The Translational Value of Secondary Pharmacology Binding Assays for Nonclinical Findings

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The findings and conclusions in this presentation reflect the views of the authors and should not be construed to represent FDA's views or policies.

Abstract

Secondary pharmacology assays are widely used by the pharmaceutical industry to determine the safety profile of a drug before entering clinical trials. The studies involve an *in vitro* assessment of a small molecule's reactivity with targets other than the primary receptor. There is currently a lack of regulatory guidance on how secondary pharmacology assays should be conducted and used for risk mitigation and hazard identification. The aim of this work is to align secondary pharmacology data with nonclinical toxicity findings from Investigational New Drug (IND) applications and clinical data from New Drug Applications (NDAs) in the near future. Secondary targets were associated to the nonclinical toxicity findings in various organs. The results indicated that many organ toxicities showed little to no correlation (Area Under the Curve (AUC) <0.7) with secondary pharmacology assays. However, target-organ pairs with known clinical or nonclinical association greatly outperformed those with no known association, particularly at the current industry threshold of 50% inhibition. This demonstrates that secondary pharmacology results can be useful for identifying some nonclinical toxicity early in development. Once the analysis of clinical findings is complete, we anticipate that these results will inform secondary pharmacology assay selection during early drug development as well as regulatory interpretation during the drug review process.

Introduction

Secondary Pharmacology Assays

- Safety profiling of drug candidates against a broad range of off-targets
- Many measure the percent inhibition of control-specific binding at the respective target with a drug concentration of 10 mM
- Prioritize candidates with similar efficacy but different clinical safety liabilities
- Allows for the assessment of translatability of secondary targets associated with adverse drug reactions

Use in Industry

- Significant agreement that secondary pharmacology assays can assist with hazard identification and risk mitigation during drug development
- Data is typically submitted in an IND application
- Guidelines produced by the International Conference on Harmonisation (ICH) describe the safety testing of new drugs. ICH S7A was designed to prevent clinical liabilities

Our Goal

- Use secondary pharmacology assays to associate off-targets with drug toxicity in nonclinical toxicity findings
- Determine the predictive performance of secondary pharmacology for toxicity in nonclinical studies
- Identify the translational value of secondary pharmacology assays to clinical adverse events
- Provide information for harmonization of secondary pharmacology assays

Materials and Methods

Data Acquisition

- 3,830 nonclinical studies and 1,120 secondary pharmacology assays from safety reports in IND application

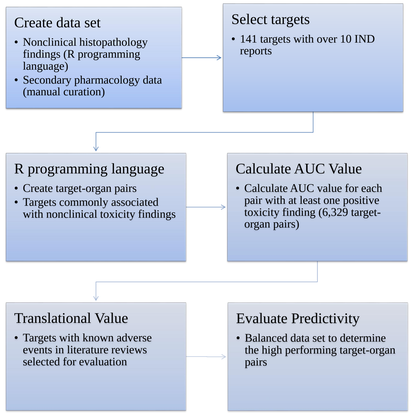

Figure 1. Workflow Diagram. Determination of target-organ pairs for evaluation of secondary pharmacology predictivity for nonclinical toxicity findings

Evaluating predictivity using R programming language

- Data sets merged by IND application number in Excel file
- Off-targets with at least 10 study reports (n target submissions >10) selected for analysis
- At least one positive toxicity finding was required to create a target-organ pair through a custom R script using the dplyr package
- Each target-organ pair returned a ROC curve with an AUC value used as an evaluation metric for predictive performance
- Translational value determined by linking the nonclinical study results to the organ systems reviewed in literature (Lynch et al., 2017)
- Further assessment of the predictive performance compared AUC values to AUC values with a percent inhibition cut-off greater than or equal to 50% inhibition

AUC Performance Category	AUC Value
Good Performance	Plots with AUROC values ≥0.8 indicates secondary pharmacology assays have a good predictive value
Fair Performance	Plots with AUROC values 0.7<AUC<0.8 indicates secondary pharmacology assays have a fair predictive value
Poor Performance	Plots with AUROC values 0.6<AUC<0.7 indicates secondary pharmacology assays have poor predictive value
Failed Performance	Plots with AUROC values AUC<0.6 indicates secondary pharmacology assays have failed performance

Results and Discussion

Predictive Performance

- Of the pairs in the good performance category, several of these pairs were identified with one or two positive toxicity findings, which suggests the dataset is imbalanced (Figure 2)
- The good performance category shows over 80% of the category has target-organ pairs that have 0-10% of positive toxicity findings.

