Non-Clinical Safety Considerations when Developing Targeted Protein Degraders

May 2022

Proteome Editing is the New Frontier of Medicine

Genome

- Essentially **static**
- Alterations are responsible for **some** diseases
- Editing is **irreversible**

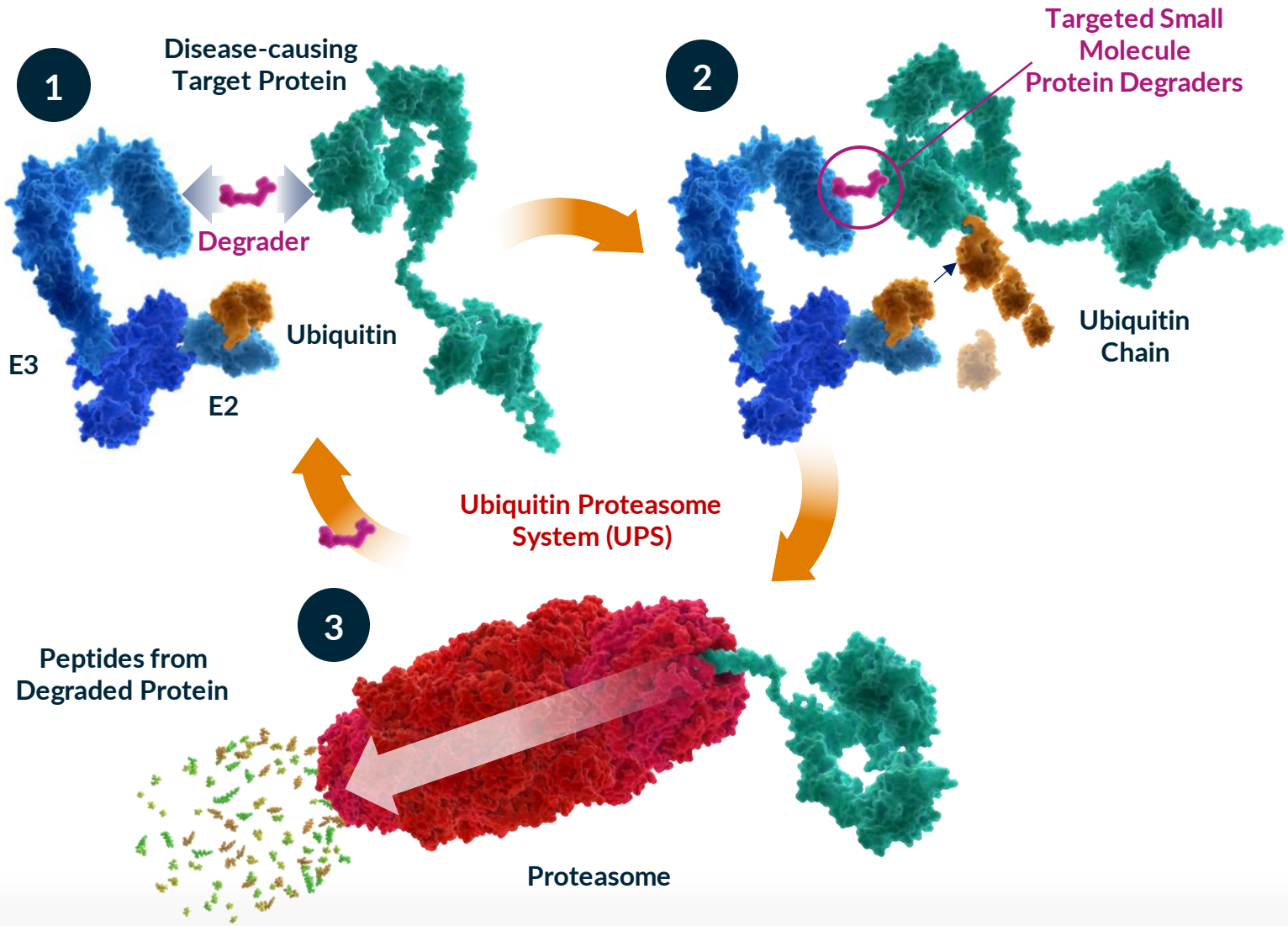
Encodes

Proteome

- **Changes** based on internal (genetic) and external (epigenetic) events
- Alterations are responsible for **all** diseases
- Editing is **reversible**

Proteome Editing with Targeted Protein Degradation

A Nobel Prize (2004) Inspired Technology



Expanded Opportunities

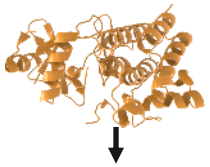
- Small molecule binds to **E3 and target protein to affect its degradation**
- Small Molecule only needs to "weakly" bind to protein: **not inhibit its function**
- Highly potent/catalytic: **Small amount of drug needed**
- Highly specific
- Genetic–like knock-down effects
- Advantage of small molecule development: **Route of administration, manufacturing**
- Agnostic to protein type and disease

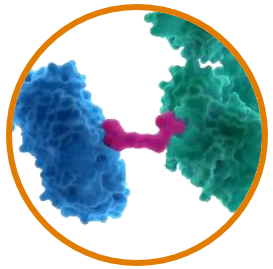
Expanding the Druggable Proteome with TPD

ID Inadequately Drugged Targets with Clear Degrader Advantage

Small molecule binders exist but unable to drug target fully

e.g. IRAK4, MDM2…



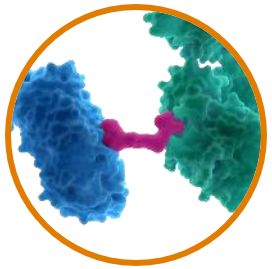


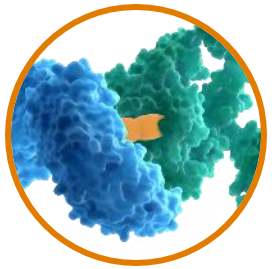
Heterobifunctional Degraders

UD Undrugged Targets

No other technology can drug

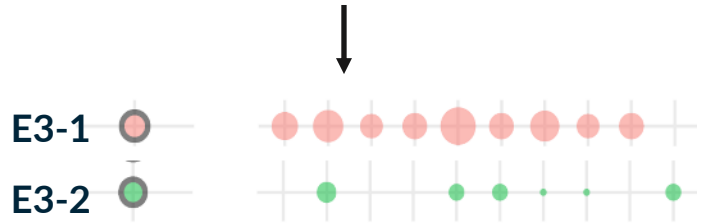
Ligandable Proteins	Un-ligandable Proteins
e.g. STAT3…	e.g. other transcription factors
Heterobifunctional Degraders	Molecular Glues





TR Clinically Validated Targets Enabled by E3 Ligase Tissue Restricted Expression

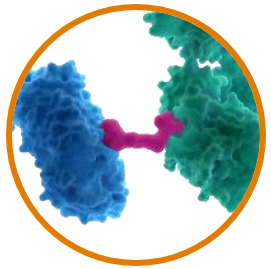
On target unwanted pharmacology limits clinical application

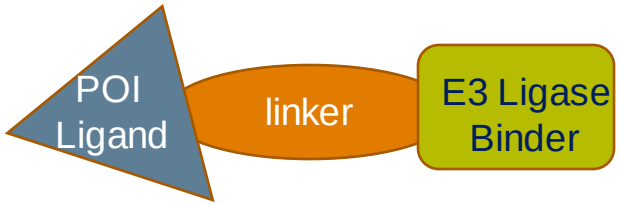


Tissue sparing or selective E3 ligases eliminate unwanted toxicity and allow full clinical potential

