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Aggregation and analysis of secondary pharmacology data from investigational new drug submissions at the US Food and Drug Administration

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ABSTRACT

Secondary pharmacology studies are utilized by the pharmaceutical industry as a cost-efficient tool to identify potential safety liabilities of drugs before entering Phase 1 clinical trials. These studies are recommended by the Food and Drug Administration (FDA) as a part of the Investigational New Drug (IND) application. However, despite the utility of these assays, there is little guidance on which targets should be screened and which format should be used. Here, we evaluated 226 secondary pharmacology profiles obtained from close to 90 unique sponsors. The results indicated that the most tested target in our set was the GABA benzodiazepine receptor (tested 168 times), the most hit target was adenosine 3 (hit 24 times), and the target with the highest hit percentage was the quinone reductase 2 (NQO2) receptor (hit 29% of the time). The overall results were largely consistent with those observed in previous publications. However, this study also identified the need for improvement in the submission process of secondary pharmacology studies by industry, which could enhance their utility for regulatory purpose. FDA-industry collaborative working groups will utilize this data to determine the best methods for regulatory submission of these studies and evaluate the need for a standard target panel.

1. Introduction

Secondary pharmacology studies are efficient and cost-effective measures to predict off-target drug effects and safety concerns (Bowes et al., 2012; Jenkinson, Schmidt, Rosenbrier Ribeiro, Delaunois, & Valentin, 2020). Often during the pre-clinical testing of potential drug candidates, molecules of interest are screened for activity at various secondary targets to determine the therapeutic target specificity and safety profile. This approach has been utilized by numerous pharmaceutical companies as part of their standard safety pharmacology screening strategy to assess potential liabilities during the forthcoming clinical trials (Papoian et al., 2015). Typically, the results of secondary pharmacology studies are included in the Investigational New Drug (IND) applications submitted to the Center of Drug Evaluation and Research at the US Food and Drug Administration (FDA). Their inclusion is recommended per International Council of Harmonisation (ICH) Guidance S7A to support the safety of new drugs (US Food and Drug Administration, 2001). Additional nonclinical studies or enhanced safety monitoring in proposed clinical studies may be voluntarily conducted by the applicant or required by the FDA upon review of these studies. For example, electrocardiograph monitoring could be requested during clinical trials for drugs that bind to cardiac-related targets, or additional animal studies or study endpoints focused on specific organ systems may need to be conducted in the case of targets associated with neurotoxicity or reproductive toxicity, among others (Papoian et al., 2017). By performing in vitro safety pharmacology profiling at different stages of the drug development process potential issues can be addressed before significant investment has been committed and further efforts can be implemented early to mitigate off-target effects (Bowes et al., 2012; Whitebread et al., 2016).

Abbreviations: 5-HT, 5-hydroxytryptamine; FDA, Food and Drug Administration; ICH, International Council of Harmonisation; IND, Investigational New Drug; μM, micromolar.

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ICH S7A does not give recommendations on the number or types of targets that should be profiled during secondary pharmacology studies. However, while the selection of targets is not universal across the industry, some pharmaceutical companies have published their methodologies for selecting targets (Bowes et al., 2012; Hamon et al., 2009; Lynch 3rd, Van Vleet, Mittelstadt, & Blomme, 2017). Targets are usually chosen based on their physiologic role and clinical implications, and while there exist overlapping screening strategies for certain targets, there remains a large range of variations in the nature and number of targets screened. However, despite these variations, there is general agreement that secondary pharmacology panel screens have significant implications for monitoring safety in the clinic (Hamon et al., 2009; Jenkinson et al., 2020; Lynch 3rd et al., 2017; Valentin et al., 2018).

Compiling secondary pharmacology data submitted at the IND stage of drug development may be a useful tool to identify trends in targets hit and their associated clinical liabilities. Herein, we have analyzed the different aspects of the in vitro pharmacology profiling in submissions to FDA. We have discovered which targets are being screened the most frequently, and among those, which ones are the most frequently hit along with their clinical significance. Moreover, we have brought attention to some of the issues that currently affect the utility of these profiles for regulatory assessment. In summary, we have established reliable methods to extract valuable information from an aggregated dataset of secondary pharmacology binding screen profiles, making the case that standardization of a submission format could greatly enhance the utility of these studies.

2. Methods

2.1. Identification of secondary pharmacology study reports and extraction of binding screen data

Secondary pharmacology study reports were identified from IND submissions received electronically in PDF format (i.e., Electronic Common Technical Document Section 4.2.1.2 Secondary Pharmacodynamics), by performing a keyword search of all study report titles using custom scripts written using the statistical computing language R (R Core Team, 2017). As these assays are typically in the same location as other safety pharmacology studies, including in vivo studies, a filter was set: study reports with a title that included the text strings “in vitro”, “panel”, “profil”, or “assay” were returned, and those with a title that included “admin”, “treat”, “modulat”, “effect”, “patient”, “serum”, “cell”, “induct”, “cytokine”, or “inflammation” were excluded. Additionally, any in vitro screens that did not contain a percent inhibition of control-specific binding, a measurement common in secondary pharmacology screens, were excluded. The returned study reports were stored in a separate folder for continued analysis.

