Large scale utilisation of dried blood spots (DBS) for the characterisation of efavirenz (EFV) pharmacokinetics (PK) in the ENCORE 1 study

[1]Amara, AB. [1]Else, LJ. [2]Carey, D. [1]Khoo, S. [1]Back, DJ, [2]Amin, J. [2]Emery, S. & [2]Puls, R
on behalf of the ENCORE1 study group

[1] Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool [2] Kirby Institute, University of NSW, Sydney, Australia

15th International Workshop on Clinical Pharmacology of HIV & Hepatitis Therapy
Washington DC, USA. 19 May 2014

encore1

UNIVERSITY OF LIVERPOOL

UNSW AUSTRALIA

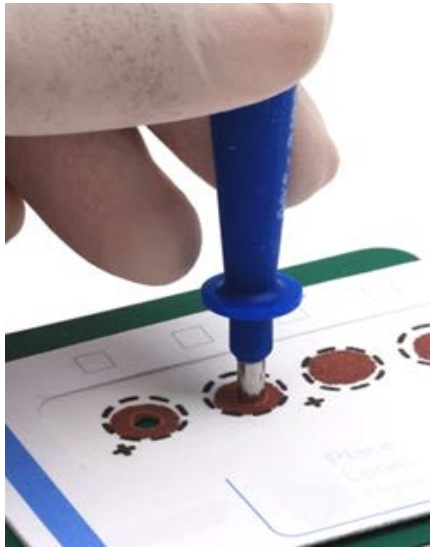
Kirby Institute

Utility of DBS in Resource Limited Settings:

- Analysis of plasma concentrations continues to be the method of choice for PK studies of antiretroviral drugs
  - trained personnel, specialised facilities, cold storage/transport

- In resource limited settings the cost of these specialised requirements may limit the collection/transport of plasma samples

- Dried blood spot (DBS) measurements provide a cheap and easier alternative
  - ease of collection, minimal processing
  - small volumes (µl)
  - less stringent storage conditions
  - availability of sensitive LC-MS/MS assays



Encore 1:

A randomised, double-blind, placebo-controlled, clinical trial to compare the safety and efficacy of reduced dose EFV (400 mg od) with standard dose EFV (600 mg od) plus (N(t)RTI) in antiretroviral-naïve HIV-infected individuals over 96 weeks[1]

- **630 ART naïve patients** (pVL >1000 copies/ml, CD4 50-500 cells/µl)
- A reduced (400 mg) dose of EFV was **non-inferior** to the standard (600 mg) dose at **48 weeks** in ART-naïve HIV-infected adults[1]
- Virological efficacy (<200 copies/ml) was comparable between doses despite lower EFV exposures[2] and mid-dose (C_{12}) concentrations[3] in the 400mg EFV arm

1. Puls R *et al* Lancet 2014; **2.** Dickinson *et al* CROI 2014, P726; **3.** Dickinson *et al* IWCPHT 2014, O1

Encore 1: *Pharmacokinetic sub-studies*

- As part of the main study all patients had paired plasma and DBS samples taken at week 4 and 12
  - sparse sampling
  - mid-dose interval (8-16 hr post-dose)

- A sub-set of patients had additional intensive (0-24 hr) PK sampling between week 4 and 8 of the study

  - ~10 participants from 4 sites
  - paired plasma and DBS (0, 2, 4, 8, 12, 16 & 24 hr post-dose)

Objectives:

- **To investigate the relationship between paired DBS and plasma samples when measuring EFV concentrations in the Encore 1 study**
  *Exploratory Endpoint

- **Sparse (mid-dose interval) concentration data from the main study population**
- **PK parameters (C_{12}, C_{16}) from the cohort undergoing intensive PK sampling**

Methods: *Sample collection*

- Whole venous blood collected in 1 x 5ml EDTA tubes
- Exactly 50µl of blood pipetted onto Whatman 903 Protein Saver cards (5 spots/card)
  - DBS air dried for 12-24 hours at room temperature
  - placed in individual zip-lock bags with humidity indicator + desiccant
  - stored at 4°C
- Remaining blood in the EDTA tube used for plasma preparation
- Plasma shipped on dry ice; DBS at 4°C
  - stored at -40°C prior to bioanalysis

Methods: *Analytical*

- EFV concentrations measured in plasma and DBS using validated LC-MS/MS methodologies[4,5]

- Plasma extracted by protein precipitation (25-10,000 ng/ml)

