Can we accurately measure free fraction of highly bound compounds for use in DDI risk assessment in the clinic?: IQ TALG Plasma Protein Binding Working group

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JANSSEN RESEARCH & DEVELOPMENT

Data Disclaimer

The data presented in this presentation constitute **preliminary data** from the IQ Plasma protein binding (PPB) working group and while final analysis continues.

Published final data and interpretations are subject to possible change.

Introduction

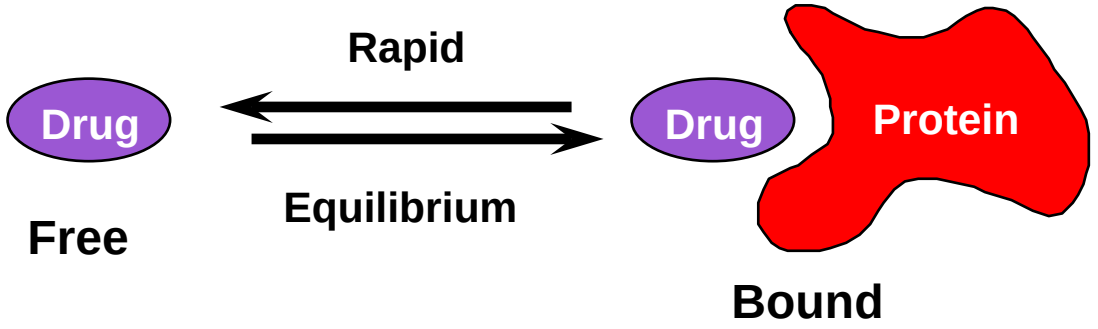
Plasma protein binding (PPB) is a fundamental DMPK parameter used in:

- PK/PD relationships
- Predict drug-drug interactions (DDIs)
- Evaluate toxicity of drugs

Regulatory agencies have historically considered 99% binding as the upper limit of high confidence measurement (FDA DDI guidance 2020)

- To be conservative in DDI prediction and avoid false negatives
- Or historically due to limits in radiochemical purity (<99%)

Acids preferentially bind to albumin while bases preferentially bind to alpha-1 acid glycoprotein (AGP)



Based on the free drug hypothesis it is the free drug that is only available to interact with a target receptor or enzyme

With present day methodologies (assays, bio-analytics), just how far beyond the 99% threshold can we reliably measure?

FDA DDI guidance for industry 2020 (p4):

*Considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.

Examples of PPB impact on industry: Montelukast Case Study

Montelukast is a strong and well-recognized **CYP2C8 inhibitor** *in vitro* with IC_{50} in HLM varying from 20 to 2000 nM depending on the final microsomal protein concentration in the assay (0.025 to 2 mg/mL).[1]

Montelukast has a **K_i of 9 nM** in rCYP2C8 where final protein concentration is minimal (*i.e.* 5 µg/mL)[2]. This K_i value can be regarded as an estimate of K_i free. The value is similar to the value derived from IC_{50} in HLM, using $f_{u,mic}$ obtained in SimCYP and assuming $K_i=IC_{50}/2$.

The above *in vitro* data triggered concerns on the risk of *in vivo* DDI with clinical substrates of CYP2C8.

[1] Walsky et al. 2005, Drug Metab Dispos 33: 413-418

[2] Walsky et al 2005, J Clin Pharmacol 45: 68-78

Montelukast Case Study (cont'd)

With a fu plasma set at 1%, DDI interactions are anticipated from *in vitro* CYP2C8 inhibition data[1,2]:

- Static modeling using unbound portal C_{max} concentrations predicted ≥2-fold change in the AUC of CYP2C8 drugs

Subsequent clinical DDI trials with CYP2C8 substrates rosiglitazone[3], pioglitazone[4] and repaglinide[5] didn't show any interaction (<5% AUC changes).

The extrapolation of the *in vitro* data using the actual f_u plasma value of **0.005%** (as opposed to the 1% default value) predicts **no risk of interaction *in vivo*** (see next slide for details).

[1] Walsky et al. 2005, Drug Metab Dispos 33: 413-418; [2] Walsky et al 2005, J Clin Pharmacol 45: 68-78; [3] Kim et al 2006, Br J Clin Pharmacol 63: 339-345; [4] Jaakkola et al 2006 Eur J Clin Pharmacol 62: 503-509; [5] Kajosaari et al 2006, Clin Pharmacol Ther 79: 231-242

Montelukast Case Study (cont'd)

With a fu plasma set at 1%, montelukast is predicted to produce a 2.2-fold change in the AUC of CYP2C8 clinical substrates. The details are described in Walsky et al 2005[1]. Briefly:

10 mg oral dose of montelukast (D); $f_{u,plasma}$ = 0.01; F_a = 0.62; k_a = 0.008/min; C_{max} = 603 ng/mL; $f_{m\ CYP2C8}$ for victim drug set at 1; Q_h = 1450 mL/min

Free portal $C_{max,u,portal} = f_{u,plasma} \times \left(C_{max} + \frac{k_a \times F_a \times D}{Q_h}\right)$

