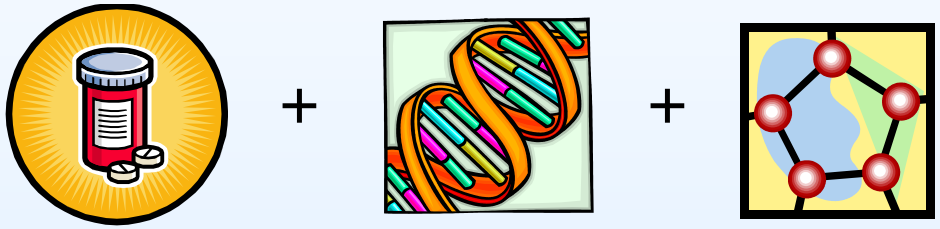
Review of the biology of emerging endogenous biomarkers of CYP3A



Yvonne S. Lin
Department of Pharmaceutics
University of Washington

CYP3A Activity Biomarkers

Marker	Sampling	Level of Evidence
Midazolam	Plasma	“Gold Standard”
Triazolam	Plasma	FDA clinical index substrate
Erythromycin	Breath test	Historically used
6β-hydroxycortisol/cortisol ratio	Urine	Frequently used
4β-hydroxycholesterol	Plasma	some data
4β,25-dihydroxyvitamin D3	Plasma	very little data
1β-hydroxy-deoxycholic acid	Urine	very little data
Other endogenous substrates	Urine and plasma	very little data

Galteau 2003 Eur J Clin Pharm, Diczfalusy 2008 BJCP, Wang 2011 Analytical Biochem, Shin 2013 CPT

CYP3A Activity "Biomarkers"

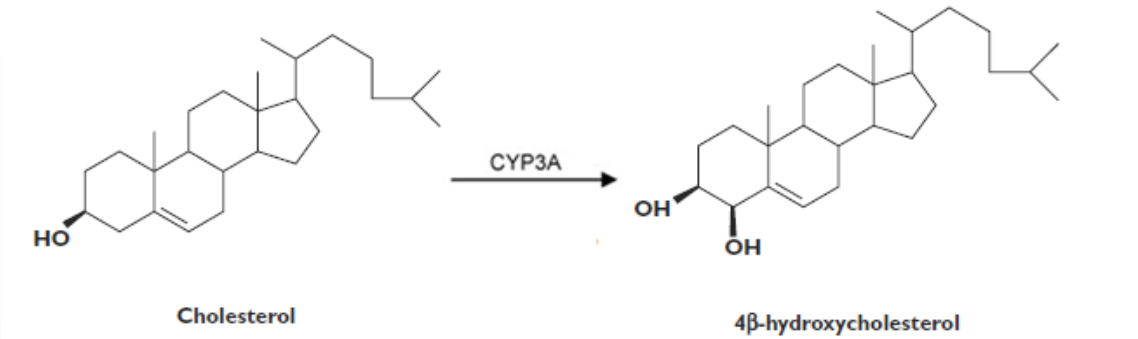
- Other endogenous CYP3A substrates: testosterone, bile acids, vitamin A, estrone, estradiol, etc.

Table 2 Fold change of mean urinary metabolite ratios in the CYP3A inhibition phase and induction phase as compared with the control phase

Metabolite ratio	Fold change (inhibition phase)	Fold change (induction phase)
6β-Hydroxycortisol/cortisol	▼0.18	▲5.88
6β-Hydroxycortisone/cortisone	▼0.21	▲5.01
16α-Hydroxy-DHEA/DHEA	1.01	▲3.97
16α-Hydroxyandrostenedione/androstenedione	▼0.72	▲2.74
4-Hydroxyandrostenedione/androstenedione	▼0.71	▲2.10
7β-Hydroxy-DHEA/DHEA	▼0.34	▲1.88
6β-Hydroxyandrostenedione/androstenedione	1.05	▲1.39
16α-Hydroxytestosterone/testosterone	▼0.62	0.90
2-Hydroxyestrone/estrone	1.06	▲1.14
2-Hydroxyestradiol/estradiol	▼0.76	1.10
11β-Hydroxyandrosterone/androsterone	▼0.59	1.07
11β-Hydroxyetiocholanolone/etiocholanolone	▲1.24	1.00
11β-Hydroxyandrostenedione/androstenedione	▼0.72	▲1.47
11β-Hydroxytestosterone/testosterone	▼0.43	0.87