- DBS using liquid-liquid extraction (ethyl acetate/n-hexane; 50:50 v/v)
  - DBS punched – whole 50µl spot
  - hexobarbital internal standard
  - calibration curve: 25-5000 ng/ml
  - % Recovery >60%

- DBS short and long-term stability
  - 24 hrs bench top, 4°C, autosampler
  - ~1 year stored at -40°C
  - 3 freeze-thaw cycles

4. Amara *et al*, TDM, 2014, in press; **5.** Amara *et al*, BMSS, 2011

Methods: *Data Analysis*

- Plasma and DBS PK parameters calculated using WinNonlin (intensive study only)

- Blood-to-plasma (B/P) ratios derived

- Predicted plasma **[plasma]^{P}** derived from DBS concentrations [DBS]

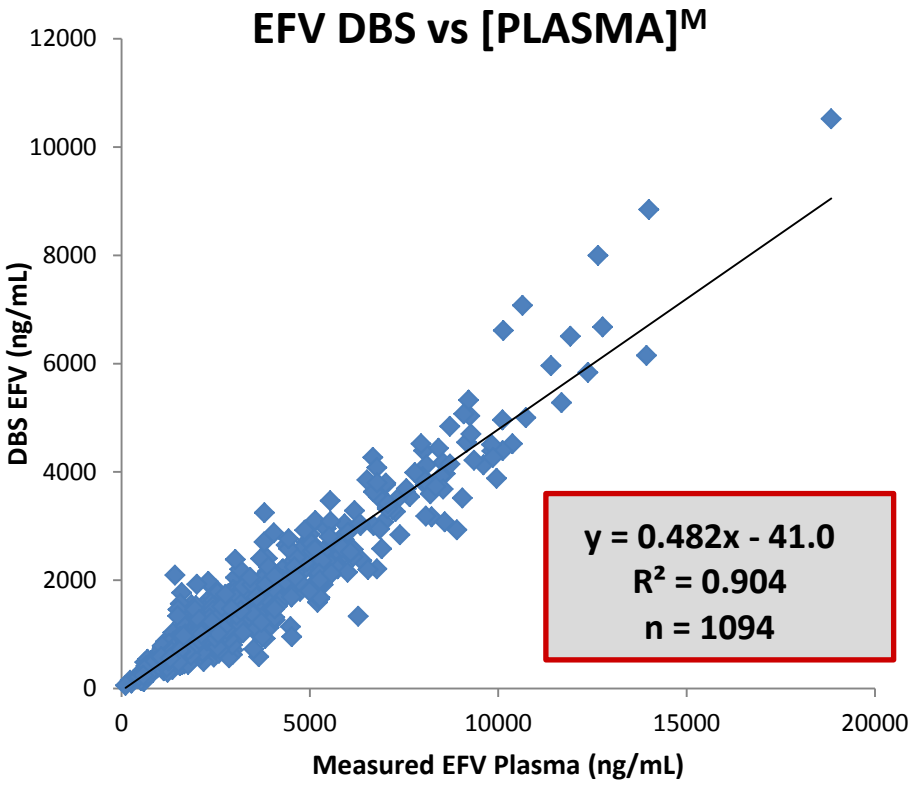
  [plasma]^{P} = [DBS]/(1-haematocrit)] × fbpp
  - standardised haematocrit of 0.45
  - EFV fbpp = 0.995 (fbpp; fraction of drug bound to plasma proteins)

