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Commentary

Preclinical Drug Metabolism in the Age of High-Throughput Screening: An Industrial Perspective

A. David Rodrigues[1,2]

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With the advent of genomics, combinatorial paradigms and high-throughput screen (HTS)-based pharmacological testing, the number of compounds flowing through the discovery pipeline is likely to escalate. At the same time, with increased knowledge of the human drug-metabolizing enzymes and the availability of *in vitro* absorption-metabolism (AM) models, Preclinical Drug Metabolism is poised to meet the challenges of HTS. In order to be successful, however, a rational HTS strategy (*vs.* serendipitous HTS) has to be employed. Such a strategy is based on *a*utomation, *v*alidation and *i*ntegration of *in vitro* AM models and *d*atabase management (AVID). A generalized strategy for rational (AVID-based) HTS in Preclinical Drug Metabolism is described briefly.

KEY WORDS: high-throughput; *in vitro* models; *in vitro-in vivo* correlations; metabolism; absorption; drug discovery; screening.

INTRODUCTION

The evolution of the drug metabolism field in the late '90s has reached a very exciting stage, primarily as a result of three major factors: first, advances in our understanding of the different human drug-metabolizing enzyme systems and the availability of various *in vitro* human models for studying drug metabolism and intestinal transport (1–3); second, the realization that these *absorption* and *metabolism* (AM) models can be used in conjunction with conventional *in vivo* PK/ADME models (1); third, the development of analytical methods (e.g., LC-MS, LC-NMR and CZE) that can be coupled to automated sample handling systems (4–8).

In a safety-, time- and cost-conscious drug development environment, most pharmaceutical companies are increasingly making use of *in vitro* information and are including it in their submissions to various regulatory agencies (1). In turn, these agencies have acknowledged the utility of this information, both in terms of assessing drug safety and approvability. Towards this end, the FDA has recently prepared a guidance package entitled: "Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies *In Vitro*." Therefore, where Preclinical Drug Metabolism investigators once had to rely solely on animal models, the same scientists can now use a variety of *in vitro* human models. This "humanization" of the processes within Preclinical Drug Metabolism, which is in keeping with the increased use of cloned *human* receptors/enzyme targets in pharmacological testing, enables one to obtain clinically relevant data early in drug discovery (Table 1 and 2).

THE TREND TOWARDS HTS

Two additional, and potentially very powerful, forces (*genomics* and *combinatorial chemistry*) are shaping the future of Drug Metabolism. Both are likely to increase the "throughput" of compounds traveling along the "discovery pipeline", as a result of more pharmacological targets (genomics) and larger numbers of structurally diverse compounds (combinatorial synthesis) (9–12). Even in the absence of combinatorial paradigms, medicinal chemists are turning to automated and parallel-compound synthesis (13). Likewise, discovery project teams are also focusing on automation coupled to 96-well microplate technology as a means of accelerating *in vitro* PT procedures. These rapid advances have given birth to a plethora of jargon phraseology such as "high-throughput screening (HTS)", "ultra HTS", "compound decks", and "bioprospecting" or "fishing" for leads (14). At the same time, given the increase in data output, there is also a growing awareness that novel experimental design and data processing strategies have to be developed (15–17).

[1] Drug Metabolism (Preclinical), Merck Research Laboratories, Sumneytown Pike, P.O. Box 4, WP26A 2044, West Point, Pennsylvania 19486-0004.

[2] To whom correspondence should be addressed.

ABBREVIATIONS: PK, pharmacokinetics; ADME, absorption-distribution-metabolism-excretion; AM, absorption-metabolism; AVID, automation-validation-integration-database; HTS, high-throughput screening; PT, pharmacological testing; LC, liquid chromatography; MS, mass spectrometry; CZE, capillary zone electrophoresis; NMR, nuclear magnetic resonance; IVIVC, *in vitro-in vivo* correlation; CYP, cytochrome P450; GLP, good lab practice; *Ah*, aryl hydrocarbon; PPAR, peroxisome proliferator activated receptor; Pgp, P-glycoprotein; NAT, *N*-acetyltransferase; UGT, UDP-glucuronosyltransferase; SAR, structure-activity relationship; NDE, new drug entity; K_m, apparent Michaelis constant; V_{max}, apparent maximal initial reaction velocity; CL_{int}, intrinsic clearance (V_{max}/K_m); SIMCA, soft independent modeling of class analogy; CoMFA. comparative molecular field analysis; P_{app}, apparent permeability coefficient.