Shin 2013 CPT

Formation of 4β-HC

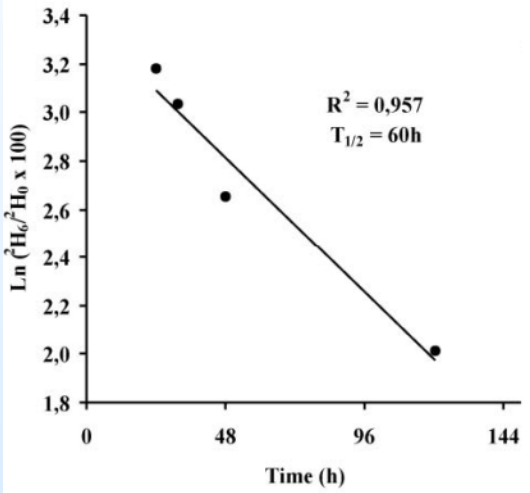


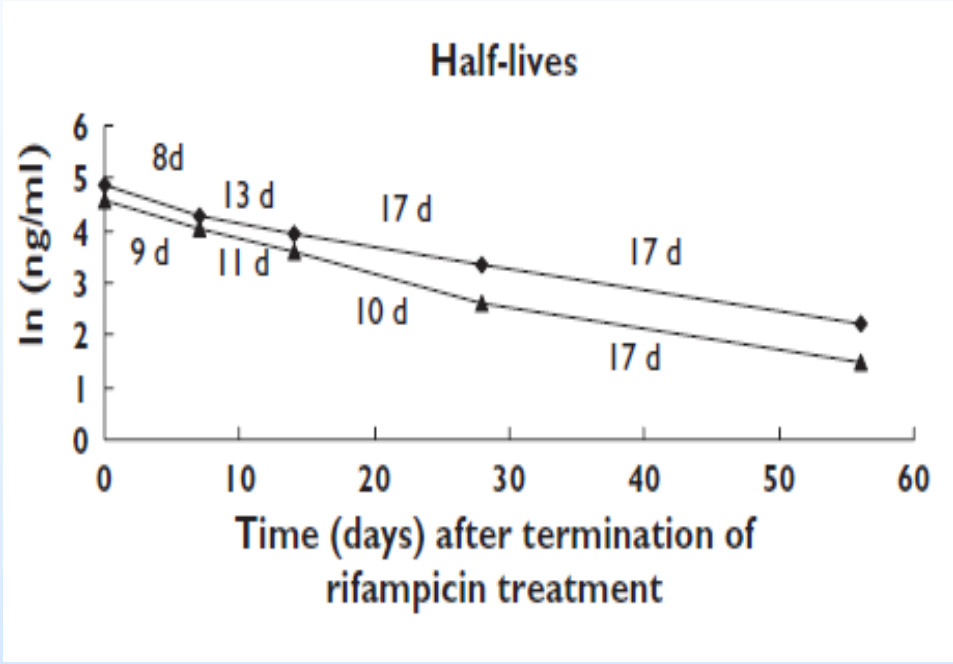
- 4β-HC formed by hepatic CYP3A
  - Recombinant CYP3A4 >> 3A5 and 3A7
  - No activity seen with recombinant CYP1A2, 2C9 or 2B6
  - Likely negligible role of intestinal CYP3A in 4β-HC formation
- Circulates as esterified 4β-HC (~83% in plasma)
- Use as a hepatic CYP3A biomarker:
  4β-HC or normalized 4β-HC (4β-HC/cholesterol)

Bodin 2001 JBC; Aubry 2008 BJCP

4β-HC has a very long half-life

- Estimated 4β-HC half-life:
  - 60 hours (N=3), may reflect distribution phase
  - 17 days (N=2)
- The long half-life has implications when assessing acute interactions with CYP3A

