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Industry Perspective on the Pharmacokinetic and Absorption, Distribution, Metabolism, and Excretion Characterization of Heterobifunctional Protein Degraders[S]

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ABSTRACT

Targeted protein degraders (TPDs), specifically the bifunctional protein degraders discussed in this manuscript, consist of two linked ligands for a protein of interest and an E3 ligase, resulting in molecules that largely violate accepted physicochemical limits (e.g., Lipinski's Rule of Five) for oral bioavailability. In 2021, the IQ Consortium Degrader DMPK/ADME Working Group undertook a survey of 18 IQ member and nonmember companies working on degraders to understand whether the characterization and optimization of these molecules were different from any other beyond the Rule of Five (bRo5) compounds. Additionally, the working group sought to identify pharmacokinetic (PK)/absorption, distribution, metabolism, and excretion (ADME) areas in need of further evaluation and where additional tools could aid in more rapid advancement of TPDs to patients. The survey revealed that although TPDs reside in a challenging bRo5 physicochemical space, most respondents focus their efforts on oral delivery. Physicochemical properties required for oral bioavailability were generally consistent across the companies surveyed. Many of the member companies used modified assays to address challenging degrader properties (e.g., solubility, nonspecific binding), but only half indicated that they modified their drug discovery workflows. The survey also suggested the need for further scientific investigation in the areas of central nervous system penetration, active transport, renal elimination, lymphatic absorption, in silico/machine learning, and human pharmacokinetic prediction. Based on the survey results, the Degrader DMPK/ADME Working Group concluded that TPD evaluation does not fundamentally differ from other bRo5 compounds but requires some modification compared with traditional small molecules and proposes a generic workflow for PK/ADME evaluation of bifunctional TPDs.

SIGNIFICANCE STATEMENT

Based on an industry survey, this article provides an understanding of the current state of absorption, distribution, metabolism, and excretion science pertaining to characterizing and optimizing targeted protein degraders, specifically bifunctional protein degraders, based upon responses by 18 IQ consortium members and non-members developing targeted protein degraders. Additionally, this article puts into context the differences / similarities in methods and strategies utilized for heterobifunctional protein degraders compared to other beyond Rule of Five molecules and conventional small molecule drugs.

Introduction

Targeted protein degraders (TPDs), specifically heterobifunctional protein degraders, more commonly known as proteolysis targeting chimeras or PROTACs, are typically high molecular weight (MW) compounds that consist of a ligand for a protein of interest (POI) and a ligand for an E3 ligase tethered together by a chemical linker. When a TPD brings a POI and E3 ligase into close proximity, the E3 ligase complex can transfer multiple ubiquitin molecules (polyubiquitination) to the POI, thereby tagging the protein for proteasomal degradation. Example clinical TPDs, ARV-110 (bavdegalutamide, oral) and DT2216 (intravenous), are shown in Fig. 1. TPDs have become an attractive "new" modality for seemingly undruggable targets because weak ligands and/or ligands for allosteric binding sites can be used and still result in substantial degradation of the target (Gadd et al., 2017). There are potential pharmacodynamic (PD) advantages to degradation, especially for targets with slow turnover (half-life >12 hour), in that the pharmacological effects can last substantially longer than the pharmacokinetic (PK) half-life of the degrader (Law et al., 2021). Additionally, degraders act catalytically (stoichiometry >1:1), thereby allowing for exquisite potency relative to traditional inhibitors (Bondeson et al., 2015).

Although TPDs may display advantageous event-driven pharmacology, they generally fall in a physicochemical space that is beyond Lipinski's Rule of Five (bRo5) or Veber's rules and therefore might be

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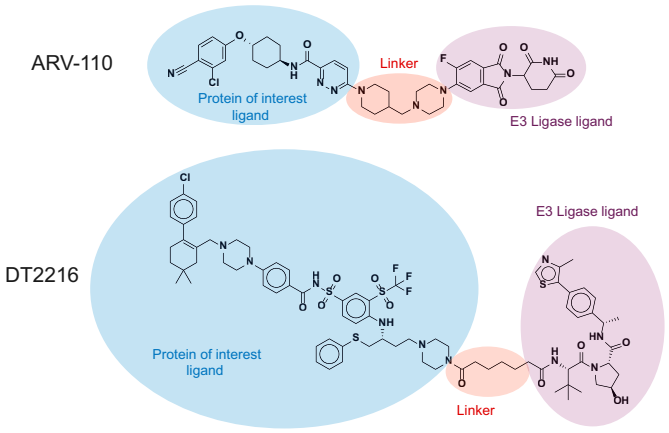

Fig. 1. Example structures of bifunctional protein degraders, with putative POI ligand, linker, and E3 ligase ligand highlighted. ARV-110 is an oral clinical CRBN ligand-containing degrader targeting the androgen receptor, and DT2216 is an intravenous clinical von Hippel-Lindau (VHL) E3 ligase ligand-containing degrader targeting BCL-XL.

expected to have poor solubility and/or permeability, leading to poor bioavailability (Veber et al., 2002; Cantrill et al., 2020). The suboptimal physicochemical properties of TPDs lead to additional challenges in generating robust and reproducible data in biologic assays, including in vitro absorption, distribution, metabolism, and excretion (ADME) assays. Therefore, modified and/or specialized assays may be required to support screening, lead selection and optimization (Pike et al., 2020).

Even with these challenges, oral bioavailability has been reported to be achievable due, in part, to a property referred to as chameleonicity, where these large molecules have been shown to adopt folded conformations that can achieve lower topological polar surface area (tPSA) and higher permeability than would be expected based on MW and calculated tPSA alone (David et al., 2021). Formation of intramolecular hydrogen bonds and shielding of amide groups from solvent have also been proposed as mechanisms to increase passive permeability and thereby oral bioavailability of TPD molecules (Atilaw et al., 2020). The observation that the clinical oral TPDs disclosed to date employ cereblon E3 ligase (CRBN) ligands (Békés et al., 2022) suggests that continuing to optimize other physicochemical properties such as MW and hydrogen bond donor count likely remains important, although there are possibly unknown special properties of CRBN-containing degraders facilitating their in vivo absorption.

TPDs entered the clinic in 2019, and now there are more than 10 oral TPDs in phase I/II clinical trials and one (AVR-110) that just entered into phase III (Chirnomas et al., 2023). These clinical advances support the notion that drug discovery teams have been able to find ways to harness the benefits and overcome the challenging properties of these molecules to achieve oral bioavailability. However, it has been shown that the discovery process for TPDs tends to be slower than traditional small molecules based on the relatively few examples in the clinic and complexities attributed to optimizing such molecules (Békés et al., 2022). In 2021, an IQ DMPK/Safety Working Group was formed and generated two comprehensive surveys to assess the current state of the DMPK/ADME and safety assessment of TPDs. In the DMPK/ADME survey, the working group asked 18 IQ member and nonmember companies questions about the properties of TPDs and how the DMPK/ADME assessment of TPDs compares to other bRo5 compounds and to traditional small molecules. Additionally, the working group requested information about the key challenges for developing TPDs. The survey also contained questions evaluating the properties or key design principles in discovering orally bioavailable TPDs since this is a focus for many companies. Because there is limited clinical data available on TPDs, the survey requested information on how tools typically used for human PK prediction of traditional small molecules performed with TPD molecules. Here, the working group presents the results of the survey, contextualizes the results in relation to other bRo5 compounds, and suggests areas where additional research will help accelerate the discovery and development of TPDs.

Materials and Methods

Industry Surveys on Safety and ADME-PK Assessments for TPD. A survey was sent to IQ member and nonmember companies in 2021. A total of 64 questions served as the basis for this publication and focused on the ideal physicochemical and ADME properties for intravenous and oral administration, ADME challenges for oral bioavailability, in vitro ADME/in vivo PK assessment, central nervous system (CNS) penetration, PK/PD, and human PK prediction. A total of 90 additional questions covered topics including the level of experience of development of degraders, stage of development, and risk perception and assessment; in vitro and in vivo toxicology assessment, including modifications to standard assays; and regulatory and starting dose setting experience. The data from these additional questions are described in a companion publication focusing on the safety evaluation of TPDs (Hemkens et al., 2023). The specific questions asked are provided in Supplemental Fig. 1. All survey responses were blinded by the IQ Secretariat before review and analysis by the authors. Information was returned from 18 companies with a range of experiences in the area of TPDs.