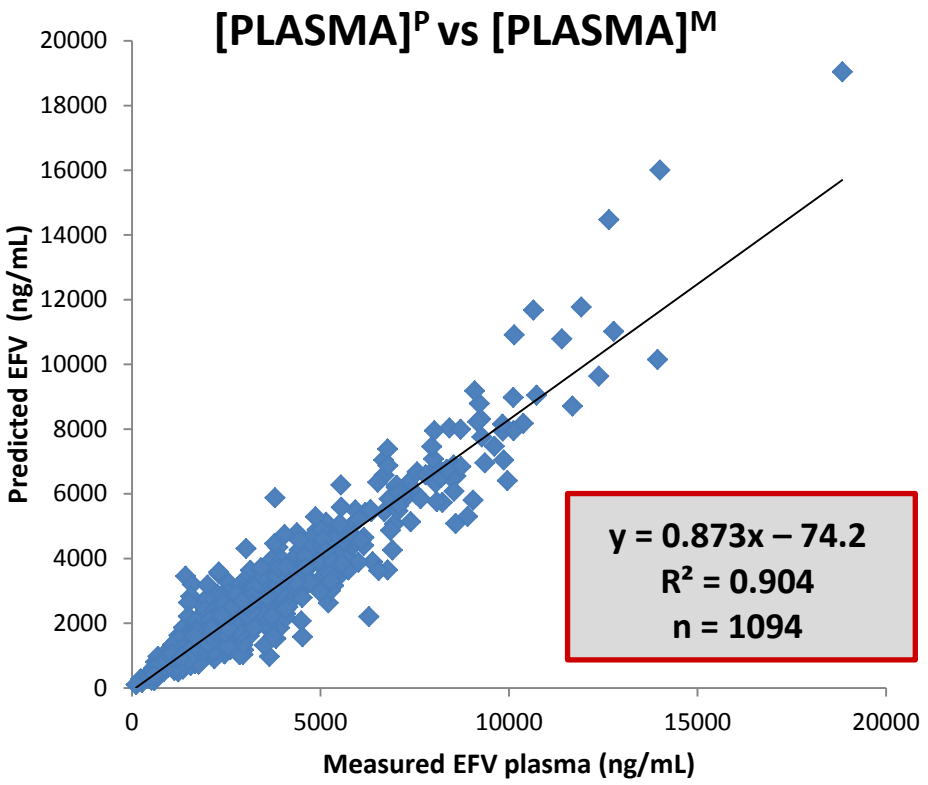
- Linear regression and Bland-Altman plots (SigmaPlot 12) used to compare measured **[plasma]^{M}** with DBS-predicted **[plasma]^{P}**

Results: *Sample population*

Main Study	TOTAL	400 mg	600 mg
Paired plasma/DBS samples, n	**1094**	579	515
Week 4, n	561	292	269
Week 12, n	533	280	253
Clinical Sites, n	39	-	-
Intensive Study	**TOTAL**	**400 mg**	**600 mg**
Subjects, n	46	28	18
Paired plasma/DBS, n	320	194	126
Clinical Sites, n	4	-	-

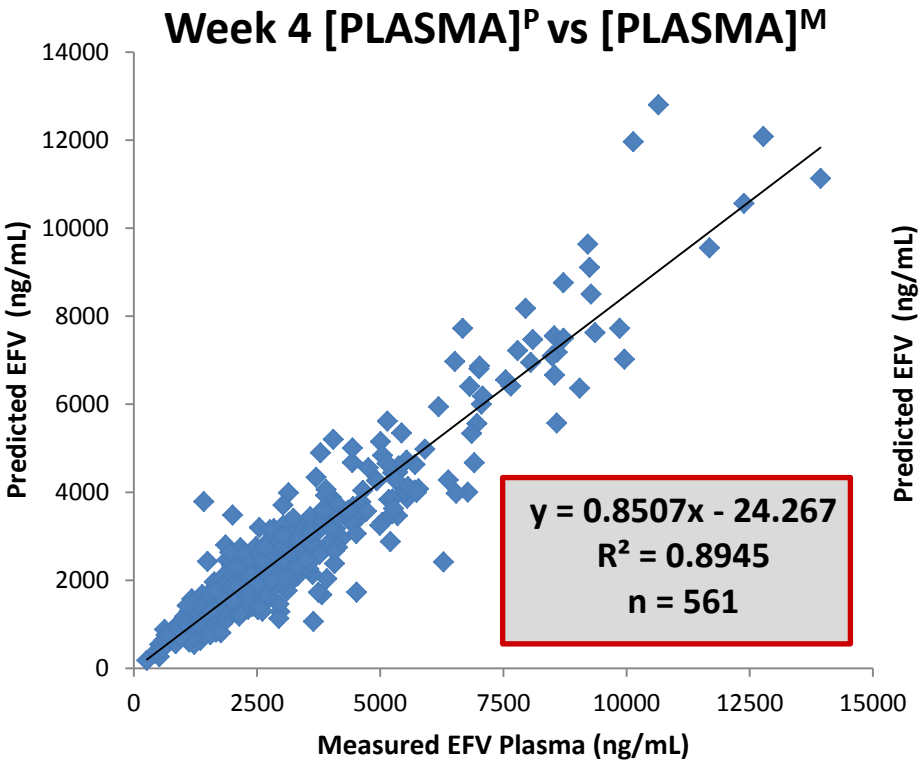
Results: *Main Study (sparse) correlations*