Change in CYP2C8 exposure was calculated as $\frac{AUC_{(inhibited)}}{AUC_{(control\grave{a}}} = \frac{1}{\frac{fm}{1+\left(\frac{C_{max,u,portal}}{Ki}\right)} + (1-fm)}$

The same approach with the $f_{u,plasma}$ set at 0.005% predicts no interaction (see table below):

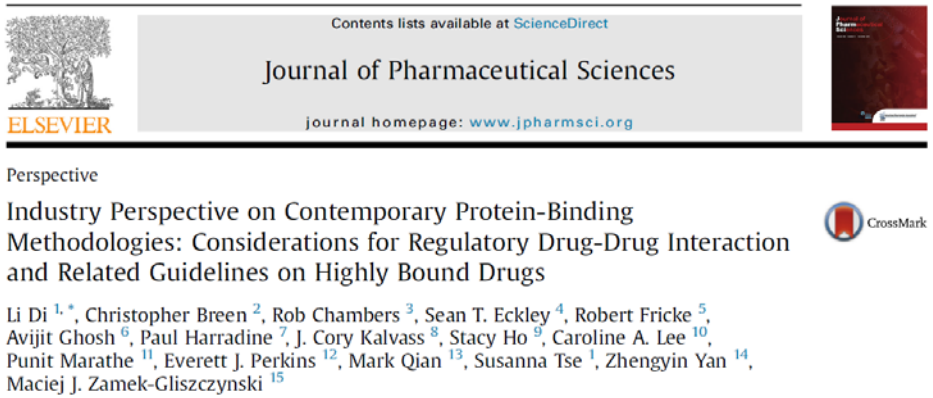
$f_{u,plasma}$ (%)	I/K_I	AUC ratio
1	1.2	2.2
0.005	0.0059	1.0

[1] Walsky et al. 2005, Drug Metab Dispos 33: 413-418

The challenge to assess highly bound compounds

- First working group assayed two compounds – warfarin & itraconazole across companies[1]
- Able to easily span one order of magnitude (i.e. 99.9% bound)

- Challenges:
  - It is challenging to identify compounds >2 orders of magnitude bound (i.e. 99.99%+)
  - Compounds with this level of binding also have analytical challenges (some folks can detect, others can't – estimated sensitivity needed without dilution: 20-50 pg/mL)
  - These compounds also tend to have high non-specific binding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Perspective

Industry Perspective on Contemporary Protein-Binding Methodologies: Considerations for Regulatory Drug-Drug Interaction and Related Guidelines on Highly Bound Drugs

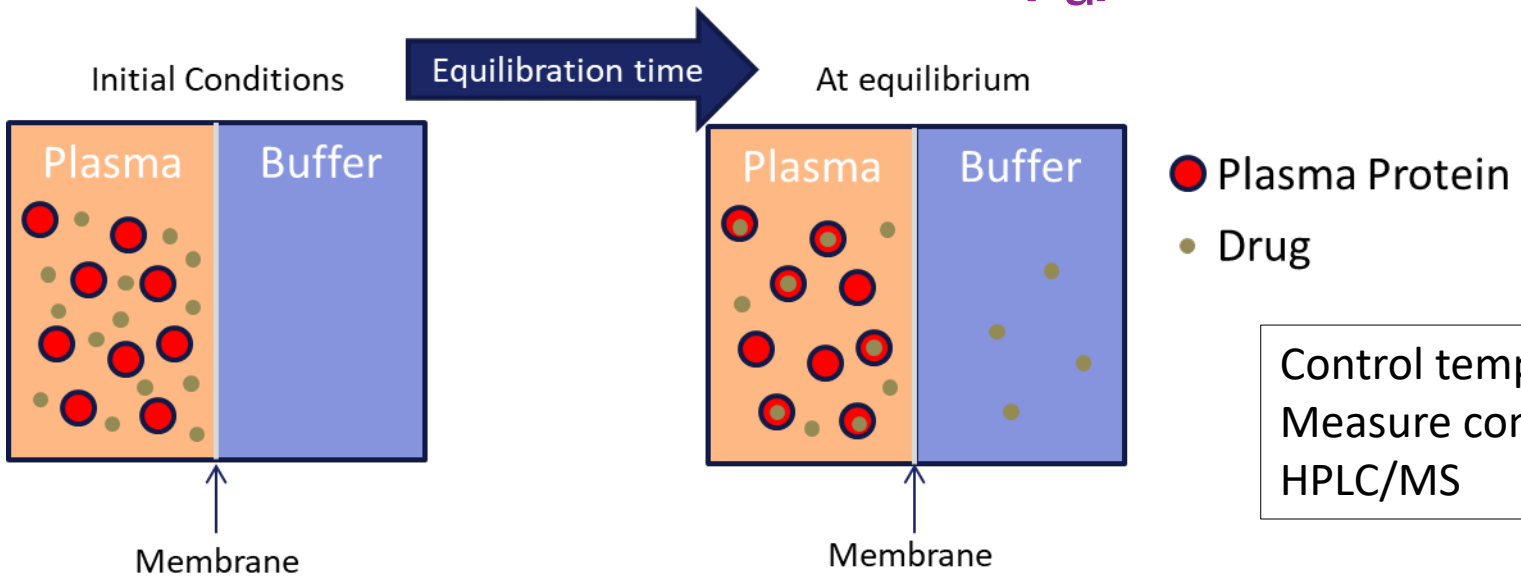
Li Di [1,*], Christopher Breen [2], Rob Chambers [3], Sean T. Eckley [4], Robert Fricke [5], Avijit Ghosh [6], Paul Harradine [7], J. Cory Kalvass [8], Stacy Ho [9], Caroline A. Lee [10], Punit Marathe [11], Everett J. Perkins [12], Mark Qian [13], Susanna Tse [1], Zhengyin Yan [14], Maciej J. Zamek-Gliszczynski [15]

