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INNOVATIONacademy

TDM of Tyrosine Kinase Inhibitors

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TDM of TKIs

- Clinical pharmacokinetics of TKIs
  - Clinical indications for TKI therapy
- TDM of TKIs
  - Use in children/adolescents
  - Target ranges
- Turboflow LC-MS/MS of TKIs
- Imatinib TDM
  - Males *vs* females
- Whole blood:plasma distributions
- Conclusions

REVIEW ARTICLE

Clinical Pharmacokinetics of Tyrosine Kinase Inhibitors: Implications for Therapeutic Drug Monitoring

Debra H. Josephs, MRCP, PhD, Danielle S. Fisher, MSc,† James Spicer, FRCP, PhD,* and Robert J. Flanagan, FRCPath, PhD†*

Therapeutic Drug Monitoring 2013; 35: 562–587

Tyrosine Kinase Inhibitors (TKIs)

- Treatment of some malignancies has moved from non-specific chemotherapy to molecular targeted therapies
- Imatinib the first TKI
  - CML, GIST
- Newer drugs: Dasatinib, erlotinib, gefitinib, lapatinib, nilotinib, sorafenib, sunitinib, etc., etc.
  - Haematological malignancies, RCC, HCC, breast cancer
- Typically fixed dose, but dose individualisation may be needed
  - Given orally, hence may be problems with adherence, absorption, interactions with transporters
  - Hepatic metabolism hence may be genetic variations in metabolism, drug-drug interactions, etc.

Some Malignancies

ALL	Acute lymphoblastic leukaemia
CML	Chronic myeloid leukaemia
GIST	Gastrointestinal stromal tumour
HCC	Hepatocellular carcinoma
pNET	Pancreatic neuroendocrine tumour
MF	Myelofibrosis
NSCLC	Non-small cell lung cancer
RCC	Renal cell carcinoma

Some Acronyms

Bcr-abl	Breakpoint cluster region-Abelson oncoprotein
PDGFR	Platelet-derived growth factor receptor
c-Kit	Mast/stem cell growth factor receptor
Src	Sarcoma oncoprotein
DDR	Discoidin domain receptor
ErbB	Erythroblastic leukaemia viral oncogene
EGFR	Epidermal growth factor receptor
HER	Human epidermal growth factor receptor
VEGFR	Vascular endothelial growth factor receptor
RET	‘Rearranged during transfection’ RET proto-oncogene

Some More Acronyms

CSF-1R	Colony-stimulating factor 1 receptor
FLT3	FMS-like tyrosine kinase 3
C-Raf	C-rapidly accelerated fibrosarcoma onco-protein
B-Raf	B-rapidly accelerated fibrosarcoma onco-protein
JAK	Janus kinase
ALK	Anaplastic lymphoma kinase
HGFR	Hepatocyte growth factor receptor
cMet	cMet oncoprotein
Ph+	Philadelphia chromosome positive

UK Licenced TKIs

Up to end-July 2012

- Axitinib
- Crizotinib
- Dasatinib
- Erlotinib
- Gefitinib
- Imatinib
- Lapatinib
- Nilotinib
- Pazopanib
- Ruxolitinib
- Sorafenib
- Sunitinib
- Vandetanib
- Vemurafenib

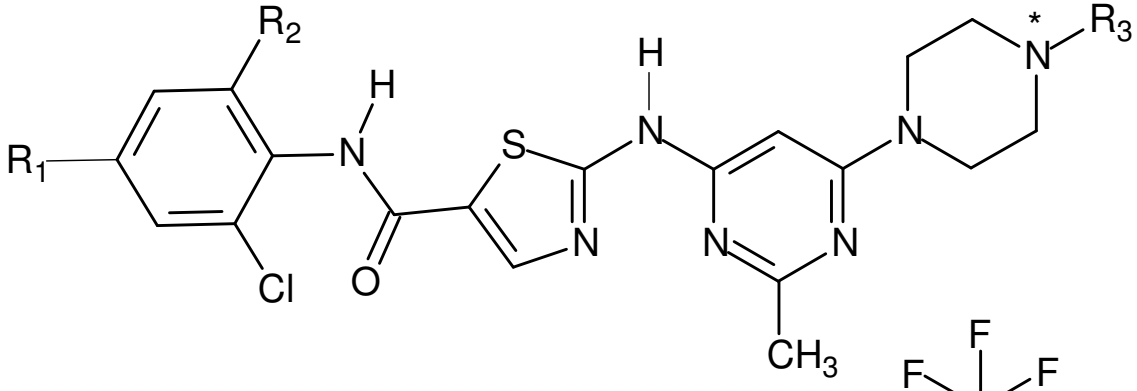
New additions

- Afatinib
- Bosutimib
- Cabozantinib
- Carfilzimib
- Dabrafenib
- Ponatinib
- Regorafenib
- Trametinib