Definitions of Stage Gates Used for the Purpose of This Survey. *Lead discovery* refers to the earliest stage gate(s), during which target validation, hit identification, early high-throughput screening, and/or investigative in vitro work is conducted.

Lead optimization/candidate selection refers to the next stage, during which a selection of identified hits/leads are optimized using structure-activity relationships to improve upon problematic characteristics (e.g., potency, selectivity, PK, etc.). During this stage, in vitro assays may be more functional and/or cell based, and tool compounds may be used in vivo for exploratory purposes. At the end of this stage, a candidate molecule is selected.

Investigational new drug–enabling/good laboratory practices studies are completed after candidate selection. This is the final preclinical stage before starting clinical trials. Typically, these in vitro and in vivo studies are conducted with the candidate to meet regulatory requirements for the planned phase I trial in the proposed therapeutic indication.

Results

Observations Relating to ADME and Physicochemical Properties for Degraders across Different Routes of Administration (Oral, Intravenous, and Subcutaneous)

Seventeen of the 18 responding companies are developing degraders for oral delivery. Of these companies, many are investigating molecules that fall outside of Lipinski's Rule of Five (Ro5) and/or Veber's rule, which describe the physicochemical properties influencing the oral bioavailability of therapeutic candidates (Fig. 2) (Lipinski et al., 2001;

ABBREVIATIONS: AB-MPS, simple multiparametric scoring function predicting oral absorption; ADME, absorption, distribution, metabolism, and excretion; BCS, biopharmaceutical classification system; bRo5, beyond Lipinski's Rule of Five; CNS, central nervous system; CRBN, cereblon E3 ligase; DMPK, drug metabolism and pharmacokinetic; EPSA, experimentally determined exposed polar surface area; HBD, number of hydrogen bond donor; IVIVC, in vitro–in vivo correlation; IVIVE, in vitro–in vivo extrapolation; $K_{puu,brain}$, unbound brain-to-plasma partition coefficient; MW, molecular weight; OATP, organic anion transporting polypeptide; P450, cytochrome P450; PBPK, physiological-based pharmacokinetic; PD, pharmacodynamic; P-gp, P-glycoprotein; PK, pharmacokinetic; POI, protein of interest; PPB, plasma protein binding; Ro5, Rule of Five; TPD, targeted protein degrader; tPSA, topological polar surface area.

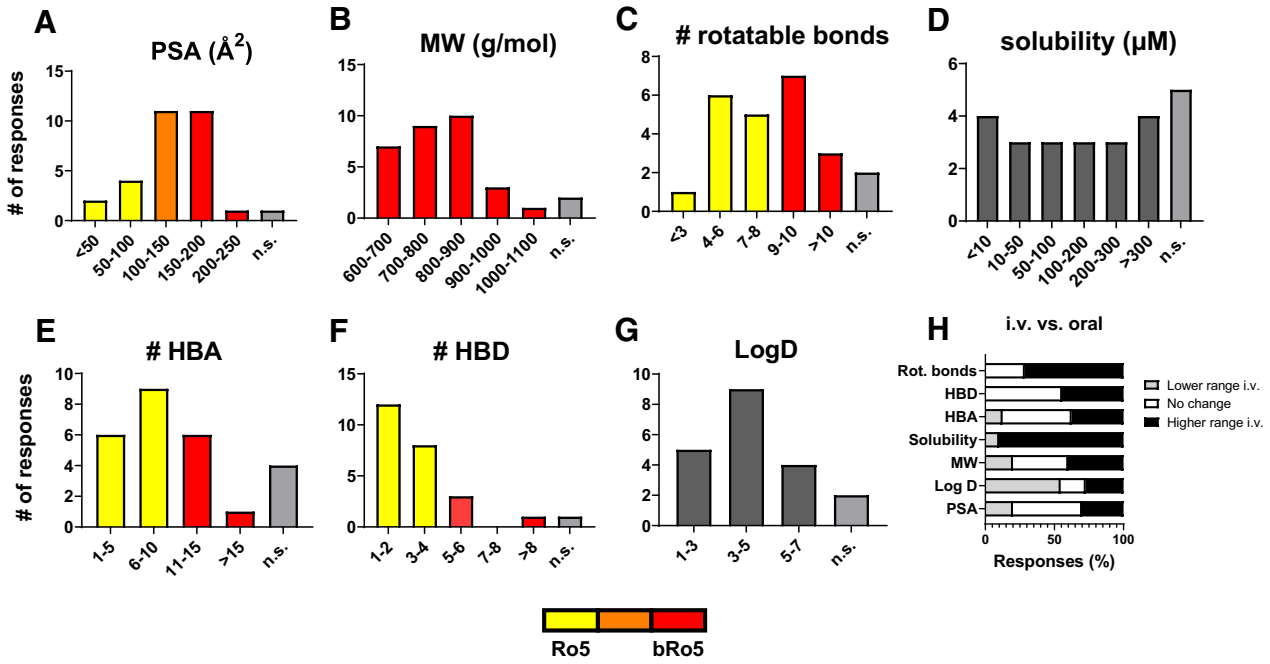

Fig. 2. Optimal property ranges for oral and intravenous TPDs. The number of responses across categories for optimal (A) polar surface area (PSA), (B) MW, (C) number of rotatable bonds, (D) solubility, (E) number of hydrogen bond acceptors (HBA), (F) number of HBD, and (G) LogD for orally bioavailable degraders. Where relevant, bars are colored to signify Ro5 and Veber's rule in yellow and bRo5 in red; orange is used when the property range falls within both the Ro5 and bRo5 rules. (H) Comparison of optimal property ranges for intravenous degraders compared with oral degraders. More than one response was allowed per respondent for each property. n.s., not specified.

Veber et al., 2002). Based on responses, the optimal chemical property space to achieve oral bioavailability for degraders was tPSA of 100–200 Å^2, MW <900 g/mol, <14 hydrogen bond acceptors (HBAs), <5 hydrogen bond donors (HBDs), and LogD 3–5 (Fig. 2, A–G). The desired solubility and number of rotatable bonds had a more uniform distribution of responses across the ranges provided in the survey.

Interestingly, of these properties, only HBD <5 is consistent with the Ro5, and this property is hypothetically related to the ability of TPDs to shield polarity and hide it in nonpolar environments, referred to as chameleonicity. One approach to hide HBDs is to form intramolecular hydrogen bonds. Chameleonicity is not unique to TPDs and was recently described in detail by David et al. (2021) for a set of 24 Food and Drug Administration–approved drugs and one proteolysis targeting chimera. In this paper, experimentally determined exposed polar surface area (EPSA) and LogP were highlighted as important experimental data to predict chameleonic nature. Although the authors did not request information on the optimal EPSA range for oral TPDs, permeability, chameleonicity, number of intramolecular hydrogen bonds, and EPSA were properties suggested by respondents as requiring optimization for orally bioavailable TPDs. Although chameleonicity is a possible explanation for permeability of oral TPDs, it cannot be ignored that the released structures of clinical oral TPDs suggest that the linker does not need to be highly flexible to achieve sufficient oral bioavailability to advance to the clinic.

Additional molecular characteristics considered important for oral degrader developability included metabolic and chemical stability, transporter-mediated efflux, permeability, solubility in physiologically relevant media (fasted simulated intestinal fluid/fed simulated intestinal fluid), conformational flexibility/chameleonicity, and EPSA (Goetz et al., 2014). Permeability, solubility, and MW were reported as being the primary challenges to overcome when developing an oral TPD. Secondary challenges included minimizing the influence of P-glycoprotein (P-gp) on absorption, metabolic instability, the number of hydrogen bond donors, and polarity/lipophilicity (Fig. 3). P-gp most likely was listed as a secondary, rather than primary, contributor in limiting oral bioavailability of TPDs as P-gp should only play a role when TPDs have low solubility and/or permeability.