Scope for second PPB working group:

- **Provide a richer data set using a common methodology and harmonized assay protocol to minimize sources of variability**
- **Consensus decision to use equilibrium dialysis based on company resourcing and inherent cancellation of non-specific binding in the assay**
  - **Not an endorsement of eq. dialysis as the only possible method, other methods are available**

[1] Di et al. 2017, J Pharm Scin 106:3442-3452

Measurement of Fraction Unbound (f_u)



Control temperature & pH
Measure concentration by HPLC/MS

At equilibrium:

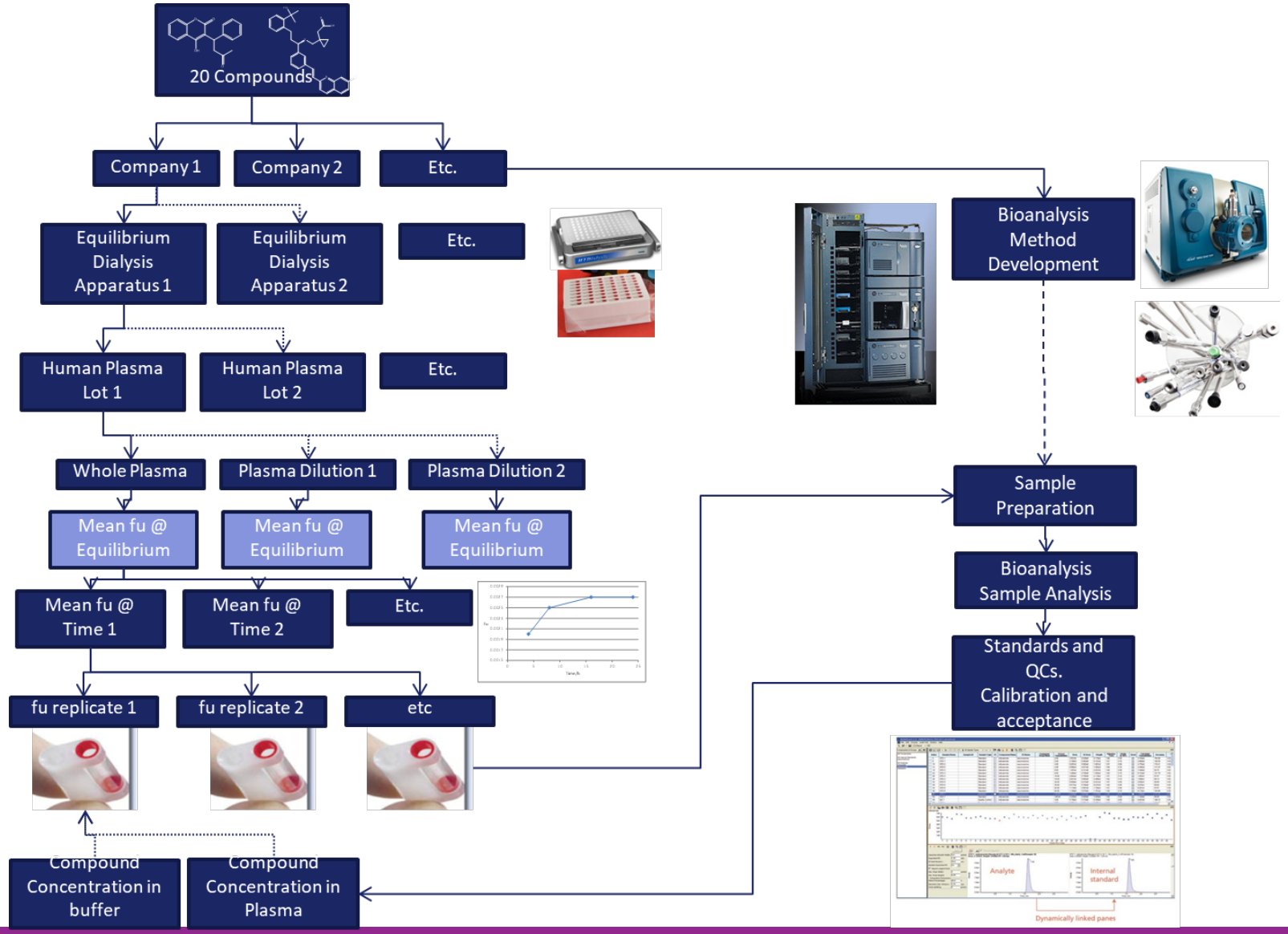
$$fu = \frac{Drug\ concentration\ in\ buffer}{Drug\ concentration\ in\ plasma}$$