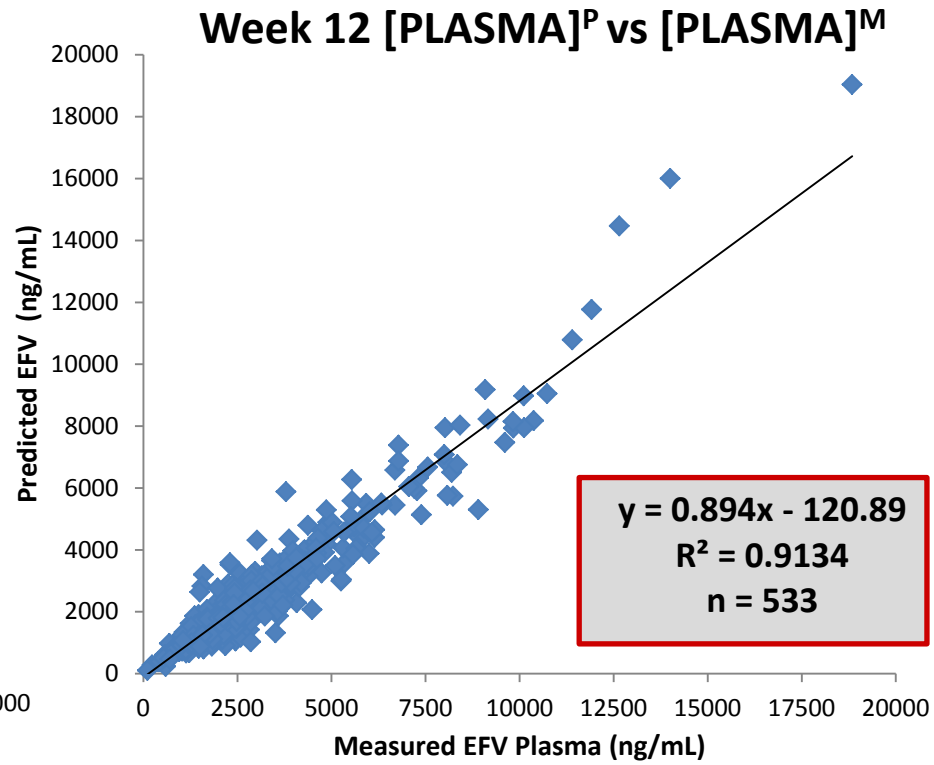




- Average (SD) B/P ratio was 0.47 (±0.11)
- [plasma]^{P} ↓ 13% vs [plasma]^{M}

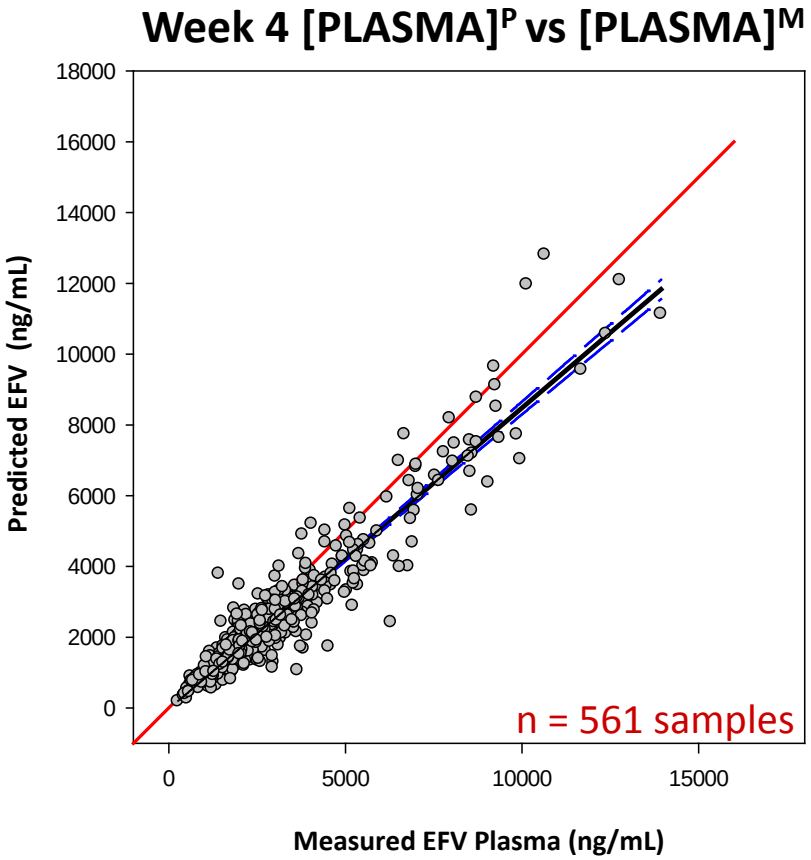
Results: *Main Study (sparse) correlations; stratified by week*