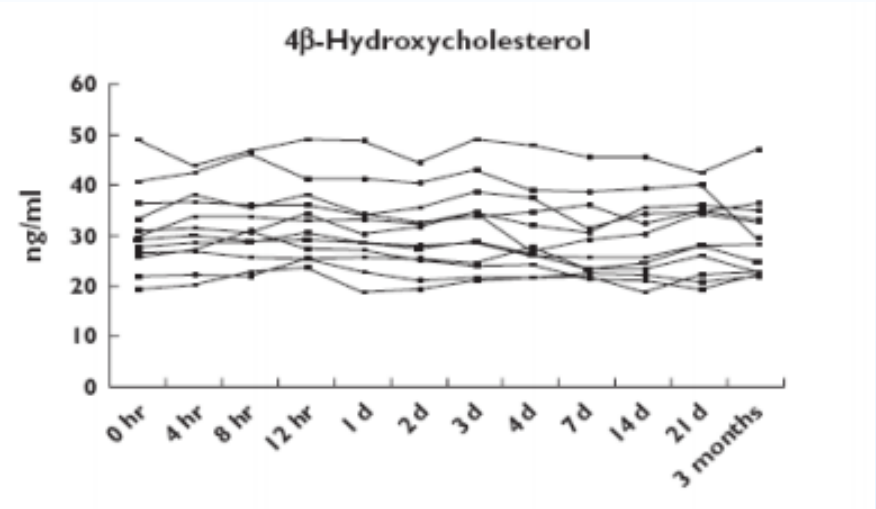




Bodin 2002 JBC, Diczfalusy 2008 BJCP

Variability in 4β-HC Levels

- Intra- and inter-individual variability of 4β-HC
  - Baseline 4β-HC ranges from 15 to 60 ng/mL
  - Low intra-individual over several months (<15%)



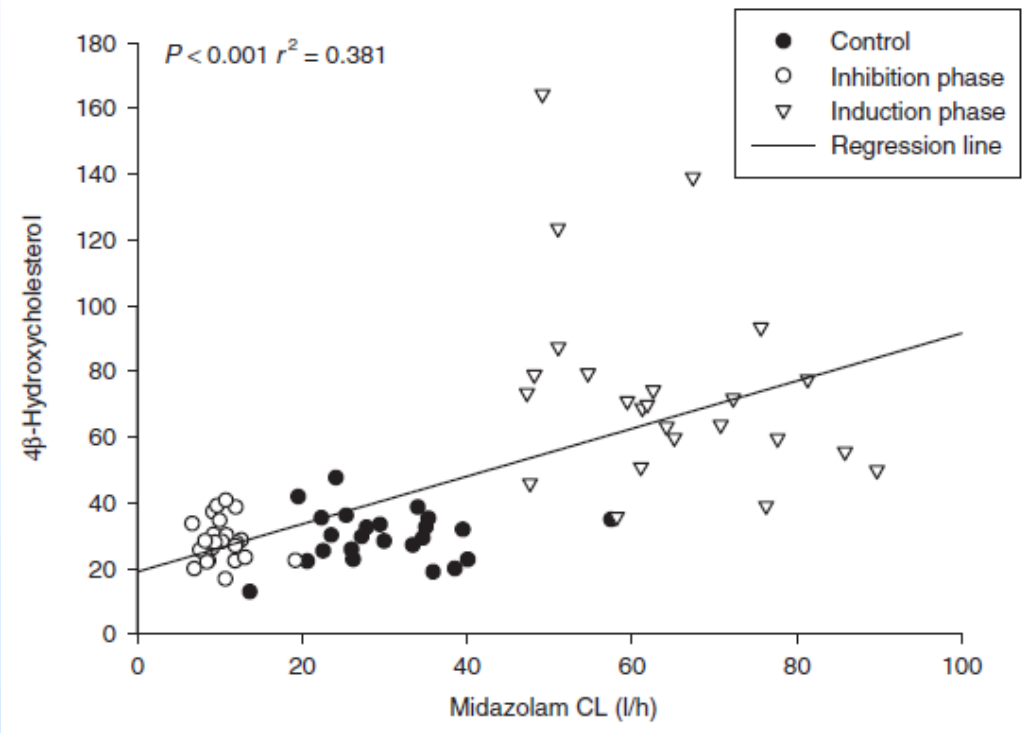
- Factors studied that affect 4β-HC:
  - Genetics (*CYP3A5*1*)
  - Trend for females to have slightly higher 4β-HC than males
  - Disease states: Crohn's, nonalcoholic steatohepatitis, and renal failure