Fifteen of the 18 responding companies have employed the intravenous route of administration with TPD compounds. Of those, seven reported issues mostly relating to formulation challenges due to the poor solubility of TPDs at the required dose levels and volumes administered. The majority of companies reported exploring a similar or wider range of physicochemical properties for the intravenous route of delivery compared with oral (Fig. 2H). Two exceptions were LogD, where over 50% of respondents reported exploring a narrower range of values for intravenous delivery compared with oral, and solubility, where 90%

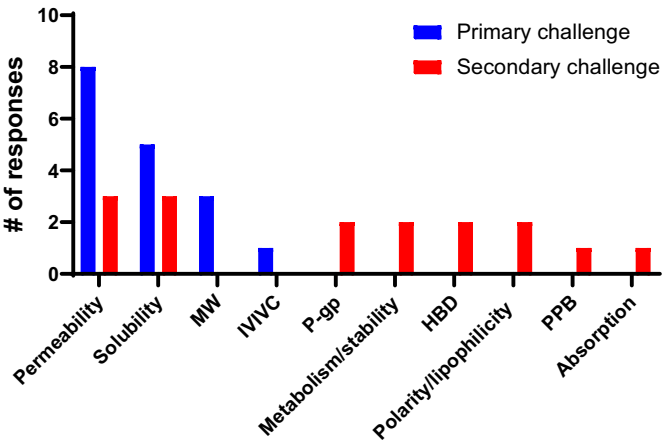

Fig. 3. Most significant ADME/physicochemical property challenges facing development of orally bioavailable (>30% oral bioavailability (% F), specified in survey) degraders.

of respondents identified a need for a higher solubility range for intravenous delivery.

Solubility was a key parameter listed by respondents for improving exposure and/or tolerability for both intravenous and oral routes of administration. For intravenous dosing, both traditional small-molecule and TPD compounds must be in solution for dosing because otherwise, acute toxicity may be observed. Low aqueous solubility can limit oral bioavailability of small molecules, even those with high permeability. Nevertheless, 40% of Food and Drug Administration–approved oral drugs fall within low solubility biopharmaceutical classification system (BCS) categories (II and IV), most being in the low solubility, high permeability category II (Bocci et al., 2022). TPDs with both permeability and solubility challenges typically fall within BCS category IV (~7% of approved drugs). When permeability or solubility is low, as mentioned previously, active efflux by P-gp can further limit oral bioavailability as illustrated by P-gp inhibitor studies in rodents (Dahan and Amidon, 2009; Wahajuddin et al., 2014; Nielsen et al., 2021). Improving fasted simulated intestinal fluid solubility and optimizing formulations were important for respondents to achieve oral bioavailability, but several respondents also indicated that they had to change the route of administration even at stages as late as lead optimization/candidate selection.

Eleven of the 18 responding companies have used the subcutaneous route of administration, which was likely employed preclinically to achieve sufficient exposure for early compounds as clinical TPDs are primarily either administered by the oral or intravenous route. Of those companies dosing TPDs subcutaneously, five reported issues including high intersubject variability, lack of exposure linearity at higher doses, formulation challenges, edema formation, necrosis, shock-like reaction (details unknown to authors), local inflammation, and lack of translatability across species.

Workflow Modifications Employed during the ADME Assessment of Targeted Protein Degraders

Respondents (17 of 18) were evenly divided on whether the ADME assessment of TPDs was different from other bRo5 compounds, with 53% suggesting modifications and 47% not. For those making changes to the ADME assessment, modifications included using in silico calculations, measuring EPSA, including assay alterations (discussed later in *Modifications Necessary for* in Vitro *Assays or* in Vivo *Studies*), prioritization of drug stability assays, correction of microsomal stability data for microsomal binding, less use or lower stringency of in vitro data to triage compounds for in vivo pharmacokinetics, and accepting lower oral bioavailability and higher clearance. In addition to adding EPSA measurements, one company added intramolecular hydrogen bonding and polar group shading assay capabilities, and another used chromatographic LogD (Lombardo et al., 2001) instead of LogP/shake-flask LogD.

The eight respondents that answered the question (no response for 10 respondents) indicated that they did not modify drug-drug interaction assessment of degraders due to the potential degradation of enzymes or transporters. The survey did not probe whether drug-drug interaction assessment was modified for any other reasons.

Half of the 18 respondents have not evaluated the epimerization/racemization of their degraders; however, one-third (33%) of respondents indicated that they have measured and observed this phenomenon, and 17% have measured and not observed epimerization/racemization. Of the six respondents that observed epimerization/racemization, none handled this feature any differently than they would for any typical small molecule. This question did not specify it was referring to the epimerization of CRBN-containing degraders; however, it is probable that the respondents understood this question to refer to this subtype of TPDs. The common chiral center on the glutarimide ring of an example CRBN ligand (thalidomide) is shown in Fig. 4. Typically, the S-enantiomer of CRBN ligands shows stronger binding to CRBN than the R-enantiomer and is mainly responsible for the polyubiquitination activity of a mixture of enantiomers (Tokunaga et al., 2018).

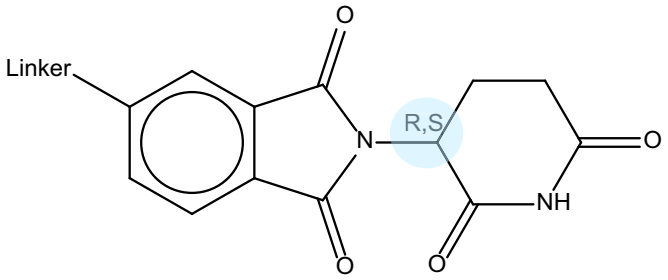

Fig. 4. Structure of a commonly used CRBN ligand (thalidomide) in TPD structures. The chiral carbon on the imide ring is highlighted on the structure. Note the linker attachment is an example and other attachment points are possible.

Absorption of Targeted Protein Degraders. As previously discussed, the oral delivery route is the most attractive route of drug administration for TPDs due to the convenience to patients undergoing chronic therapy and the readily adjustable dosing schedule. This route also provides significant challenges for high MW compounds, yet numerous high MW oral TPD compounds are in the clinic. To understand how this was achieved, information on properties of TPDs that have been optimized for oral bioavailability was requested from respondents. These properties are presented in Table 1.

One possible alternative mechanism for absorption of oral TPDs is through lymphatic absorption. Some BCS class IV and/or bRo5 compounds, such as venetoclax, have moderate oral bioavailability in part because of lymphatic absorption (Choo et al., 2014). Therefore, it was asked whether lymphatic absorption has been observed for TPDs. Lymphatic absorption of oral compounds occurs when a compound associates with the lipid/triglyceride of chylomicrons in the gastrointestinal tract and is processed along with chylomicrons in enterocytes for secretion into the lymphatic circulation, thereby bypassing first-pass metabolism (Trevaskis et al., 2015). Typically, lymphatically absorbed compounds have a high hydrogen bond acceptor count, PSA, Log P, and/or MW (Lu et al., 2015). Four of 18 companies have assessed the potential for lymphatic absorption of degraders. Of those, only two observed lymphatic absorption (species and other details are unknown). No follow-up was completed to identify which route of administration was associated with which outcome in these lymphatic absorption studies, but based on the experience of the authors, lymphatic absorption could be observed with oral, sublingual, or subcutaneous administration. Lymphatic absorption via chylomicrons is relevant for oral drugs and is likely the route associated with the studies referenced by the respondents as most are focusing on oral administration. Further investigation is needed to determine whether oral TPDs can be lymphatically absorbed and if this mechanism can be harnessed to increase oral bioavailability.

Tissue Distribution of Targeted Protein Degraders. A total of 18 companies responded to questions regarding tissue distribution of TPDs (besides brain, which was the focus of another set of questions). Eight companies indicated they did not invest resources to measure tissue concentrations, whereas nine of the 10 companies that pursued tissue bioanalysis indicated that the tissue concentrations approximated or were greater than expected. The remaining respondent indicated tumor levels were lower than anticipated and suggested that this may have been explained by P-gp–mediated efflux limiting distribution into tumor cells.