At equilibrium in dilute plasma with dilution factor D:

$$fu_{undiluted} = \frac{{}^{1}/_{D}}{\left({}^{1}/_{fu_{measured}} - 1\right) + {}^{1}/_{D}}$$

$$\%\ Recovery = \frac{Drug\ concentration\ in\ buffer + Drug\ concentration\ in\ plasma}{Initial\ Drug\ Concentration\ in\ plasma} \times 100$$

Workflow for PPB assessment (11 companies)



Assay protocol and acceptance criteria

Plasma

- Pool size at least 5 donors mixed gender from healthy non-fasted volunteers
- pH 7.4 should be controlled in assay (see below)
- Frozen no more than once
- No heat inactivation
- Anticoagulant up to each company

Incubation

- Apparatus either HTD (4 members) or RED device (8 members)
- Buffer = Phosphate buffered saline @ pH 7.4
- Gentle shaking at 37°C with high humidity
- Test whole plasma (100%) initially with plasma dilutions allowed as necessary
- Compound concentrations < 2 µM
- pH should be controlled
  - Guarantee pH of plasma throughout incubation. Suggestions:
    - Using pre-dialyzed plasma
    - Using 100 mM phosphate buffer
    - Performing incubations under 5% CO_2

Equilibrium time

- Determine compound equilibrium using a time course (4-24 hr)
- Upper limit of apparatus (both HTD & RED) = 24 hr

Controls

- Warfarin or itraconazole included with each run as a reference control

Acceptance

- **Replicates should have <30% CV**. Replicates with >30% should be excluded from analysis
- At least three acceptable replicates needed per incubation

Recovery

- Assess recovery

Bioanalytical criteria more stringent than typical discovery level assays

- Calibration curves (min 7 pts)
- +/- 25% LLOQ, +/- 20% otherwise
- Etc.

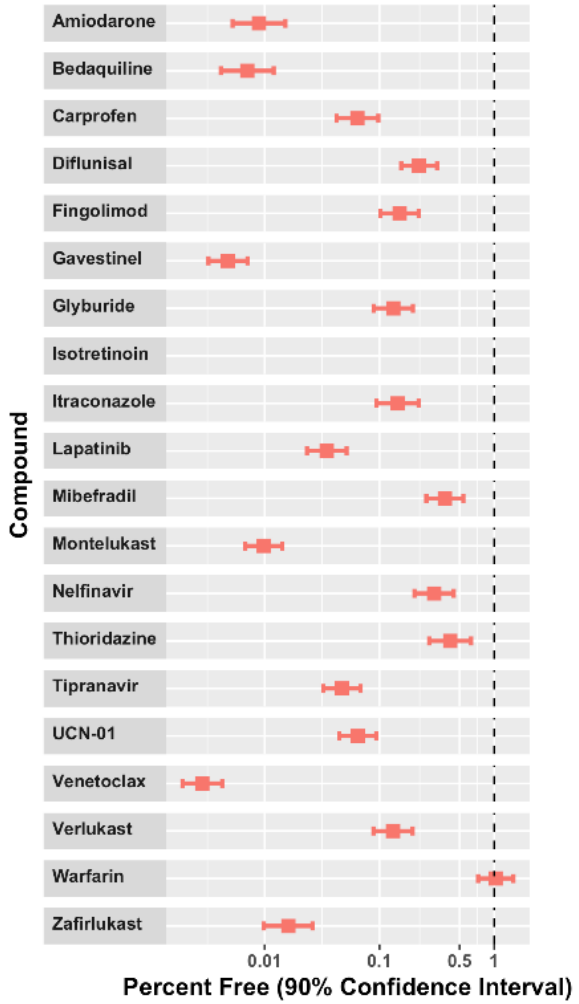
Compounds studied

Compounds (1-10)	Compounds (11-20)
Amiodarone	Mibefradil
Bedaquiline	Montelukast
Carprofen	Nelfinavir
Diflusinal	Tipranavir
Fingolimod	Thioridazine
Gavestinel	UCN-01
Glyburide	Verlukast
Itraconazole	Venetoclax
Isotretinoin	Zafirlukast
Lapatinib	Warfarin

20 Compounds were identified with diverse properties:

- Spanning multiple physchem space (acid, base, etc)
- Binding to albumin, Alpha-1 acid glycoprotein or lipoprotein
- Provided at least a two-order of magnitude f_u range (1% to 0.01%)