Reviewed in Mao 2016 Drug Met Rev

How well does 4β-HC predict CYP3A activity?

- 4β-HC was weakly correlated with IV and oral midazolam clearance under baseline conditions (r^2 ~ 0.24 - 0.29)
- Stronger correlation with IV MDZ CL in the presence of inhibitors/inducers

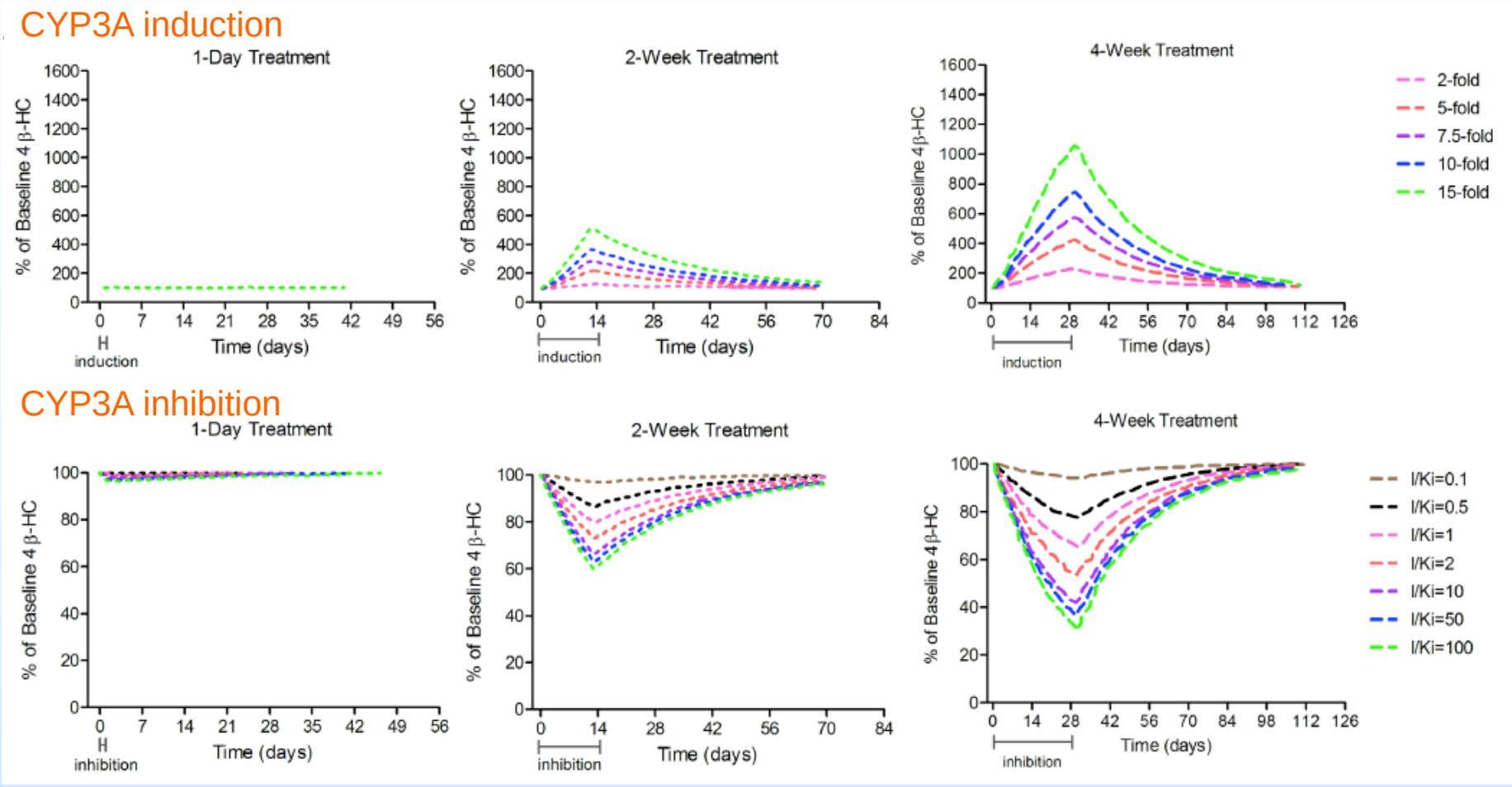
