The Boost of ADME, PK and Bioanalysis for Drug Discovery and Development

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Abstract

The main aim of drug development is to get a compound that has a therapeutic effect into the form of a medicine we can dose to patients that is safe and effective. A drug must reach the site of action in a quantity sufficient to exert its pharmacological effects, and be eliminated in a reasonable timeframe. ADME stands for absorption, distribution, metabolism and excretion of chemicals and drugs and is used to define the drug's impact in a human body. ADME test results can be used to predict how the drug will behave in the body and to assess its potential for adverse interactions with other drugs.

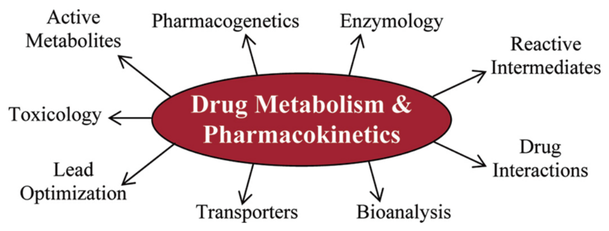
Characterization of ADME properties help to explore and explain how pharmacokinetic processes happen, so as to provide safety considerations of a new drug on which risk-based assessments can be made. ADME studies are key at each preclinical stage of the drug discovery process, from high throughput screening (HTS), hit identification, lead optimization and finally the selection of a candidate molecule for clinical development. In discovery and lead optimization, drug developers may make chemical modifications to drug candidates to optimize ADME properties. An early characterization of these properties will ensure appropriate selection of compounds with acceptable pharmacokinetic characteristics to guarantee efficacy while limiting adverse effects and optimizing development time. Bioanalytical assays that reliably quantify biotherapeutics and biomarkers in biological samples play pivotal roles in drug discovery and development.

Medicilon's DMPK&BA department offers our clients a broad spectrum of high quality services in the areas of *in vitro* ADME, *in vivo* pharmacokinetics and bioanalytical services, for both small and large molecule drugs, such as proteins, antibodies, oliogonucleotides, ADC and new modalities. We have available all common laboratory animal species such as non-human primates, canines, minipigs, mice, rats, rabbit and etc.

Background

According to industry reports, over 80% of investigational new drugs fail during development because of unsatisfactory ADME characteristics. Therefore, ADME research can greatly impact clinical success, and early assessment of ADME characteristics has real value in improving the effectiveness of the drug discovery and development process.

Pharmacokinetics is a subject that quantitatively studies the absorption, distribution, metabolism and excretion of drugs in organisms, and uses mathematical principles and methods to explain the changes in blood drug concentration over time. Drug metabolism and pharmacokinetics (DMPK) is an important branch of pharmaceutical sciences. The nature of ADME and PK inquiries during drug discovery and development has evolved in recent years from being largely descriptive to seeking a more quantitative and mechanistic understanding of the fate of drug candidates in biological systems. Dramatic increases in investments in new modalities beyond traditional small and large molecule drugs, such as peptides, oligonucleotides, and antibody-drug conjugates (ADC), necessitated further innovations in bioanalytical and experimental tools for the characterization of their ADME properties. Bioanalytical support plays a vital role during the lead optimization stages. The major goal of the bioanalysis is to assess the over-all ADME characteristics of the new chemical entities (NCE's) and biologics. Bioanalytical tools can play a significant role and impact the progress in drug discovery and development.