Assessment of PPB determination for all compounds across companies



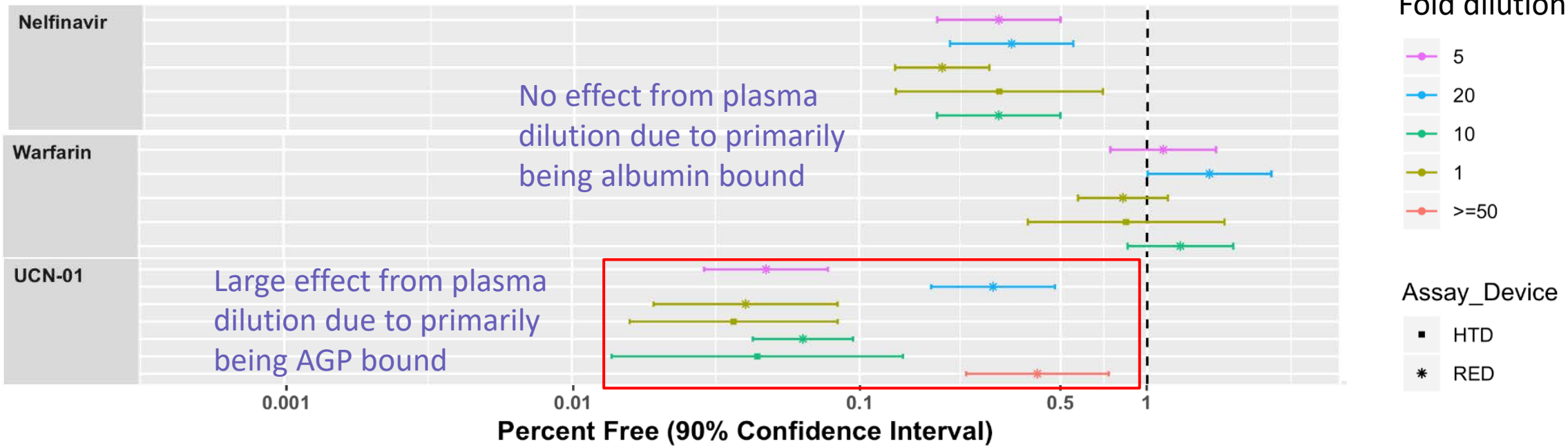
Statistical model of PPB data from all companies with both company and assay device (RED or HTD) set as random effects

- 20 compounds assessed for PPB spanning three orders of magnitude of PPB below 99% threshold
- 90% confidence interval spans up to half a log order or less across all compounds.

Suggests there is no technical limitation and PPB values less than 99% are readily achievable with good precision

Note: Does not account for the effect of plasma dilutions!

Examples of the effect of plasma dilutions on PPB values

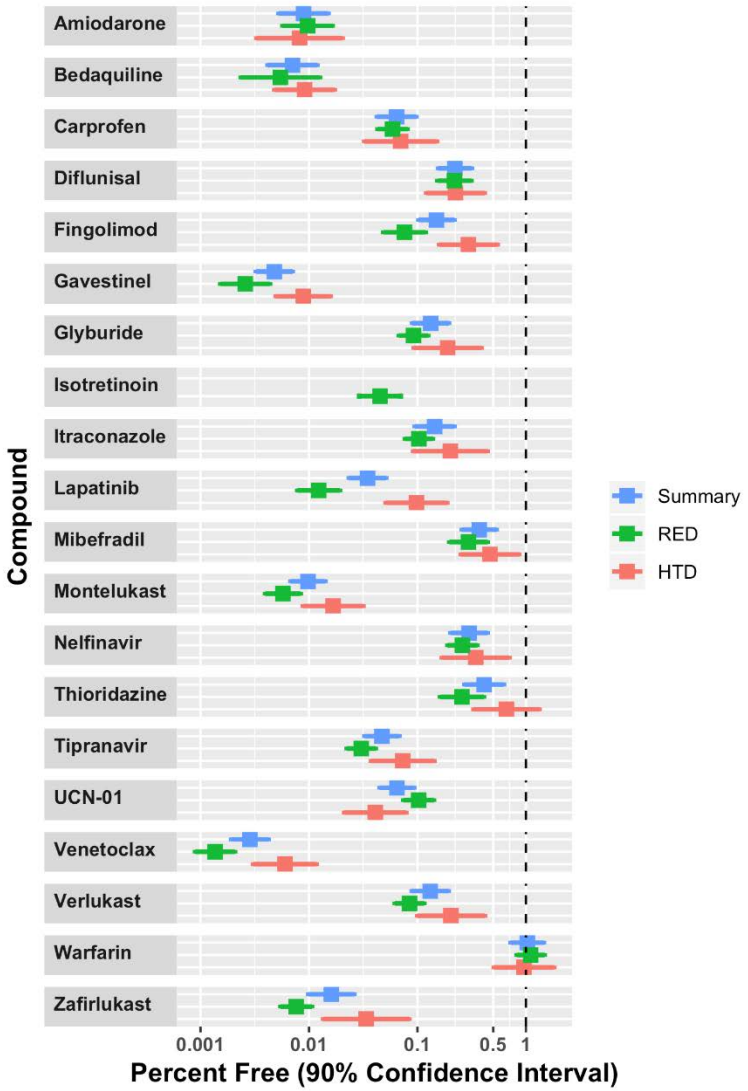


Suggests that large plasma dilutions can greatly skew PPB results when compounds reach saturable binding conditions (i.e. bind to AGP)

No significant differences in AGP levels in plasmas sourced by 6 companies that had residual samples for analysis (i.e. any saturation effects should be consistent across companies)