4β-HC vs. IV MDZ CL



Tomalik-Scharte CPT 2009
Björkhem-Bergman DMD 2013
Shin CPT 2013

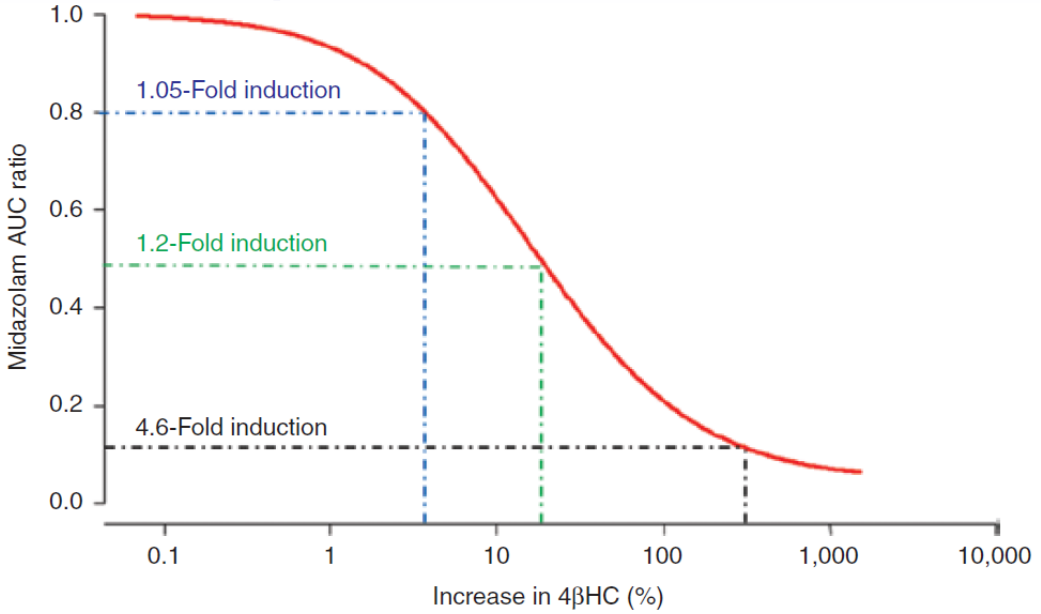
Static Modeling and Simulation of 4β-HC Response to CYP3A DDIs

- Magnitude of change depends on induction/inhibition potency and treatment duration