Data tables containing targets, compound information, and binding data were extracted from each PDF file using the open source software tool Tabula (Aristaran, Tigas, Merrill, & Das, 2019) into CSV files. CSV files were manipulated into a standardized format and subsequently combined for additional analysis using custom R scripts.

2.2. Accuracy check and harvesting of additional information

Screening assay data was combined from all study reports selected from each IND and was then checked to ensure the accuracy of each extraction. Since multiple compounds are often tested in the same study report during the early stages of drug development and then subsequently submitted to FDA in an IND application, it was necessary to isolate the data associated with the investigational compound of interest. This was done by ascertaining the company code name and/or testing site code name and matching it with the submitted formula and molecular weight of the compound. Salts of the primary compounds that were submitted for in vitro testing were assumed to demonstrate similar activity features of the submitted drug, even if the submitted compound was a free base or a different salt from the substance that was tested. Additionally, since secondary pharmacology screening was performed by several different contracted companies, the receptor naming conventions were not always consistent across all submissions. As such, it was necessary to standardize the target names in order to properly aggregate the data.

Additional data was manually harvested from the IND applications and the FDA’s Global Substance Registration System (Peryea et al., 2021), including: molecule name or codename, therapeutic indication (subsequently mapped to Office of New Drugs division name), application status, sponsor, and submission date (when available).

Once the data was collected and checked for accuracy, each binding screen result was assigned a binary classification of either positive or negative. For this project, the industry standard was adopted in that a positive, or “hit”, was classified as possessing greater than or equal to 50% inhibition of control specific binding at the respective target with a drug concentration of 10 μM (Bowes et al., 2012; Jenkinson et al., 2020).

2.3. Collection of IC50 (Binding) and IC50/EC50 (Functional) assay data

The INDs were checked for additional binding assay data and functional assay data that were tested via a dose-response relationship. Data tables containing any binding (IC50 or Ki) or functional (IC50 or EC50) assay data were manually extracted and compiled into two tables. If a dose-response relationship was evaluated for binding activity and/or functionality, that information was extracted from each drug report and reported in molar concentration. The binding and functionality assays were then compared against the targets that received greater than or equal to 50% inhibition of control specific binding (“hit targets”) in their respective binding screen result. The correlation between targets “hit” and targets tested for binding and/or functionality was then assessed. If the binding or functionality assays reported a significant value, it was considered “positive” for binding or functionality. The binding and functional assay data that was reported was qualitatively assessed.

2.4. Concordance of hit rates with published data

The hit rates of targets provided within the secondary pharmacology study reports submitted across 226 IND applications were compared to those recently reported by Bowes et al. (Bowes et al., 2012). These authors reported the hit rates in discrete ranges (i.e., very high [> 20%], high [5–10%], medium [1–5%], or low [0–1%]). In our analysis, we calculated the hit rates of targets provided within the secondary pharmacology study reports as $\frac{n\ hits}{n\ target\ submissions}$. Only targets submitted in at least ten study reports (n target submissions >10) were compared. Targets listed in Bowes et al. with multiple classifications were combined into a single range; for example, low/medium were combined into 0–5%.

2.5. Target similarity analysis

Target hit similarity was evaluated using target hit responses across the submitted INDs to identify correlations between receptors. Due to the sparsity of hit responses, the analysis was limited to targets with at least five active responses. Similarity between targets was calculated using the Jaccard Distance, defined as $Jaccard\left(Target_i, Target_j\right) = \frac{\left|Target\_Hits_i \cap Target\_Hits_j\right|}{Total\_INDs}$, where $|Target\_Hits_i \cap Target\_Hits_j|$, represents the number of hits shared between $Target_i$ and $Target_j$, and $Total\_INDs$ represents the total number of INDs in the present study. Target similarity was visualized as a similarity network plot where targets are nodes and edges are the calculated Jaccard distances between them. The software package Gephi (https://gephi.org/) (version 0.9.2) was used to create, manipulate, and visualize the network similarity plot. To identify

clusters within the network, the Louvain modularity algorithm (Blondel, Guillaume, Lambiotte, & Lefebvre, 2008) available within Gephi was used with default parameters (i.e., resolution equal to 1).