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Table 1. Some Potential Issues Encountered in Preclinical Drug Metabolism: *In Vivo* Studies with Animal Models

Issue	Cause
1) Poor oral bioavailability	a) Poor absorption b) Pronounced gut first-pass metabolism and/or Pgp-mediated efflux c) Pronounced hepatic first-pass metabolism and/or biliary secretion
2) Short plasma half-life	High hepatic (systemic) and/or renal clearance
3) Long plasma half-life	Low hepatic, or low renal clearance, and/or large volume of distribution
4) Large volume of distribution	High tissue/plasma ratio
5) Predict poor PK profile in man	*In vivo* allometric scaling
6) Non-linear pharmacokinetics	a) Autoinhibition of metabolism b) Saturation of first-pass metabolism
7) Induction of drug-metabolizing enzymes (usually as part of short term rodent toxicology studies)	

Table 2. Some Potential Issues Encountered in Preclinical Drug Metabolism: *In Vitro* Studies with Human and/or Animal Models

Issue	Model
1) Induction of drug-metabolizing enzymes	a) Primary cultures of hepatocytes b) HepG2 cells
2) Metabolic lability	a) Hepatocyte suspensions b) Precision-cut liver tissue slices c) Subcellular fractions (e.g., microsomes) d) cDNA-expressed drug-metabolizing enzyme(s)
3) Inhibition of drug-metabolizing enzymes: (mechanism-based or potent/azole-type reversible inhibition)	a) Subcellular fractions (e.g., microsomes) b) cDNA-expressed drug-metabolizing enzyme(s) c) Hepatocyte suspensions
4) Poor drug absorption/uptake	a) Caco-2 cells b) HT29-18-C_1 cells
5) Metabolism by polymorphically expressed drug-metabolizing enzyme(s) (e.g., CYP2D6 CYP2C19, CYP2C9, NAT2, UGT1.1)	a) cDNA-expressed drug-metabolizing enzyme(s) b) Subcellular fractions (e.g., microsomes)
6) Formation of reactive (electrophilic) metabolite(s)	a) Subcellular fractions (e.g., microsomes) b) cDNA-expressed drug-metabolizing enzyme(s) c) Precision-cut liver tissue slices d) Hepatocyte suspensions/cultured hepatocytes
7) Structure of NDE conforms to known SAR or pharmacophore	a) Drug-metabolizing enzyme (e.g., CYP2D6, CYP2C9) b) Receptor (e.g., *Ah*, PPAR).
8) Predict poor PK in man (*in vitro* allometric scaling)	a) Subcellular fractions (e.g., microsomes) b) Hepatocyte suspensions c) Precision-cut liver tissue slices

The net effect of these "upstream" events is to force drug metabolism investigators to reevaluate the way they perform their studies. No doubt that traditionally "downstream" activities, such as toxicological testing, will also have to keep up with medicinal chemists and pharmacologists. At the end of the day, no one department wants to become a "bottleneck."

In general, the allure of HTS is strong because one potentially can obtain data with large numbers of compounds, while saving time, manpower, cost and the need for large amounts of drug. Therefore, an increasing number of publications are beginning to appear which describe; 1) integrated (AM-PT) *in vitro* metabolism screening, where drug analysis by LC or LC-MS is replaced with receptor binding or enzyme assays (18); 2) *in vivo* "cassette" (or "*n*-in-one") dosing strategies, where multiple compounds are simultaneously administered to a single animal (6,7); and 3) automated PK analysis (8). While these advances are exciting, it is important to realize that HTS should be performed within the context of a *rational* screening strategy.

A FRAMEWORK FOR RATIONAL HTS IN PRECLINICAL DRUG METABOLISM

Rational HTS (as opposed to serendipitous HTS) can be built on a framework of four-interlinked-elements (Figure 1): *automation*, *validation*, *integration* and *database management* (AVID).