Adapted from Yang 2010 J Clin Pharm

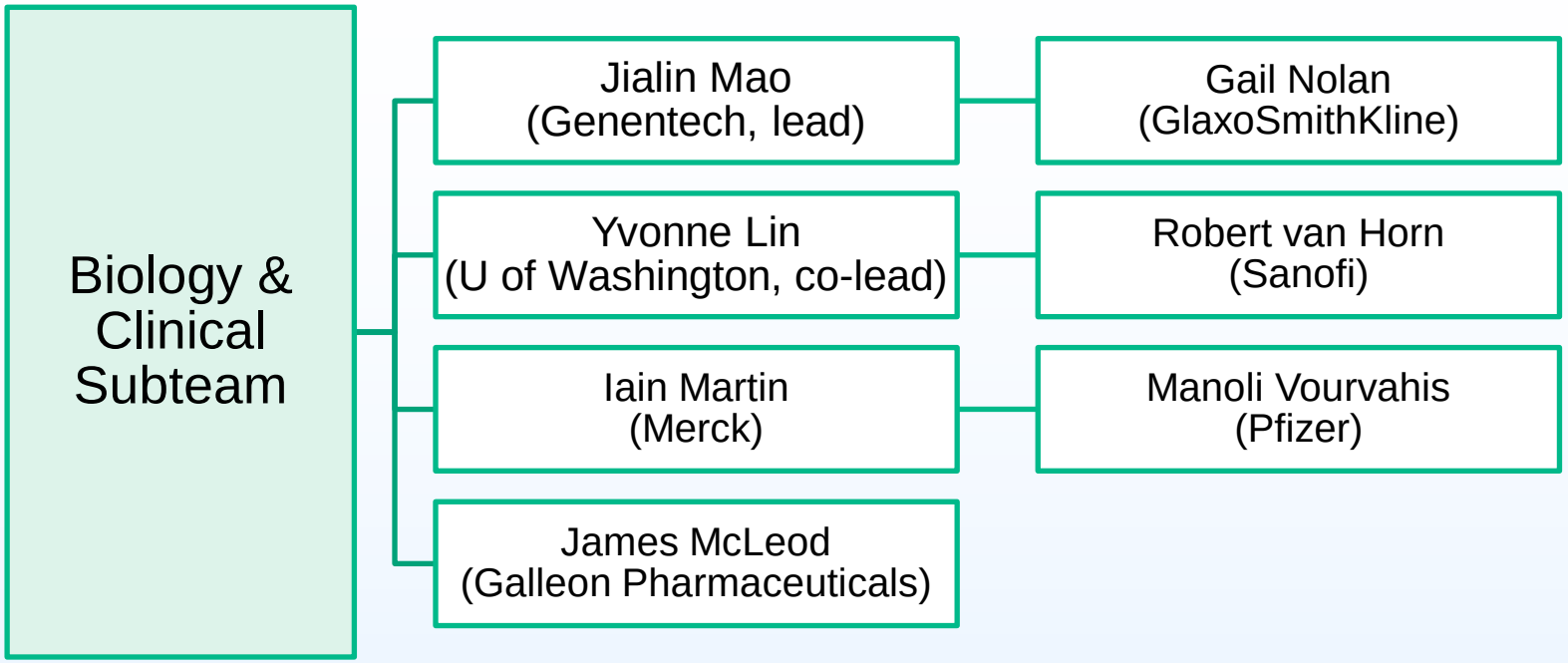
Other Modeling and Simulation of 4β-HC Response to CYP3A induction



Regulatory CYP3A Inducer Classification	Midazolam AUC ratio	Predicted fold-change in 4β-HC		
		Population PK/PD	Emax-Imax	Bayesian Mechanism-Based PK/PD
Weak	0.5 - 0.8	< 1.13	1.09 - 1.37	1.05 - 1.20
Moderate	0.2 - 0.5	1.13 - 2.10	1.37 - 2.46	1.20 - 2.05
Strong	0.2	> 2.10	> 2.46	> 2.05

Leil 2014 CPT Pharmacometrics Syst Pharmacol; Mangold 2016 Clin Pharm in Drug Dev

IQ 4β-hydroxycholesterol Working Group



- Evaluation of 4β-HC as CYP3A biomarker
  - What are the unique features of 4β-HC?
  - How can it be used in drug development?
- Does 4β-HC reflect:
  - CYP3A activity at baseline
  - Change in CYP3A activity following induction or inhibition

Mao 2016 Drug Metab Rev

Gaps in 4β-HC Knowledge

Intrinsic Characteristics

- Verify the long half-life of 4β-HC
- Characterize intestinal CYP3A contribution (if any) to the formation of 4β-HC
- Understand if transporters are involved in the disposition of 4β-HC

Application

- Basal 4β-HC concentrations in special populations (pediatrics, pregnancy and elderly), in patients (hepatic or renal disease), and patients where the illness affects CYP3A expression
- Determine the relationship of 4β-HC with midazolam and/or other CYP3A probes administered intravenously at baseline