Common Approaches For Designing TPDs

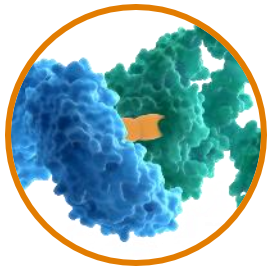
Heterobifunctional Degraders



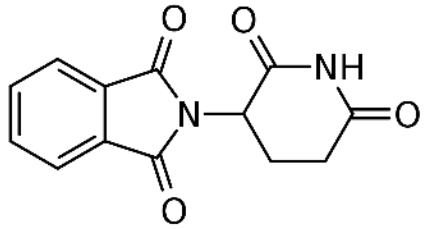


- SM molecules w/ ligand for POI, linker region, and ligand for E3 ligase
- Rationally designed molecules that create ternary complex formation between protein of interest and an E3 ligase leading to degradation

Molecular Glues

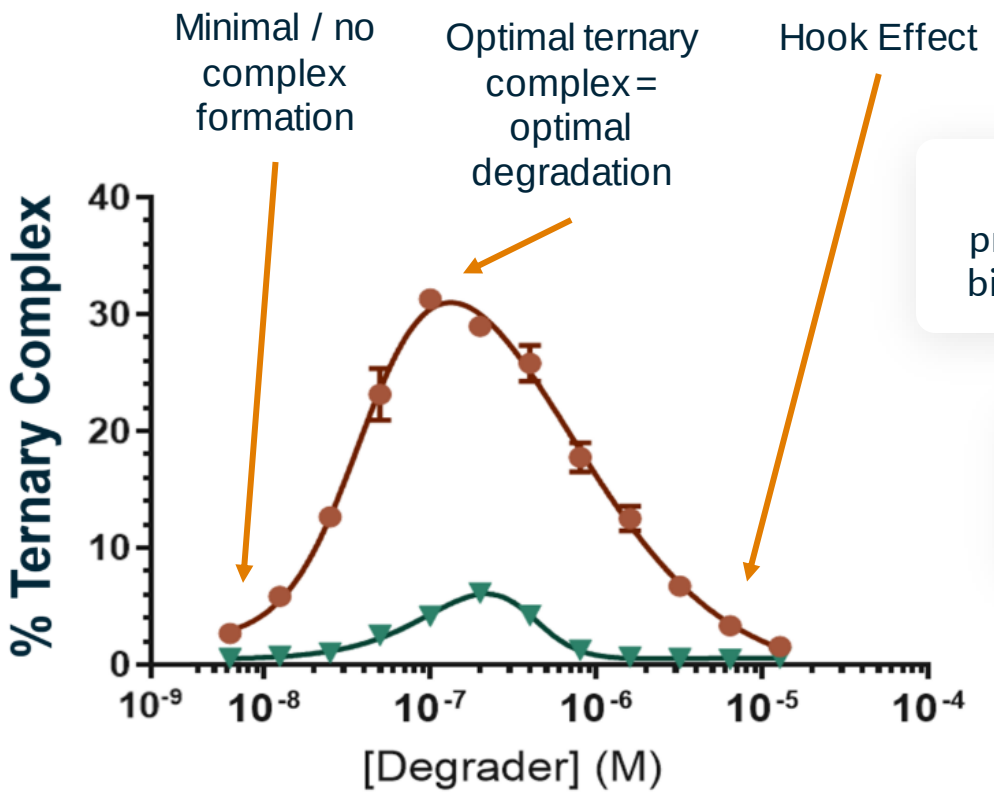


IMiDs (e.g.Thalidomide)



- SMs that bind to natural protein-protein interaction sites between a POI and E3 ligase
- Often discovered serendipitously

Mechanism of Action Requires Efficient Ternary Complex Formation



Hook-Effect - predominance of binary complexes

Degrader A: DC_{50}~10nM

Degrader B: DC_{50}>1000nM

Quantitation of Ternary Complex by AlphaLISA

- Efficiency of ternary complex formation drives potency – reason for high selectivity observed for TPDs
- Loss of degrader activity at high concentrations needs to be considered for interpretation of toxicology studies (e.g., off-target profiling, in vivo findings…)

Heterobifunctional Degraders

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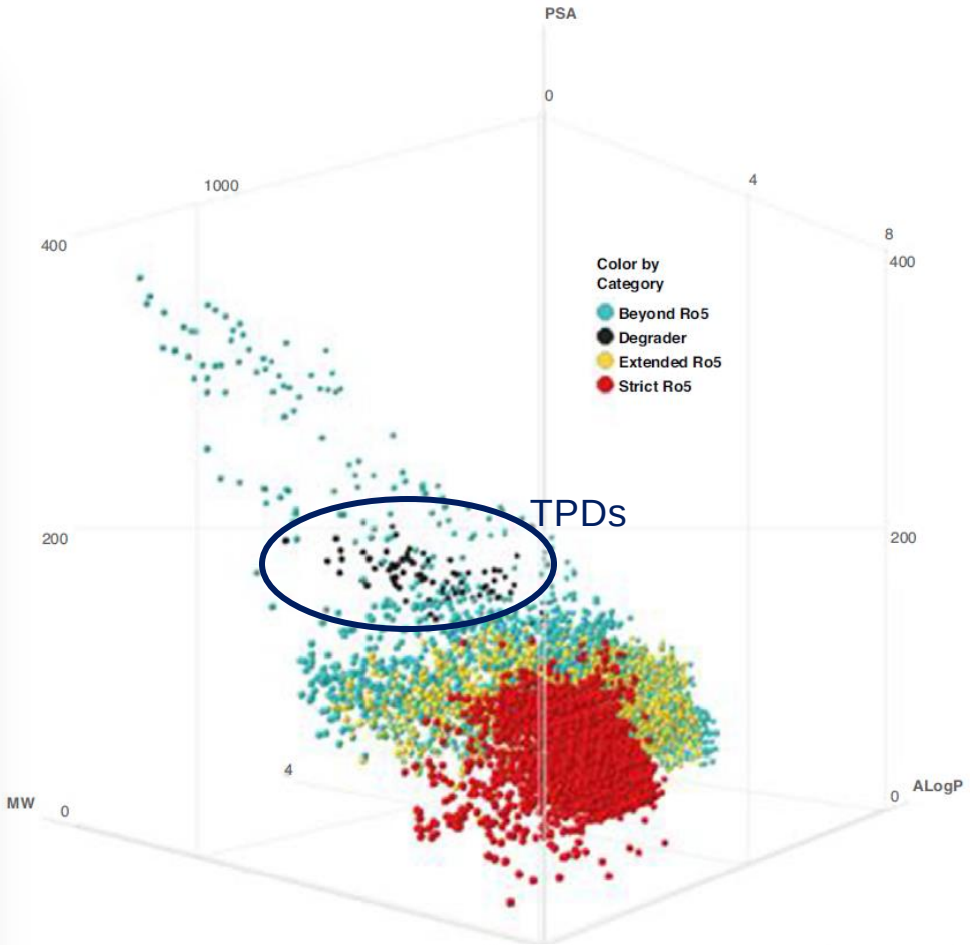
Heterobifunctional Degraders Often Exist in Unique Chemical Space

- High MW / lipophilicity can lead to challenges for oral absorption
- Non-specific binding of compounds to plastic/labware is possible
- Intrinsic solubility can be limiting for both in vitro and in vivo studies
  - In vitro screening data can be misleading
  - "Formulatability" can be difficult
- High plasma binding and significant tissue partitioning (Kp) may be observed