3. Results

3.1. Identification of secondary pharmacology study reports and extraction of binding screen data

A total of 539 INDs were collected containing 968 individual compound screening assays. After data cleaning, a total of 226 INDs with assay information from the years 1986 through 2019 were usable for our analysis. A total of 15 drugs were approved as of 2021. These INDs contained 316 distinct G-protein coupled receptors (107), transporters (6), ion channels (24), enzymes (4), and other target types (15) that have been screened by 89 different firms. The submission year did not correlate with the number of targets tested, number of targets hit, or the hit rate (targets hit/targets tested), including before and after the Bowes et al. paper. Additionally, no correlation was seen between hit rate and approval status or the number of adverse events present on an approved drug's label.

The most common therapeutic areas represented in this study were oncology (39 applications), neurology (31 applications), and psychiatry (28 applications) (Fig. 1). At the time of this analysis in 2019, 135 (60%) IND applications were active, while the remaining 91 (40%) were not active (i.e. inactive, withdrawn, on hold, or terminated). Among therapeutic areas with at least 10 INDs, cardiovascular had the highest number of active applications (9/11, 82%) while endocrinology had the lowest (3/11, 21%).

Tables 1, 2, and 3 list the most commonly screened receptors, most frequently hit receptors, and receptors with the highest hit percentage among targets with at least 10 INDs screened. On average, drug companies screened 53 targets (range: 1–122 targets) and reported one to two hits per drug. Since the primary pharmacology was not indicated in the study reports for each molecule, the binding of a target greater than 99% at 10 μM was assumed to be indicative of the drug's primary mechanism of action. Most of the receptors associated with primary pharmacology had clinical implications in the central nervous system and the cardiovascular system. Of note, several targets with important clinical implications, such as mu opioid receptor and 5-HT_{2B}, known to be associated with addictive behaviors and cardiac valvulopathy, respectively (Roth, 2007; Trescot, Datta, Lee, & Hansen, 2008), were in the top ten receptors with the highest hit percentage. This discovery suggested that drugs allowed to reach Phase 1 still possessed inherent toxicity that will be important to screen against. We additionally evaluated testing rates (times tested/226 total targets) before and after the Bowes et al. study, and found that of the targets that had a change greater than 10% in testing rate, most had a reduced testing rate after Bowes, even targets that were included in their list (two had increased testing after Bowes et al., 13 had decreased testing). Information about all targets screened more than 10 times may be found in Supplementary Table 1.

3.2. Analysis of IC50 (Binding) and IC50/EC50 (Functional) assay data

Of the receptors that received 50% or more inhibition of control specific binding ("hit") in the binding screens, 254 tested positive for binding (had an IC50 value), three tested negative, and 287 were not tested. Receptors most commonly tested to obtain an IC50 value were the adenosine transporter, Cl^- channel, dopamine transporter, vascular endothelial growth factor receptor 2, and neurokinin 1 receptor. Moreover, 129 compounds had at least one "hit" from the secondary pharmacology assays that was not tested for binding. Conversely, 70 compounds were tested at least one target for an IC50 value that was not "hit" in the secondary pharmacology assays. In addition, there were 436 compound-target pairs that did not produce "hits" in secondary pharmacology binding screens but were still tested for binding. In examining the functional assays that were performed, of those "hit" in secondary pharmacology binding screens, five tested positive for functionality, two tested negative, and 537 were not tested for functionality. The most common targets tested for functionality were serotonin 5-HT_{2A}, serotonin 5-HT_{2B}, adrenergic α1A, and the mu opioid receptor. There were 296 additional compound-target pairs that were not "hit" in the secondary pharmacology screens but were still tested for functionality. Several of these targets were on the Bowes et al. list; however, multiple Bowes et al. targets were not tested for functionality at all in our dataset. Furthermore, 78 compounds had at least one "hit" from secondary pharmacology screens that was not tested for functionality. Conversely,