Automation

With the development of sophisticated and robust "x-y-z" robotic systems, it is possible to automate solid phase and liquid-liquid extraction procedures as a prelude to sample analysis. However, HTS paradigms demand that automation can accommodate 12-, 24-, 96-well and even 384-well microplates. When this is achieved, it is possible to perform more routine *in vitro* AM studies in a HTS format (Table 2). On the other hand, one cannot lose sight of the fact that weighing of drug, measurement of *in vitro* plasma protein binding, and aspects of tissue culture and animal handling (e.g., dosing and blood draw), can and should be automated. In addition, in a future where microplate-based HTS becomes obsolete, it has become apparent that genomics and combinatorial chemistry will lead the way with *microchip* technology (14,19–21). For instance, first- and second-generation microchip-based combinatorial chemistry systems are being built with the capability of synthesizing up to 10,000 compounds per chip and the rush is on to develop analogous screening systems. Such systems will need to integrate real-time, sensitive-detection assays and submicroliter fluid-handling methods onto microchips. This trend towards "miniaturization" is being fueled by the belief that one can greatly decrease the demand for drug and the cost of assay reagents. Therefore, one can envision future drug metabolism investigators performing *in vitro* incubations using microchip arrays!

Validation

An important part of HTS is the use of *in vitro* AM models, which ultimately necessitates that they be validated and that the right model is applied to the right problem (1). This means that the strengths and weaknesses of each model are fully evaluated. For instance, the prediction of overall metabolic lability using a cDNA-expressed enzyme has its limitations (Figure 2). At the same time, in the absence of the corresponding cDNA-expressed proteins and selective immunoinhibitory antibodies, it is difficult to catalytically differentiate between allelic variant forms of some enzymes (e.g., CYP2C9) or between members of the same gene subfamily (e.g., CYP3A4 *vs.* CYP3A5).

Validation not only encompasses the precision, accuracy and reproducibility of the data being generated, but also the quality of the ensuing IVIVCs. Does this mean that one has to

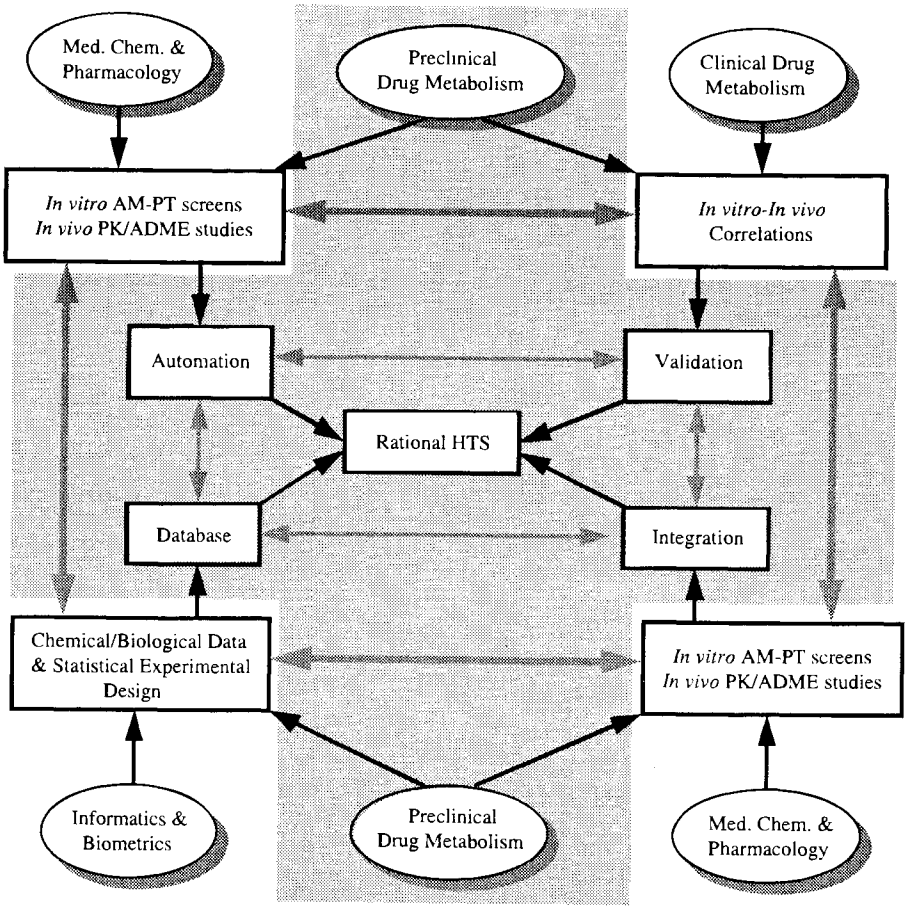

Fig. 1. AVID-Based HTS in Preclinical Drug Metabolism.