Method

In vitro ADMET

- Liver microsomes / S9 / Hepatocyte stability
- CYP450 enzyme inhibition & TDI
- CYP450 enzyme induction
- Enzyme phenotype analysis
- Plasma protein binding
- Plasma (serum) stability
- *In vitro* MetID and metabolic pathways
- GSH-trapping
- Whole blood / plasma distribution
- Permeability and efflux
- Transporters
- (Pgp/BCRP/OATs/OCTs/OATPs/MATEs/BSEP/MRPs)
- BBB penetration,Kp,uu
- hERG
- Mini-Ames

In Vivo PK & Non-GLP Tox

- Species: Mouse (ICR, C57, balb/c, SCID, Nude mouse), Rat (SD, Wistar), Guinea pig, Mini-pig, Rabbit, Canine (beagle dog), Cynomolgus monkey
- Administration Routes: Intravenous (IV), Oral (PO), Subcutaneous (SC), Intramuscular (IM), Intraperitoneal (IP), Topical, Transdermal, IT etc.
- Dose Strategies: Single, multiple and cassette dosing
- Serial blood microsampling
- *In vivo* metabolite identification and quantitation
- Tissue distribution
- Mass balance with excretion
- Pre-formulation screening
- PK/PD & human PK modeling
- Non-GLP Tox, MTD, DRF (Safety window)
- $^{125}I/^{14}C/^{3}H$ labeled isotope drug metabolism and mass balance studies
- Surgical techniques: Venous cannulation, biliary cannulation, infusion pump, liver/muscle biopsy and implantation

Results

Case 1: ADME and Toxicology Evaluation

TOP5300 is an orally active follicle stimulating hormone receptor allosteric agonist that provides a preferred treatment for over 16 million infertile women of reproductive age in low complexity methods or in high complexity methods.

TOP5300 represents a new allosteric agonist with potential for ovarian stimulation in women. The safety profile demonstrated lack of toxicity.

TOP5300 was evaluated in standard ADME, including Cytochrome P450 inhibition, clearance and pharmacokinetic profiles. Toxicological evaluations were performed in both rat and dog as the second species according to the guidance from FDA. These assays were performed by **Medicilon**.

Parameter	TOP5300
Clint (r,h,d,monkey, mice) (μL/min/mg protein)	11,37,37,165,19
CYP inh@10 μM	CYP3A4 (midazolam)
CYP TDI (3A4)	Negative
Rat PK (AUC, $T_{1/2}$, C_{max}, F%)	2,655, 5.1 h, 237, 20%@10mg/kg
Mouse PK (AUC, $T_{1/2}$, C_{max}, F%)	5,533, 2.5 h, 1,133,22%@5mg/kg
Dog PK (AUC, $T_{1/2}$, C_{max}, F%)	8,719, 9.4 h, 391,32%@10mg/kg

ADME properties of TOP5300[1]

Case 2: ADME Study

Aberrant activation of the PI3K pathway has been intensively targeted for cancer therapeutics for decades. In this work, researchers designed and synthesized a novel photocaged PI3K inhibitor 1, which could be readily activated by UV irradiation to release a highly potent PI3K inhibitor 2.

To elucidate the difference in ADME properties between compounds 1 and 2, several studies were conducted including plasma protein binding assays, plasma, and liver microsomal stability assays as well as Caco-2 permeability assays. Both compounds showed high plasma protein binding (>98%) as well as a long plasma half-life (>120 min) in rat and dog.

In the liver microsomal stability assay, compound 1 showed a much shorter half-life than the uncaged compound 2. In addition, compound 1 was much less permeable compared to compound 2 in the Caco-2 assay, which may be attributed to its largely increased molecular size. ADME studies of compounds 1 and 2 were conducted by **Medicilon**.

	plasma protein binding (%)		plasma stability $T_{1/2}$ (min)		liver microsomal stability $T_{1/2}$ (min)		Caco-2 permeability P_{app} (10^{-6} cm/s)	
cmpd	rat	dog	rat	dog	rat	dog	A to B	B to A
1	99.4	99.5	>120	>120	1.82	4.62	0.49	1.95
2	98.3	98.3	>120	>120	103.64	>120	21.19	26.51

ADME Properties of Compounds 1 and 2[2]