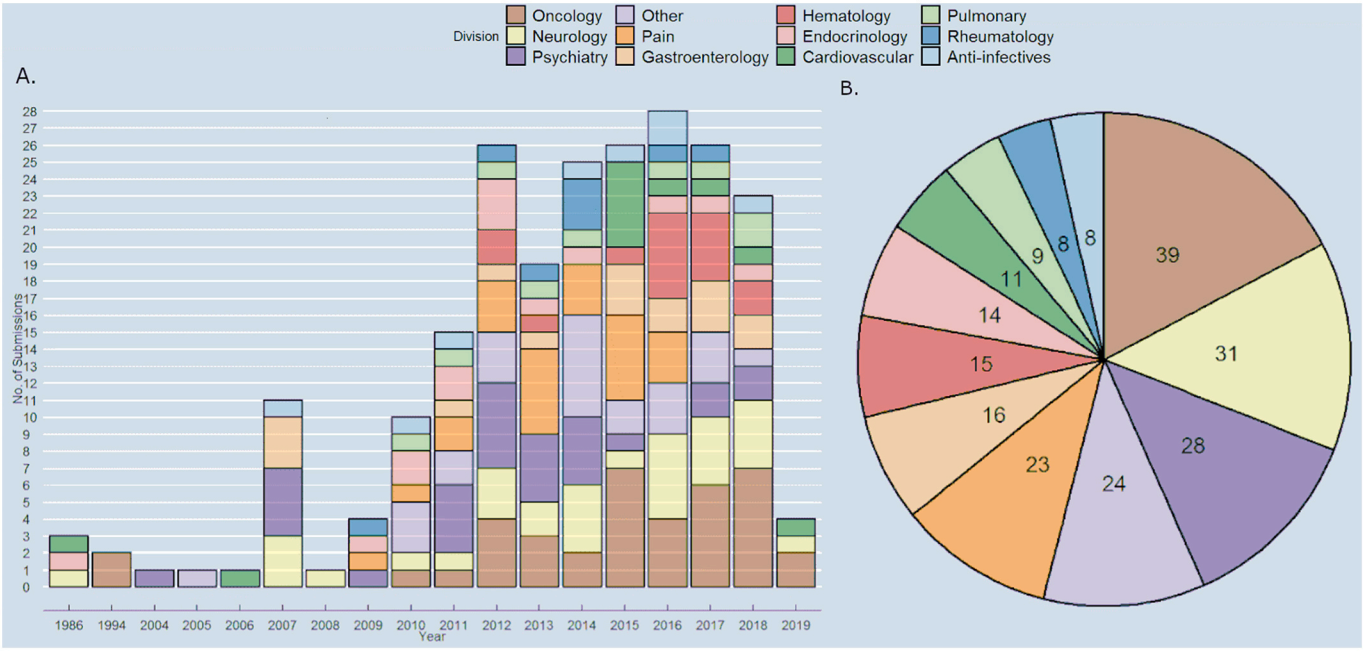

Fig. 1. Distribution of the 226 INDs by therapeutic area. (A) Division totals by year 1986–2019. (B) Cumulative division totals from years 1986–2019.

Table 1
Targets screened most frequently.

Most Tested Targets							
Receptor (Gene Name)	Receptor (Protein Name)	UniProt ID	#Tested	#Hits	Hit %	#Potential Primary Pharmacology (>99% Inhibition)	Clinical Implications
GABRA	Gamma-aminobutyric acid receptor subunit alpha	N/A	168	6	4	0	Central Nervous System (Lader, 2008)
SLC6A2	Norepinephrine transporter	P23975	165	13	8	0	Central Nervous System, Cardiovascular (Mayer et al., 2006)
SLC6A4	Serotonin transporter 1	P31645	154	7	5	1	Central Nervous System, Cardiovascular (Stahl, 1998)
SLC6A3	Sodium-dependent dopamine transporter	Q01959	154	18	12	0	Central Nervous System (Bannon, 2005)
HTR3	5-hydroxytryptamine receptor 3	N/A	153	3	2	0	Gastrointestinal, Central Nervous System (Thompson & Lummis, 2007)
DRD1	D(1A) dopamine receptor	P21728	153	6	4	2	Central Nervous System, Cardiovascular (Peacock & Gerlach, 2001)
CACNA1C	Voltage-gated calcium channel subunit alpha Cav1.2	Q13936	151	4	3	0	Cardiovascular, Central Nervous System (Lynch 3rd et al., 2017)
ADRB1	Beta-1 adrenergic receptor	P08588	150	1	<1	0	Cardiovascular, Gastrointestinal (Lohse, Engelhardt, & Eschenhagen, 2003)
CCKAR	Cholecystokinin receptor type A	P32238	150	5	3	0	Gastrointestinal (Okonkwo, Zezoff, & Adeyinka, 2020)
ADRB2	Beta-2 adrenergic receptor	P07550	149	2	1	0	Respiratory, Cardiovascular (Cazzola, Matera, & Donner, 2005)

A total of 226 INDs were screened at 316 receptors. A screen was defined as an assay performed at that target, regardless if the assay was positive or negative for binding. All targets displayed in this table were screened in a minimum of 10 INDs.

Table 2
Targets with the greatest number of hits.

