*i*HUMITE®: A TARGETED METABOLITE PROFILING WORKFLOW

PHASE I: METABOLITE PREDICTION FOR FIRST-IN-MAN AND PRECLINICAL COVERAGE STUDIES

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OVERVIEW

*i*Humite = targeted identification of major human metabolites
*i*Humite phase 1, *i.e.* metabolite predicition, is evaluated here.

INTRODUCTION

Predictive value of early human metabolite ID studies is low:
- *in vitro* (incomplete biotransformation)
- cold compound in First-in-Man (LC-MS operator dependent profiling)

False negative human metabolites results involve a serious risk:
- uncertainty until ^{14}C human ADME data become available (clinical phase 3).
- exceeding timelines due to new metabolite findings
- additonal metabolite ID work and tox studies

The *i*Humite® Workflow is specifically designed to identify all major human metabolites, already during First-in-Man:

Phase 1: Drug Metabolite Prediction
- finding all potential metabolite targets

Phase 2: Plasma Sample Pooling
- dosed plasma samples
- placebo dosed or pre-dose

Phase 3: Accurate MS measurements
- potential metabolites are confirmed

Phase 4: MS Data Processing
- ID of remaining unpredicted metabolites

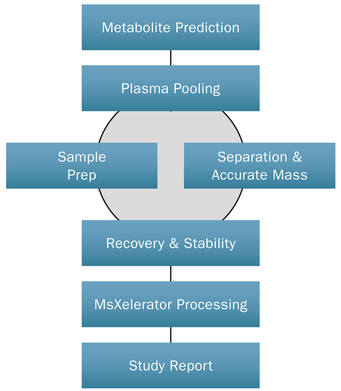


FIGURE 1: *i*Humite® workflow for plasma metabolite identification

METHODS

Drug Metabolite Prediction
- input: 2D chemical structure
- rule based prediction: three subsequent human metabolic reaction steps
- output: list of metabolites with calculated monoisotopic masses

Accurate MS measurements of Pooled Plasma
- confirmation of predicted metabolites and characterization of unexpected metabolites.

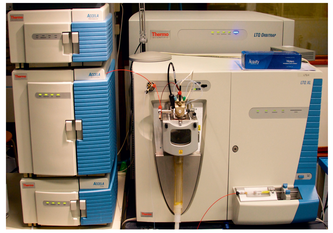


FIGURE 2: Thermo Scientific LTQ Orbitrap

MS Data Processing
- detected peaks in LC-MS data of in-vitro or in-vivo metabolism studies, are compared against the accurate masses of predicted metabolites, and a control sample using MsXelerator processing software (MsMetrix).

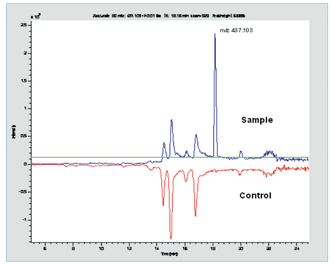


FIGURE 3: Example of differential analysis for sample and control in MsXelerator. EIC analysis over the full mass range, the result for m/z 437.103 is shown.

The sequential use of multiple filters of MsXelerator ensure real metabolites:
- chromatographic peak shape
- isotope signature match
- product ion and neutral loss ion scanning relative to the parent drug

RESULTS

To estimate the current coverage of our targeted *i*Humite approach, we evaluated the predictions based on 14 ^{14}C-ADME published studies (Tabel 1)

This dataset included 14 compounds, with different modes of action, giving 20 metabolites (of which 17 major and 3 non-major according to FDA/ICH) in human plasma.

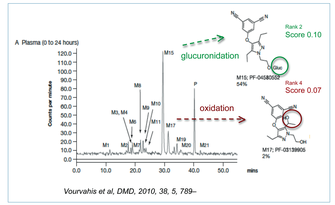
Tabel 1: Selected ^{14}C labeled compounds with human plasma data published
(^{14}C in title of publication in Drug Metabolism and Disposition, 2008-2012)

