Computational Prediction of Off-Target Related Risks of Molecules: Cardiotoxicity, Hepatotoxicity and Reproductive Toxicity

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Introduction

Drug efficacy and toxicity are controlled by interplay of pharmacokinetic, pharmaco-dynamic and genetic factors. Most pharmaceutical compounds are active against **more than a single target**. Off-target profiling is a recently established tool to identify critical liabilities, which can lead to drug toxicity. Combined in silico/in vitro profiling strategies are found to be most effective when conducting compound safety assessments.

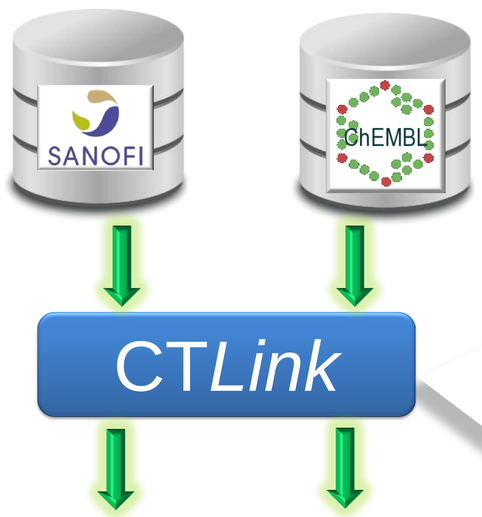
In-silico off-target profiling

We have developed a tiered strategy to optimally support drug safety profiling. Computational models for numerous off-target interactions allow for systematic prediction of drug-target interactions. Target engagement in adverse pathways can be analyzed with pathway databases, supporting the toxicity risk assessment and mode-of-toxicity evaluation of drugs.

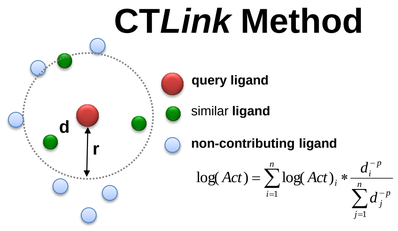
Tier I: Off-Target Prediction

Concept: Chemo-centric mining of protein-ligand interactions

- Sanofi and public bioassay data (CHEMBL, Iuphar, PDSP, BindingDB)
- **Chemotargets CT*Link* as a platform** to build and apply predictive off-target models
  - Activity prediction using kNN models with distance weighting interpolation



CT*Link* Method



Validation*

		Predicted: inactive	Predicted: active		
Actual	inactive	1019	234	1253	Accuracy: 81.1%
	active	43	171	214	Error: 18.9%
		1062	405	1467	Sensitivity: 79.9%
					Specificity: 81.3%

***81.1% predictive accuracy based on 1467 Sanofi samples**

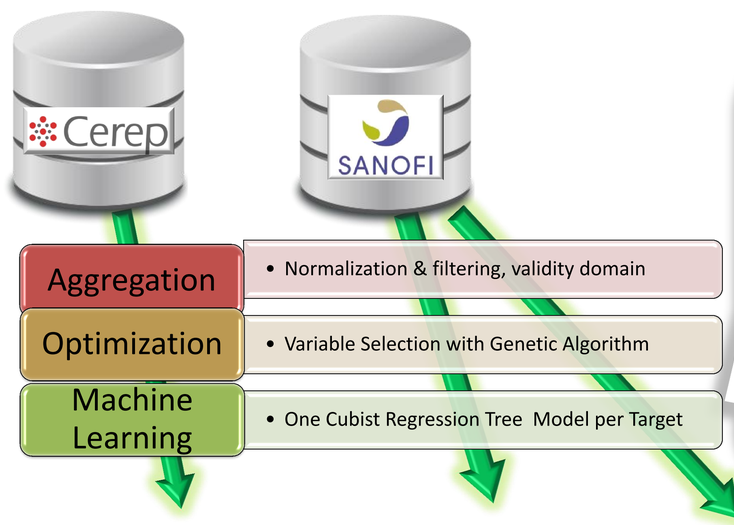
Sanofi		Public	
Models:	1,811	Models:	4,229
Cpds:	557,000	Cpds:	358,169
Interactions:	2,207,000	Interactions:	808,057

The model applicability domain can be substantially enhanced by integration of Sanofi in-vitro data.