Bcr-abl TKIs

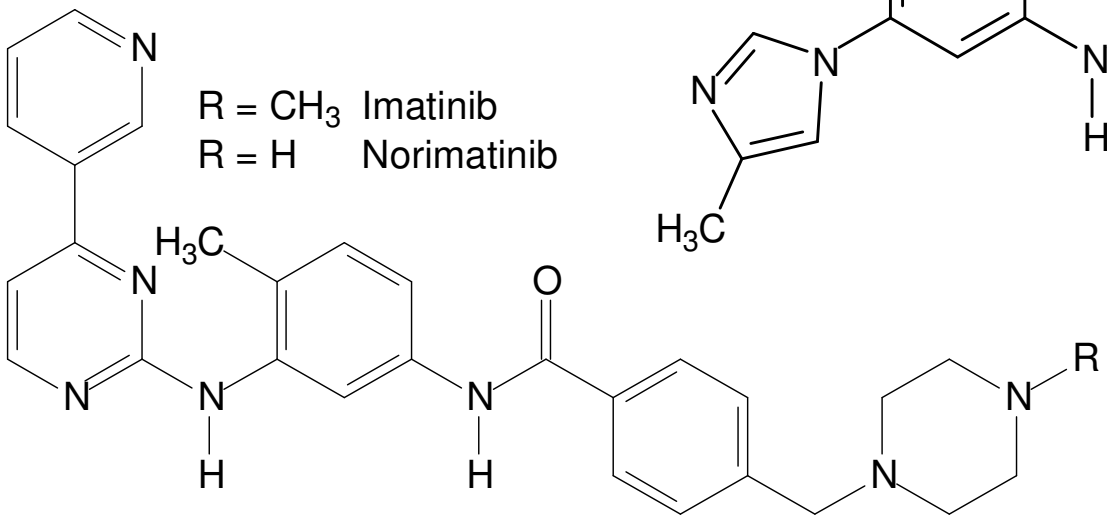
Name	Registration date	Tyrosine kinase target	Indication
Dasatinib (*Sprycel*; BMS)	28 June 2006	Bcr-abl, Src-family kinases, PDGFR-β, c-Kit, ephrin receptor kinases	Newly diagnosed Ph+ CML in chronic phase, and Ph+ CML and ALL resistant or intolerant to imatinib
Imatinib (*Glivec*; Novartis)	10 May 2001	Bcr-abl, PDGFR-α, -β, c-Kit	Ph+ CML, c-Kit positive GIST
Nilotinib (*Tasigna*; Novartis)	29 Oct 2007	Bcr-abl, PDGFR-α, -β, c-Kit, DDR-1, -2	Newly diagnosed Ph+ CML in chronic phase Chronic/accelerated phase Ph+ CML resistant or intolerant to imatinib