Plasma Protein Binding of Targeted Protein Degraders. Plasma protein binding (PPB) is a drug metabolism and pharmacokinetic

TABLE 1

Additional considerations for the optimization of orally bioavailable TPDs

Category	Responses
Molecular properties	EPSA, AB-MPS, intramolecular H-bond formation, chameleonicity, choice of functional groups
Solubility	FaSSIF solubility, properties that facilitate formulation including self-emulsifying nanosuspension
Metabolism	Good linker stability favoring ternary complex formation
Other	Daily dose, stability in SGF

AB-MPS, a simple multiparametric scoring function predicting oral absorption; FaSSIF, fasted simulated intestinal fluid; SGF, simulated gastric fluid; TPD, targeted protein degrader.

(DMPK) parameter critical to interpreting drug properties, such as clearance and volume of distribution, and to developing drug-drug interaction predictions and pharmacokinetic-pharmacodynamic relationships. Numerous techniques have been developed to measure drug PPB and free drug concentration. The most commonly used techniques for traditional small molecules are equilibrium dialysis, ultracentrifugation, and ultrafiltration (Howard et al., 2010). Drug molecule challenges such as high nonspecific binding (i.e., to incubation plates or tube plastics), plasma instability, or long incubation times required to reach equilibrium are common for small molecules. It is important to consider the various merits and caveats for each of the PPB techniques and the various assay adaptations that may be needed to improve outcomes (Riccardi et al., 2015).

In total, 18 companies responded to questions related to PPB of TPDs. Based on respondents' experience, a wide range of PPB values of TPDs have been observed, from <50% to >99.9%, although the majority of binding exceeded 90%, with the highest incidence of PPB in the 99%– 99.9% range (Fig. 5).

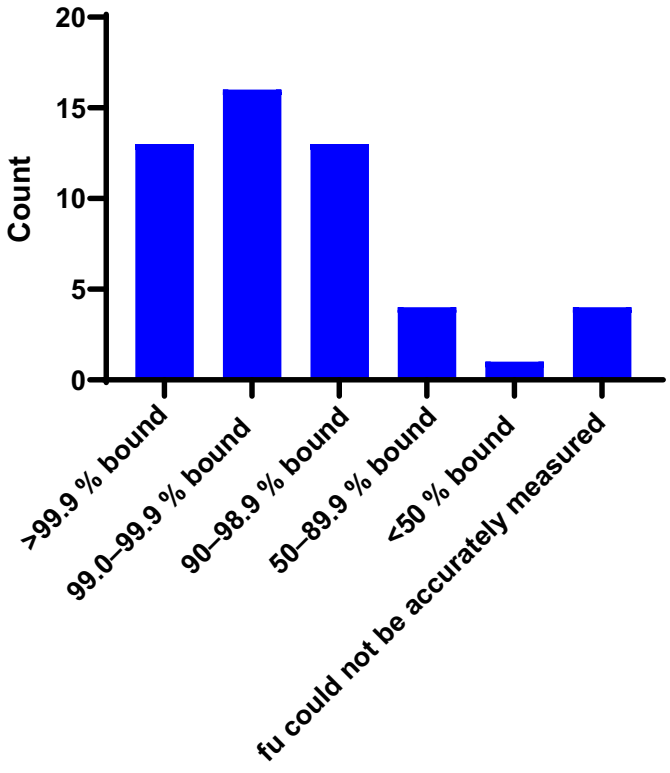

Fig. 5. Range of observed PPB of target protein degraders. Multiple selections were allowed for the observed range. fu, fraction unbound in plasma.

The Free Drug Principle and Targeted Protein Degraders. The free drug principle is a well established concept for small molecules that is widely applied in drug discovery and development in the interpretation of PK/PD relationships (Isbell et al., 2019). The survey responses indicated that most companies agreed that a clear relationship exists between the steady-state unbound concentration at the therapeutic target site and pharmacodynamic effects for TPDs. Of the 17 respondents, three indicated that although there were no major pharmacokinetic-pharmacodynamic disconnects, minor disconnects were observed, which may be due to low rates of membrane permeation of TPDs, resulting in longer times to reach equilibrium across the plasma membrane of target cells, or may be addressed by measuring unbound cellular potencies in in vitro assays. Accurate determination of PPB is required to fully understand both PK and PK/PD relationships of traditional small molecules as well as bRo5 compounds like TPDs because unbound concentrations of drug dictate not only drug pharmacological effect (PD) but also its total clearance and distribution in the body (Summerfield et al., 2022).

It is important to note that TPDs elicit event-driven pharmacology, whereby the TPD transiently binds to a target protein, tagging it for degradation (ubiquitination) in a catalytic manner (Békés et al., 2022), so the pharmacodynamic effect may persist even after drug has been cleared (Law et al., 2021). POIs that have a long half-life will also influence the duration of response; therefore, the dosage regimen may need to be adapted to modulate the desired level of POI degradation. This PK/PD disconnect is one of the key features of TPDs that can balance some of the ADME challenges of the molecules. Because of this apparent PK/PD disconnect, it may seem as if the free drug principle may not hold true for TPDs, but the initial binding event of the TPD to the POI was likely driven by unbound concentrations, which is consistent with the observations of most respondents.

Target Half-Life

Among the 14 respondents that have measured target protein resynthesis rates, the most observed range was 9–24 hours (9 of 14), followed by >24 hours (5 of 14). Multiple respondents indicated that they rarely work on targets with resynthesis rates in the <2-hour and 2–8-hour ranges.

Hook Effect

The "hook" effect is observed at high in vitro concentrations of TPDs when binding to the POI or E3 ligand is saturated, resulting in formation of inactive binary complexes, which results in a bell-shaped half-maximal degradation concentration or more commonly called DC_{50} curve with apparent loss of activity at high concentrations (Moreau et al., 2020). In the TPD field, it is commonly accepted that the hook effect is observed in vitro, but there have been conflicting reports of observations of the hook effect occurring in vivo. Across all respondents, more than 350 in vivo PK/PD or efficacy studies were conducted with TPDs. Of these studies, only six examples of hook effect were observed in vivo from two respondents; however, unbound plasma or tissue concentrations were not directly measured in these studies to confirm the data could be explained by a hook effect. These respondents indicated that the presumed hook effect did have an impact on data interpretation, PK/PD modeling, and human efficacious dose projection.

Brain Penetration Potential of Targeted Protein Degraders. The blood-brain barrier consists of endothelial cells with very tight intercellular junctions that limit the paracellular flux of drugs. Passage of drugs across the blood-brain barrier is highly dependent on a drug's physicochemical properties, passive diffusion, and uptake and efflux transporters (Di et al., 2013). Due to their ability to target proteins that

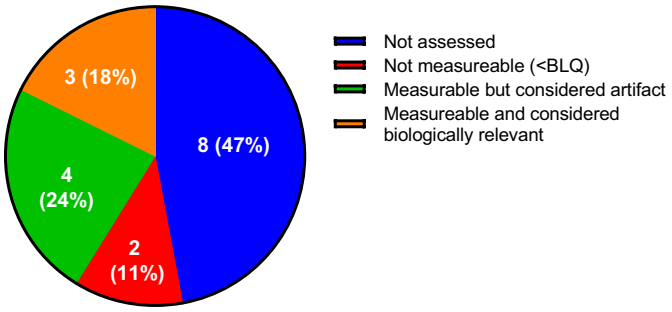

Fig. 6. Assessment of brain concentrations of TPDs. BLQ, below the limit of quantification.

are largely undruggable, TPDs may have significant potential in the treatment of CNS disorders, but a number of limitations must be overcome before they can be considered a viable option for CNS disorders (Farrell and Jarome, 2021; Lospinoso Severini et al., 2022). Achieving pharmacologically relevant brain or central nervous system concentrations of TPDs presents a significant challenge due to their physicochemical properties. Seventeen companies responded to questions related to assessment of brain concentrations of TPDs, and these data are represented in Fig. 6. Nine of the respondents indicated that they had conducted analysis of brain concentrations following an in vivo assessment with TPDs, seven of which could quantify TPD brain concentrations, but only three of the seven deemed the measurements biologically relevant, with the remaining four respondents indicating concentrations were considered an artifact (reasons are not known by the authors). Hence, responses from this survey generally suggested low brain penetration potential of TPDs. For the three companies that measured biologically relevant brain concentrations, the corresponding unbound brain-to-plasma partition coefficients were 0.1–1.0, 0.1–2.0, and 0.1–35. The authors anonymously received additional information from the respondent who provided a brain-to-plasma partition coefficient of 35, and the respondent confirmed that this ratio was the unbound brain-to-plasma partition coefficient or $K_{puu,brain}$ (not total partition coefficient or K_p) for one compound and that this result was confirmed in multiple follow-up studies, with a mean $K_{puu,brain}$ of 17. Additionally, the respondent noted that all of their other $K_{puu,brain}$ measurements were in the 0.1–1 range.

