Extensive utilization of Dried Blood Spot sampling in early clinical development studies:
pharmacokinetics, pharmacogenomics and safety assessments

EBF workshop – Connecting Strategies on Dried Blood Spots
Brussels - June 17-18 - 2010

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Content

- Design and Objectives Clinical Study
- Sampling
- Analysis of samples
- Results
- Conclusions

General justification of DBS sampling:

1. For bioanalysis of drugs or genotyping, DBS sampling can be an alternative for venous sampling. Possible advantages include:
   - small blood volume
   - stabilization of the analyte by the DBS matrix
   - no need for dry ice during shipment
   - storage without the need of freezers in less storage room

2. The technique allows for less invasive sampling in non-clinical, i.e. ambulant, conditions.

3. DBS addresses to the demands of society to reduce, replace and refine the use of laboratory animals (3R's) in drug development and to minimize the use of invasive techniques in clinical R&D

Justification for setting up a phase I clinical trial at Xendo using DBS sampling:

1. "Validation" of our Clinical Pharmacology Unit - Bioanalysis Unit combination regarding DBS sample collection, handling and analysis
2. To investigate if DBS can be applied to early clinical development
3. To investigate if DBS can be applied to other fields of interest:
   - Genotyping
   - Elemental Analysis (ICP-MS, ongoing)

Challenges:

Is DBS sampling possible in Clinical Phase I trials as low sensitivity is often required due to low starting doses.

Set-up Clinical Study:

An open-label study to determine the usefulness of the dried blood spot method for phenotyping and genotyping of CYP450 enzymes in healthy volunteers (METC approved).

Study Drug:

Dormicum® tablet 7.5 mg
(midazolam; Substrate for CYP3A4 activity)

Design:

12 healthy volunteers (male/female - age 18-55)

Subjects received single dose. Midazolam concentrations were determined up to 12 h post dose

Primary Objective:

To compare the results of analysis of midazolam between DBS method and plasma as well as whole blood, all drawn by venopuncture.

CYP3A4 activity is accuractely predicted by AUC midazolam concentrations (not the ratio of 1'-hydroxymidazolam to midazolam) [lit: Lois S. Lee et al. J. Clin. Pharmacol. 46 (2006) 229]

Secondary Objectives:

1. To compare the results of DBS analysis of midazolam in whole blood drawn by fingerpuncture (FP) and venopuncture (VP)
2. DBS analysis of midazolam using 1x3mm, 2x3mm and 1x6mm discs
3. To evaluate effect of storage time on DBS analysis
4. To compare genotyping CYP2D6, CYP2C19, CYP3A4 and CYP3A5
5. To investigate the feasibility of measuring 1'-OH midazolam by DBS analysis

Exploratory Objectives:

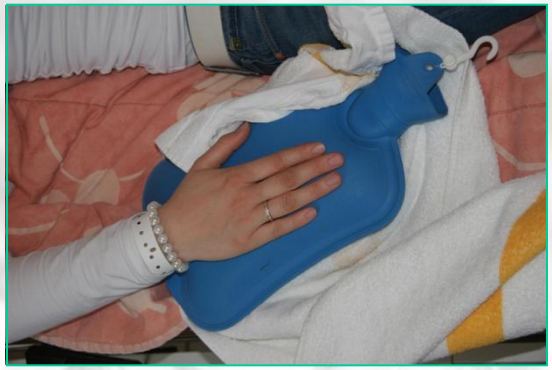
1. To evaluate subject and phlebotomist satisfaction of DBS method (FP) vs. venous blood sampling using a standardized questionnaire

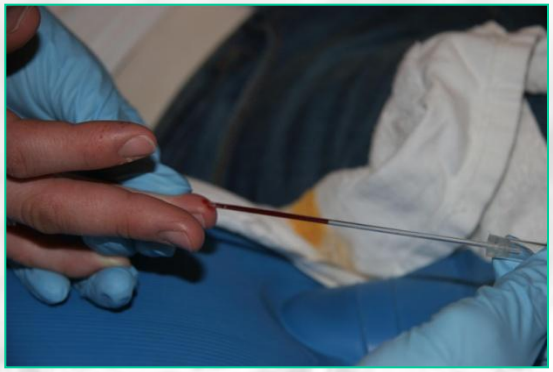
Venous sampling:

2x 2 mL venous blood was collected
– one for whole blood and one for plasma -

Finger puncture:

Puncture: Single-use, automatic, lancing device
[procedure: Edelbroek et al. Ther Drug Monit, 2009;3;327]
Collection: EDTA filled Capillary

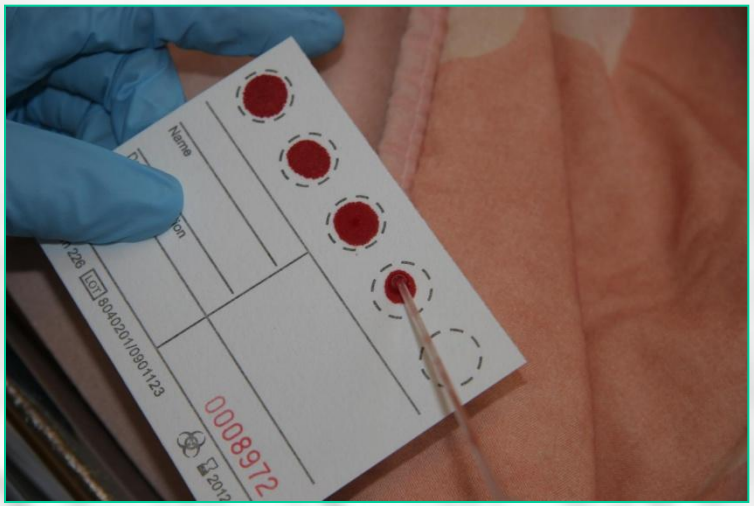


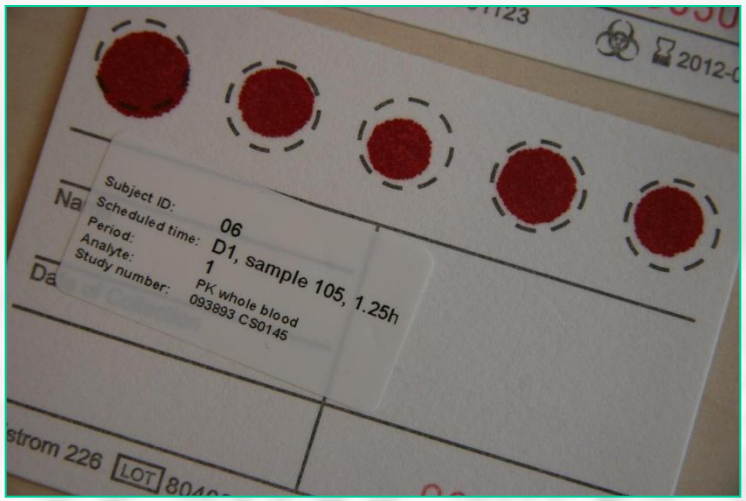


[Blood Gas Analysis 100 µl EDTA (CAP-BGA-100-EDTA)]

Dried Blood Spots:

Whole blood from veno- or fingerpuncture is pipetted (20 µL) on an IDBS Bioanalysis card (ID Biological Systems, Greenville, SC).





DBS card, Ahlstrom 226

- All cards have a unique number
- After cards have been labeled, spots were dried for 2h at RT
- Cards were stored at RT until analysis in sealed plastic bags containing dessicant

Fully validated assays:

Assay 1: midazolam and 1'-hydroxymidazolam in human EDTA plasma
Assay 2: midazolam and 1'-hydroxymidazolam in human EDTA whole blood
Assay 3: midazolam and 1'-hydroxymidazolam in human EDTA dried blood spots (6mm)

(LLOQ: 0.100 ng/mL; HLOQ: 100 ng/mL) – including on card stability at RT

Note that no compromises were made to the LLOQ of the DBS assay

Partially validated assays:

Assay 3b: midazolam and 1'-hydroxymidazolam in human EDTA dried blood spots (2x3mm)

Assay 3c: midazolam and 1'-hydroxymidazolam in human EDTA dried blood spots (1x3mm)

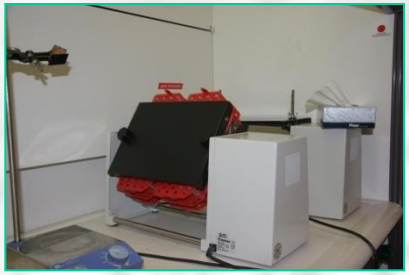
LLOQ: 0.300 ng/mL (1'-hydroxymidazolam)

Extensive utilization of Dried Blood Spot sampling in early clinical development studies

	Plasma / Whole blood	DBS
PREPARATION	Thaw sample	
EXTRACTION	1.Pipette sample 2.Add IS 3.Extraction 4.Transfer	1.Punch disc 2.Add IS 3.Extraction & Shake tube for 1h 4.Transfer
ANALYSIS	LC-MS/MS	

1.