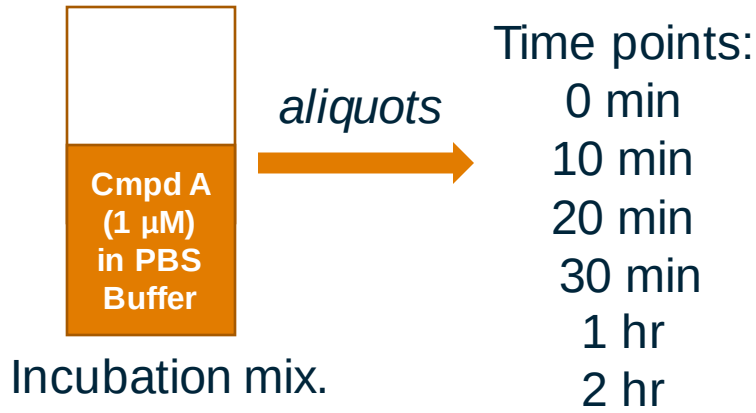


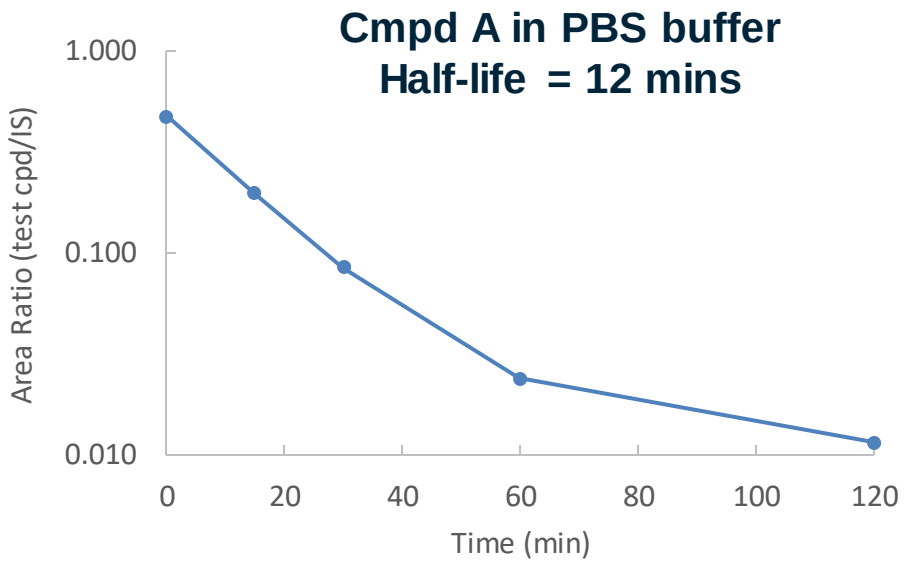
Compound class	MW	cLogP	HBD	HBA	*N*
Strict Ro5	300-500	≤5	≤5	≤10	5000
Extended Ro5	500-700	0-7.5	≤5	≤10	5000
Beyond Ro5	>500	All	All	All	5000
Degraders	All	All	All	All	77

Adapted from: Cantrill, C. et al. *Drug Discov Today*. 2020 Jun;25(6):969 982

In Vitro Study Considerations that Can Impact Data Interpretation

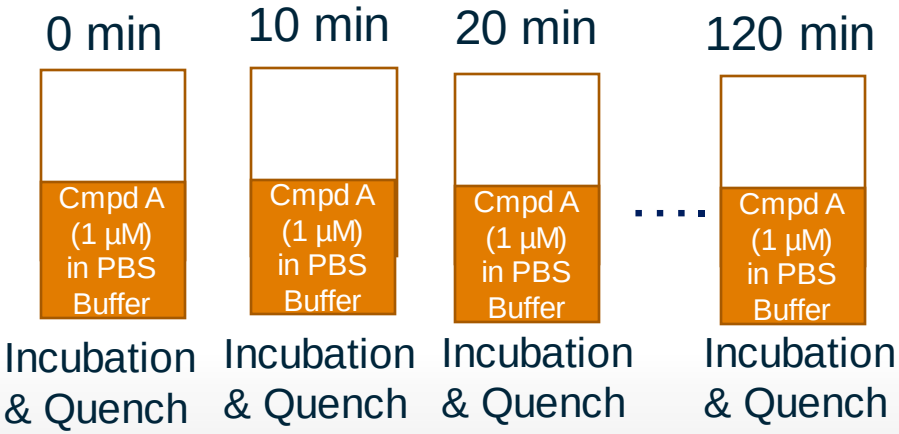
- **Conventional approach: sequential sampling**

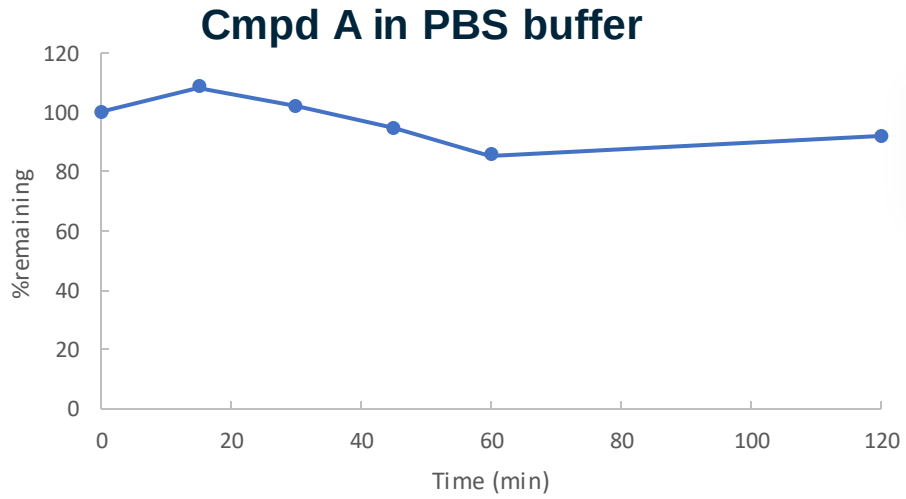




Chemical instability?

- **Parallel incubation and sampling**

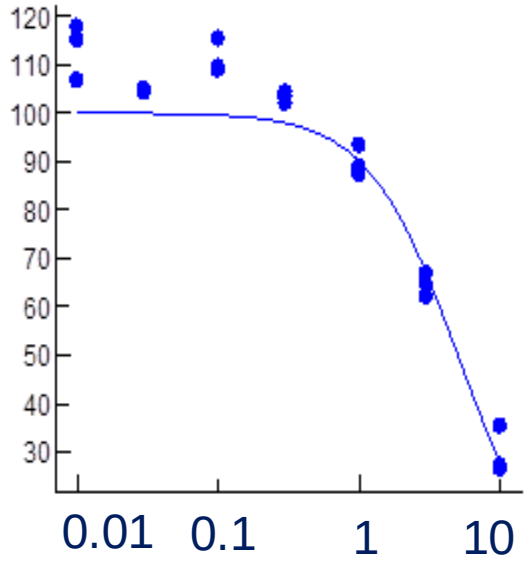




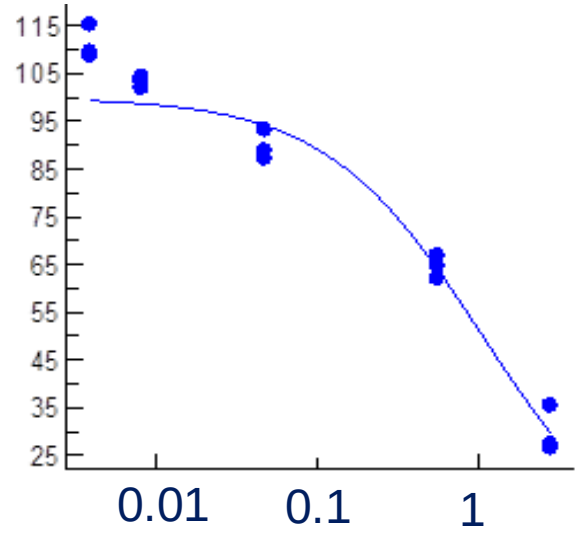
Stable

Nominal and Measured IC_{50} Values – Impact on Screening Data vs. Definitive In Vitro Studies