Low $K_{puu,brain}$ (0.1) values are not surprising and are likely due to active efflux at the blood-brain barrier. Kurimchak et al. (2022) recently showed that P-gp short interfering RNA and inhibitors can reduce tumor cell line resistance mediated by overexpression of Mdr1 (P-gp) for three structurally distinct TPD molecules, and although they did not directly demonstrate direct P-gp-mediated efflux of the compounds, this report, along with survey responses, indicates that TPDs can be P-gp substrates. A $K_{puu,brain}$ value of 1 is encouraging for developing CNS-penetrant TPDs. The response of a $K_{puu,brain}$ value of 35 is quite interesting and may suggest active uptake into the brain by transporters (Huttunen et al., 2022). The bRo5 compound bromocriptine (MW 655 Da) is reported to be a substrate of both organic anion transporting polypeptide (OATP)-2B1 and OATP1A2, suggesting that these transporters can transport high MW compounds (Schäfer et al., 2020). One of the respondents noted that they have observed active uptake of TPDs by OATP transporters as a major clearance mechanism, so active uptake by OATPs appears feasible. Several studies have demonstrated brain penetration of TPDs in rodents, but to the best of our knowledge, only brain pharmacological effects with non-oral doses or total brain-to-plasma ratios with oral doses have been reported, not unbound concentrations (DeMars et al., 2019; Wang et al., 2021; Liu et al., 2022a,b). The authors would also like to highlight that the potential technical challenges associated with accurate brain and plasma protein binding measurements of TPDs (mentioned previously) and confounding effects of blood in the brain tissue must be considered when interpreting $K_{puu,brain}$ data. Clearly, more work is needed in this area, but there seems to be some promise of oral CNS-active TPDs.

Metabolism and Elimination of TPDs. Like for traditional small molecules, understanding metabolism and clearance pathways is important in TPD optimization. In vitro–in vivo correlation (IVIVC) and metabolism/stability were highlighted by a few respondents as primary and secondary challenges of TPDs, respectively (Fig. 3). It is possible that the survey responses were low in these areas because these challenges are not that different from other small molecules. A total of 18 respondents selected major mechanisms of elimination from the survey list (Fig. 7). Respondents were asked to select all that apply. From 18 respondents, a total of 64 answers were selected, or more than three selections per respondent.

Of these, 17 of 18 (94.4%) observed cytochrome P450 (P450)-mediated metabolism. Glucuronidation by UDP-glucuronosyltransferase enzymes was observed as a major metabolic route by seven of 18 (38.9%) respondents. P450 and UDP-glucuronosyltransferase enzymes are also common metabolic pathways for traditional small molecules. Amidase and esterase activities were each selected by eight respondents as major metabolizing enzymes, whereas three of 18 respondents selected hydrolysis as a major route of elimination. Hydrolysis was provided as a separate option to allow collection of data for this clearance route when the enzyme responsible for hydrolysis was unknown. As TPDs have two functional groups connected with a linker, sometimes by amide or ester bonds, it is not unexpected that these hydrolytic metabolic pathways are observed as significant clearing mechanisms. Linker cleavage can result in the release of the POI ligand as well as the E3 ligand, which could inhibit the intact TPD from binding to the target or E3 in addition to rendering the TPD inactive (Pike et al., 2020). As shown by Goracci et al. (2020), P450 enzymes can also be involved in the dealkylation and/or hydrolysis of TPD linkers. Other enzymes involved in TPD

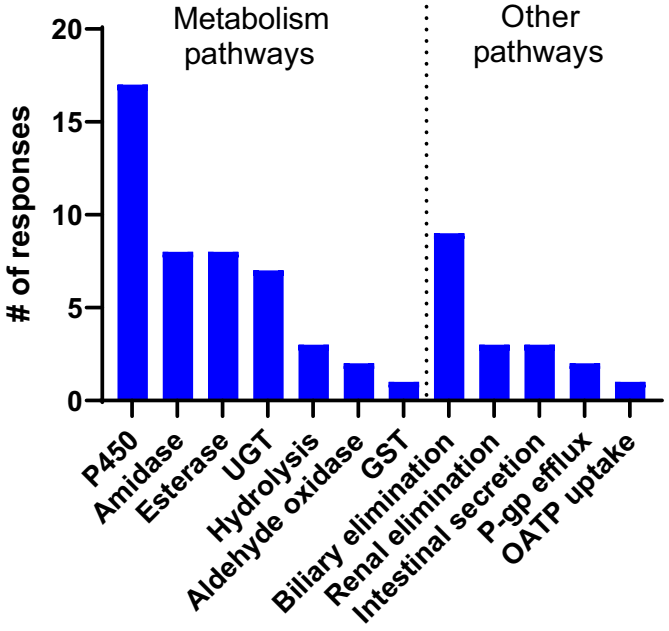

Fig. 7. Major mechanisms of elimination observed by respondents. More than one response was allowed per respondent. GST, glutathione *S*-transferase; UGT, UDP-glucuronosyltransferase.

clearance were cited by a small number of respondents; aldehyde oxidase was cited by two (11.1%) and glutathione *S*-transferase was cited by one respondent (5.6%). Thus, enzymatic clearance represents 45 of 64 selected major clearance pathways observed, or >70% of all selections.

The literature on nonmetabolic elimination of TPDs is limited to a single report of a high-clearance molecule administered to rats, with ~9% of the intravenous dose being excreted into the bile as parent and a negligible amount excreted into the feces or urine (Cantrill et al., 2020). However, half of the respondents (9 of 18) reported that they have observed biliary excretion, and approximately 17% (3 of 18) have observed renal elimination. In one report, renal elimination was assumed to be unlikely for TPDs due to their high PPB and limited potential for transport by renal transporters (Pike et al., 2020), but based on survey responses, renal excretion is possible. Transporter-mediated elimination was selected by three of 18 respondents (15.6%) as an observed elimination mechanism, with two of the three referring to P-gp efflux and one out of three referring to OATP-mediated hepatic uptake. Intestinal secretion was cited as a major route of elimination by three of 18 respondents (15.6%) without referring to a specific transporter. In the intestine, several transporters could be involved in active secretion in the intestine, but coupled with the high rate of observation of biliary excretion and known P-gp efflux of TPDs, P-gp is likely involved, although involvement of MRP2 and BCRP cannot be ruled out. In total, elimination as unchanged TPD was selected 18 times (28%). Nonenzymatic hydrolysis was mentioned in one of the other pathway selections (1.6% of all selections).

Additionally, respondents were asked if metabolites affected the pharmacology of TPDs. Of the respondents, 12 of 18 (67%) did not measure metabolites, whereas one of 18 (5.6%) measured and did not detect any pharmacological effects, but 5 of 18 respondents (27.8%) measured metabolites and observed effects on pharmacology. Responses to this follow-up question indicated that the pharmacologic interactions were, in four of five responses, characterized as derived from separation of the ligase binding motif from the target binding motif. Observations from the four respondents could be summarized as target binding and/or ligase binding motifs led to competition with TPD for ligase binding or for target binding. One respondent that observed a unique pharmacological impact of the metabolites of TPDs only replied that the metabolite(s) interfered with pharmacology. Because of the potential for metabolites to interfere with primary pharmacology, metabolite identification prior to in vivo pharmacology studies is recommended to be considered for TPDs to aid in PK/PD study interpretation.

