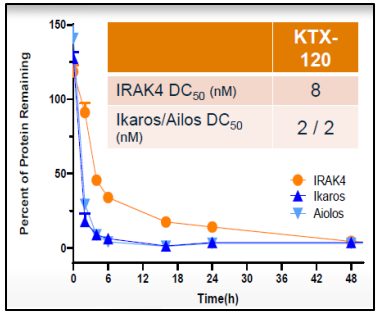
Internal CRBN-PROTAC can degrade IKAROS



Some clinical CRBN-PROTAC intend to degrade IKAROS



[Therapeutic Pipeline - Kymera Therapeutics (kymeratx.com)]

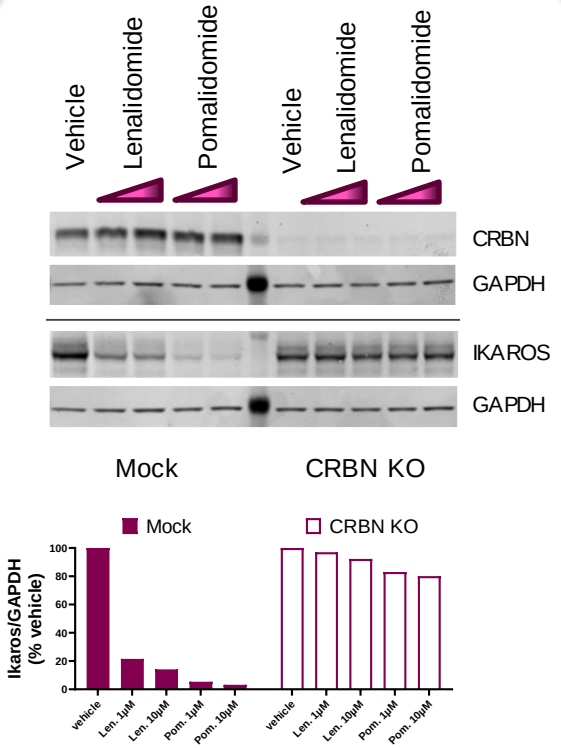


Conclusion

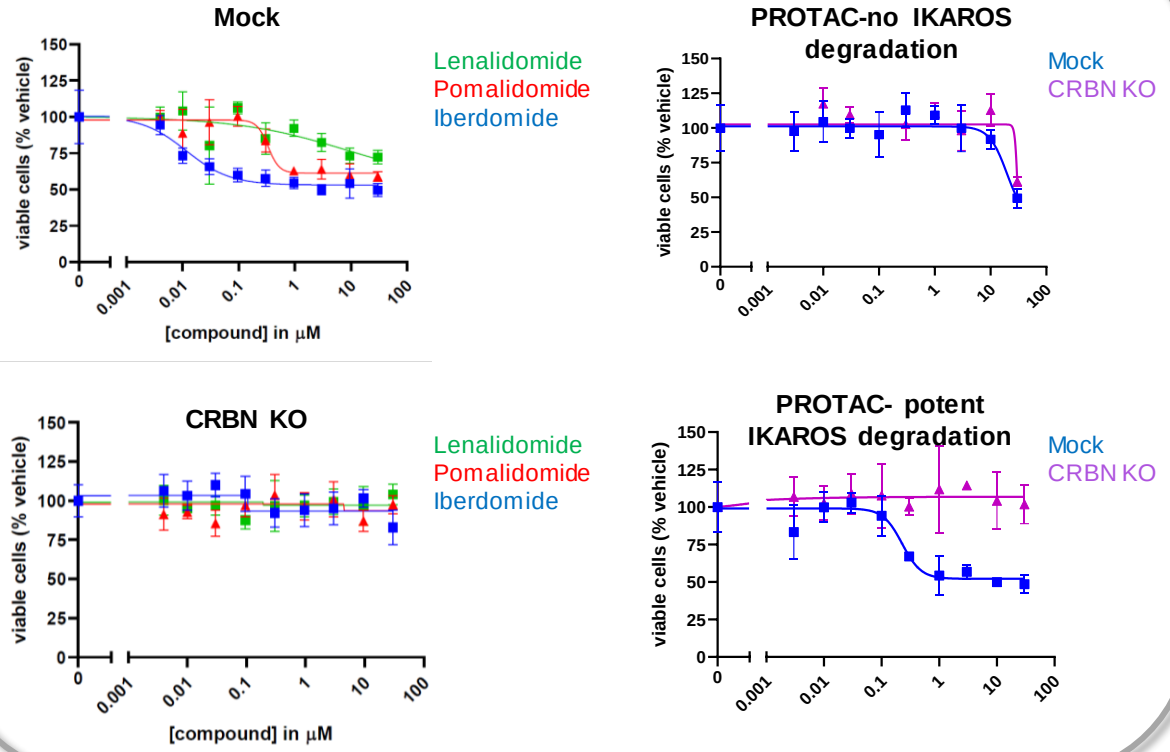
CRBN-PROTAC can degrade IKAROS/AIOLOS. What are the consequences on IMiD induced hemetox?