Nominal IC_{50}: 5 µM



Measured IC_{50}: 1 µM

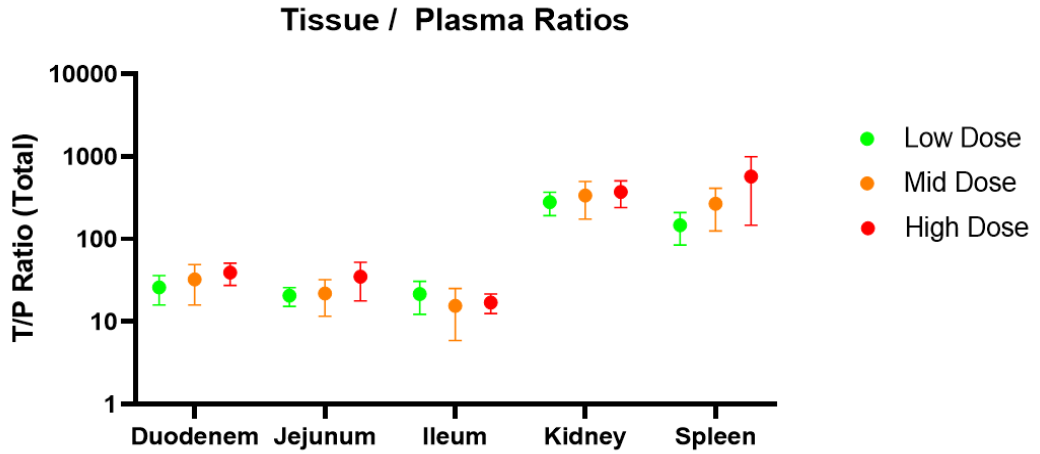


- Need for determining / estimating in vitro solubility and nonspecific binding/adsorption
  - Impacts are broad and can affect 2ndary pharmacology, cytotoxicity, PPB, and other in vitro assays
  - Evaluating compound recovery from in vitro systems may be needed
  - Added cost and complexity to screening approaches – balance between speed vs. accuracy

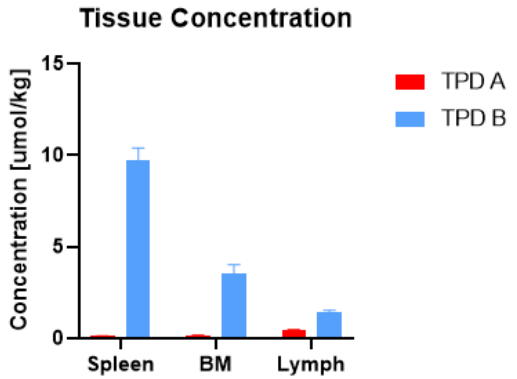
High Tissue Partitioning Can be Observed with Heterobifunctional Degraders

- High tissue to plasma ratios can be observed
- Important to context relevance in relation free-drug levels (e.g., Kp,u,u)
- Can be beneficial for PD/activity, prolonged terminal $T_{1/2}$, …

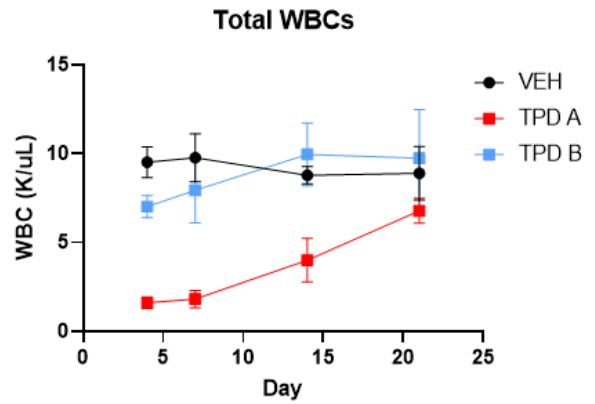
- Tissue levels do not always correlate w/ increase safety risk / toxicity
  - Accessibility to target/site of action could be limited due to increased binding or organelle sequestration (e.g., lysosomotropism)
- Species differences in tissue distribution can be observed further confounding interpretation



Tissue to Plasma (T/P) ratios for select tissues 24h post-single dose



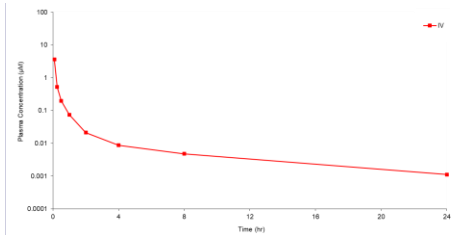
Tissue concentrations in lymph organs/BM for equipotent TPD A & B in rats 24h post dose



WBC changes following single dose of TPD A and TPD B in rats

Factors that Determine Relevance of Preclinical Species for TPD Safety Assessment

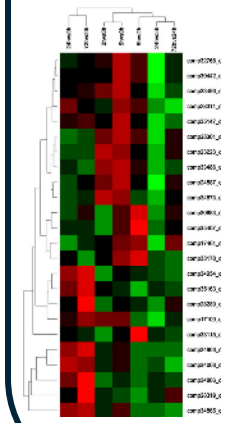
1. Exposure



Select species that can achieve sufficient exposure to characterize on- and off-target pharmacology

- Solubility / absorption
- cross-species potency differences

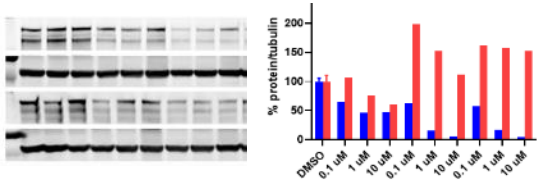
2. Target Expression



Select species where the **POI** & **E3 ligase** are expressed in the same cells / tissues / compartments as humans

- Species differences in E3 ligase activity
- Expanding to novel E3 ligases with restricted expression potential to increase complexity

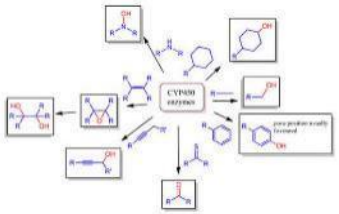
3. Pharmacology



Complete target degradation (>90%) and exposure multiples beyond that in at least one non-clinical species

- Ternary complex formation critical for PD / activity
- In vitro degradation values (e.g., DC_{50}s) can be highly context dependent

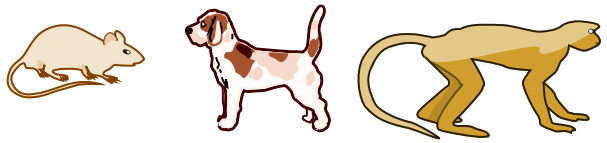
4. Metabolism



Metabolite coverage in preclinical species: need to cover major (>10%) human c irculating metabolites non-clinically (MIST)

In Vitro Cross-Species Reactivity / Pharmacology Assessments

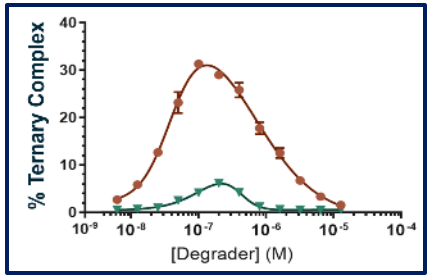
- **Overall Goal:** Complete target degradation (>90%) in at least one non-clinical species and exposure multiples above that in general toxicology studies

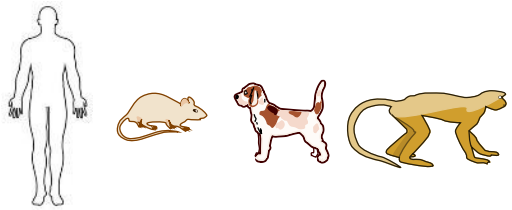


- **In practice:** need to identify species early on (e.g., during early lead-optimization stages) using predominantly in vitro data
  - Need to demonstrate **comparable** degradation in vitro across species that supports potential to achieve complete degradation in vivo
    - Primary cells (hepatocytes, PBMCs, splenocytes, …) preferred over cell lines, but cell availability, donor variability, assay drift, and other technical issues may present challenges for this approach
  - Difficult to pivot quickly due to long and unpredictable study lead-times and shortages of non-rodent animals (e.g., NHPs)
  - Depending on exposures that are achievable in a tox species, it may (or may not) be possible to overcome some species differences with DC_{50} values

Example: Significant Cross-Species Potency Shifts Between Lead-like Molecules

- Efficient ternary complex formation required for pharmacologic activity (e.g., degradation)
- Subtle changes across species with POI and/or E3 ligase structures/homology may lead to significant impacts on formation of ternary complexes
- Subtle changes to structures of TPDs can lead to significant cross-species changes in potency/degradation