Modifications Necessary for in Vitro Assays or in Vivo Studies

When asked if respondents employed a modified screening cascade for bifunctional degraders, an approximately even distribution was observed (9/8, yes/no); however, 89% of respondents reported using modified versions of in vitro ADME assays. Surprisingly, no respondents reported modifications to their drug-drug interaction assessment strategy, but the response rate was only 44%. Typical challenges encountered with degraders in standard ADME assays included nonspecific binding, low stability, and lack of solubility. Hence, assay modifications aiming at overcoming these restrictions have been employed to improve recovery, reduce nonspecific binding, and/or adjust buffers and readouts (Table 2).

Low solubility can impact the maximum concentration that can be tested in an assay, increase the chance of observing variable results, and result in submaximal degradation or a perceived hook effect in DC_{50} dose responses. Nonspecific binding to labware and protein binding to incubation components can also result in lower free concentrations than nominal, particularly at low compound concentrations. These two factors result in a narrow concentration range at which in vitro experiments can be performed without confounding effects. Addition of modifiers like bovine serum albumin or solvents can be used for certain assays, such as permeability or hepatocyte intrinsic clearance or CL_{int}, but care must be taken to not interfere with assay performance.

Besides these modifications, some respondents established additional assays to assess physicochemical properties of degraders (i.e., EPSA, intramolecular H bond formation, and polar group shading capability) or to determine lipophilicity and permeability [i.e., chromatographic-based assays such as chromatographic LogD or surrogates (e.g., EPSA as correlate of Caco-2 permeability)]. Such assays can avoid the nonspecific binding and solubility issues commonly described. In addition, the AB-MPS score, a simple multiparametric scoring function predicting oral absorption, was also mentioned as an approach to evaluate the propensity of degraders toward acceptable bioavailability (DeGoey et al., 2018). One respondent noted that parallel artificial membrane

TABLE 2

Common reported issues and respective modifications for in vitro ADME assays investigating TPDs

Assay	Reported issues	Assay modifications	Comments
Plasma protein binding	Stability (chemical and/or enzymatic), solubility, nonspecific binding and recovery, failure to equilibrate, very low buffer compartment concentration	Plasma dilution, increased incubation time, use of ultracentrifugation, increased assay concentrations, increased number of replicates, addition of NaF to improve metabolic stability	
MDCK or Caco-2 permeability/ bidirectional transport assays	Solubility, nonspecific binding	Pre-incubation, increased organic content, addition of protein e.g., Bovine Serum Albumin or Fetal Calf Serum, and/or pH adjustment	PAMPA not recommended, monitoring of recovery and solubility in all assays
Solubility		Use of biorelevant media, e.g., FaSSIF, FeSSIF	Kinetic solubility not used
LogD	Dynamic range too low, solubility	Chromatographic LogD as alternative approach	
CL_{int}		Increased incubation times to achieve measurable turnover	Hepatocytes more reliable for IVIVE than microsomes

CL_{int}, intrinsic clearance; FaSSIF, fasted simulated intestinal fluid; FeSSIF, fed simulated intestinal fluid; PAMPA, parallel artificial membrane permeability assay.

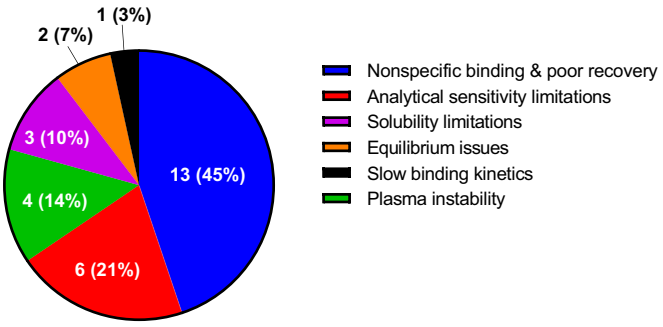

Fig. 8. Main challenges faced with measuring PPB of targeted protein degraders. More than one response was allowed for each respondent.

permeability assay was not recommended (reason not specified), yet literature both confirms and refutes the utility of parallel artificial membrane permeability assay for predicting permeability of TPDs in MDCK or Caco-2 cell monolayers (Klein et al., 2020; Scott et al., 2020).

Specifically for PPB, respondents adopted multiple PPB methodologies to address known assay challenges. Respondents' first choice for PPB methods was rapid equilibrium dialysis or high-throughput dialysis, with 65% of responses followed by ultracentrifugation (25%), traditional equilibrium dialysis (15%), and flux dialysis (5%) (Supplemental Fig. 2). Another method rarely used was ultrafiltration, likely due to the issues with nonspecific binding with this methodology. Of respondents, 55.6% suggested that their methods of measuring plasma protein binding for degraders was different than traditional small molecules; however, no indication was given as to whether the device used to measure plasma protein binding differed from those used for traditional small molecules.

Respondents were asked to list their top challenges associated with measuring PPB for TPDs, which are illustrated in Fig. 8. There were 29 responses (more than one response allowed) in total, with the most common challenge reported as high nonspecific binding to plasticware/dialysis membranes and associated poor assay recovery (45%), followed by analytical sensitivity limitations, particularly for very high binders (21%), plasma instability (14%), and finally, issues with not reaching equilibrium (7%) or due to slow binding kinetics (3%).

Additionally, respondents listed the general methodology adaptations employed to improve outcomes when measuring PPB of TPDs, many of these modifications were implemented to address the challenges noted above. These included using longer incubation times to achieve equilibrium with dialysis-based methods, adding sodium fluoride to plasma to mitigate plasma instability, and the use of diluted plasma or higher drug concentrations if permitted by solubility. The latter can be useful when there are limitations with analytical sensitivity, although care should be taken not to saturate plasma protein binding sites. Another respondent stated that they often used flux dialysis to measure PPB (Kalvass et al., 2018), which helps address the common issues caused by nonspecific binding, including the need for longer equilibrium times and the inability to measure unbound concentrations for highly bound drugs (TPDs often have PPB in the >99% range) when using equilibrium dialysis.

Chemical and metabolic stability of TPDs also can confound the interpretation of in vitro ADME assay results. Specifically, cereblon ligands such as thalidomide have been shown to undergo chemical hydrolysis at neutral pH and also species-dependent metabolic hydrolysis of the glutarimide ring (Lepper et al., 2006; Hoffmann et al., 2013). Rapid equilibrium dialysis with addition of metabolic inhibitors (e.g., sodium fluoride for preventing amide hydrolysis, mentioned by one respondent), flux dialysis, and ultrafiltration also provide options for measuring PPB of metabolically unstable TPD compounds (Kalvass et al., 2018; Toma et al., 2021). Many, if not all, of these assay modifications were originally implemented to address needs for non-TPD bRo5 compounds.

From a formulation perspective, a range of oral formulation strategies are routinely employed for initial PK studies across companies, mostly during lead discovery and lead optimization. Details of these strategies and the stage in which they are typically employed are shown in Supplemental Fig. 3. Based on the authors' experiences, these formulation strategies are comparable to traditional small molecules, particularly for those that are bRo5.

In Vivo Species Selection

Species selection for toxicology studies with traditional small molecules is usually accomplished by comparing in vitro profiling metabolism and metabolites and/or pharmacology in preclinical species to human (Prior et al., 2020; Namdari et al., 2021). The majority (11 of 17) of respondents indicated that a compound being a TPD did not influence their species selection for in vivo experiments. The most common reason given for selecting a specific species was pharmacology driven, either cross-species expression/selectivity of the E3 ligase or ability of the TPD to degrade the POI in the respective species. Other reasons included cross-species differences in linker or E3 ligand metabolic stability and metabolite profile, as well as species differences in oral bioavailability due to permeability or gastrointestinal fluid composition, suggesting that exposure of TPDs may also differ across species and play a role in species selection. Selecting a PK/PD species with pharmacology similar to humans for the target POI was critical for one respondent. The companion IQ safety TPD publication (Hemkens et al., 2023) has more details on this topic, but species selection for TPDs is not fundamentally different than traditional small molecules.