Top Targets with the Greatest Number of Hits (>50% Inhibition)							
Receptor (Gene Name)	Receptor (Protein Name)	UniProt ID	#Tested	#Hits	Hit %	#Potential Primary Pharmacology (>99% Inhibition)	Clinical Implications
ADORA3	Adenosine receptor A3	P0DMS8	115	24	21	0	Central Nervous System, Respiratory (Chen et al., 2012)
CFTR	ATP-binding cassette sub-family C, member 7	N/A	144	23	16	1	Central Nervous System (Belelli et al., 2019)
SCN2A	Voltage-gated sodium channel subunit alpha Nav1.2	N/A	145	22	15	2	Central Nervous System (Catterall & Waxman, 2019)
HTR2B	5-hydroxytryptamine receptor 2B	P41595	129	20	16	0	Cardiovascular, Respiratory, Congenital (Roth, 2007)
OPRM1	Mu-type opioid receptor	P35372	128	19	15	5	Central Nervous System, Gastrointestinal, Cardiovascular (Trescot et al., 2008)
SLC6A3	Sodium-dependent dopamine transporter	Q01959	153	18	12	0	Central Nervous System (Bannon, 2005)
N/A	Sigma receptor non-specific	N/A	92	18	20	1	Central Nervous System (Guo et al., 2015; Sanchez et al., 1997)
OPRK1	Kappa-type opioid receptor	P41145	144	17	12	3	Gastrointestinal, Central Nervous System, Cardiovascular (Walsh, Strain, Abreu, & Bigelow, 2001)
MTNR1A	Melatonin receptor type 1A	P48039	120	16	13	2	Central Nervous System (Liu et al., 2016)
OPRD1	Delta-type opioid receptor	P41143	141	15	11	2	Central Nervous System, Cardiovascular (Barron, 2000)
TACR2	Substance-K receptor	P21452	122	15	12	1	Gastrointestinal, Immune (Lofgren, Qi, & Lundeberg, 1999; Tramontana, Maggi, & Evangelista, 1994)

A hit was defined as >50% inhibition of control specific binding at 10 μM. All targets displayed in this table were screened in a minimum of 10 INDs.

65 compounds tested at least one target that was not "hit" in the secondary pharmacology screens for functionality. After examining the targets tested for IC50 binding assays against EC50/IC50 functional assays in each report, it was concluded that there was no observable quantitative correlation between the two data sets.

3.3. Concordance of hit rates with published data

While several recent publications have published panels of potential targets suitable for screening (Bowes et al., 2012; Hamon et al., 2009; Lynch 3rd et al., 2017), only Bowes et al. provided the relative hit rates (i.e., the rate at which a target will produce greater than 50% inhibition at 10 μM), allowing comparison against the current study results . The hit rates of targets provided within the secondary pharmacology study reports submitted in 226 IND applications were compared to the hit rates of the 44 targets provided by Bowes et al. For each of these 44 targets, Bowes et al. classified their hits rates as being either very high (>20%), high (5–10%), medium (1–5%), or low (0–1%). Of the 44 targets in Bowes et al.'s publication, 42 were tested within at least one of the 226 IND applications, and 32 targets were tested in at least 10 applications. The hit rates of these 32 targets were compared to the classification ranges proposed by Bowes et al. (Fig. 2). The majority (22/33)

Table 3
Targets with the highest percentage of hits.

Targets with the Greatest Hit% (#Hits/#Tested)							
Receptor (Gene Name)	Receptor (Protein Name)	UniProt ID	#Tested	#Hits	Hit %	#Potential Primary Pharmacology (>99% Inhibition)	Clinical Implications
NQO2	Quinone reductase 2	P16083	24	7	29	0	Central Nervous System (Oxenkrug, Bachurin, Prakhie, & Zefirov, 2010)
ADRA1D	Alpha-1D adrenergic receptor	P25100	14	4	29	2	Cardiovascular (Jensen et al., 2009)
TMEM97	Sigma-2 receptor	Q5BJF2	15	4	27	2	Central Nervous System (Sanchez et al., 1997)
ADORA3	Adenosine receptor A3	P0DMS8	115	24	21	0	Central Nervous System, (Chen et al., 2012)
ADRA1A	Alpha-1A adrenergic receptor	P35348	50	10	20	1	Cardiovascular, Gastrointestinal, Central Nervous System (Michelotti, Price, & Schwinn, 2000)
N/A	Sigma receptor non-specific	N/A	92	18	20	1	Central Nervous System (Guo et al., 2015; Sanchez et al., 1997)
KCNH2	hERG	Q12809	21	4	19	0	Cardiovascular (Rampe & Brown, 2013)
N/A	Muscarinic acetylcholine receptor non-selective	N/A	12	2	17	0	Central Nervous System (Carlson & Kraus, 2020)
CFTR	ATP-binding cassette sub-family C, member 7	N/A	144	23	16	1	Central Nervous System (Belelli et al., 20199)
HTR2B	5-hydroxytryptamine receptor 2B	P41595	129	20	16	0	Cardiovascular, Respiratory, Congenital (Roth, 2007)
SCN2A	Voltage-gated sodium channel subunit alpha Nav1.2	N/A	145	22	15	2	Central Nervous System (Catterall & Waxman, 2019)
OPRM1	Mu-type opioid receptor	P35372	128	19	15	5	Central Nervous System, Gastrointestinal, Cardiovascular (Trescot et al., 2008)

A hit was defined as >50% inhibition of control specific binding at 10 μM. Hit percentage was defined as the number of hits/the number of compounds tested at each target. All targets displayed in this table were screened in a minimum of 10 INDs.