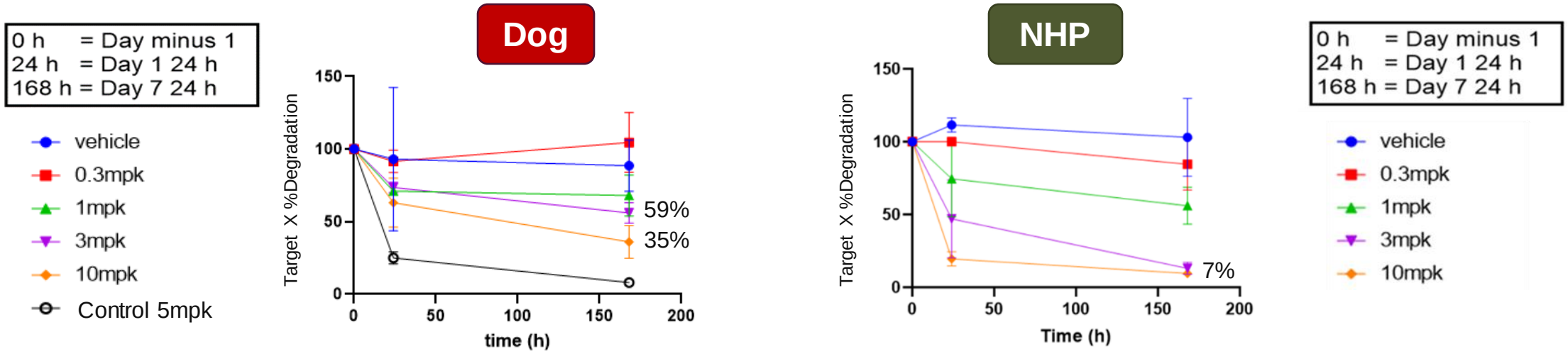
Example: In-Vitro Cross-Species activities for two lead-like TPDs with equivalent potency in human cells

Compound	Human	NHP	Dog		Mouse		Rat
	PBMC DC50 (nM)	PBMC DC50 (nM)	MDCKII DC50 (nM)	PBMC DC50 (nM)	RAW264.7 DC50 (nM)	Splenocytes DC50 (nM)	Splenocytes (DC50) (nM)
TPD 1	1.4	1.2	7.2	9.8	81 ± 27	4.2	2.5
TPD 2	0.8	0.3	415.3 ± 258.2	N/A	597 ± 187	8.9 (71%)	>500

- Subtle structural changes caused shift in canine and rodent potency between TPD 1 and TPD 2
- Differences between murine RAW264 and mouse splenocytes highlight context dependence of DC50 values

Importance of Assessing Supra-Pharmacologic Doses (> In Vivo DC_{90}) Can Impact Species Selection

Species Cross-Reactivity – In Vivo



Target degradation was assessed in PBMCs isolated from animals treated with for 7d TPD 2 at varying doses. Control to context achievable in vivo depth of degradation in dogs.

TPD 2 demonstrated significant potency shift in vivo in dogs relative to NHPs

- Approximately **10x** shift observed in vivo in comparison to **60x** shift in vitro (e.g., MDCK)
- Complete degradation (e.g., >90%) was not achieved in dogs w/ TPD 2 up to 10mpk
- Ability to cover complete degradation in vivo as well exposure multiples above not possible in dogs
- NHP was selected as non-rodent species for TPD 2

Off-Target Assessment for TPD

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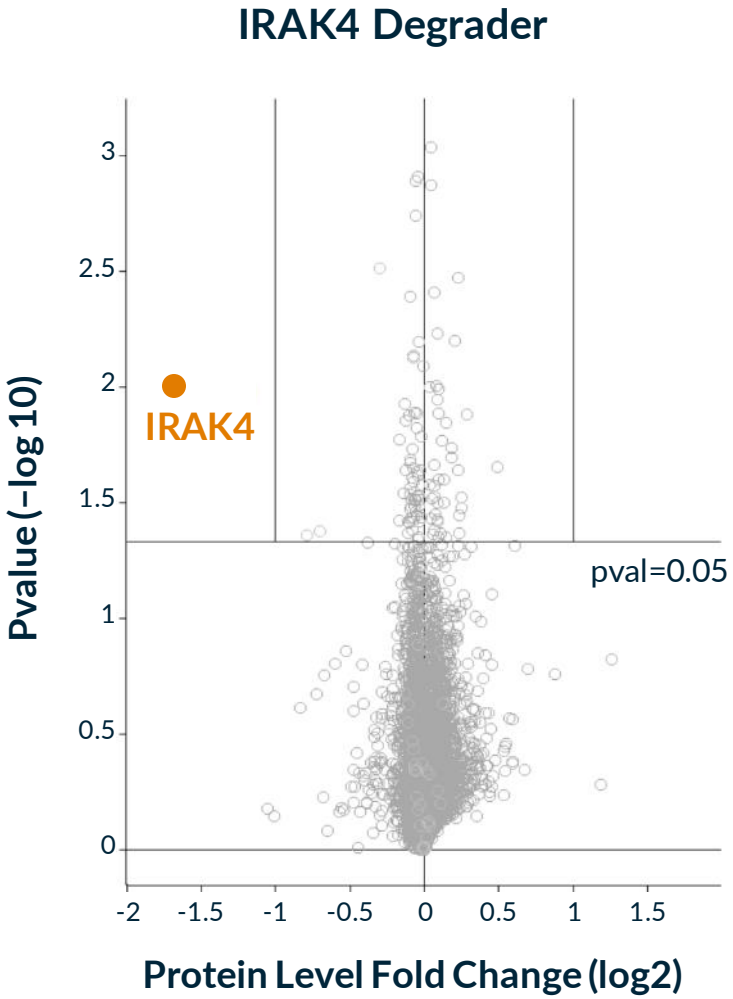
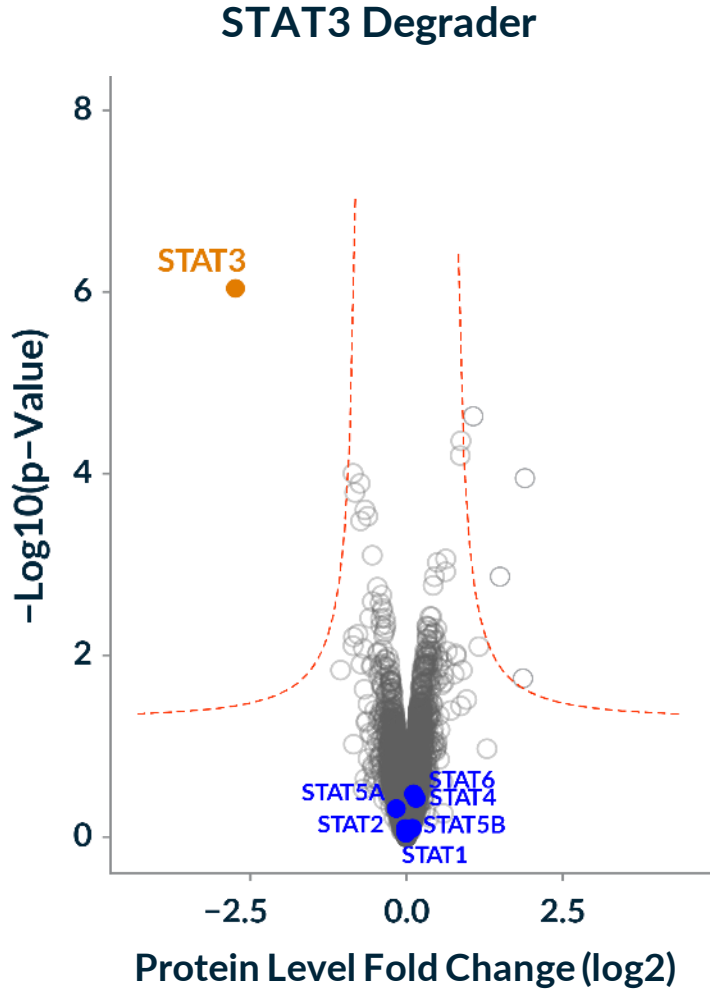
Advantage of Heterobifunctional TPD is the High Degree of Selectivity that Can Be Achieved