Design of Degraders to Optimize PK/ADME Properties. Respondents were asked if they had examined the contribution of components of TPDs (E3 ligand, POI ligand, linker) on the overall PK properties of the TPD. Seven of 18 (38.9%) indicated that they had not tried to evaluate component properties. Three of 18 (16.7%) indicated that they had compared properties of individual components to those of the TPD and were not able to establish a correlation. However, eight of 18 respondents (44.4%) examined the role of physical properties of individual components of the TPD and were able to make correlations. There is one report where metabolism of components was compared with the intact TPD, and in that report, metabolism of the capped components (attachment site capped by an alkyl group) often occurred on the cap structure and was not relevant to the TPD molecule (Pike et al., 2020). Based on this report, it is possible that the lack of correlation between properties of TPD and its components could be due to not being able to compare the best matched pairs, but it cannot be excluded that TPDs behave completely differently than the sum of its components.

Respondents were also asked if they had observed TPDs with improved PK properties relative to the components within the TPD. Of 11 responses, seven (63.6%) said no, whereas four (36.4%) said yes. This indicates that TPDs often do not have better PK properties than their components.

Respondents were asked to list how they have optimized components of TPDs. Nine responses were received. One respondent simply listed the three components of the TPD (ligase binder, linker, target binder). Three respondents listed ADME/physicochemical properties (permeability, HBD, MW, metabolic stability, solubility) without mentioning a specific component, whereas one additional respondent listed "transporter properties" as focus for improvement. Three respondents mentioned properties of the linker, specifically attachment of the linker to other components, linker length and type, linker lipophilicity, linker impact on

three-dimensional shape, and linker metabolic stability as important areas for improvement. Although only three respondents mentioned the linker for optimization of a TPD, 10 of 11 respondents (90.9%) indicated that improvement of the linker resulted in overall improvement of the entire TPD. Additionally, one respondent cited stability of ligase binding motif to hydrolysis as the focus for improvement.

Respondents were asked whether ADME properties or drug-ability were considered in the selection of the E3 ligase. Of the 18 responses, 14 (77.8%) indicated that yes, ADME and drug-ability were key considerations in selecting an E3 ligase, whereas four (22.2%) respondents indicated that ADME or drug-ability was not a key concern in selecting an E3 ligase.

Prediction of TPD Human PK

TPDs are just entering the clinic, so little has been publicly disclosed about the success of predicting human pharmacokinetics, specifically human clearance. The majority of respondents (14 of 17) indicated that their approach for prediction of human PK with degraders did not differ from that taken for traditional small molecules. Despite three of the 17 respondents indicating that an alternative strategy was employed, no alternative method to traditional strategies [i.e., in vitro–in vivo extrapolation (IVIVE), allometry, or physiological-based pharmacokinetic (PBPK) modeling] was specified. Interestingly, the most common strategy for human PK predictions was allometry, followed by IVIVE, with nine respondents indicating that they have used PBPK modeling (Fig. 9A). It is important to note that the questions on human PK prediction did not explicitly ask about approaches used for specific human PK parameters such as clearance (CL) or volume of distribution at steady-state (Vss), and it is possible, for example, that IVIVE could be used for CL prediction whereas allometry was used for Vss prediction, or vice versa, to generate final human PK predictions.

Achieving IVIVC when scaling in vitro intrinsic clearance or CL_{int} to in vivo CL for small molecules is desirable to increase confidence in predicting human clearance; however, IVIVC is often not observed when non-P450 clearance pathways are involved. In that case, allometry is usually the alternative approach, followed by or coupled with PBPK modeling, although all three can be, and often are, used simultaneously. IVIVE may be used by respondents less than allometry for TPDs because of involvement of alternative clearance pathways (non-P450 or transporter mediated) or because of the challenges with accurately determining the inputs for IVIVE. Microsomal, hepatocyte, and plasma protein binding can all be difficult to measure as binding is often high for TPD molecules and subject to experimental error from nonspecific binding, solubility, and stability. Also, low TPD permeability could potentially limit the use of hepatocytes for IVIVE. Nevertheless, good IVIVC has been reported (most within threefold) for a series of TPDs using mouse hepatocytes, indicating that with careful measurements, IVIVE can be a useful/valid approach for TPDs (Pike et al., 2020).

When asked if these standard preclinical approaches work as well for protein degraders as they do for typical small molecules, more than 75% of the 18 respondents suggested that there was not enough experience to compare based on currently available data (Fig. 9B). Indeed, disclosures of clinical pharmacokinetic data for degraders have been very limited to date. In response to a question from the parallel survey (Hemkens et al., 2023), only three respondents had active ongoing clinical trials, with the majority having clinical studies planned in the next 5 years (Fig. 9C). As more clinical data becomes available, this will be an important area of investigation to follow up on.

In Silico Tools and PBPK Modeling for Prediction of TPD ADME/PK

Twelve of the 18 respondents had some experience with using in silico/machine learning tools to predict ADME properties of degraders, with one respondent having extensive experience and whose experience suggested that they sometimes accurately predicted the ADME properties of TPDs.

In contrast, the use of PBPK for PK prediction appears limited, with 11 of the 17 respondents having no experience, approximately one-third having some experience, and one respondent having extensive experience. The one respondent with extensive experience reported that PBPK methods could accurately predict the PK of TPDs.

Discussion

The goal of the survey prepared by the IQ working group on the DMPK/ADME of protein degraders was to gain insight into the discovery and development practices and experiences across IQ member and non-member companies. The response data collected met this goal and allowed a comprehensive overview of the current state of TPD workflows.

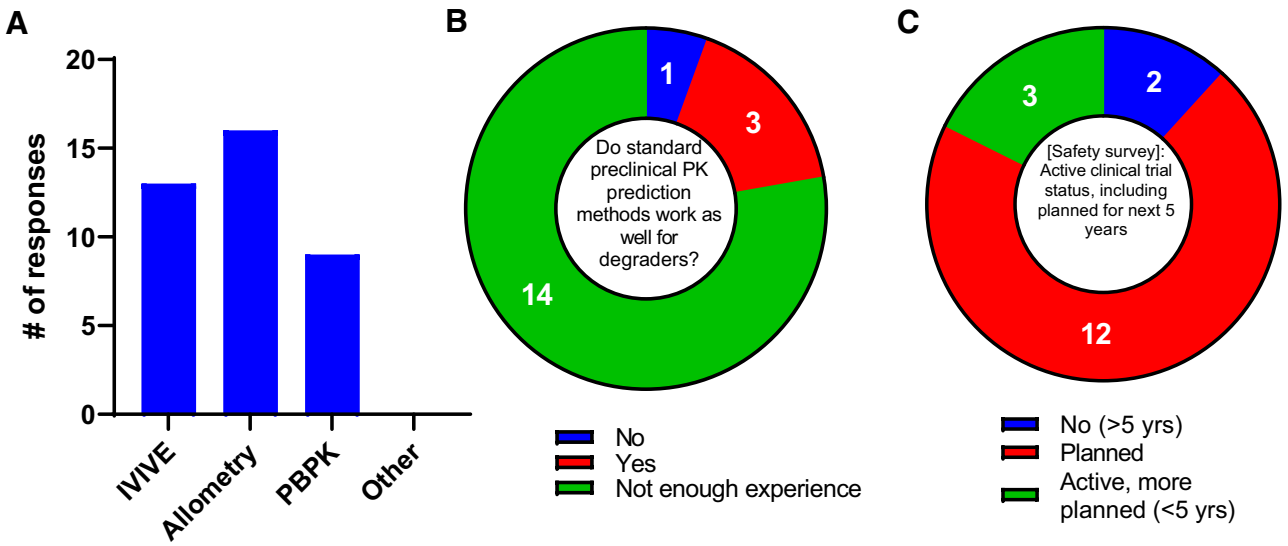

Fig. 9. Observations relating to the strategies for prediction of human PK of protein degraders: (A) strategies employed, (B) reliability, and (C) current active clinical status (Hemkens et al., 2023). More than one response was allowed per respondent for the question resulting in (A).