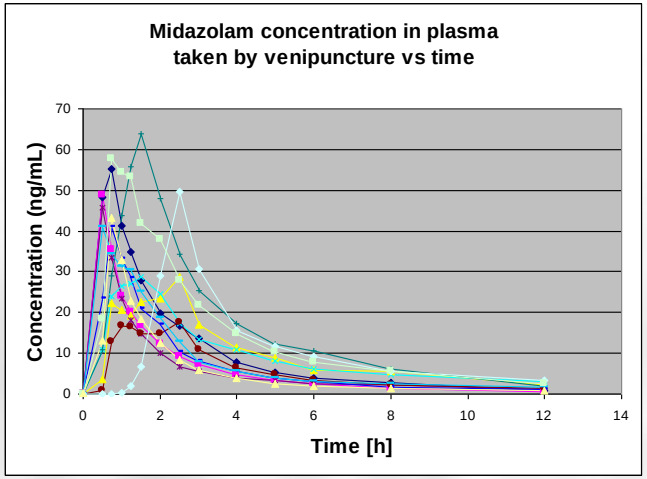
1+2.



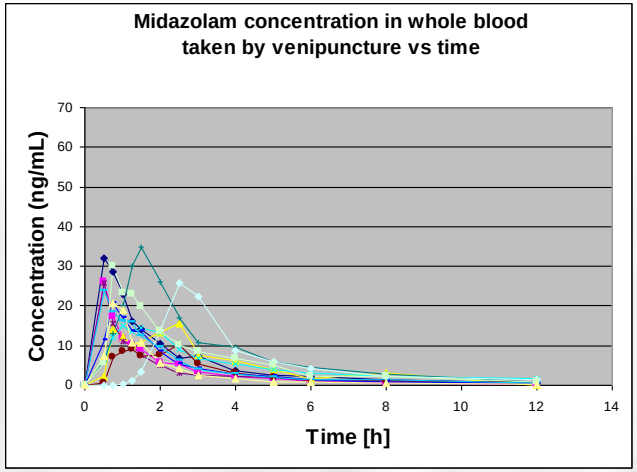
3.

4.