Bcr-abl TKIs

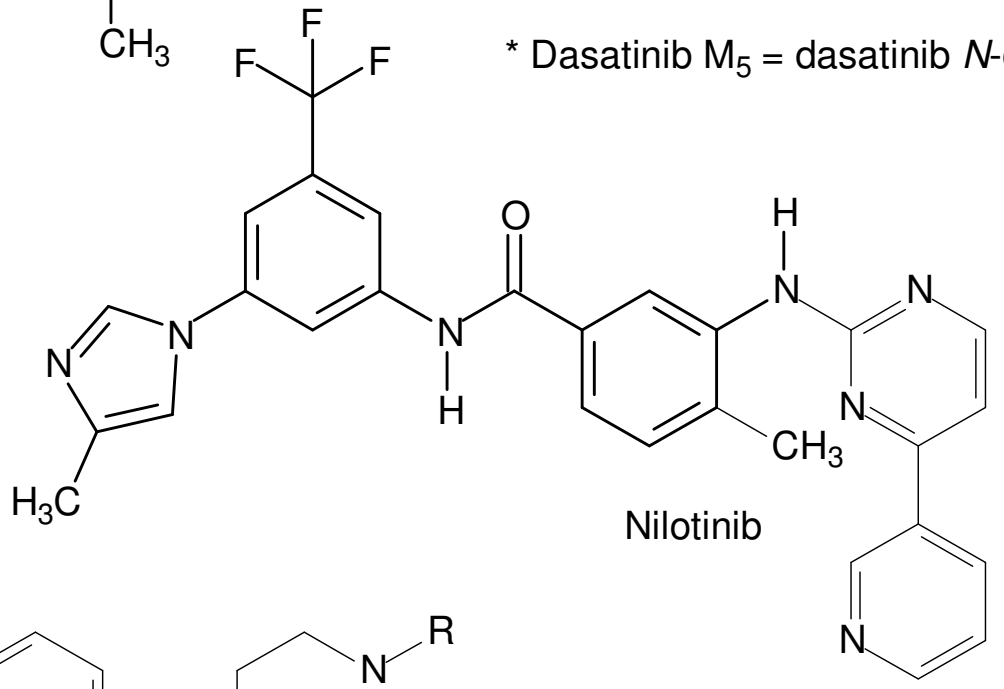


	R_1	R_2	R_3
Dasatinib	H	CH_3	CH_2CH_2OH
Dasatinib M_4	H	CH_3	H
Dasatinib M_6	H	CH_3	CH_2COOH
Dasatinib M_{20}	OH	CH_3	CH_2CH_2OH
Dasatinib M_{24}	H	CH_2OH	CH_2CH_2OH

* Dasatinib M_5 = dasatinib *N*-oxide



R = CH_3 Imatinib
R = H Norimatinib



Nilotinib

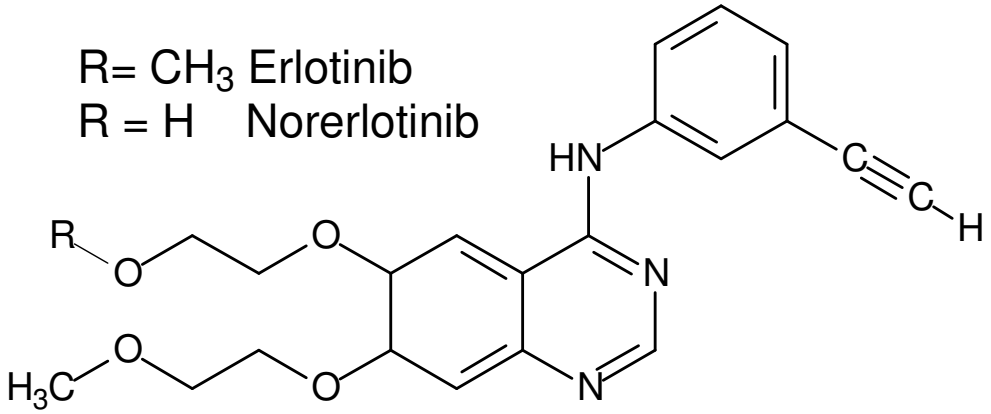
	Dasatinib	Imatinib	Nilotinib
pK_a	10.3, 6.8, 3.1	8.1, 3.7, 2.6, 1.5	13.5, 5.4, 2.1
Log P (octanol/water)	2.5–2.8	3	4.2–4.5
t_{max} (h)	0.5–3	2–4	3
Bioavailability (oral, %)	< 34	98	30
Concomitant food intake effect on bioavailability	Increases AUC (14 %)	No effect	Increases C_{max} (112 %) and AUC (82 %)
Concomitant food intake: FDA recommendation	With/without food	With food	Without food
V **(L/kg) ***	30-40	2-6 (imatinib) 15-40 (norimatinib)	10-15
Primary enzymes involved in metabolism	CYP3A4; FMO-3	CYP3A4, CYP3A5, CYP2C8	CYP3A4, CYP2C8
Plasma half-life (h)	3–5	12–20 (imatinib) 40–74 (norimatinib)	15–17
Plasma protein binding (%)	92–97	95 (imatinib and norimatinib)	98
Plasma:whole blood ratio	0.7	1.4–1.7 (imatinib) 1.4 (norimatinib)	1.4