Company	AGP Concentration [g/l]
BL	0.93
DK	0.87
NR	0.64
SN	0.74
TP	0.9
XS	0.81

The effect of assay device on PPB values



For most compounds both HTD and RED device have similar results

However, assay device may play a role in contributing to the overall variability for certain compounds

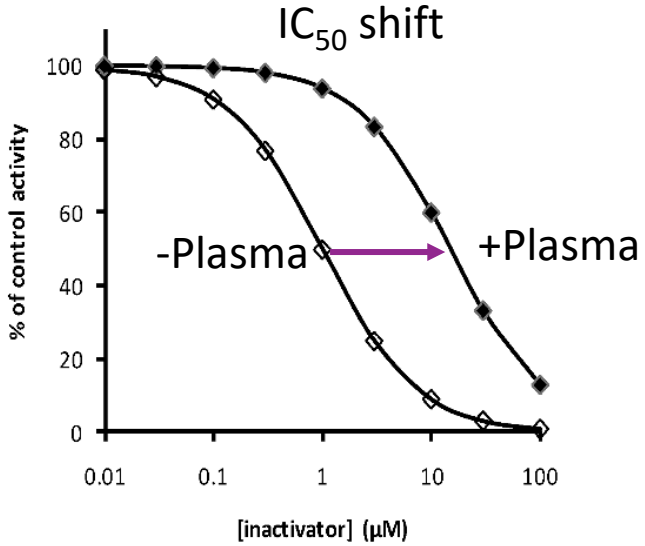
- Unclear what is driving HTD right shift relative to RED
  - Possible effect of incubation volumes?
- May suggest RED may be more sensitive in certain cases?

Accurate or not?

- Literature PPB values suffer from similar (or more) variability as values generated in this study (alternative methods, conditions, etc.) and thus are not ideal to use for an accuracy comparison
- Ideally, working group would generate data with orthogonal PPB methods
  - Resource intensive, project time limitations
- Have flux dialysis data (n = 1) that suggests good correlation (data not shown)

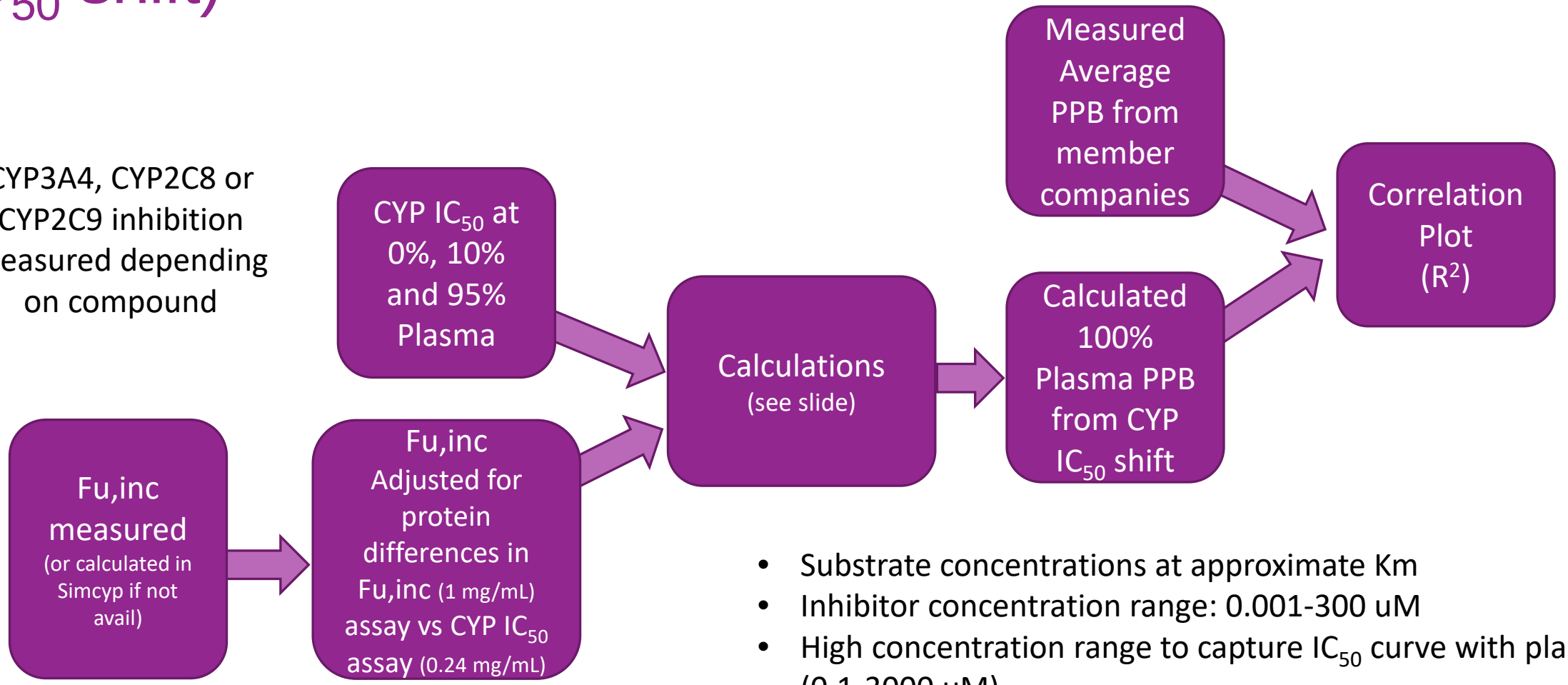
Is there non-PPB endpoint to validate the accuracy of our results? – Yes!