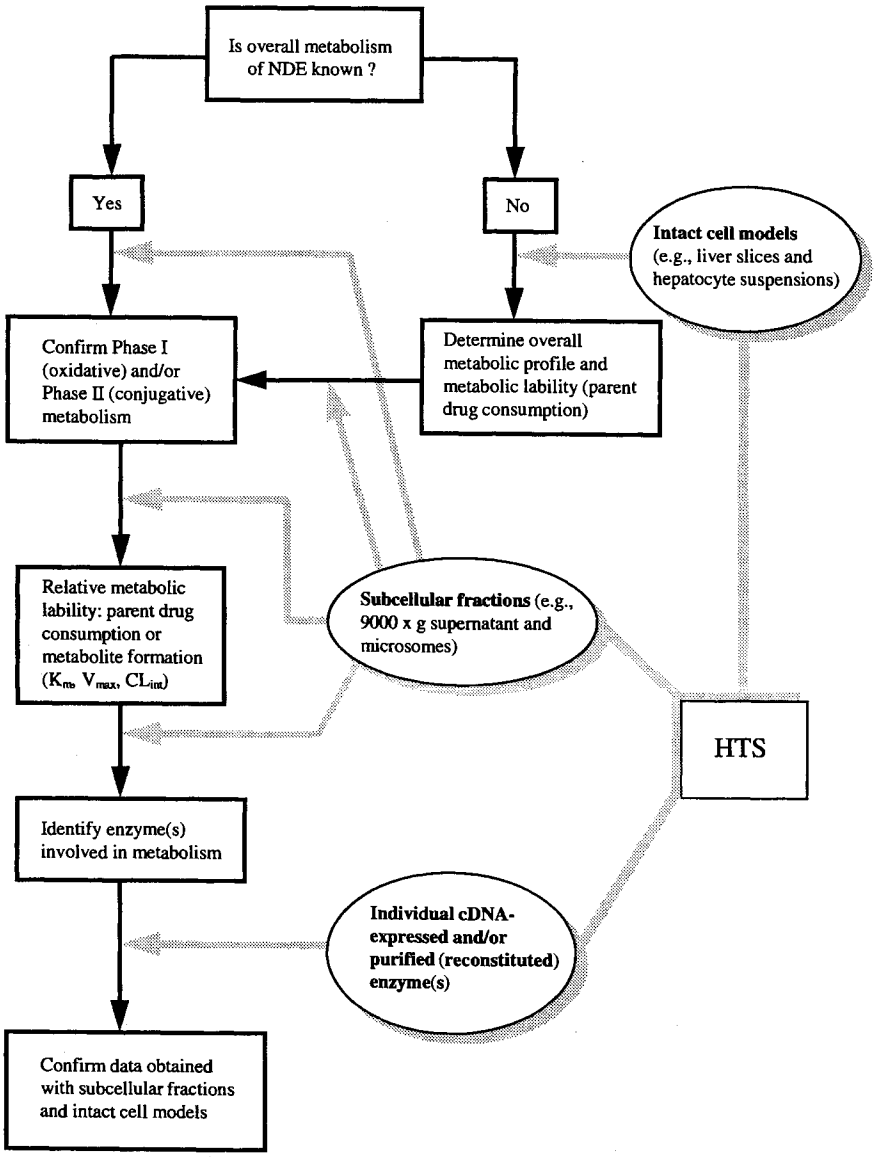

Fig. 2. Integrated use of *in vitro* metabolism models.

"standardize" all *in vitro* AM procedures and IVIVC approaches? The answer may be "yes", especially if one enters data into a corporate-wide (e.g., intranet-linked) or national (e.g., internet-linked) database. However, many questions remain. For instance, should one focus on absolute *in vitro* data or use relative rank order data? How will one define successful *vs.* unsuccessful *in vitro* HTS approaches? If the number of discovery compounds continues to escalate to the point when even *in vivo* cassette dosing regimens are obsolete, then Preclinical Drug Metabolism may need to rely solely on *in vitro* AM models as a first tier screen. How well validated will these models be?