Evidence of IKAROS driven bone-marrow toxicity in human hematopoietic stem and progenitor cells (HSPC)

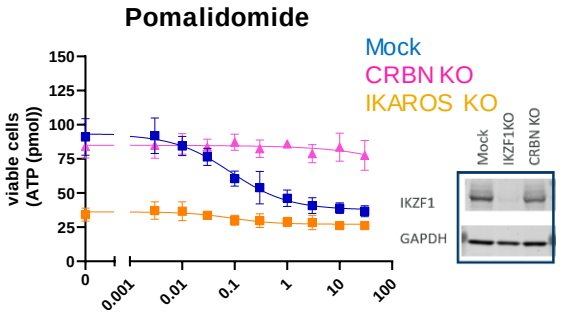
IMiD induced IKAROS degradation through CRBN



IMiD induced HSPC proliferation arrest through IKAROS degradation



IKAROS KO affects HSPC proliferation



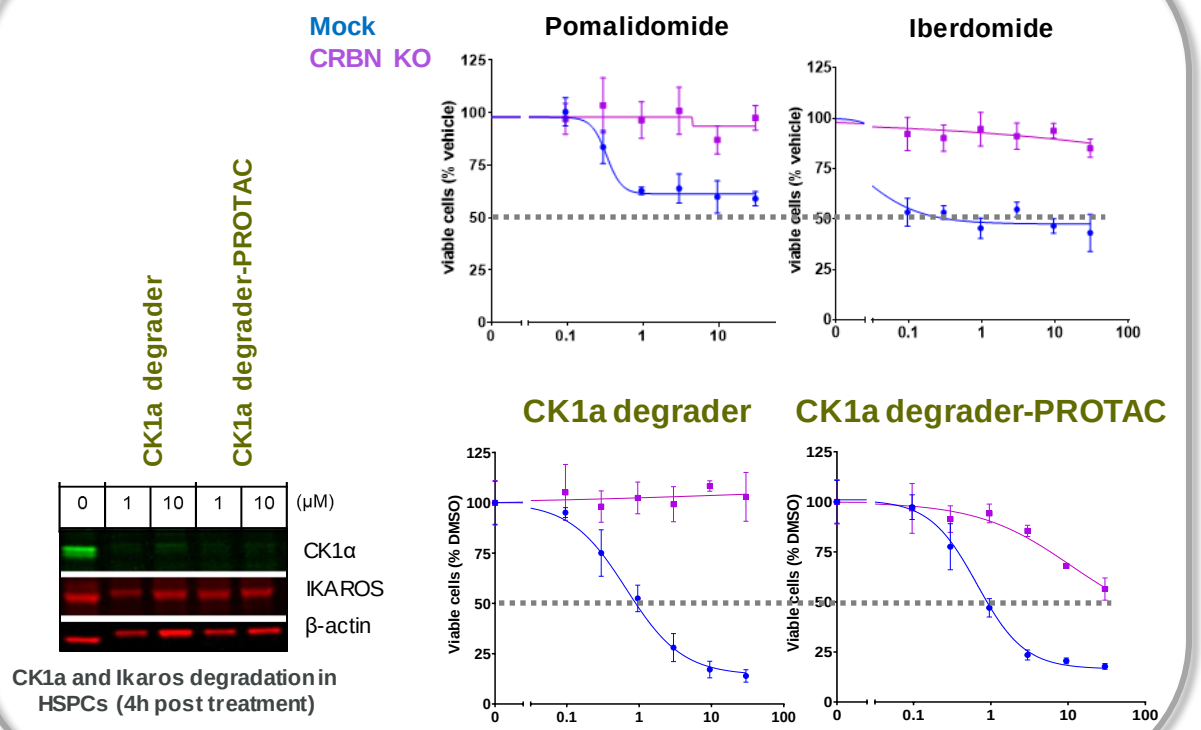
Cell Viability	HSPC
Vehicle	94.7%
Iberdomide	92.1%
Pomalidomide	93.9%
IKAROS KO	91.5%