Case 3: PROTAC PK and Plasma Stability Studies

ARD-2128 is a bona fide PROTAC AR degrader and strongly suppresses AR-regulated genes in a dose- and time-dependent manner in AR^+ prostate cancer cell lines. The PK data show that ARD-2128 has a low clearance (1.2 mL/min/kg) and a moderate to high steady-state volume of distribution (V_{ss}) of 2.7 L/kg. ARD-2128 exhibits a long half-life following both intravenous (2 mg/kg, i.v.) and oral administration (5 mg/kg, p.o.) with the $T_{1/2}$s of 27.6 h and 18.8 h, respectively. ARD-2128 (5 mg/kg, p.o.) achieves 67% oral bioavailability in mice, effectively reduces AR protein and suppresses AR-regulated genes in tumor tissues after oral administration, leading to the effective inhibition of tumor growth in mice without signs of toxicity. PK studies were performed in **Medicilon**.

compound	route	dose (mg/kg)	$T_{1/2}$ (h)	AUC_{0-t} (h·ng/ml)	Cl (ml/min/kg)	V_{ss}(L/kg)	route	dose (mg/kg)	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/ml)	AUC_{0-t} (h·ng/ml)	F (%)
26	IV	2	17.8	11,035	1.9	2.7	PO	5	12.0	4.0	1389	20,600	75
27	IV	2	11.5	15,759	1.7	1.5	PO	5	11.2	4.0	980	14,588	37
28 (ARD-2128)	IV	2	27.6	13,299	1.2	2.7	PO	5	18.8	4.7	1304	22,361	67
33	IV	1	21.0	4334	2.2	3.8	PO	3	12.4	6.0	207	3127	24
34	IV	1	25.5	2565	3.2	6.8	PO	3	67.8	4.7	134	2550	33

Summary of PK Data for five highly potent AR degraders in Male ICR Mice[3]

The plasma and microsomal stability data show that ARD-2128 has excellent plasma and microsomal stability in in mouse, rat, dog, monkey, and humans.The stability was studied in **Medicilon**.

species	plasma stability ($T_{1/2}$, min)
mouse	>120
rat	>120
dog	>120
monkey	>120
human	>120

Plasma Stability of ARD-2128 in Five Species[3]

Case 4: PROTAC Microsomal Metabolic Stability, hERG and Plasma Stability Studies

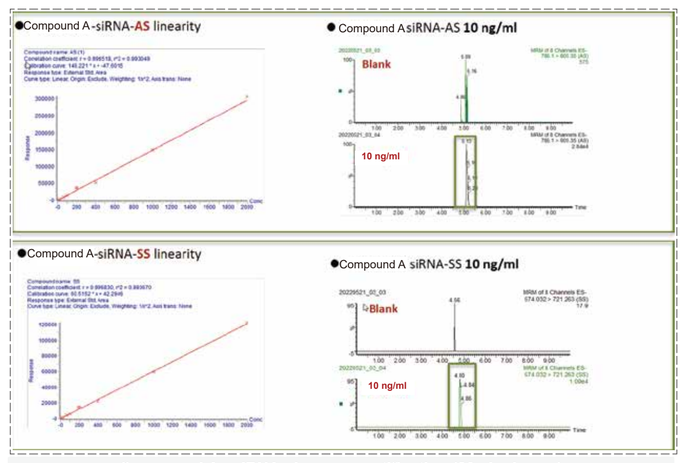
ARD-2585 is an exceptionally potent and orally active AR degrader. ARD-2585 is a promising androgen receptor (AR) degrader suitable for further extensive evaluations for the treatment of AR^+ prostate cancer and other human diseases in which AR plays a key role.

Researchers evaluated ARD-2585 for its liver microsomal stability in five different species (human, mouse, rat, dog, and monkey). ARD-2585 showed excellent stability in liver microsomes in all the five species with $T_{1/2}$>120 min. The excellent mouse microsomal stability data are consistent with the slow clearance of ARD-2585 seen in the PK data in mice. The liver microsomal stability assay was performed by **Medicilon**.