Tier II: Off-Target Profiling by QSAR

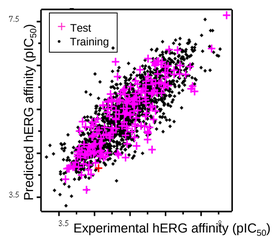
Concept: Global QSAR models for critical off-Targets

- Sanofi and CEREP bioassay data
- ***Cubist* regression trees** based on *MOE* descriptors
  - Evaluation of applicability domain by similarity to training set



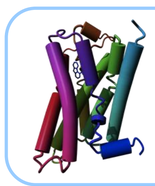
Cubist Method

Rule-based decision tree
Terminal nodes with linear models



Example: global hERG inhibition
r^2: 0.72, pred. r^2: 0.67

In-silico Lead Side Effect Profile

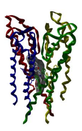


LSE1 Models: 33
LSE2 Models: 74
LSE3 Models: 109

In-silico Kinase Profile

Core Models: 60
Ext Models: 135

Ion Channels



Models: 9

Tier III: ADR Prognosis based on Biological Networks and Mining in Pathway Databases

Concept: Pathway mining to identify relationships between predicted off-targets and modes of toxicity for novel drug candidates

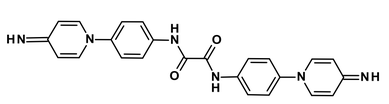
- Particularly for cardiac and reproductive toxicity, causal relationships between off-target interaction and mode of toxicity can often be assumed

IPA® (Ingenuity), MetaCore®, WikiPathways, Reactome are systematically exploited for off-target engagement in adverse drug reactions

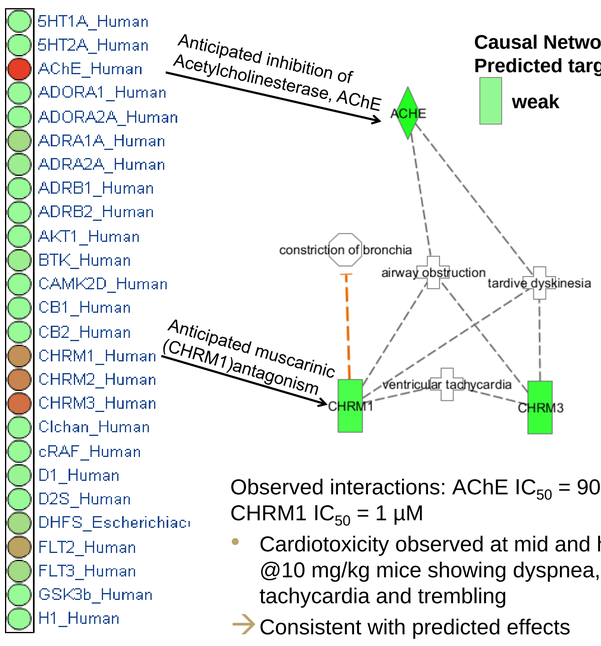
- Typical off-target activity threshold <10 µM to focus on effective interactions

Cardiotoxicity

Antifungal Lead Compound
CT*Link* off-target heatmap (part)



10 µM IC50/EC50/KI 0.1 µM



Causal Network
Predicted target inhibition
weak strong

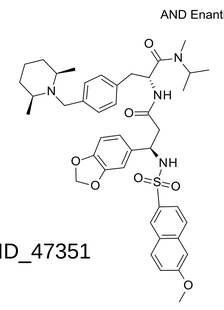
Observed interactions: AChE IC_{50} = 90 nM, CHRM1 IC_{50} = 1 µM

- Cardiotoxicity observed at mid and high dose, @10 mg/kg mice showing dyspnea, tachycardia and trembling