Conclusion

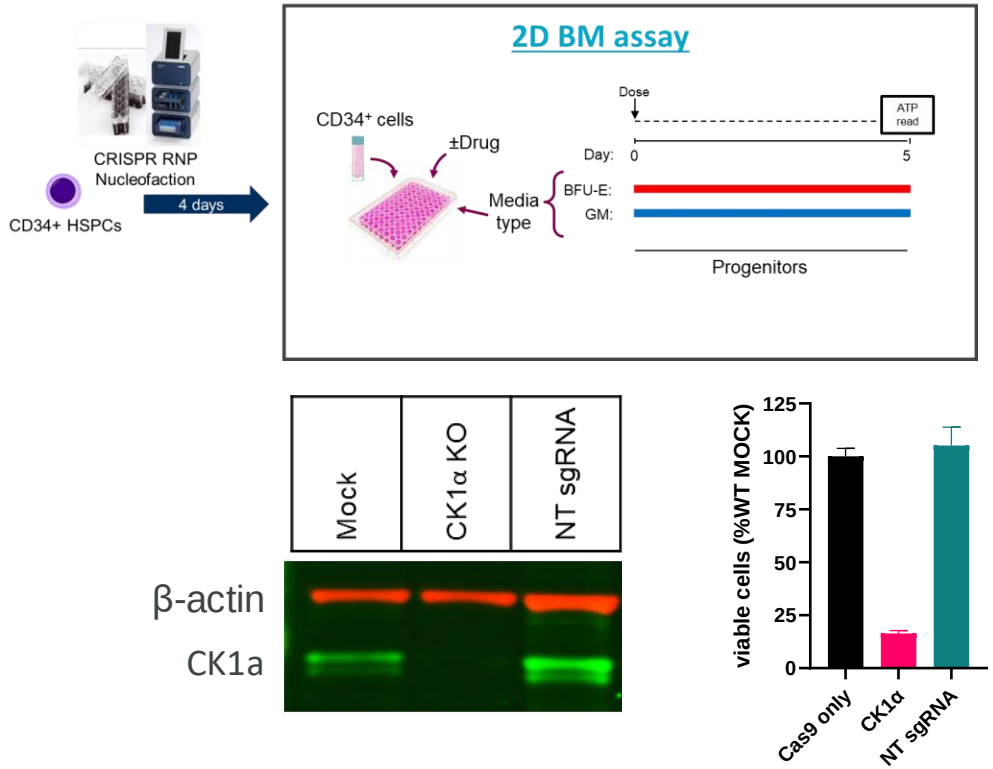
IKAROS degradation leads to reduction in hematopoietic stem and progenitor cells proliferation

Evidence of CK1a driven bone-marrow toxicity in human hematopoietic stem and progenitor cells (HSPC)