- Global proteomic approaches offer unbiased view into on- and off-target pharmacology of TPD
- Compounds assessed for off-target effects at 10x $DC_{90/95}$ concentrations
  - Concentrations selected at highest level w/o potential for hook effect
- Kymera degraders were shown to be highly selective degraders of their respective targets by global proteomics



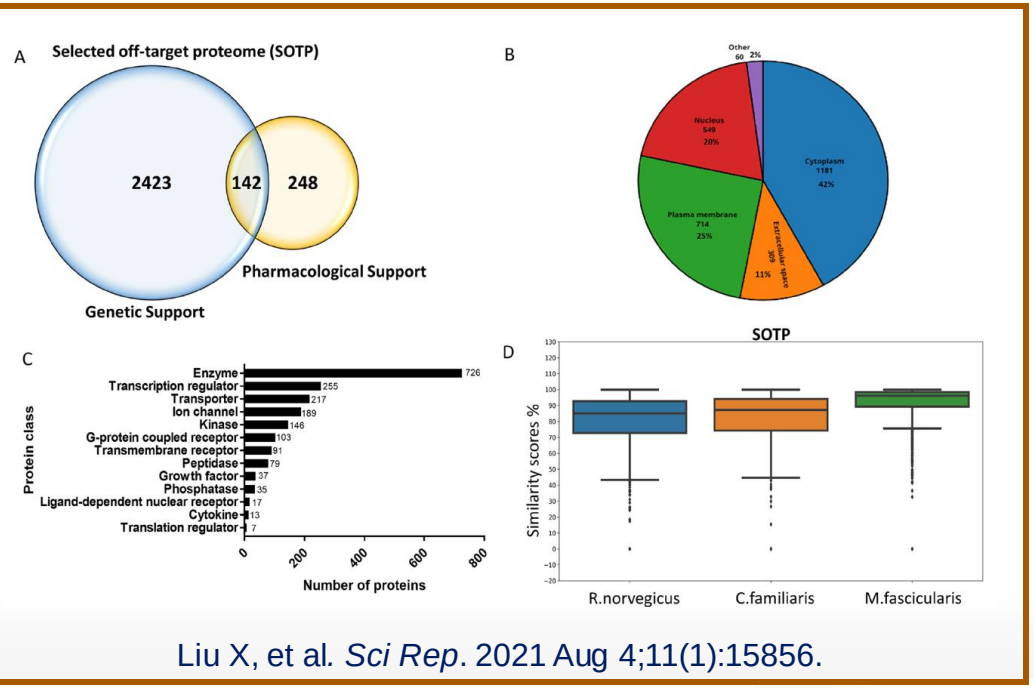
Volcano plot of global proteomics from human immune cells at 10× DC_{90} at 24h



Volcano plot of global proteomics from SU-DHL-1 cells at 10× DC_{95} at 8h

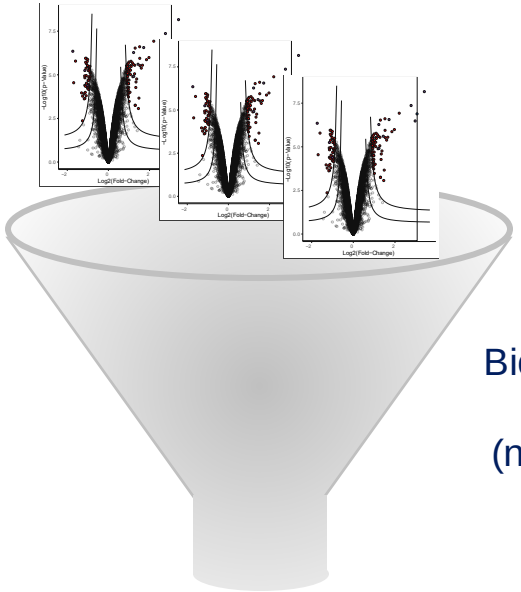
What degree of Off-Target Proteome Coverage is Necessary for Safety Assessment of TPD?

- There is a need to comprehensively assess off-targets of TPD due to catalytic nature, potency, and drivers for reversibility of off-target effects (e.g., protein re-synthesis)
  - Departure from traditional SM approaches that focus on only a subset of important targets for safety assessment
  - Concentrations, cell models, and incubation times all can impact protein changes that may be observed
  - Protein changes does not inform on functional consequence and requires follow-up approaches (in vitro / in vivo) to properly context and interpret



Liu X, et al. *Sci Rep*. 2021 Aug 4;11(1):15856.

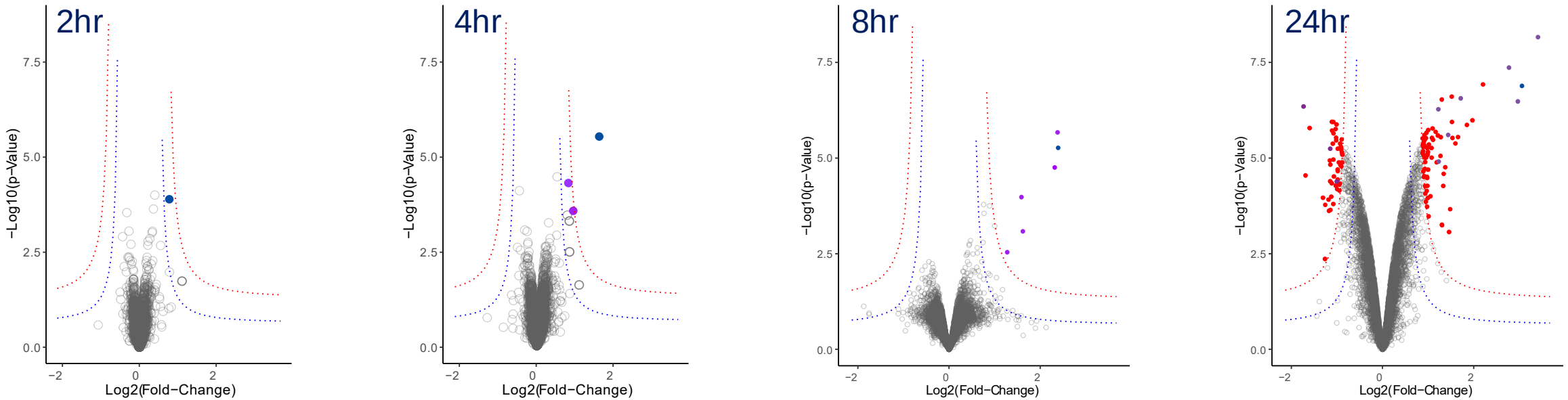
Comprehensive Off-Target Profiling



Bioinformatics w/ expert review required (not all off-targets carry the same weight)

Meaningful off-targets and approaches to assess / mitigate

Time-Dependent Effects on Off-Target Profiling: Elucidating Off-targets from Downstream Biology



Volcano plots of global proteomic changes at various incubation timepoints from cell line X with an example TPD at 10x DC90 concentrations. **Blue** dot represents biomarker of response, **purple** dots represents pathway biology, **red** dots represents other protein changes

- Selecting the appropriate timepoint for assessing off-targets is important to deconvolute primary and secondary pathway biology from true off-target effects

- Down-regulated targets vs. up-regulated targets – both are important, but are they equally important?
  - Down-regulated proteins could be due to off-target degradation vs. downstream pathway biology
  - Up-regulation could be due to an impact on the degradation of the natural E3 ligase substrate, saturation of the proteosome via increased target occupancy, or downstream pathway biology