Integration

The "integration" element of rational HTS is very critical and ties together a number of issues:

1. The coordinated use of the different *in vitro* models themselves (1). This is because a given problem often requires the use of more than one model (Figure 2). For instance, HTS may lead to compounds with improved absorption, only to find that metabolic lability now becomes the issue. Furthermore, there are many examples of "metabolic switching", where HTS effectively results in "dampening" metabolism at one site on the molecule, but increasing metabolic lability at another site. Moreover, decreases in metabolic (hepatic) clearance may be accompanied by increased renal clearance.

2. The integration of animal model-based IVIVCs and human model-based IVIVCs, in order to obtain cross-species correlations. It is essential to avoid the optimization of PK/ADME in animals, without regard for *in vitro* human data.

3. The integration of *in vitro* AM data with *in vitro* PT data is imperative, so that Preclinical Drug Metabolism coordinates their efforts with medicinal chemists and pharmacologists.

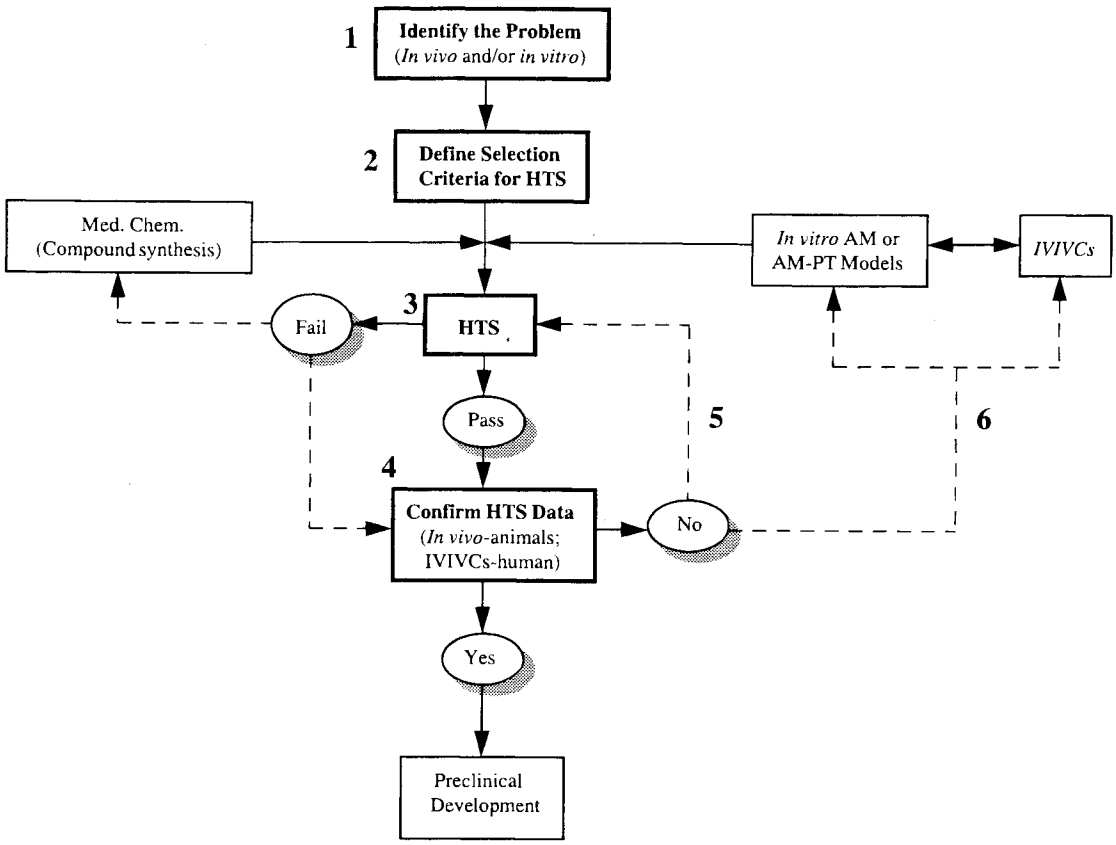

Fig. 3. Rational HTS in Preclinical Drug Metabolism.