CK1a degradation reduces HSPC survival



CK1a CRISPR KO reduces HSPC survival

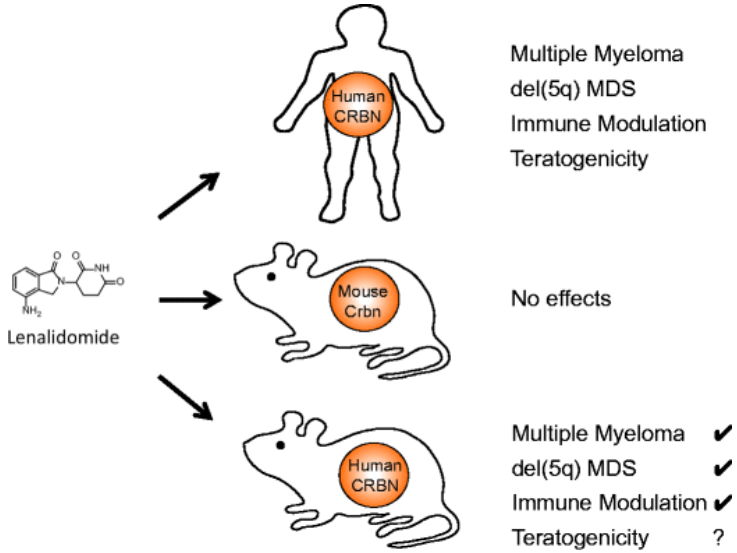


Conclusion

CK1 degradation leads to hematopoietic stem and progenitor cells cytotoxicity

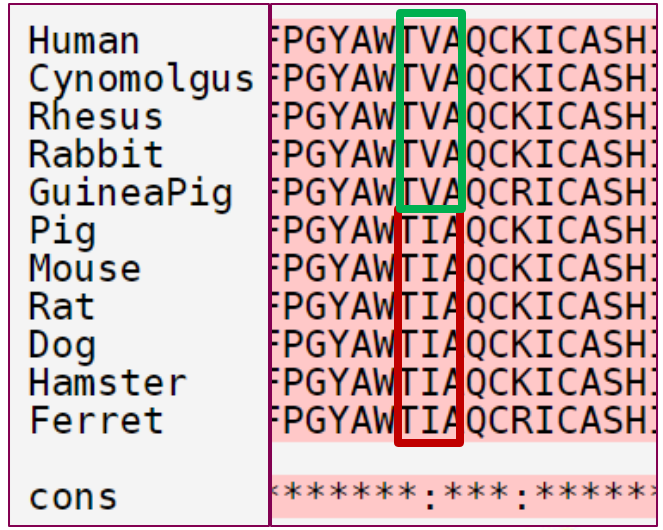
CRBN polymorphism impacts IMiD-induced hemetox studies

- **Thalidomide teratogenicity is species specific**
  - Passed safety tests in mice but proved teratogenic in human
  - CRBN polymorphism (aa391) explains IMiD species sensitivity
  - In human it induces degradation of SALL4, Ikaros (but not mouse)



Lindner and Kronke, J Mol Med 2016

- **In vivo tox studies may under-estimate protein degradation and toxicity**
  - Un/anticipated off-target risk may be difficult to discover in rat or dog
  - Guinea pig, rabbit, and NHP are conserved at aa391
  - Potential to use CRBN transgenic mice



CRBN polymorphism at aa391

Lindner and Kronke, J Mol Med 2016

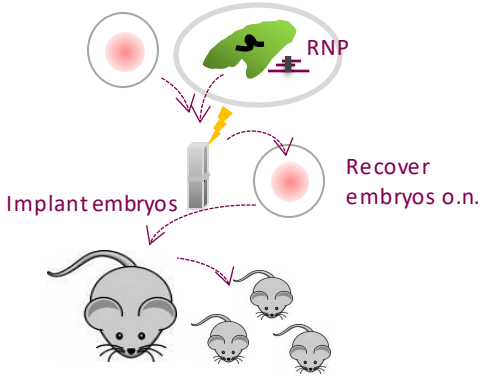
Conclusion

CRBN-PROTAC safety would need to be tested in IMiD sensitive species or engineered models to assess IMiD-induced toxicities

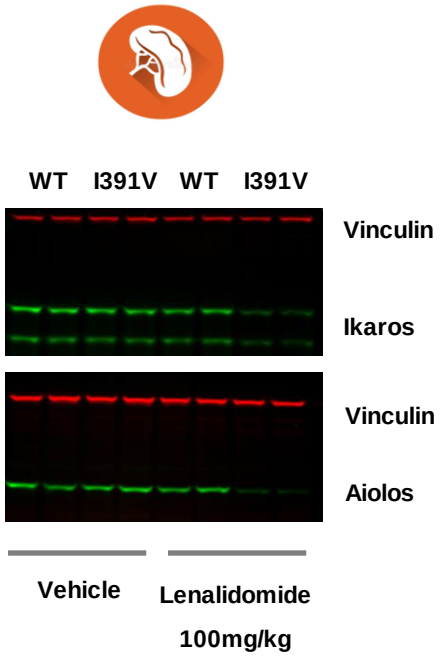
CRBN I391V Tg mice for IMiD-PROTAC hematotoxicity risk

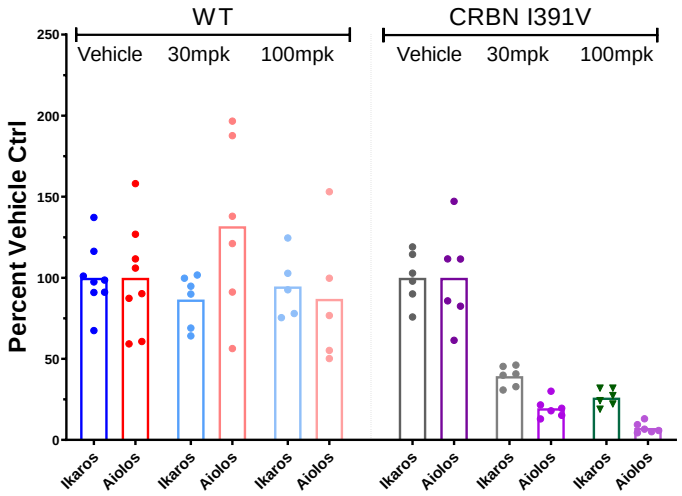
- "Humanized" mice (I391V) degrade Ikaros (not Sall4) and replicate some levels of toxicities including bone-marrow toxicity (Fink et al. 2018)
- We have developed our own model using a CRISPR knock-in strategy