E3 Ligase Selection and Aspects to Consider for Safety Assessment

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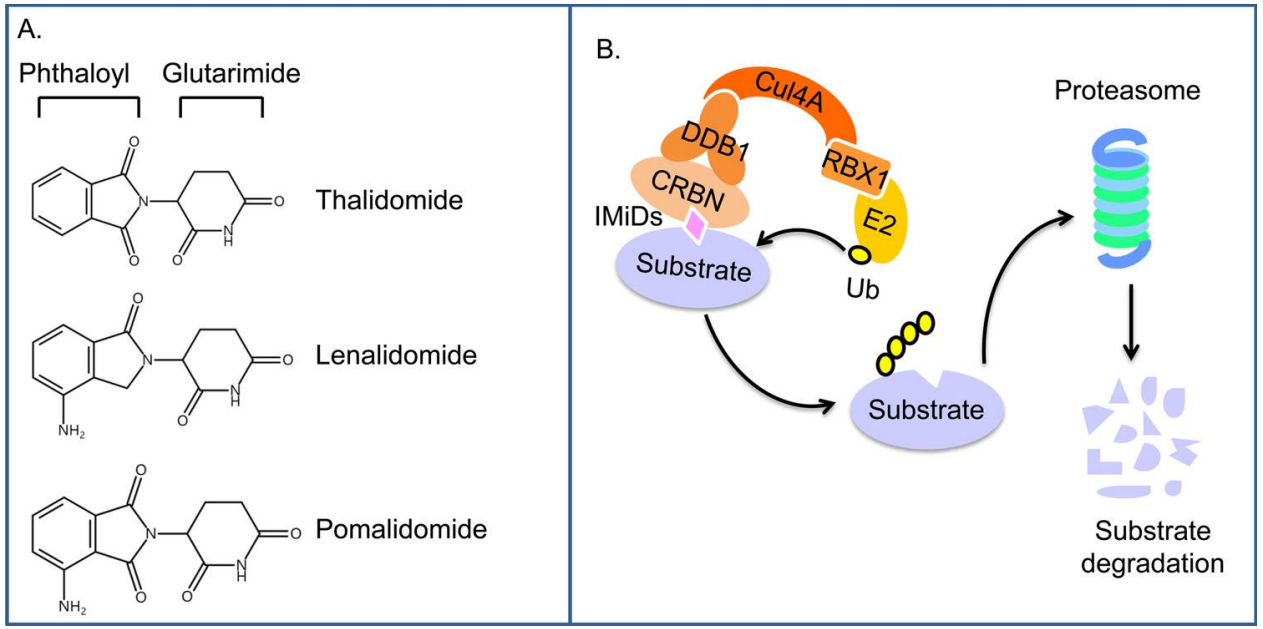
IMiDs and Mechanism of Teratogenicity



Gao et al. *Biomed Pharmacother*. 2020 Jul;127:110114.

- Cereblon modulation determined to be responsible for thalidomide-mediated teratogenicity

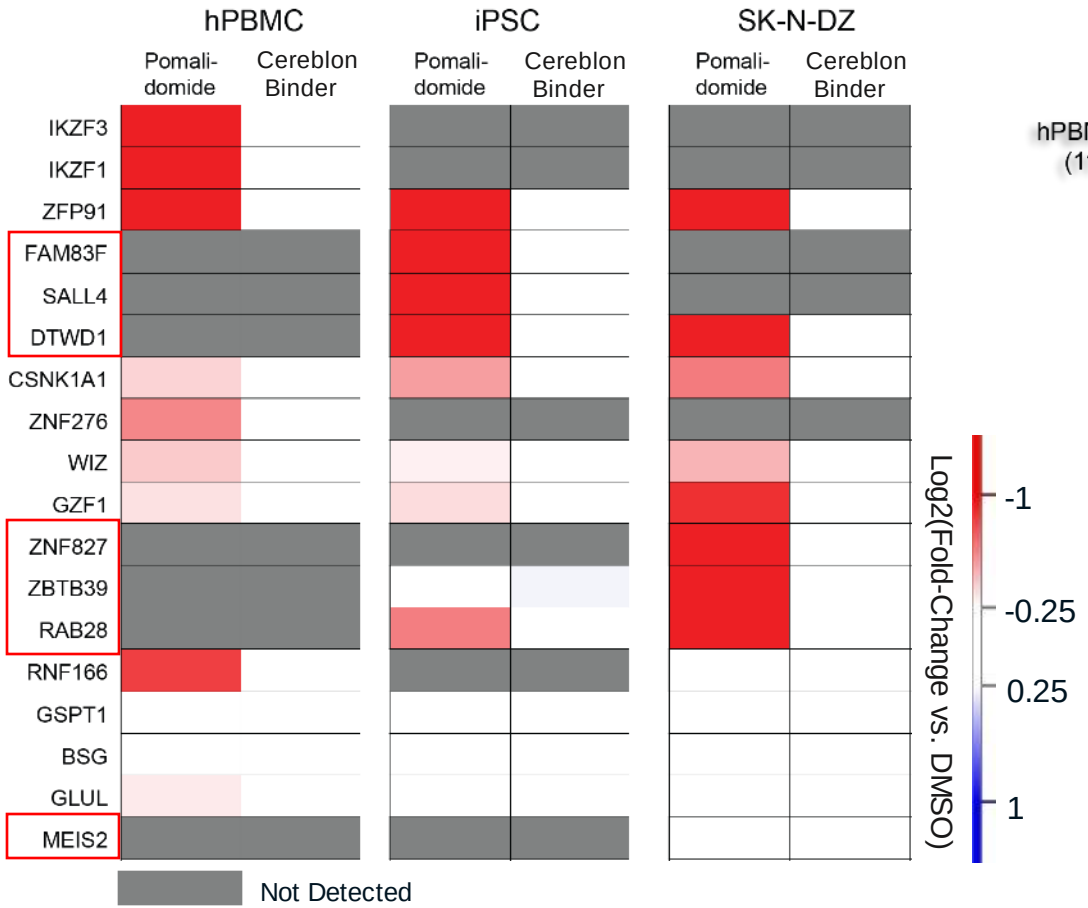
  (Ito T. et al. *Science*. 2010 Mar 12;327(5971):1345-50).

- Degradation of SALL4 via IMiD-CRBN leads to teratogenicity

  (Matyskiela ME, et al. *Nat Chem Biol*. 2018 Oct;14(10):981-987.)

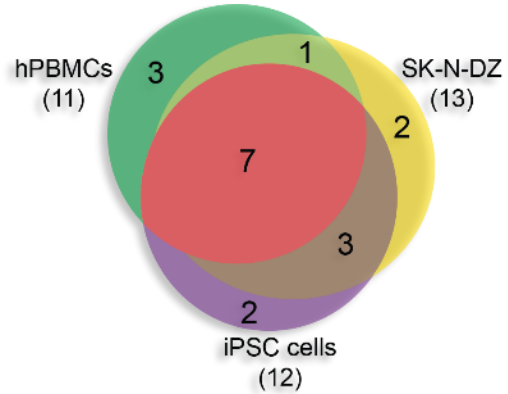
- Cereblon ligands are commonly employed for heterobifunctional TPD as they lend better oral bioavailability than others of ubiquitously expressed E3 ligases (e.g., VHL)
- Immunomodulatory imide drugs (IMiDs) are cereblon-modulators associated with hematopoietic effects as well as teratogenicity
- Reducing IMiD activity may be required/desired depending on indication and intended pharmacology

Proprietary Cereblon-Binder Containing TPDs Do Not Degrade SALL4 or other IMiD Neosubstrates