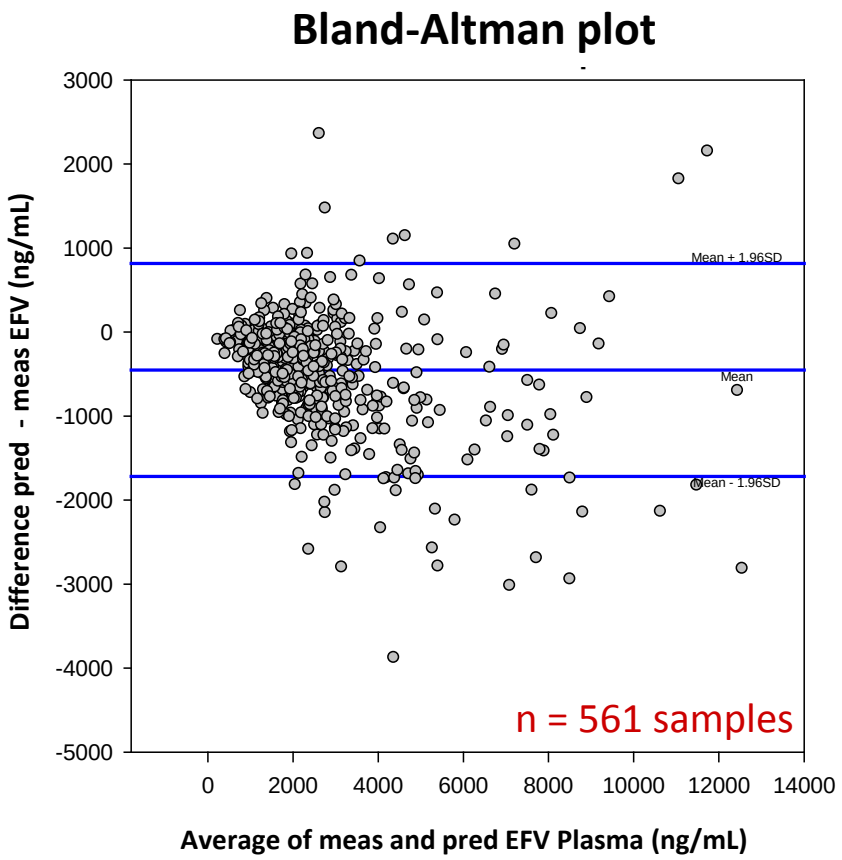




- No difference between predicted and measured plasma at week 4 & 12

Results: *Main Study (week 4) Bland-Altman plots*

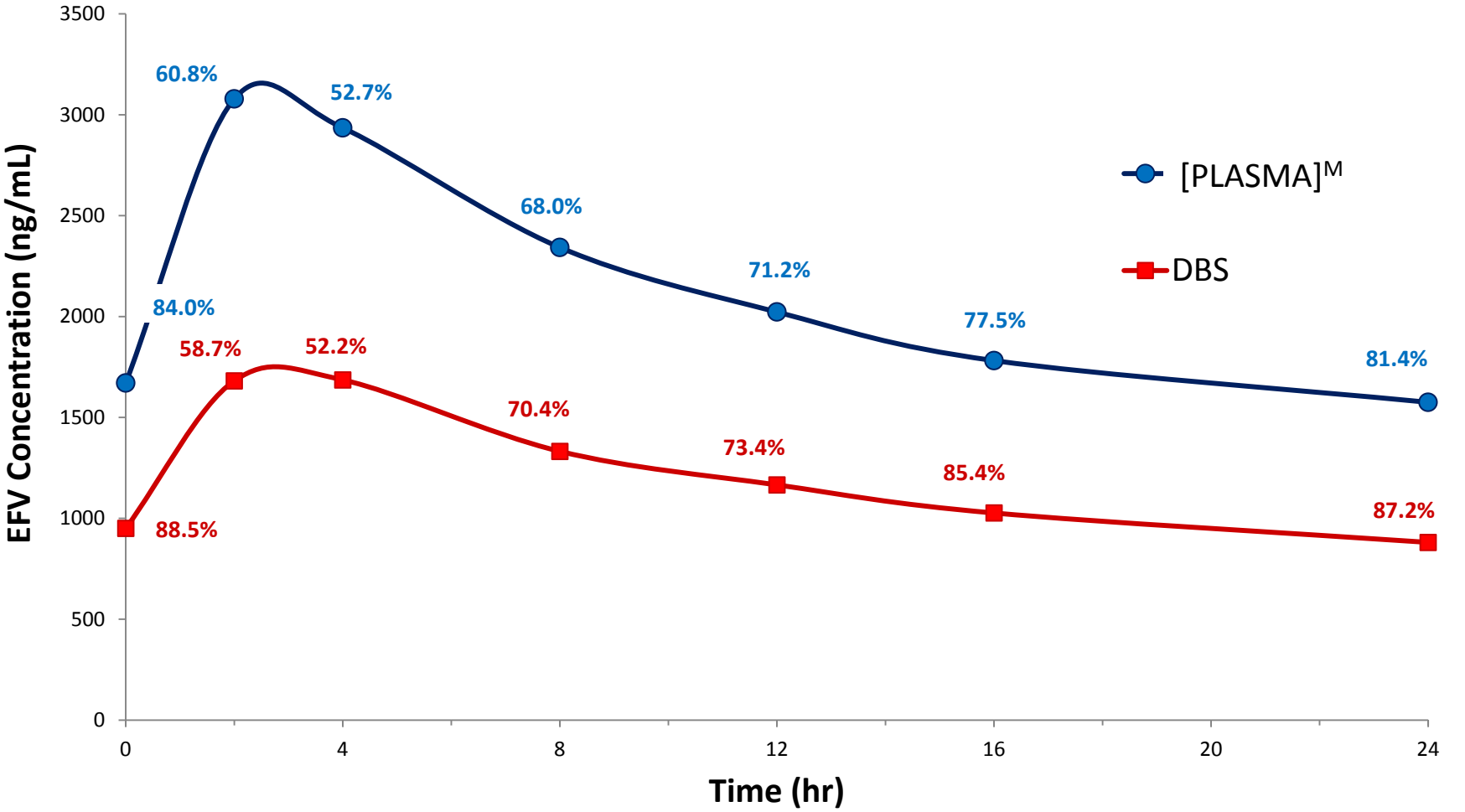