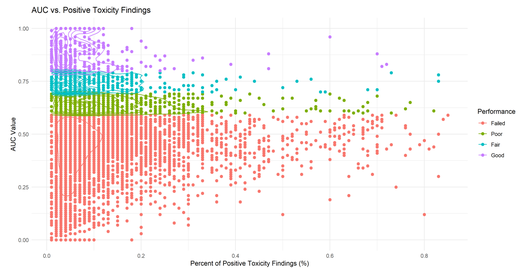

Figure 2. Imbalanced Class Distribution. There is a large imbalance in the ratio of the number of tested targets with positive toxicity findings. 569 (8.9%) target-organ pairs fell into the good performance category. 501 (7.9%) target-organ pairs had fair performance. 4,406 (69.6%) target-organ pairs fell into the failed performance. 853 (13.5%) target-organ pairs had poor performance.

Translational Value

- A total of 3,016 target-organ pairs were created from 59 associated targets and tagged as associated (855) or not associated (2,161)
- Compared target-organ pairs with balanced data (30-70% positive toxicity findings) and at 50% inhibition threshold (industry standard) for performance of the target-organ pairs
- More target-organ pairs with a known association in Lynch et al fell into the good performance category (31.3%) compared to those without a known association (19.5%) (Figure 3)
- The 50% inhibition threshold resulted in more balanced target-organ pairs (206 balanced pairs when no cutoff was used vs. 252 balanced pairs when a 50% inhibition threshold was used)

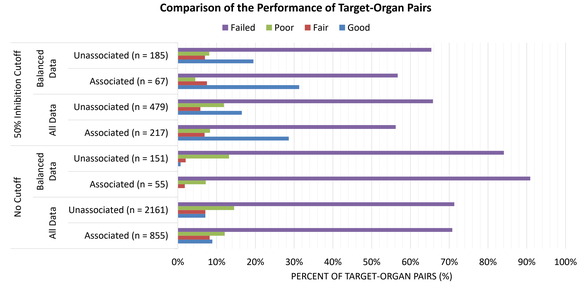

Figure 3. Cut-off Comparison**.** By decreasing the number of tests for a particular target with the 50% inhibition cut-off, primarily negative toxicity findings are eliminated and result in a more balanced ratio of positive toxicity findings to the total number of tests (IND applications).

- The effects of the 50% inhibition cutoff are demonstrated in Table 1, which includes the 217 pairs that were associated in Lynch et al when a 50% cutoff was used
- 20 pairs that demonstrated failed performance without a cutoff demonstrated good performance when a cutoff was used
- 13 pairs that demonstrated good performance without a cutoff demonstrated failed performance when a cutoff was used
- The 50% cut-off often altered the balance of positive toxicity findings and distribution with relation to the percent inhibition.

		50% Inhibition Cut-Off			
		Good Performance	Fair Performance	Poor Performance	Failed Performance
No Cut-Off	Good Performance	**14**	1	3	13
	Fair Performance	12	**2**	1	8
	Poor Performance	16	1	**4**	19
	Failed Performance	20	11	10	**82**

Table 1. Change in Performance. Target-organ pairs below the bold values show an increase in performance and those above the bold values show a decrease in performance. This shows the potential of applying a cut-off where pairs performing well with the cutoff benefiting from the current industry standard and pairs performing poorly potentially benefiting from further exploration of the optimal threshold to better capture positive data.

Conclusion

- Target-organ pairs with a known association performed better than those that are not associated when the industry standard 50% inhibition threshold was applied
- Future studies will further evaluate the relationships identified here and how this information can be used to enhance secondary pharmacology practices
- Additionally, future studies will evaluate the optimization of the percent inhibition threshold for high performing target-organ pairs
- Finally, an examination of the correlation of clinical data and secondary pharmacology can enhance the safety monitoring of clinical studies and mitigate off-target effects

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This project was supported in part by an appointment to the Research Participation Program at the Office of New Drugs/Center for Drug Evaluation and Research, U.S. Food and Drug Administration, administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and FDA.