- The use of a CYP inhibition IC_{50} shift assay +/- the addition of plasma
- The IC_{50} shift due to the addition of plasma under "ideal conditions" can be directly used to calculate the magnitude of plasma protein binding
- Ideal conditions:
  - Accounting for non-specific binding to human liver microsomes (HLM)
  - Having sufficient concentration range to define IC_{50} curves under +/- plasma conditions



Assessment of PPB accuracy with an orthogonal method (IC_{50} shift)

CYP3A4, CYP2C8 or CYP2C9 inhibition measured depending on compound



- Substrate concentrations at approximate Km
- Inhibitor concentration range: 0.001-300 uM
- High concentration range to capture IC_{50} curve with plasma (0.1-3000 uM)

Test compounds for IC_{50} shift assay

Pilot IC_{50} data from 0% plasma dataset

Compound	U. Wash IC_{50}	Pilot IC_{50}	% Std Dev (N=3)
Amiodarone (3A4)	15 - >50	195.2922	36.8
Itraconazole (3A4)	0.02 – 0.1	0.0623	8.6
Mibefradil (3A4)	0.2 – 4.6	0.2432	12.0
Montelukast (2C8)	0.010 – 0.022	0.5476	3.0
Nelfinavir (3A4)	1.3 – 8	0.3404	10.6
Tipranavir (3A4)	30	0.8297	5.5
Warfarin (R+S) (2C9)	12	7.5835	9.1
Venetoclax (2C9)	0.14	0.5476	12.1
Zafirlukast (2C9)	0.5	0.6766	4.90
Ketoconazole (3A4)	0.006 - 0.03	0.076	10.6
Quercetin (2C8)	0.6 - 3	3.9	4.5
Sulphaphenazole (2C9)		0.269	13.6

Confirmed solubility issue – excluded from analysis

Higher IC_{50} than literature however data variability low

Lower IC_{50} than literature however data variability low

Control compounds not run with earlier PPB set

Calculations

Fu_{inc} Correction

(1 mg/mL to 0.24 mg/mL)

$$Fu,inc\ 2 = \frac{1}{\left(\frac{C2}{C1} * \left(\frac{1 - Fu,inc\ 1}{Fu,inc\ 1}\right)\right) + 1}$$

P_{total} = 570 µM albumin or percentage therein

AIM: Equation describing 'free fraction'

$$[BD] \overset{B}{\leftrightarrow} [D_{free}] \overset{P_{free}}{\longleftrightarrow} [PD]$$

Equation 1 (non-specific binding)

$$[D_{free}] = fu_{inc0} * (D_{total} - [PD])$$

Equation 2 (binding to albumin)

$$K_1 = \frac{[PD]}{[D_{free}] * [P_{free}]} = \frac{[PD]}{[D_{free}] * (P_{total} - [PD])}$$

Rearrangement and adding Eq. 1

$$fu_{inc0} * (D_{total} - [PD]) * (P_{total} - [PD]) = {}^{[PD]}/_{K_1}$$

Grouping of terms

$$fu_{inc0} * [PD]^2 + fu_{inc0} * [PD] * \left(-P_{total} - D_{total} - \frac{1}{K_1 * fu_{inc0}}\right) + fu_{inc0} * P_{total} * D_{total} = 0$$

Divide by Fu,inc0

$$[PD]^2 + [PD] * \left(-P_{total} - D_{total} - \frac{1}{K_1 * fu_{inc0}}\right) + P_{total} * D_{total} = 0$$

Solve for [PD]

$$[PD] = \frac{\left(P_{total} + D_{total} + \frac{1}{K_1 * fu_{inc0}}\right) - \sqrt{\left(P_{total} + D_{total} + \frac{1}{K_1 * fu_{inc0}}\right)^2 - 4 * P_{total} * D_{total}}}{2}$$

Free Fraction in assays w/ non-specific & albumin binding

$$fu_{assay} = \frac{[D_{free}]}{D_{total}} = \frac{fu_{inc0} * (D_{total} - [PD])}{D_{total}} = fu_{inc0} * \left(1 - \frac{[PD]}{D_{total}}\right)$$

Substitute in [PD] above

$$fu_{assay} = fu_{inc0} * \left(1 - \frac{\left(P_{total} + D_{total} + \frac{1}{K_1 * fu_{inc0}}\right) - \sqrt{\left(P_{total} + D_{total} + \frac{1}{K_1 * fu_{inc0}}\right)^2 - 4 * P_{total} * D_{total}}}{2 * D_{total}}\right)$$