- Mean difference (95% CI) of -451 ng/ml (-504 to -398)
- Limits of agreement (±1.96 SD) of -1632 to 769 ng/ml

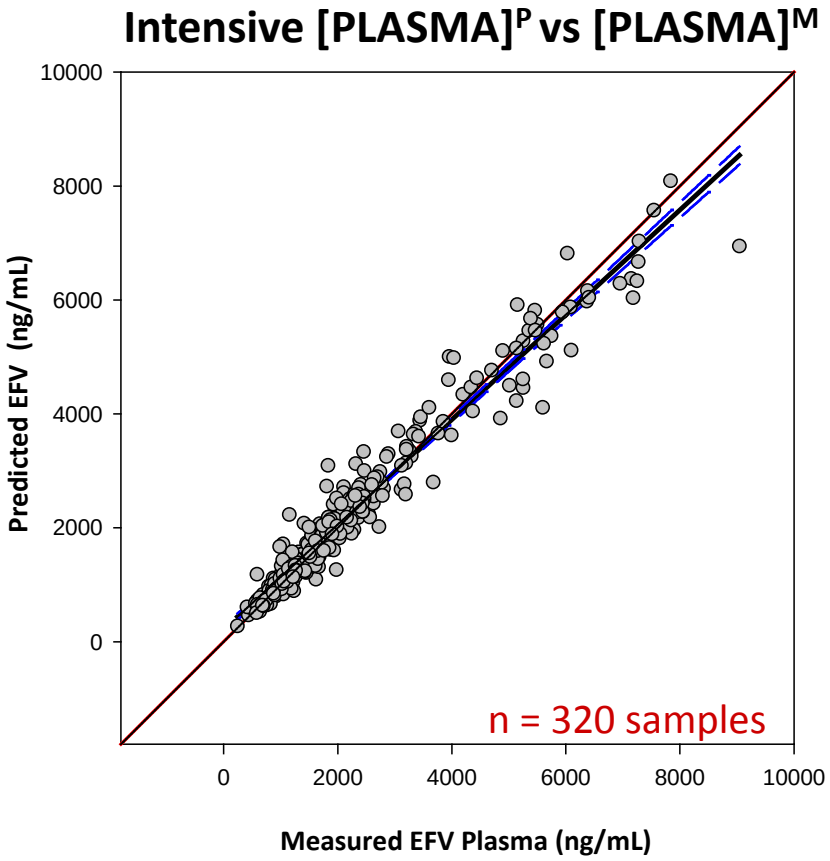
Results: *Intensive PK sub-study: EFV DBS vs [PLASMA]^{M}*