I391V CRBN transgenic generation

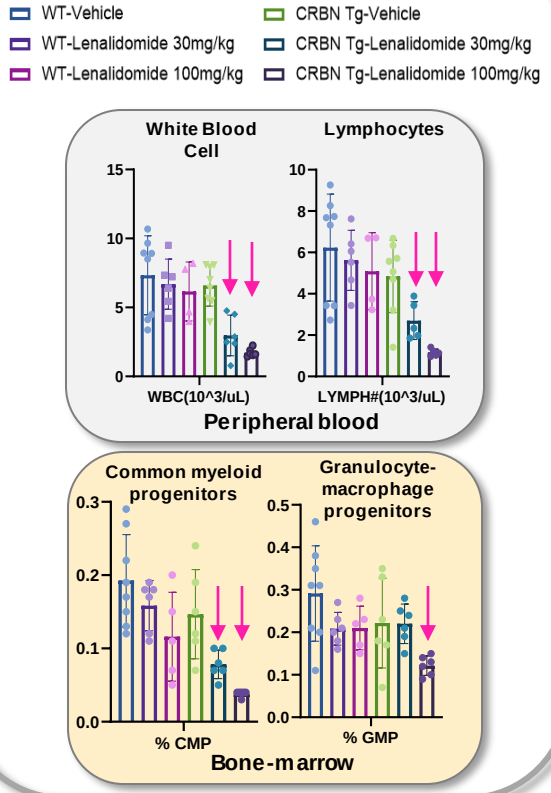


IMiD induced Ikaros/Aiolos degradation in I391V CRBN transgenic mice





Neutropenia seen after 21 days repeat dosing

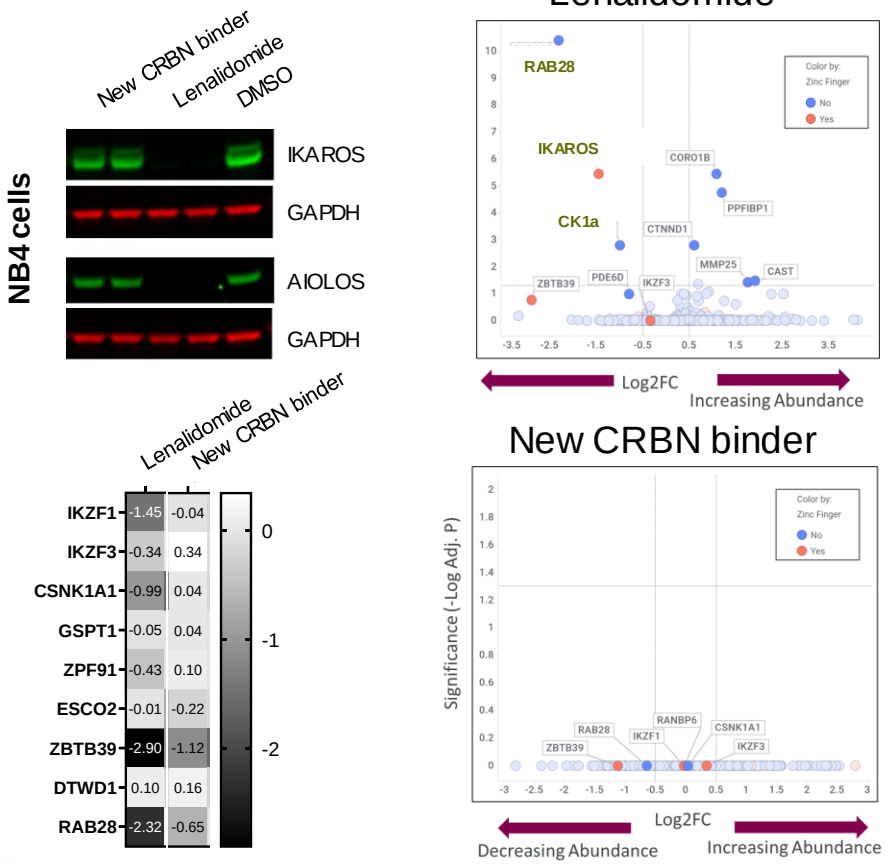


Conclusion

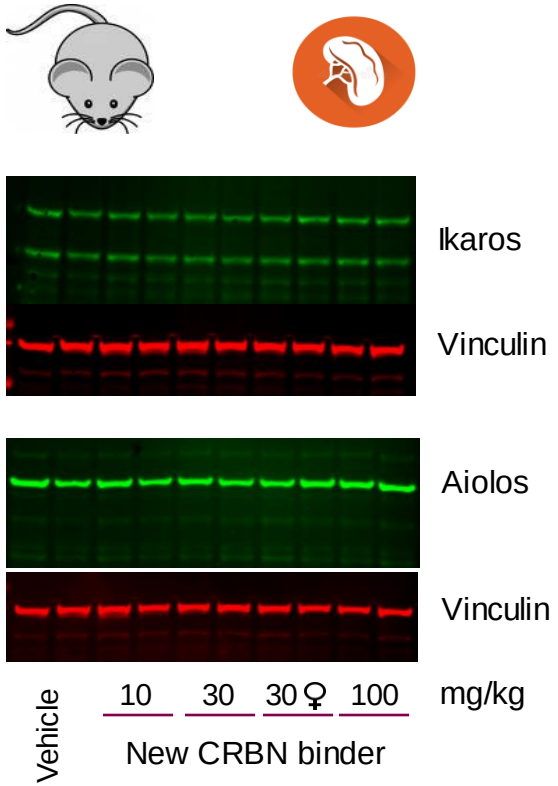
IMiD induced hemetoxicity recapitulated in CRBN I391V mice