Researchers tested ARD-2585 for its plasma stability in five different species (human, mouse, rat, dog, and monkey). ARD-2585 showed excellent plasma stability in all 5 species with $T_{1/2}$>120 min. The plasma stability assay was performed by **Medicilon**.

In vitro inhibition of the human ERG (the human ether-à-go-go-related gene) channel has been used as an important assay to assess potential cardiotoxicities of a drug molecule. We evaluated ARD-2585 for its inhibition of the hERG channel and found that ARD-2585 exhibits no hERG inhibition up to 30 μM, the highest concentration tested. The hERG assay was performed by **Medicilon**.

Case 5: Oligonucleotide Drugs Bioanalysis



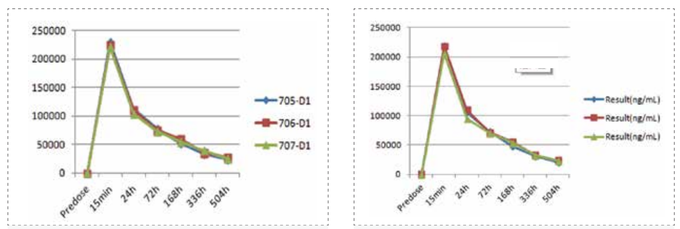
Compound A –siRNA plasma quantification (20 μL plasma)

- Sensitivity: 10 ng/ml
- 20 μL plasma!
- 1344 injections (runs)
- Good reproducibility
- CV< 10%
- Waters Xevo TQ-XS
- 1 ng/ml feasible by MS!

Case 6: ADC Pharmacokinetic Study

The ADC molecule raises the difficulties of PK study as each component of the ADC molecule has unique PK characteristics. **Medicilon** provides high quality quantification assays for key parameters in ADC pharmacokinetic evaluations, presenting accurate results.

Analyte	Description	Common analysis methods
Conjugated Anitbody	Antibody with minimum of DAR >= 1	LBA
Total Antibody	Conjugated, partially unconjugated and fully unconjugated (DAR >= 0)	LBA
Small Molecules	Released/free samllmolecule and its metabolities	LC-MS/MS
ADA	Antibodies against antibody of ADC, linker or drug	LBA



Total Ab ADC

Benchmarking with global lab standard for results with high consistency. Developing stable and reliable methods for results with high correlation.

Summary

- We offer a full suite of *in vivo* ADME and PK services, conducted by a team with near 20 years of experience.
- We maintain an AAALAC -accredited facility with clean rooms for cell culture, an animal care vivarium, and a large variety of instrumentation to perform IND-enabling studies to support your compound's development.
- We offer you our expertise in DMPK & non-GLP/GLP bioanalysis and toxicology, to support you in the complete characterization of the ADME properties and the evaluation of the toxicity of your future clinical candidate.
- From our global locations, we serve many of the largest pharmaceutical, specialty pharmaceutical and biotechnology companies in America, Europe and Asia. Our highly trained scientists utilize an extensive range of leading-edge technology, automation and state-of-the-art techniques.

References

[1] Selva Nataraja, et al. Discovery and Preclinical Development of Orally Active Small Molecules that Exhibit Highly Selective Follicle Stimulating Hormone Receptor Agonism. Front Pharmacol. 2021 Jan 14;11:602593. doi: 10.3389/fphar.2020.602593.

[2] Kehui Zhang, et al. Design, Synthesis, and Biological Evaluation of a Novel Photocaged PI3K Inhibitor toward Precise Cancer Treatment. J Med Chem. 2021 Jun 10;64(11):7331-7340. doi: 10.1021/acs.jmedchem.0c02186.

[3].Xin Han,et al. Strategies toward Discovery of Potent and Orally Bioavailable Proteolysis Targeting Chimera Degraders of Androgen Receptor for the Treatment of Prostate Cancer.J Med Chem. 2021 Sep9;64(17):12831-12854. doi: 10.1021/acs.jmedchem.1c00882.

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