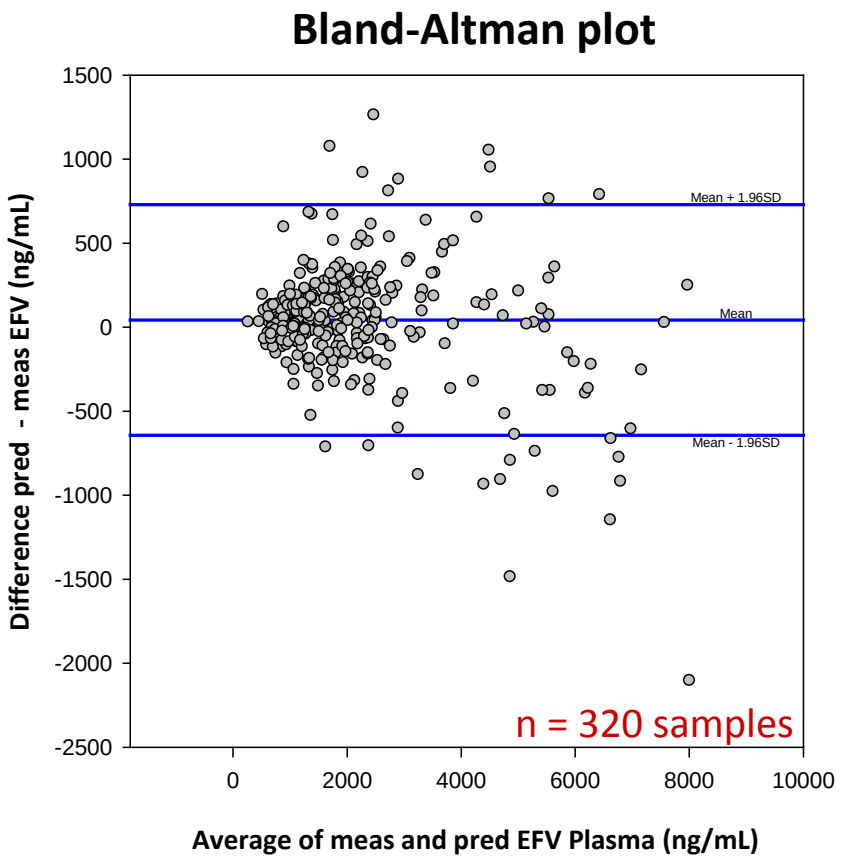
Mean B/P Ratio ± SD						
0.58 ± 0.09	0.56 ±0.07	0.59 ± 0.06	0.59 ± 0.10	0.59 ± 0.09	0.59 ± 0.12	0.57 ± 0.09



4. Amara *et al*, TDM, 2014, in press

Results: *Intensive study Bland-Altman plots*





- Mean difference (95% CI) of 42 ng/ml (-4.4 to 81.1)
- Limits of agreement (±1.96 SD) of -643 to 729

Discussion:

- DBS-predicted plasma from the **large-scale multicentre trial** resulted in significant underestimation of EFV concentrations
  - inter-operator (clinic personnel) differences in accuracy of sampling volumes or spotting technique
  - handling and transit times

- However, DBS was a better predictor of plasma exposure in the **intensive PK study** performed at a limited number of sites

- DBS sub-punches of a **pre-defined diameter** (3-6 mm) may help to normalise sample-to-sample variations in DBS volume
  - homogeneity/viscosity of the spot
  - variations in haematocrit (age, gender, disease state)

Conclusion:

- Routine utilisation of DBS as a stand-alone method in large-scale clinical trials should be judged on a **case-by-case basis**:

  - drug
  - sample integrity
  - clinic expertise
  - bioanalytical considerations