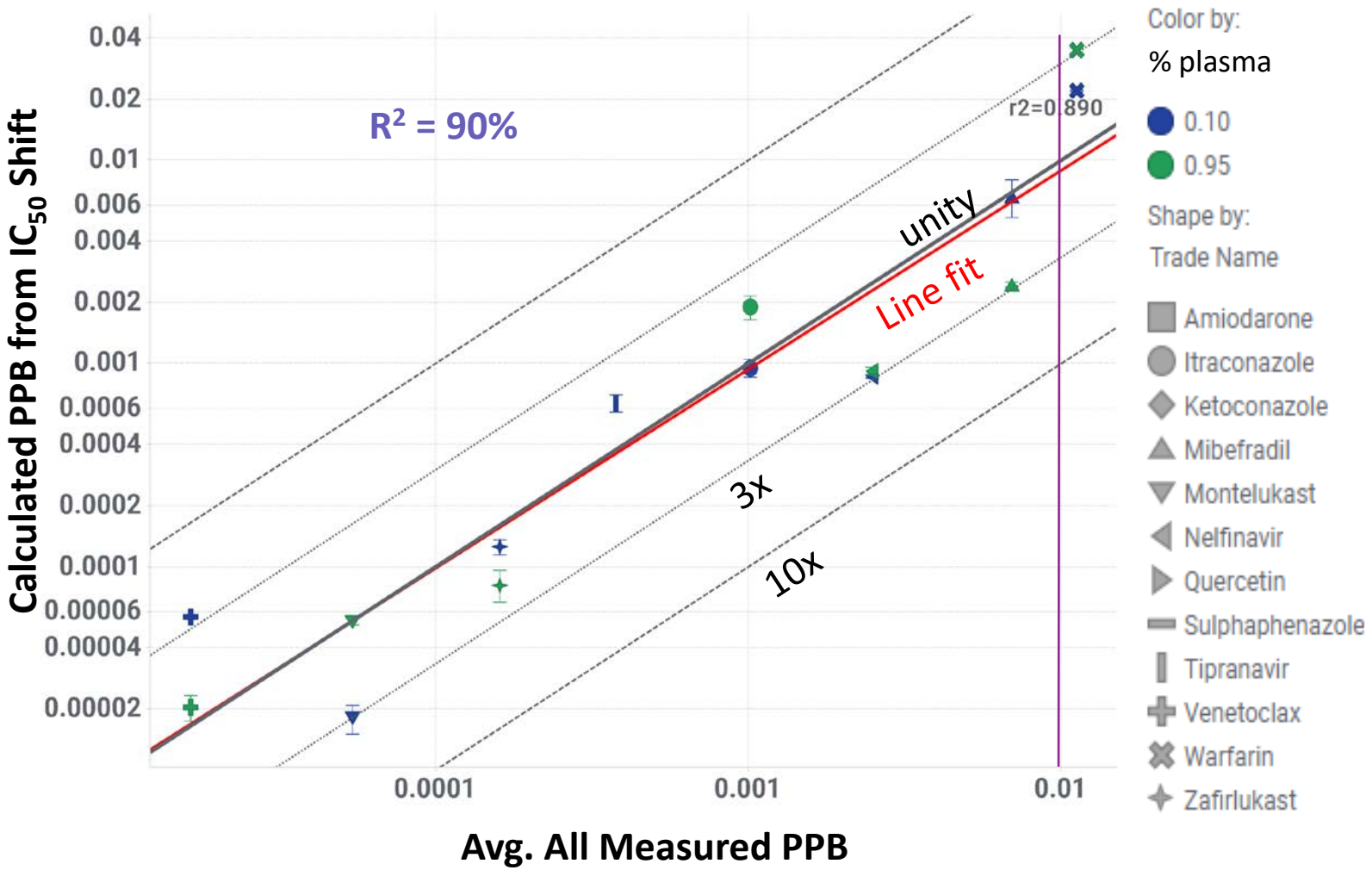
Estimation of K_1 from IC_{50} Shift

$$K_1 = \frac{(Shift - 1)}{fu_{inc0} * \left(P_{total} + D_{total} * \left(\frac{1}{Shift} - 1\right)\right)}$$

Calculating Fu from K_1

$$f_u = \frac{\left(D_{total} - P_{total} - \frac{1}{K_1}\right) - \sqrt{\left(P_{total} - D_{total} + \frac{1}{K_1}\right)^2 + 4 * \frac{D_{total}}{K_1}}}{2 * D_{total}}$$

Correlation of PPB from IC_{50} shift method and measured PPB values



Indicates good concordance (within 3-fold) between IC50 shift derived PPB values and mean values measured by working group.

Suggests that values generated by PPB working group have good accuracy

Preliminary conclusions and recommendations

Results from statistical model suggest that there is no technical limitation to determining PPB greater than 99%

- Requires robust assay design with good bio-analytics

Be wary of dilutions

- Know your compound – albumin vs AGP binding and the potential for saturable binding
- If insufficient analytical sensitivity requires dilution, try to minimize as much as possible

Plasma based CYP Inhibition assays show accuracy of PPB data

- Good correlation of IC_{50} shift derived PPB values and mean working group values

Will publish final conclusions and recommendations this year

Acknowledgements

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Others

Numerous Bench Scientists

Former WG members