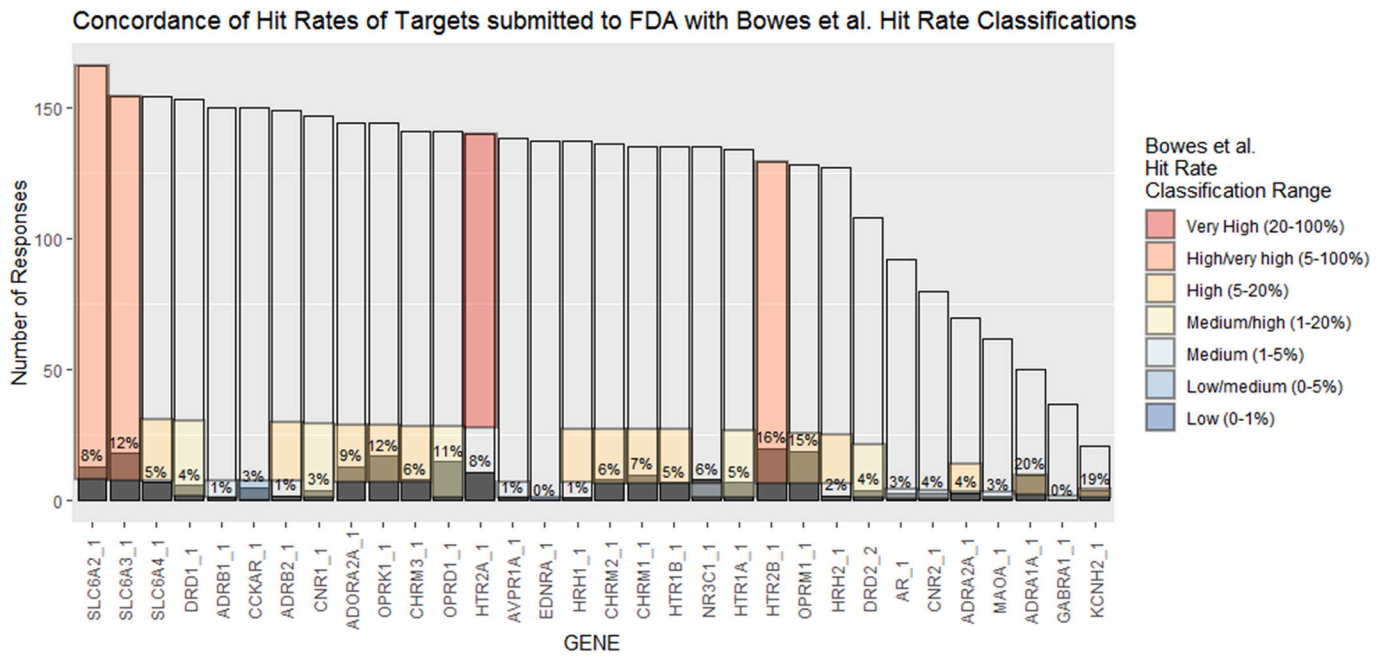

Fig. 2. Concordance of hit rates of targets submitted via IND applications to those proposed in Bowes et al. Transparent bar heights correspond to the number of compounds tested with black bar heights corresponding to the number of compound hits with hit rate labeled just above. The colorized overlapping bars correspond to the relative Bowes classification range.

of these targets fell within their respective Bowes classification range. Nine of the ten misclassified targets had hit rates lower than those classified, but more than half (5/9) were within 1% or less of their proposed range. Notably, the 5-HT_{2A} receptor had the largest difference with the Bowes classification (very high, >20%) compared to the hit rate of responses submitted to the FDA (8%). Only one target showed a higher hit rate in FDA data (6%) than in the Bowes classification (1–5%), the glucocorticoid receptor (NR3C1), which is involved in regulating metabolic functions including glycemic control and weight (McMaster & Ray, 2008).

3.4. Target similarity analysis

To identify similar targets and assess concordance among the receptor families, we calculated similarities using the Jaccard distance via the submitted target hit profile (limited to targets with at least five hits across the 226 IND submissions, see Methods). Upon visually inspecting individual target hit profiles, there appeared to be good concordance within families of receptors (e.g., muscarinic acetylcholine receptors, Fig. 3A). In order to determine this empirically, the Jaccard distances between all 35 targets were represented as a network graph and