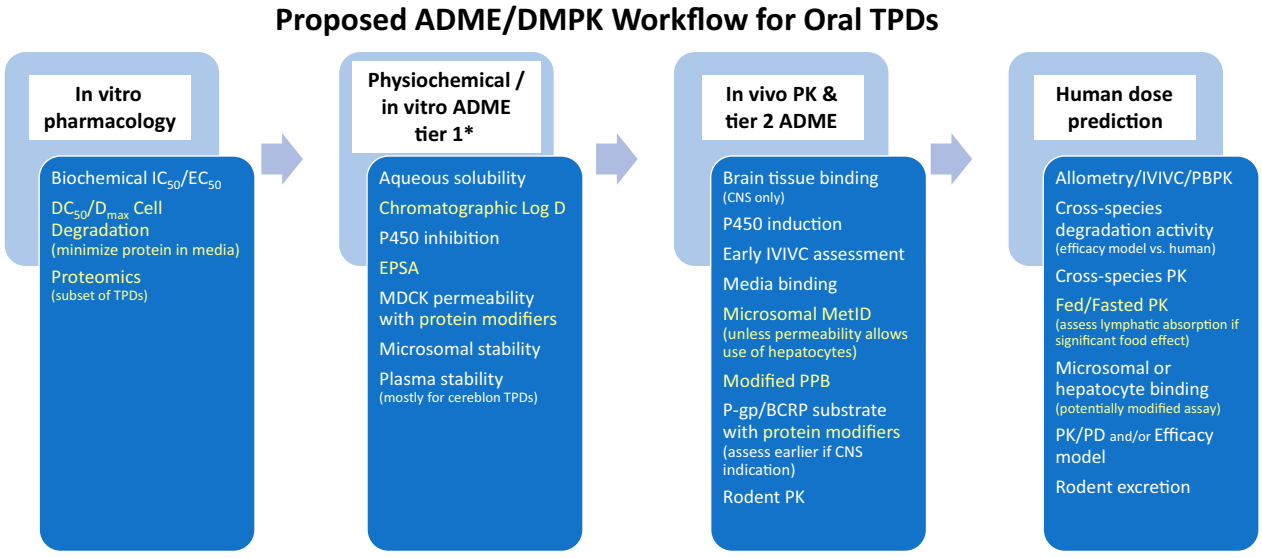

Fig. 10. Proposed ADME/DMPK workflow for oral TPDs. Assays differing or requiring modification for most TPDs compared with small molecules are highlighted in yellow. *An initial assessment of the utility of assays in the tier 1 category should be performed. If assays/data are not predictive of rodent PK and specifically oral bioavailability, assay data should not be gating for PK selection. But, if correlations develop later, assays should be added back as gating assays. Protein modifiers include but are not limited to bovine serum albumin or serum. DC_{50}, concentration with 50% target degradation.

The results are limited by the scope of the survey questions and the respondents' interpretation of the questions. Additionally, although nonmember companies were included in the survey to capture a wide range of TPD chemical and target space, it is possible that the survey responses did not reflect the most innovative methodologies and discoveries as proprietary approaches or observations may not have been shared.

Two main themes were apparent in the survey responses: 1) oral delivery was preferred and achievable with TPDs and 2) the DMPK/ADME evaluation of TPDs does not differ significantly from other bRo5 compounds. Although the hurdles for TPDs are significant due to the molecules falling outside Ro5 properties, most respondents are still pursuing oral delivery. Not unexpectedly for bRo5 compounds, permeability, solubility, transporter-mediated efflux, metabolic stability, and physiochemical properties such as HBD and lipophilicity were reported to be important for optimizing TPDs for oral delivery in preclinical species. To date, there is limited information available to know how well these preclinical optimization efforts translate to the clinic. Additionally, limited clinical PK data also precludes assessment of typical human PK prediction methods for TPDs to determine if they work as well as for traditional small molecules.

Previously unreported new information on TPDs was revealed as part of this survey. Due to the high MW and large PSA of TPDs, elimination of TPDs via biliary, intestinal, and particularly renal routes was not anticipated; however, several respondent companies have observed clearance by these pathways. Additionally, TPDs were not expected to be brain penetrant with $K_{puu,brain}$ values of 1, let alone 17 (mean $K_{puu,brain}$) as reported by one respondent, but three companies reported pharmacologically relevant brain exposures of TPDs. This respondent with the high $K_{puu,brain}$ observation confirmed that the result was observed across multiple studies, so it would appear that active brain uptake of TPDs is possible as this is the most plausible explanation for this observation. Active uptake or efflux of TPDs by transporters could be an explanation for biliary, intestinal, and renal elimination of TPDs as well as high unbound brain exposures, and further work is needed to determine if these transporter mechanisms can be optimized.

With the known ADME challenges of TPD molecules and increasing experience in industry and academia, survey questions were provided to respondent companies to determine if there were any learnings about TPD design to allow reduced drug design-test cycle times and rounds. Although most responses indicated that properties of the components and the TPD were related, several responses suggested that there was no correlation. A key series of responses on TPD optimization highlighted the importance of linker optimization for improving properties of the TPD, especially for the proper ternary complex formation between target protein, E3 ligase, and the degrader molecule. The linker can also alter MW, permeability via folding/PSA, metabolism, and many other aspects of on- and off-target pharmacology; therefore, significant focus should be placed on generating better linker starting points and rapidly evolving linker structure-activity relationships. One report investigated metabolism of the individual components compared with the intact TPD and observed that the majority of TPD metabolism occurred on the linker and that certain metabolic hot spots on the components were not metabolized as part of the TPD (Goracci et al., 2020). It is interesting that major metabolites of the ligands were not observed. This could be due to the chameleonic nature of TPDs, where the molecules can fold back upon themselves, thereby blocking sites of metabolism and exposing the linker for metabolism. Alternatively, linker metabolism may be so dominant that other secondary/ternary metabolites were not detectable. In addition to the linker, selection of the E3 ligase was highlighted by respondents as a key consideration when optimizing properties of TPDs. It is generally known that the prototypical von Hippel-Lindau E3 ligase (VHL) ligand imparts lower permeability and oral bioavailability compared with its cereblon ligand counterparts, in part due to its peptide-like structure. The data and experiences of respondents illustrate that there are specific optimization strategies that can be employed to move TPDs to the clinic more rapidly. A general workflow for oral TPDs is proposed in Fig. 10.

To overcome poor pharmacokinetic properties of bifunctional protein degraders, new delivery methods are emerging, such as through conjugating them to delivery vehicles like antibodies (Dragovich et al., 2021a,b). Progress has also been made in developing new nanoparticle formulation to improve oral bioavailability (Gao et al., 2022). These technologies could prove useful to deliver TPD molecules in the future.

Much has been accomplished in the last 20 years of TPD research, especially the last 5–10 years of highly active pharmaceutical discovery and

development of TPD molecules. Similar to any bRo5 compound, TPDs pose challenges for the ADME/DMPK community to achieve orally bioavailable compounds, but the promise of extended and potent pharmacology outweighs those challenges, especially for "classically" undruggable targets. As more TPDs advance into the clinic, the field will inevitably learn more about ADME/DMPK knowledge gaps that were identified as part of this IQ survey, which include the potential of CNS penetration; role of active transport, renal elimination, and lymphatic absorption; in silico/machine learning of degraders; and clinical translation, specifically regarding human PK prediction and drug-drug interactions. These IQ survey data are an important step to advance the field by sharing learnings and bringing together a larger set of experiences to inform rational drug design of bifunctional TPD drugs, thereby bringing medicines to the clinic more rapidly, but also suggests that additional work is needed.

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