Acknowledgements

This study was funded through an investigator-driven grant by the National Health and Medical Research Council (NHMRC) of Australia. The Kirby Institute is funded in part by the Australian Government Department of Health and Ageing. Pharmaceutical support was provided by Gilead Sciences, Mylan (formerly Matrix Laboratories)

Australia: *Alfred Hospital* – Julian Elliot, Michelle Boglis, Janine Roney. *Melbourne Sexual Health Centre* – Tim Read, Helen Kent, Julie Silvers. *Northside Clinic* – Richard Moore, Thai Lim, Jeff Wilcox. *AIDS Medical Unit* – Mark Kelly, Abby Gibson, Lucy Bird. *Royal Perth Hospital* – David Nolan, Julie Robinson, Laura Barba. *Albion St Centre* – Don Smith, Jega Sarangapany, Denise Smith. *Burwood Road General Practice* – Nicholas Doong, Nam Phan, Jeff Hudson. *Taylor Square Private Clinic* – Emanuel Vlahakis, Amrita Patel, Isobel Prone. *St Vincent's Hospital* – David Cooper, Sarah Pett, Karen MacRae. **Argentina:** *CAICI* – Sergio Lupo, Liliana Trape, Luciana Peroni. *Hospital Italiano* – Waldo Belloso, Marisa Sanchez, Mariana de Paz Sierra. *Hospital Ramos Mejia* – Marcelo Losso, Mariana Kundro, Patricia Burgoa. *Hospital Posadas* – Hector Laplume Lucia Daciuk. *Hospital Rawson* – Norma Luna, Daniel David, Laura Nieto. *FUNCEI* – Valeria Confalonieri, Emiliano Bissio, Pablo Luchetti. *CEADI* – Oscar Garcia Messina, Olivia Gear, Adrian Rodriguez. *CEIN* – Liliana Calanni, Susana Calanni, Daniel Clafunao. **Chile:** *Hospital San Borja-Arriaran* – Marcelo Wolff, Claudia Cortes, Gladys Allendes. *Hospital de la Universidad Catolica Pontificia* – Carlos Perez, Jimena Flores. **Germany:** *Medical Group Practice Berlin* – Heiko Jessen, Arne Jessen, Luca Stein, Carmen Zedlack, Maria Koch. *Bonn University* – Jurgen Rockstroh, Brigitta Späth, Christoph Boesecke. **Israel:** *Rambam Medical Centre* – Eduardo Shahar, Eynat Kedem, Garmal Hassoun. **Hong Kong:** *Queen Elizabeth Hospital* – Man-Po Lee, Patrick CK Li, Pansy PC Yu. **Malaysia:** *UMMC* – Raja Iskandar Shah Raja Azwa, Sharifah Faridah Syed Omar, Sasheela Sri La Sri Ponnampalavanar. *Hospital Sungai Buloh* – Suresh Kumar, Yasmin Gani, Henry Chang. **Mexico:** *Hospital General de Guadalajara* – Jaime Andrade Villanueva, Angeles Gonzalez, Lucero Gonzalez. *INCMNSZ* – Brenda Crabtree Ramirez, Juan Sierra Madero, Maru Zghaib. **Singapore:** *Tan Tock Seng Hospital* – Barnaby Young, Evelyn Chia, Andy Loh. **South Africa:** *Desmond Tutu HIV Centre* – Catherine Orrell, Richard Kaplan, Robin Wood. *Josha Research* – Sharne Foulkes, Neo Tsikoane, Nadia Grace. *Chris Hani Baragwanath Hospital* – Lerato Mohapi, Maureen Mohata, Ashaan Naidoo. **Nigeria:** *BUTH* – Chidi Nwizu, George Chima, Vera Labesa. *PSSH* – Chidi Nwizu, Henriatta Selle Tiri, Jonathan Bulu. **Thailand:** *HIV-NAT* – Praphan Phanuphak, Amanda Clarke, Anchalee Avihingsanon. *Khon Kaen University* – Ploenchan Chetchotisakd, Siriluck Anunnatsiri, Parichat Seawsirikul. *RIHES* – Khuanchai Supparatpinyo, Patcharaphan Sugandhavesa, Patchanee Samutarlai. **United Kingdom:** *St. Mary's Hospital* – Alan Winston, Borja Mora-Peris, Siobhan McKenna. *Chelsea and Westminster Hospital* – Brian Gazzard, Marta Boffito, Chris Higgs.