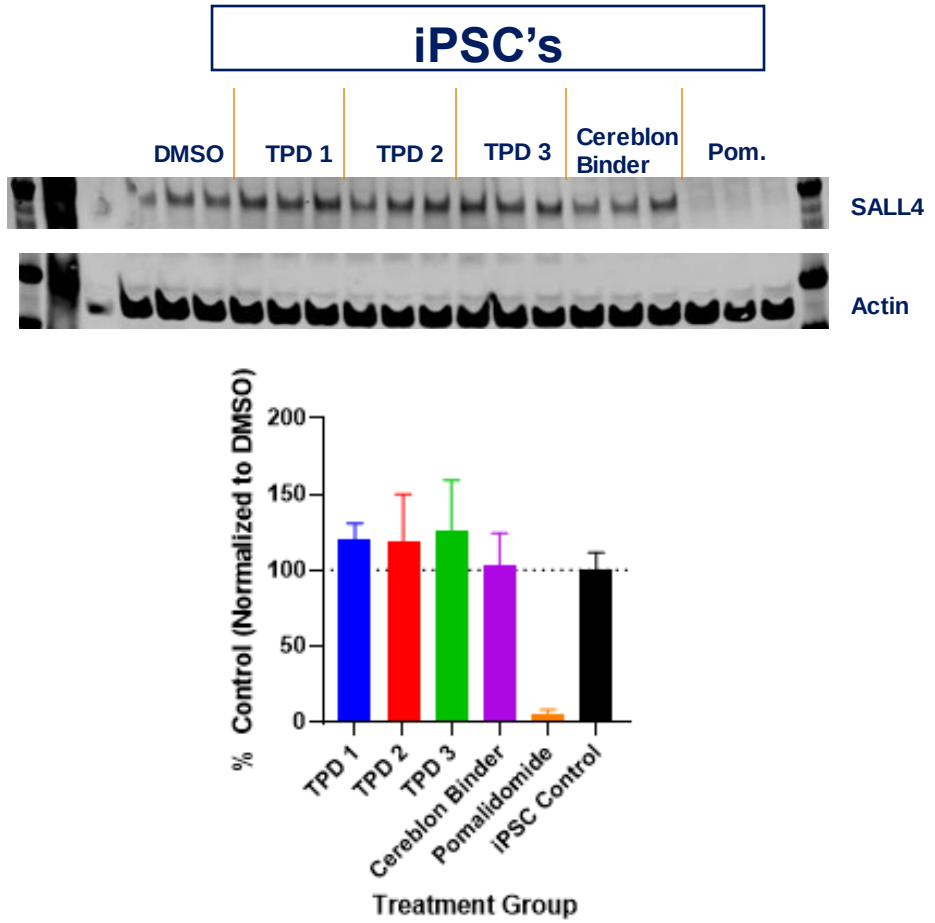
Note: ZNF653, ZNF692, ZNF98 and ZBTB16 were not detected in any of the three cell types

18 *IMiD* substrates covered



7 additional *IMiD* substrates assayed in iPSC and SK-N-DZ, including SALL4

IMiD substrates extracted from global proteomic assessment of human PBMCs, iPSC cells, and SK-N-DZ cells treated with pomalidomide and proprietary cereblon binder at 1uM for 24h



WB of SALL4 levels in iPSC cells treated with TPDs at 10x DC90 concentrations, pomalidomide (1uM), and proprietary cereblon binder (1uM) for 24h

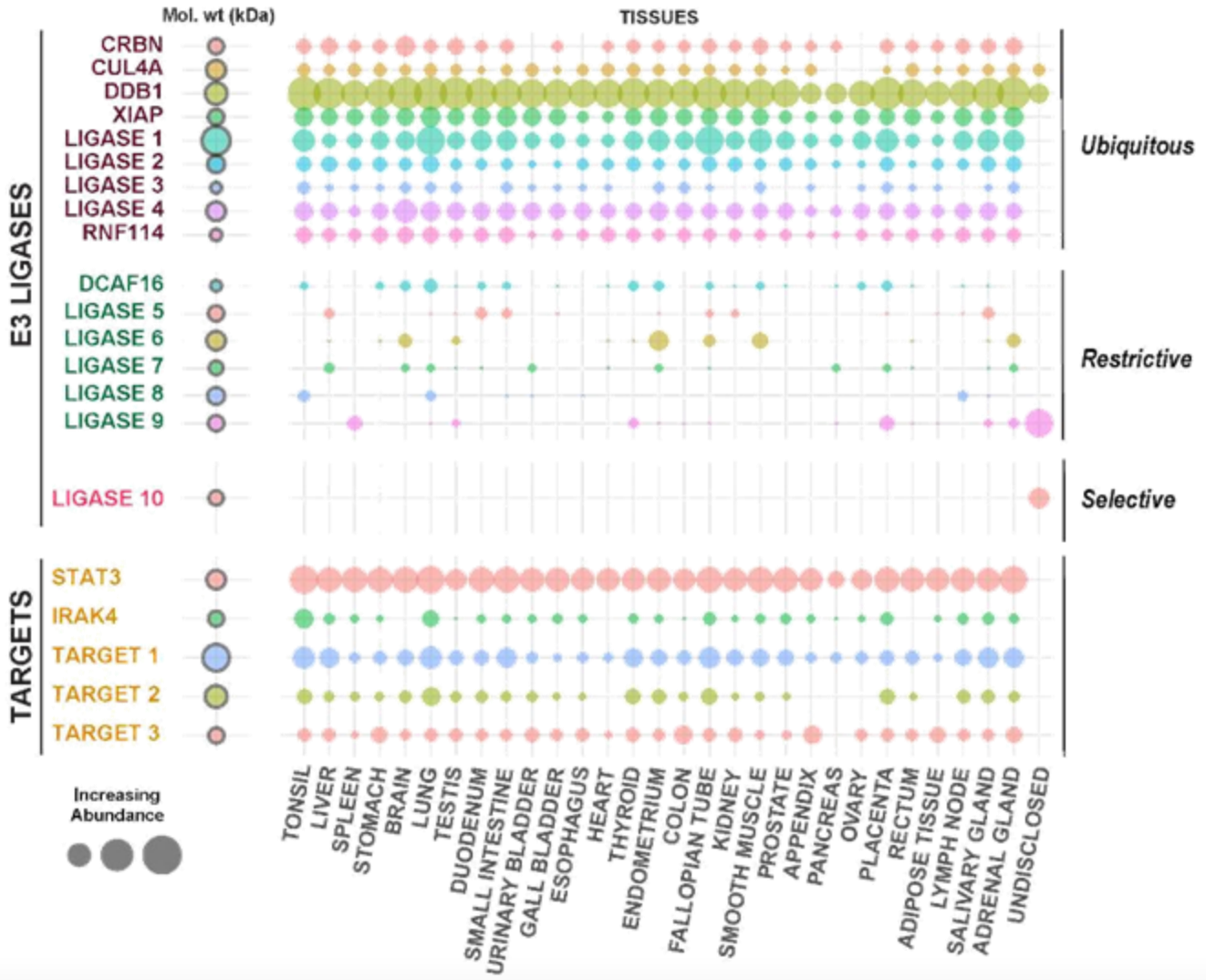
Novel E3 Ligases to Drug a New Generation of Targets

TR

Clinically Validated Targets Unlocked by E3 Ligase Differential Expression

On target unwanted pharmacology limits clinical application

- Focused on determining the expression profiles **of ~600 unique E3 ligases**
- Patterns mapped in both **disease and healthy contexts**
- Ability to match a **target protein with appropriate E3 ligase** based on expression and biology via **a machine learning algorithm**
- **Vision to develop tissue-selective or tissue-restricted degraders** to enable novel therapeutic opportunities



Source: Kymera's Proprietary E3 Expression Atlas

Overall Conclusions and Future Directors

- TPD offer unprecedented ability to selectively edit the proteome to impact human health and transform healthcare

- This emerging modality requires special considerations when addressing exaggerated pharmacology, species selection, off-target profiling, and other aspects of nonclinical safety from that of traditional SM therapeutics
  - Physiochemical properties of heterobifunctional degraders further add to these considerations
  - E3-ligase selection important component for assessing safety risk as well as opportunities for reducing on-target safety liabilities

- Expansion into novel tissue-restricted E3-ligases and molecule glues have the potential to markedly expand the utility of known-drug targets and as well as unlocking previously undruggable targets

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QUESTIONS
KYMERA