New CRBN binder reduces IMiD-neosubstrates degradation

New CRBN binder reduces IMiD neosubstrates degradation in NB4 cells treated for 24h



New CRBN binder showed no Ikaros/Aiolos degradation in I391V CRBN mice



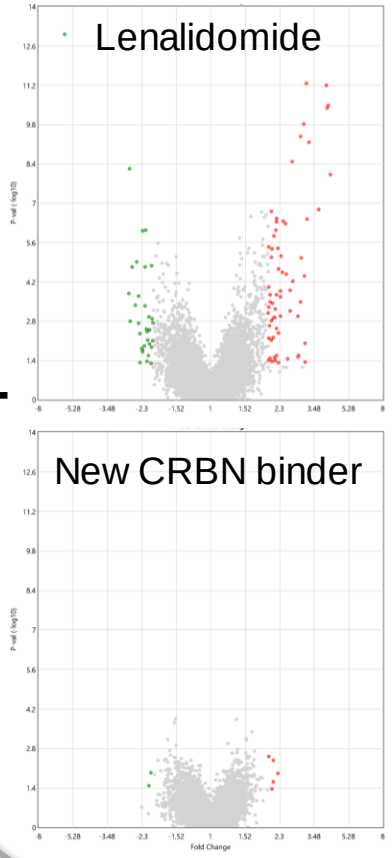
Conclusion

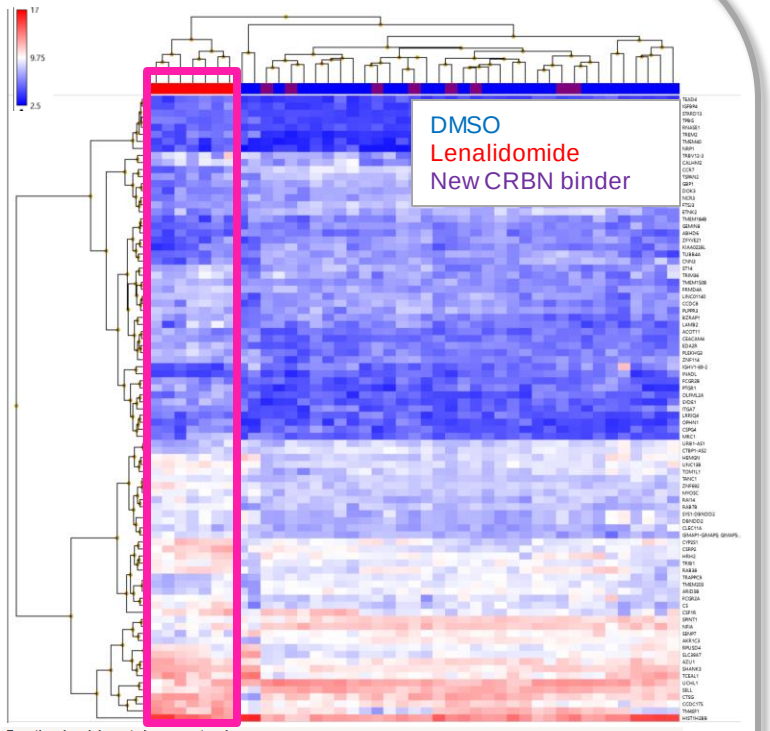
No neosubstrates degradation seen in vitro and in vivo with a new CRBN binder