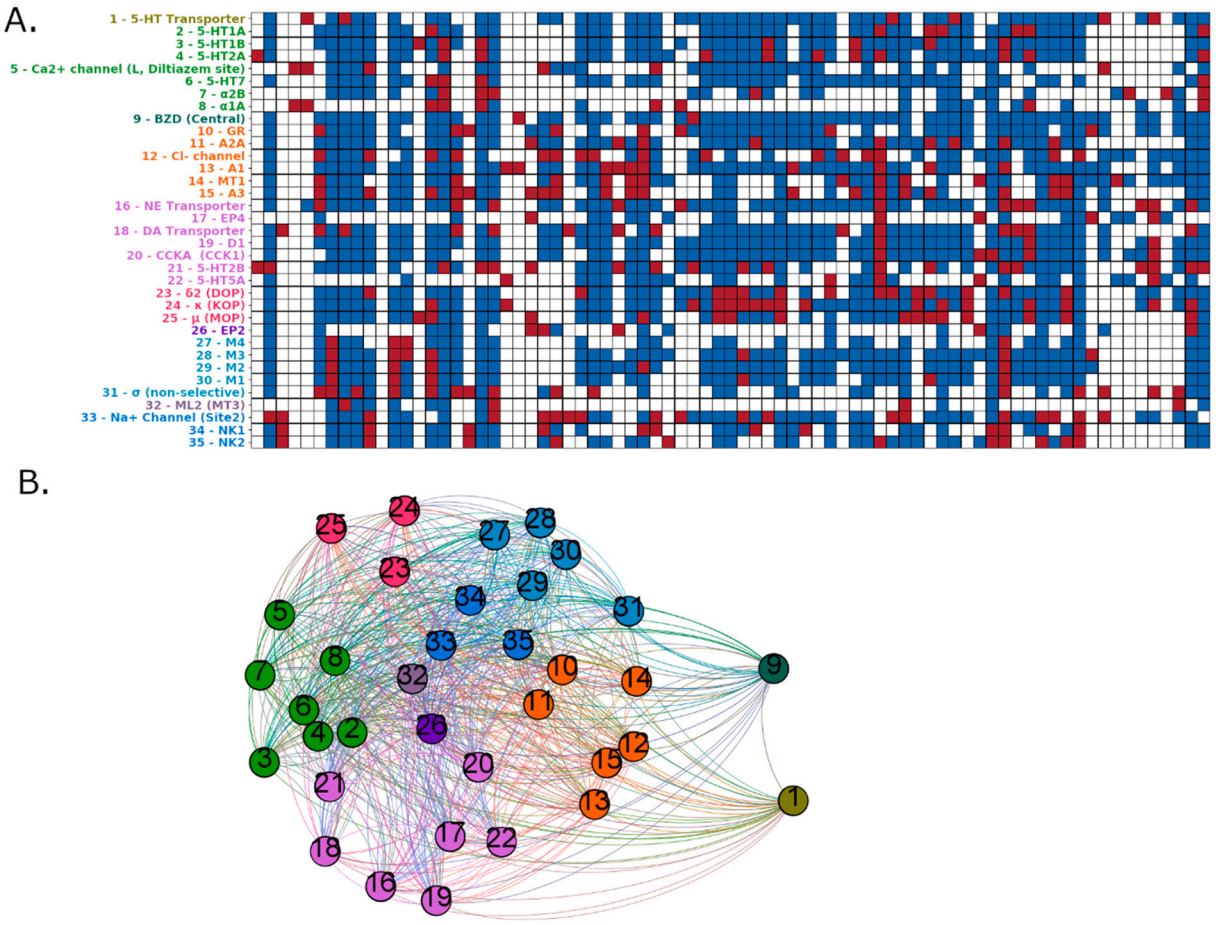

Fig. 3. Target similarity based on hits across IND submissions. (A) Heatmap of 35 targets (rows) with more than five hits across IND submissions (columns), Red: Hit, Blue: Tested, not a hit, White: Untested. (B) Network similarity plot of 35 targets colored by modularity class. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

visualized (Fig. 3B). Using the Louvain modularity algorithm, the 35 targets could be clustered into 9 distinct colored groups (Fig. 3A and B). Most receptors of the same family were clustered into the same group (e.g, muscarinic acetylcholine receptors and adenosine receptors). Notably, the serotonin receptor family was separated into two groups (5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2A} in green and 5-HT_{2B}, 5-HT_{5A} in pink).

4. Discussion

In the current study, secondary pharmacology study reports were examined from 226 different IND applications. Among these applications, 60% were currently active and 17.2% were indicated for oncology, the top indication in this set. The most screened target in our set was the GABA benzodiazepine receptor, the most hit target was adenosine 3, and the target with the highest hit percentage was the NQO2 receptor. Additionally, fewer than half of the hit targets were tested for an IC50 value, although several non-hit targets were tested. Finally, the results of the current study demonstrated high concordance with similar studies in the published literature.