#	Compound	Company	DMD	First author	Mode of Action	Main plasma metabolite reported
1	Mirabegron	Astellas Pharma	2012	Takusagawa	β3-Adrenoceptor agonist	O-glucuronide
2	Brivanib Alaninate	BMS	2011	Gong	Dual inhibitor of vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF)	O-dealkylation, sulfation, carboxylation
3	Peliglitazar	BMS	2011	Wang	Dual α/γ peroxisome proliferator-activated receptor activator	1-O-β-acyl-glucuronide
4	SB-649868	GSK	2011	Renzulli	Orexin 1 and 2 receptor antagonist	hemiaminal
5	Sunitinib	Pfizer	2011	Speed	Oral multi-targeted tyrosine kinase inhibitor	des-ethyl
6	BMS-690514	BMS	2010	Christopher	ErbB/vascular endothelial growth factor receptor inhibitor	O-glucuronide
7	INCB018424	Incyte	2010	Shilling	Selective janus tyrosine kinase 1/2 Inhibitor	2-hydroxy-cyclopentyl
8	Lersivirine	Pfizer	2010	Vourvahis	Next-generation non-nucleoside reverse transcriptase inhibitor	glucuronide
9	Stavudine	BMS	2010	Zhou	Orally active nucleoside reverse transcriptase inhibitor	+O +Glucuronide
10	Apixaban	BMS	2009	Zhang	Reversible and direct inhibitor of coagulation factor Xa	O-demethyl sulfate
11	Bazedoxifene	Wyeth	2009	Chandrasekaran	SERM	Indole glucuronide
12	Vildagliptin	Novartis	2009	He	Dipeptidyl peptidase 4 inhibitor	carboxylic acid
13	Brasofensine	BMS	2008	Zhu	Inhibitor of the synaptic dopamine transporter	O-demethylation
14	Brivaracetam	UCB Pharma	2008	Sargentini-Maier	SV2A ligand	n-propyl side chain hydroxylation

Of this set of 20 selected metabolites
- 17 (85%, including all of the eleven 1-step metabolites) would have been covered in the mass chromatograms derived from elemental formula of predicted metabolites.
- 15 (75%) of the reported metabolite structures were in the predicted set (including all of the 1-step metabolite structures).

Example 1

The main observed human plasma metabolites are ranked 2 and 4 (Figure 4a). A subset of metabolites predicted for Lersivirine, ranked 1-10, is show in Figure 4b and both are covered *i*Humite.



Vourvahis et al, DMD, 2010, 38, 5, 789–

FIGURE 4a: ^{14}C-Lersivirine human plasma profile from literature with prediction scores and ranks for main metabolites

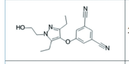
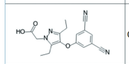
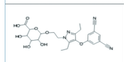
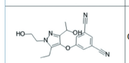
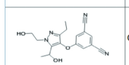
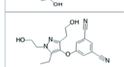
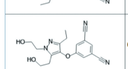
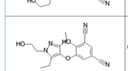
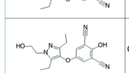
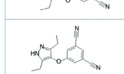
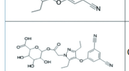
Structure	Priority score	N steps	Reaction sequence
	1.0	0	PARENT
	0.199536	1	primary alcohol oxidation
	0.102502	1	O-glucuronidation
	0.073395	1	benzylic hydroxylation
	0.073395	1	benzylic hydroxylation
	0.063228	1	aliphatic hydroxylation
	0.063228	1	aliphatic hydroxylation
	0.059605	1	aromatic hydroxylation
	0.058762	1	aromatic hydroxylation
	0.048508	1	N-dealkylation
	0.031946	2	primary alcohol oxidation O-glucuronidation

FIGURE 4b: Predicted metabolites of Lersivirine (rank 1-10)

Example 2

The main human plasma metabolite for Brivanib (M32) is the result of 3 subsequent biotransformation steps (Figure 5). Structures of both M32 and its intermediates are in the predicted metabolite set. Rank numbers will increase with the number of subsequent steps, as MsXelerator data processing is very fast 3-step data processing is routinely used.

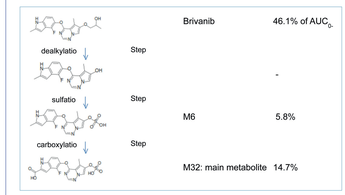


FIGURE 5: Multi-Step prediction for the main reported human plasma metabolite of ^{14}C-Brivanib after oral administration of its alanine prodrug

CONCLUSIONS

Metabolite prediction in the *i*Humite workflow:
- is a key step for identification of human metabolites
- allows a comprehensive targeted approach to identify major human metabolites
- in pooled human plasma and their coverage in preclinical species

*i*Humite identifies both expected and unexpected metabolites in a single processing run. Incorporation of new biotransformations will further increase the efficiency of our *i*Humite workflow by reducing the time spent for the non-targeted approach.

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