New CRBN binder mitigates IMiD-induced hemetox

IMiD induced a transcriptional change in HSPC after 12h treatment not seen with the new CRBN binder

Transcriptome





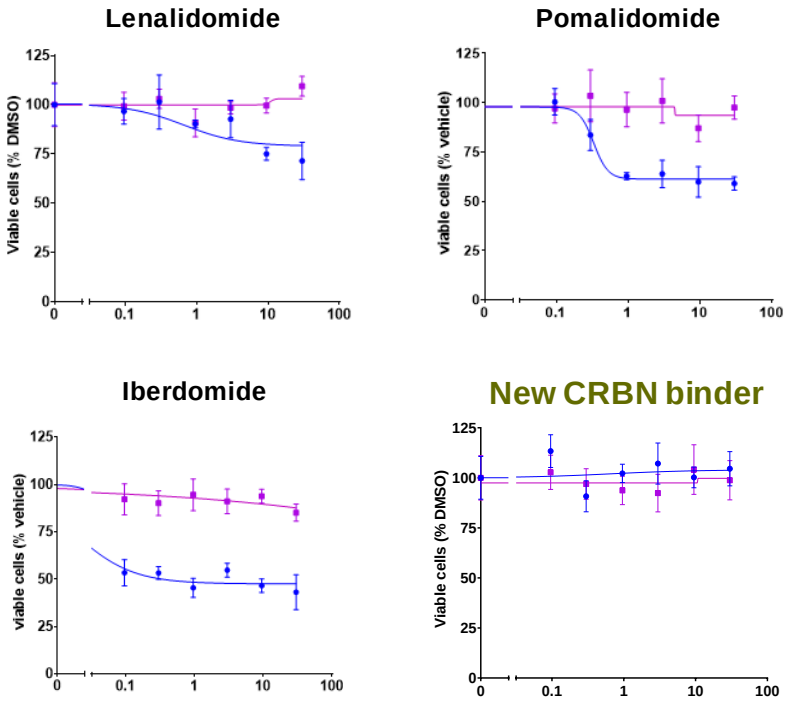
Functional enrichments in your network

explain columns

Biological Process (Gene Ontology)				
GO-term	description	count in network	strength	false discovery rate
GO:0002605	negative regulation of dendritic cell antigen processing and p…	2 of 2	2.36	0.0056
GO:0097029	mature conventional dendritic cell differentiation	2 of 4	2.06	0.0103
GO:0002580	regulation of antigen processing and presentation of peptide …	2 of 5	1.96	0.0133
GO:0002604	regulation of dendritic cell antigen processing and presentation	3 of 9	1.88	0.0020

New CRBN binder has no effect on HSPC proliferation after 5 days

Mock
CRBN KO



Conclusion

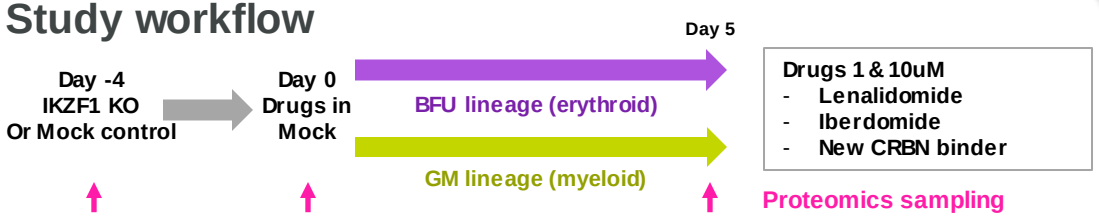
No transcriptional changes and no proliferation effect seen in HSPC with a new CRBN binder