Bowes et al. published a list of targets that had been compiled by four major pharmaceutical companies as the 44 primary targets linked to clinical liabilities that all sponsors should consider testing during preclinical development before entering Phase 1. To date, their analysis is the most comprehensive analysis of secondary pharmacology testing and binding screen data publicly available. However, we found that few of their recommendations have been implemented. We did not identify any significant changes to screening panels before and after Bowes et al. While there was significant overlap between Bowes et al. and the current study, there were other receptors not listed in Bowes et al. that are currently commonly screened. Perhaps more interestingly, many of the receptors with high hit rates in our findings were not listed on the Bowes et al. proposed list, with only two of the top 10 receptors with the highest hit rate being singled out for recommended screening. For consideration, not all of the candidates evaluated in Bowes et al. may have reached IND stage. The current study only examined data that has been submitted to FDA within IND applications, and therefore it is possible that some compounds (and their respective target profiles) with unfavorable safety, efficacy, or pharmacology profiles have been excluded from the current analysis. For example, Bowes et al. noted that the companies frequently tested and hit the 5-HT_{2A} receptor. As this receptor has clinical implications in psychiatry, it is possible that a positive hit at this target results in a company choosing not to advance the compound further in development, which results in fewer hits in our dataset. Therefore, while it is interesting to note the similarities and differences between the results, it is difficult to directly compare the methodologies and results.

This study has illuminated current concerns with secondary pharmacology submissions for regulatory review, in agreement with previous studies (Bowes et al., 2012; Jenkinson et al., 2020; Valentin et al., 2018). In this study, it was difficult to perform analysis on this set, as there is currently limited standardization in data location, target names, or table structure as the data is submitted in PDF format. Many screening assays are performed with other drug candidates and the products are given code names, resulting in the need to utilize other files to determine which candidate is the drug of interest. All of these factors made automatic extraction and input into a single dataset difficult and time

consuming. In alignment with Papoian et al.'s, 2015 review, it is likely that the regulatory review process would benefit from format standardization and utility of a common nomenclature, and this study supports the conclusion from Bowes et al. that a small, standard panel of safety-based targets may assist industry and regulatory reviewers in identifying potential safety concerns early. Multiple collaborative working groups involving FDA, the pharmaceutical industry, and other stakeholders are currently exploring methods to improve the submission of secondary pharmacology data. The data generated in this study along with other published data (such as Bowes et al.) will inform these efforts to potentially update the format for secondary pharmacology submissions and identify if there are certain targets that should always be tested in a screening panel.

This study had several limitations. First, only small molecules were evaluated in this study as larger molecules, such as biologics, were excluded. Additionally, the current study was limited by the number of applications that were available in the FDA's Electronic Document Room (EDR), which only began receiving electronic, PDF-formatted submission documents in 2004. Therefore, most submissions before this period were not included, except for a small subset of applications or studies received before this date that were scanned into the EDR at a later time. We also limited the study to in vitro secondary pharmacology data that was available in the EDR folder 4.2.1.2 Secondary Pharmacodynamics. Manual inspection occasionally found these binding studies in other EDR folders. Additionally, as mentioned above, data were limited to what was submitted to FDA, and compounds with unfavorable profiles may have been excluded, resulting in slight differences between our set and the Bowes set. Finally, we used the industry standard of 50% inhibition at 10 μM as the cutoff for a hit, which may not correlate directly with in vivo models or clinical effects. While 50% is the industry standard, others have discovered that hit rates between 30% and 50% may also hold biological relevance (Bowes et al., 2012; Jenkinson et al., 2020). While we plan to mitigate some of these factors with an expanded manual curation and analysis effort, the ongoing collaborations between the pharmaceutical industry and the FDA to standardize the structure of secondary pharmacology study data submissions will significantly improve the breadth and depth of similar studies in the future.

Overall, this is the first large scale investigation of secondary pharmacology data submissions across nearly 90 pharmaceutical companies. We noted that while individual pharmaceutical companies often had a standard panel of targets that were screened, these panels were not consistent across companies. Additionally, several recommendations from Bowes et al. have yet to be incorporated into screening practices, supporting the need for submission format and target panel standardization. We intend to expand this dataset via manual curation and utilize this data for better understanding clinical liabilities linked to off target effects. Additionally, with this larger dataset we plan to further investigate the association between secondary pharmacology and IND status. In the meantime, our analysis has shed light on potential best practices for submissions of secondary pharmacology data for review by the regulatory authorities. Many efforts to standardize secondary pharmacology screening and submissions have been made previously by both FDA and the pharmaceutical industry, and cooperative working groups are ongoing between FDA, industry, and other stakeholders to identify best practices. The results in this study will inform these collaborations and will improve early identification of potential safety liabilities in drug development.

Author contributions

MS, KS, RR: Designed Research; AD, KM, DR, CS, KS, RR: Performed Research and Analyzed Data; AD, KM, DR, CS, MS, KS, RR: Wrote Manuscript.

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Disclosure

This article reflects the views of the authors and should not be construed to represent the views or policies of the FDA. The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

Data availability

Due to the sensitive nature of the data in this analysis, individual assay or drug data is not publicly available. A full list of targets that were screened more than 10 times and their associated data is available in the supplemental file.

Declaration of Competing Interest

All authors declare no conflicts of interest.

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Appendix A. Supplementary data

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