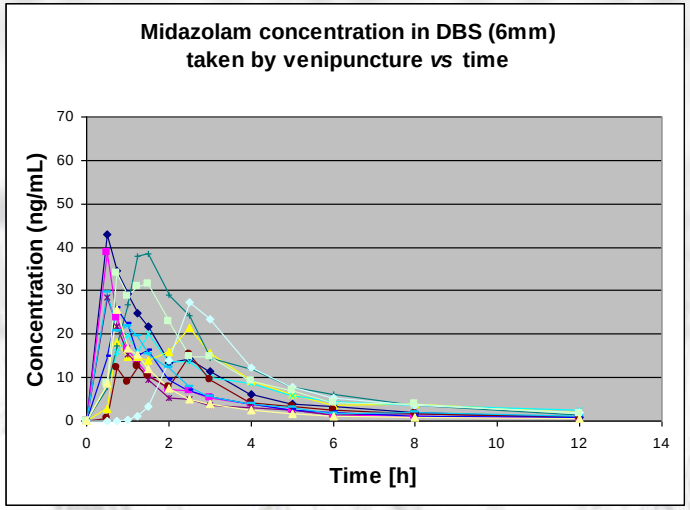
Bioanalytical Results



Plasma



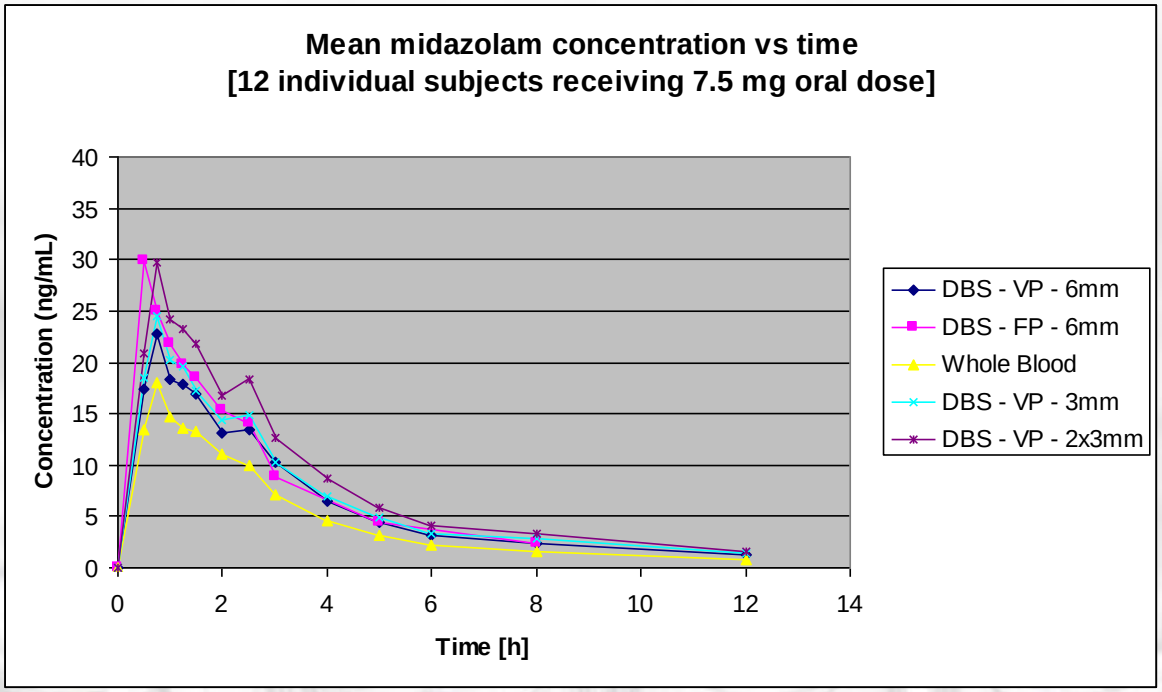
Whole blood (factor 2)



DBS (factor 1.5)

Primary objective: Plasma *vs.* whole blood *vs.* dried blood spots

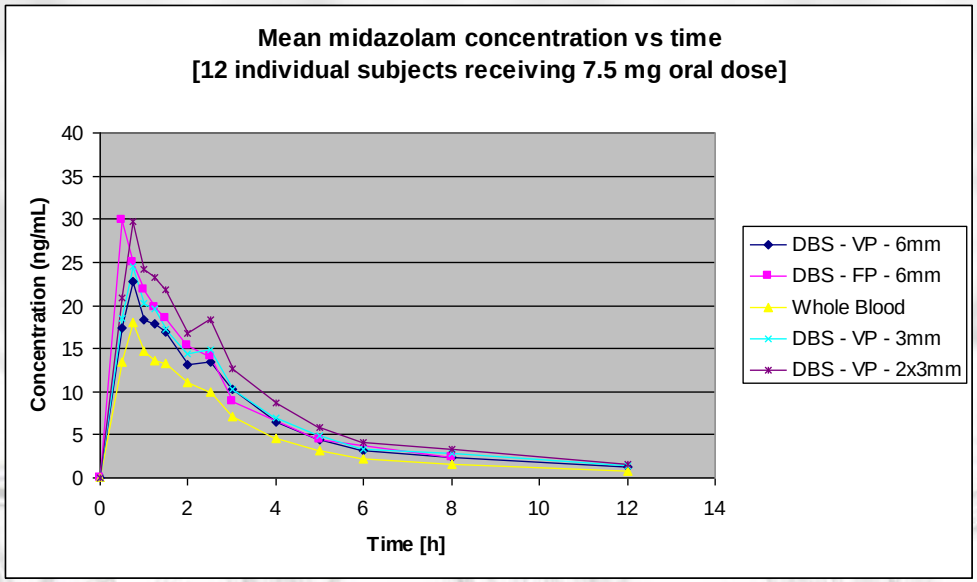
DBS sampling can be considered as a complementary method.