New CRBN binder mitigates IMiD-induced hemetox

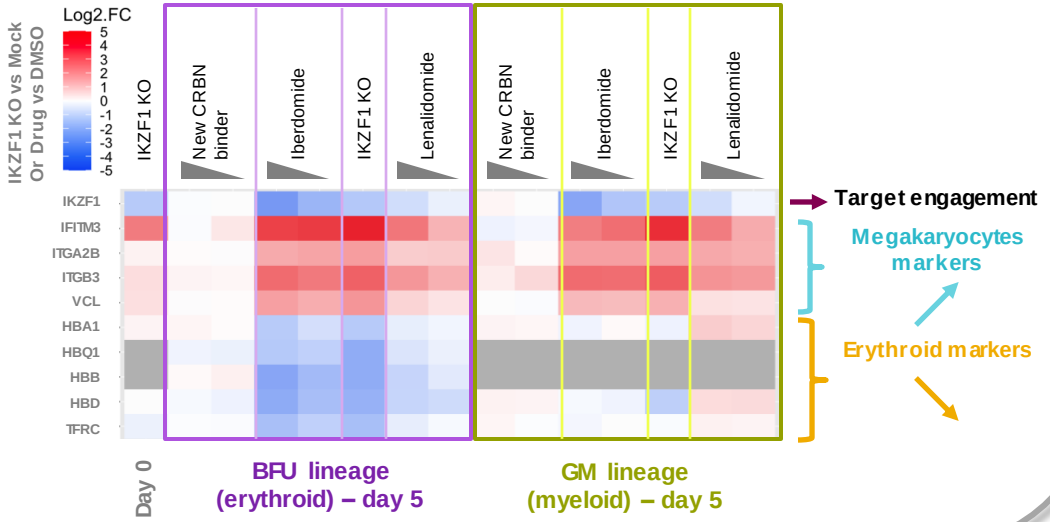
IMiD induced a proteome rewiring in HSPC not seen with the new CRBN binder

Fast Proteomics

Study workflow

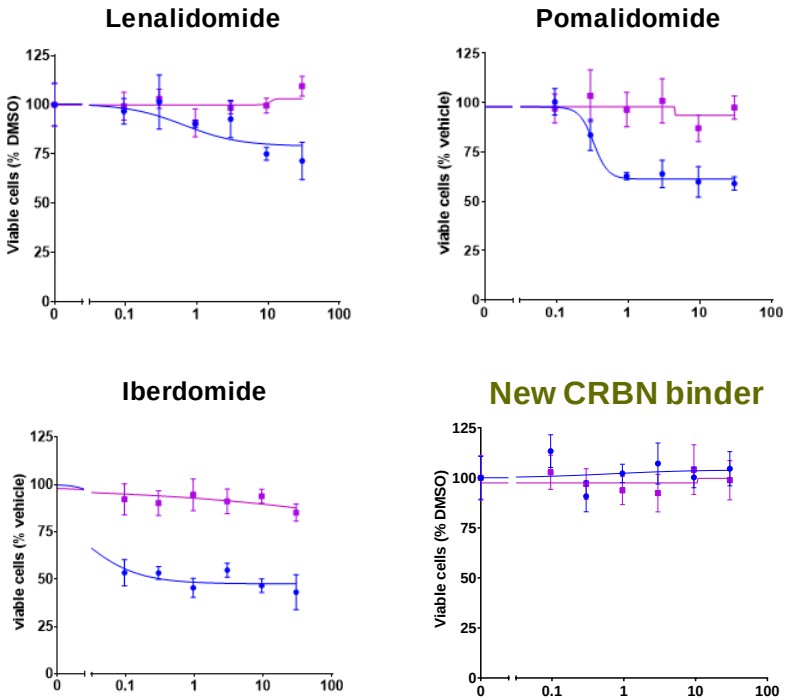


Focused analysis on key up/down regulated proteins



New CRBN binder has no effect on HSPC proliferation after 5 days

Mock
CRBN KO



Conclusion

No proteome rewiring and no proliferation effect seen in HSPC with a new CRBN binder

Key learning

"Every drug has two actions – the one you know about, and the one you don't"

Sir John Gaddum 1900-1965

Acknowledgements

AstraZeneca

Clinical Pharmacology and Safety Sciences:

- Safety Innovation and PROTAC Safety: Monica Rodrigo & Sophie Reagan
- Oncology Safety: Frida Gustafsson, Suzanne Randal & Jasper Komen, Clare Hoover
- Animal Sciences and Technologies: Jonathan Rose, Ian Purvis, Ruth Macdonald
- Integrated Bioanalysis: Emilyanne Leonard, Jayati Basak
- Imaging and Data Analytics: Marianna Trapotsi

Discovery Sciences

- Discovery Biology: Fiona Pachl, Andrew Zhang, Ghaith Hamza, Eric Miele, Katja Madeyski-Bengtson, Johan Forsström, Anna-Lena Loyd
- Hit Discovery: Iacovos Michaelides, Guy Williams, Elizabeth Mouchet, Ian Storer