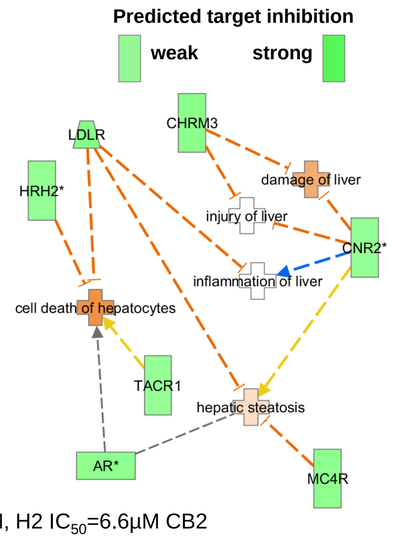
→ Consistent with predicted effects

Hepatotoxicity

Bradykinine B1 antagonist
Analysis of predicted interactions



Predicted target inhibition
weak strong



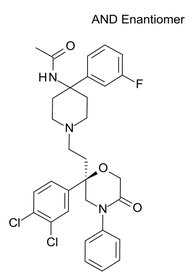
Observed interactions: AR KI=0.7µM, H2 IC_{50}=6.6µM CB2 IC_{50}=7µM, CHRM3 IC_{50}=0.5µM LDLR IC_{50}=0.9µM

- GSID_47351 was shown to promote treatment-related liver injury in two speciea (rats and dogs)
- Hepatocellular necrosis and vacuolation was accompanied by and inflammatory cell infiltrates and significant ALT and bilirubin increase
- Bile duct hyperplasia

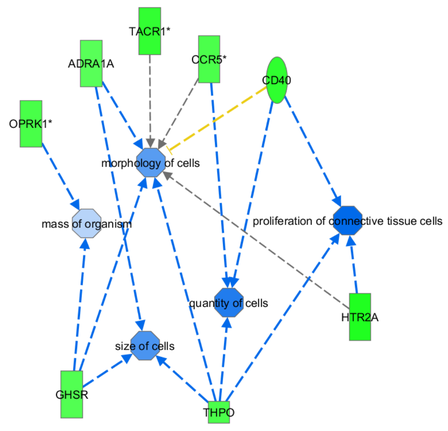
→ Several off-target interactions of GSID_47351 might be accountable for the observed hepatotoxic effects

Reproductive Toxicity

Neurokinine antagonist
Analysis of predicted interactions



Predicted target inhibition
weak strong



Observed interactions: 5HT2A IC_{50}=0.07µM

- Developmental toxicity observed in rabbit
- GSID_47387 does not cause general or hepatocellular necrosis, but ALP increase was observed

→ Mixed on-/off target effects might be accountable for developmental toxicity

Network relationships
- Leads to activation
- Leads to inhibition
- Inconsistent literature findings
- Effect not predicted

Summary

In-silico off-target profiling at Sanofi includes a growing portfolio of models, currently including > 6000 similarity-driven CTlink models and 414 QSAR models. These models are regularly curated and the model applicability domain is controlled to ensure a maximum of correct predictions. Evidence for toxicity of drugs is generated by data mining in pathway databases. In-silico toxicity analyses inform about potential safety concerns of new molecules. Off-target profiling and PredictFX are regularly applied. These methods serve as cost-effective tools to select compounds prior to screening.

In discovery, in silico predictions help to initiate focused experimental follow-up studies and to enhance hit and lead selection. These methods are also applied in development to support risk assessments for regulatory purpose, e.g. to create hypotheses and to better understand mechanisms of toxicity.
Further method development will foster a better characterization of clinically relevant adverse effects based on published knowledge and relevant target-side effect networks and, as a next evolution step, considering inter-individual genetic differences.

References
[1] Schmidt et al. *Future Med Chem.* 2014 ; 6(3):295-317.
[2] Vidal et al. *Methods Mol Biol.* 2011; 672:489-502.
[3] IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity.
[4] MetaCore®,http://thomsonreuters.com/metacore/

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