DBS = dried blood spot; VP = venopuncture; FP = fingerpuncture;

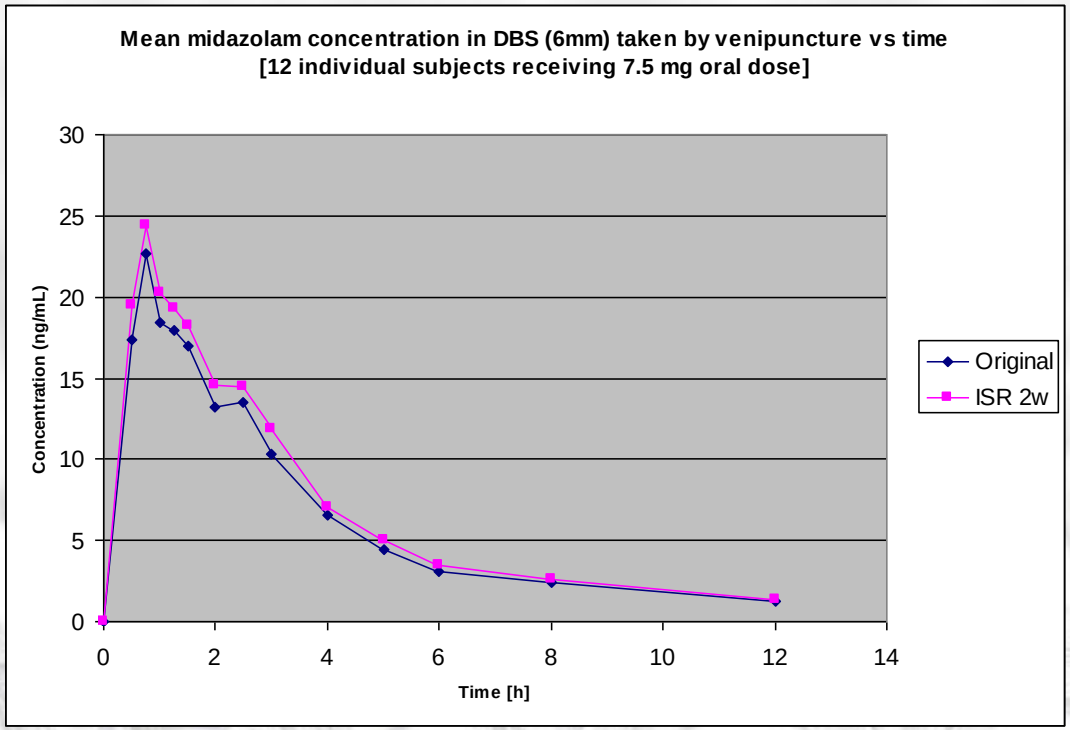
Secondary objectives:

1. DBS - FP vs VP:	*No effect on AUC between capillary blood (FP) and venous blood (VP)*
2a. DBS – 1x3mm vs 1x6mm:	*No effect on AUC with respect to punchwidth*
2b. DBS – 1x3mm vs 2x3mm:	*No effect on AUC with respect to number of dots*



Secondary objectives:

3. Effect of storage time DBS: No effect on AUC when stored for 2 weeks at RT



Secondary objectives:

4. Genotyping:

Methods

DNA isolated from DBS and whole blood and qualified for DNA recovery

SNP genotyping for a series of allele-specific polymorphisms

CYP2D6	*3, *4, *6
CYP2C19	*2, *3
CYP3A4	*1B
CYP3A5	*3C

Results

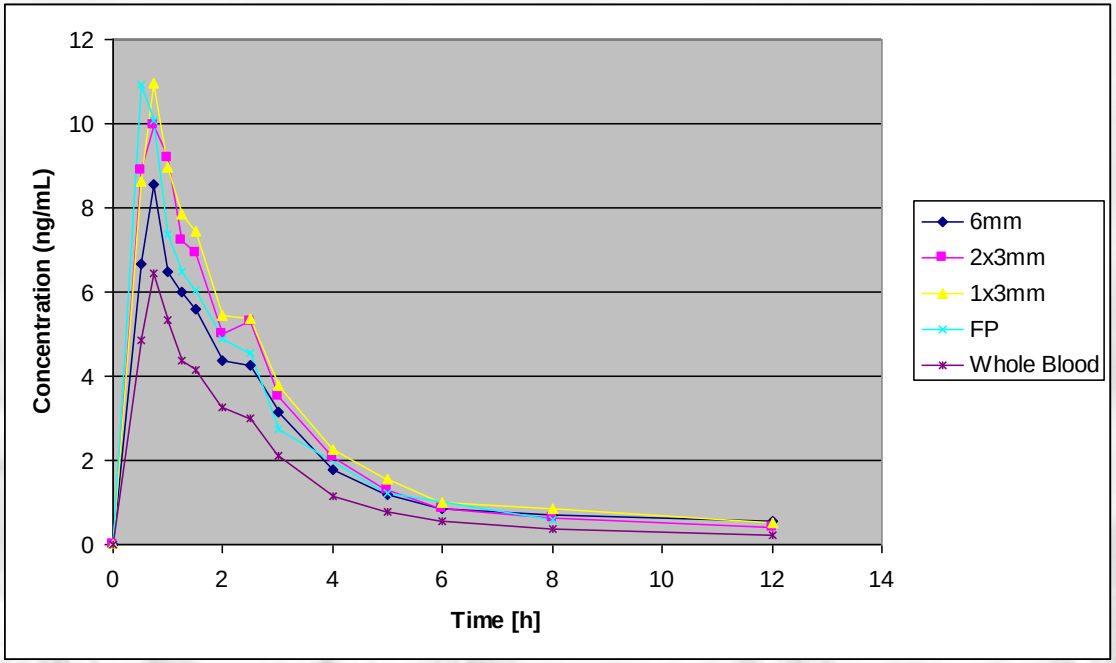
Whole blood and DBS genotyping: 100% match for each polymorphic gene allele examined

DBS samples generally have a slightly lower genomic DNA yield and

DBS samples generally have slightly lower A260/A280 ratios

Secondary objectives:

5. Feasibility measuring 1'OH midazolam by DBS analysis



Exploratory objectives:

1a Evaluation phlebotomist satisfaction of DBS (fingerpuncture) method vs. venous blood sampling

• Spotting on paper	25.0% (EE); 75.0% (E)
• Drying blood spot	50.0% (EE); 37.5% (E); 12.5% (NED)
• Storing DBS in bag	25.0% (EE); 25.0% (E); 50% (NA)
• Sample collection/processing	12.5% (EE); 50.0% (E); 12.5% (NED); 25.0% (NA)
• Enough space for drying on bench	25.0% (yes); 50.0% (no); 25.0% (NA)
• Prior training/experience with DBS	87.5% (no); 12.5% (some)
• Ease of blood collection by FP	62.5% (E); 37.5% (NED)
• FP per timepoint	1 pin prick (100%)

Number of Staff: 8

EE = Extremely Easy; E = Easy; NED = Neither easy or difficult; NA = Not applicable

Exploratory objectives:

1b Evaluation volunteer satisfaction of DBS (fingerpuncture) method vs. venous blood sampling

• Finger puncture (rating pain/discomfort)	16.7% (none); 8.3% (moderate); 75.0% (mild)
• Finger punctures tolerated per time point	16.7% (1); 25.0% (2); 58.3% (3)
• Finger punctures allowed per day	75.0% (11-15); 25.0% (6-10)
• Participate again?	100%
• Cannula (rating pain/discomfort)	58.3% (none); 41.7% (mild)
• Preferred sample type	75.0% (cannula); 25.0% (FP)

Number of volunteers: 12

Summary

- No differences in AUC between sampling methods (VP, FP)
- No disk size/disk number limitations
- No compromises regarding LLOQ between analytical methods
- Phenotyping/genotyping possible with DBS sampling
- Satisfying and promising responses from phlebotomists and subjects

Conclusion

- We achieved our goal of conducting a clinical study with DBS sampling within our CPU/bioanalytical laboratory.
- We achieved our goal to use DBS for genotyping purposes.
- No critical objections from phlebotomists and subjects.
- Easy to implement in bioanalytical operations.

- As blood is an acceptable biological matrix for the measurement of drug exposures, DBS bioanalysis has proven to be a promising alternative for plasma bioanalysis.
- DBS sampling can be applied to early clinical development studies: pharmacokinetics, pharmacogenomics and safety assessments.

Acknowledgements

- GSK for valuable (on-site) discussions and for kindly providing the evaluation forms
- QPS for performing the genotyping studies
- ID Biological Systems for providing specimens for our research purposes

Thank you for your attention: Questions?