After all, many compounds may pass AM screens only to fail potency *vs.* selectivity PT screens! Therefore, it may be advantageous to establish hyphenated (AM-PT) HTS methods whenever possible (18).

Database

HTS approaches are only as good as the database that is used to store and process rapidly generated (real-time) data. In going to great lengths to develop HTS methods, one should avoid the stockpiling of data, where data acquisition, entry and analysis itself becomes a bottleneck. It is important to set up a database that is manageable, user friendly, amenable to data input and retrieval, and can be used iteratively. A database would contain information such as the structure of a given drug (e.g., *ISIS*, *Merlin* and *Unity* databases), its *in vitro* and *in vivo* PT profile, *in vivo* PK/ADME data and *in vitro* AM data. The structure *vs.* function information contained therein would then be amenable to: 1) analysis using commercial (e.g., SIMCA and CoMFA) or in-house computational tools for data modelling and pattern recognition; and 2) the application of active site (e.g., CYP2D6 and CYP2C9) or binding site (e.g., *Ah* and PPAR receptor) pharmacophores and SARs (9, 22–24).

Furthermore, a database should also incorporate novel data *and* data obtained with older compounds that have already gone into the clinic and where *in vivo* human PK/ADME information is already available. This would make the retrospective validation of *in vitro* AM models more systematic and would greatly add to the power of existing IVIVC approaches (25–27). One possibility would be to analyze IVIVCs using artificial neural networks and use the database as "training" data (28–30).

SIX STEPS TO RATIONAL HTS

Although the ultimate goal of HTS in Preclinical Drug Metabolism is to avoid relatively costly and time consuming single dose *in vivo* animal studies, such studies still play an important role: first, in terms of defining the problem; second, as a means of confirming HTS data. Within an AVID-based framework, the iterative process of rational HTS can be described in six steps (Figure 3):

1. Identify the problem (e.g., oral bioavailability, plasma $t_{1/2}$, absorption, clearance, etc) with an appropriate *in vivo* animal model. In case of mechanism-based inhibition or induction of human CYP, the problem can be defined *in vitro* (Table 2).
2. Define selection criteria for HTS of compounds. For example, to pass HTS a compound has to be a weak non-mechanism-based inhibitor (e.g., IC_{50} or $K_i \geq 50$ μM) and inducer (≤two-fold increase) of CYP. Similarly, successful compounds have to be characterized by a P_{app} (e.g., Caco-2 cells) that is above, or a CL_{int} (e.g., hepatocyte suspensions) that is below, a predefined (validated IVIVC-based) "threshold" value.
3. Perform *in vitro* HTS with an appropriate, validated, animal and human AM or AM-PT model(s). Screen out com-

pounds that do not meet the selection criteria. If a series of compounds fail to pass HTS, then Medicinal Chemistry can make appropriate structural changes while attempting to maintain optimal pharmacological activity.

4. For compound(s) that pass step 3, confirm data with an *in vivo* animal model and/or with a validated database of human IVIVCs. The objective is to "screen out" *false positive* compounds. At the same time, it may also be important to confirm HTS failures and "screen in" *false negative* compounds.

5. If confirmation (step 4) is unsuccessful, repeat steps 3 and 4.

6. If step 4 continues to be unsuccessful, then *in vitro* models, the HTS approach, selection criteria or IVIVCs need to be reevaluated.

CONCLUSIONS

As the 1990s draw to a close, Preclinical Drug Metabolism is bracing itself for the full impact of genomics and combinatorial paradigms that will give rise to larger numbers of compounds passing through the discovery or preclinical pipeline. While adapting to this new environment, Preclinical Drug Metabolism will have to perform its tasks more rapidly, efficiently, cost-effectively and employ HTS paradigms in the shadow of a GLP environment. Moreover, if these HTS approaches are to be successful, there will have to be even greater cooperation between *technology*-based groups and *project*-based groups within Preclinical Drug Metabolism, cooperation between Preclinical and Clinical Drug Metabolism, and cooperation between Preclinical Drug Metabolism and other groups such as Medicinal Chemistry, Pharmacology and Bio-/Chemo-Informatics (Figure 1). In doing so, the vision of Preclinical Drug Metabolism employing rational (AVID-based) HTS paradigms can be realized.

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