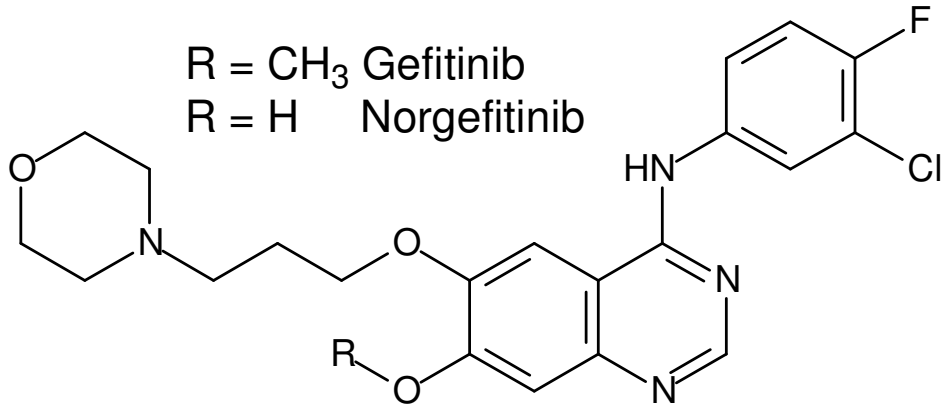
* 70 kg subject assumed

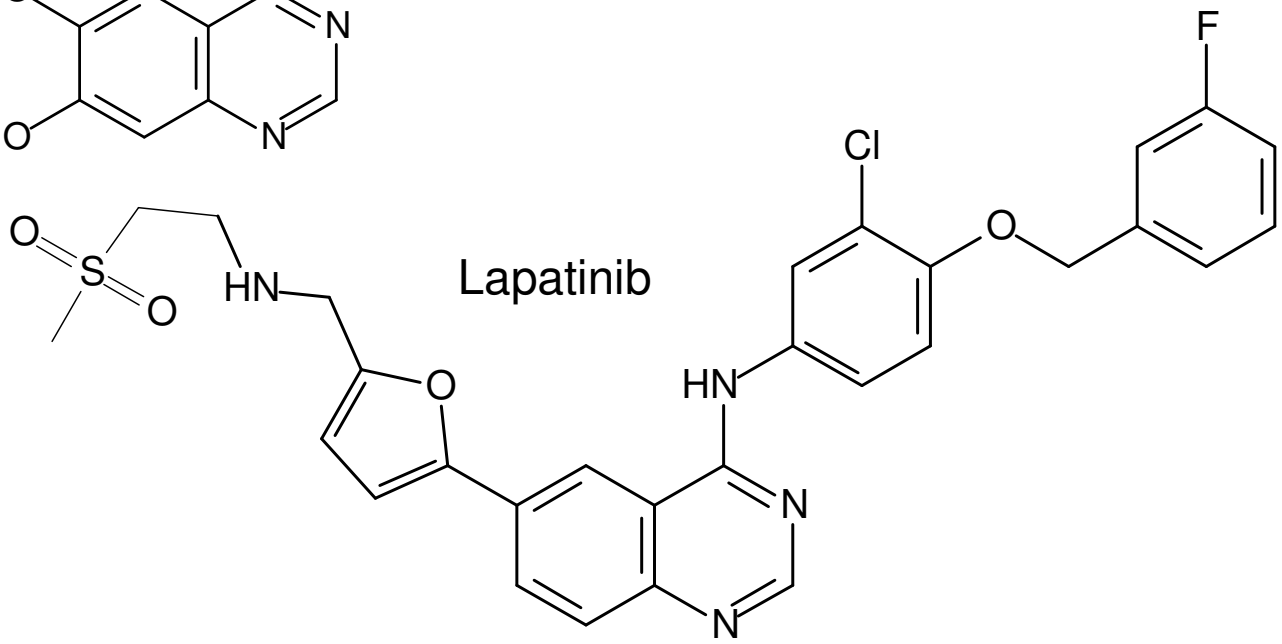
ErbB TKIs

Name	Registration date	Tyrosine kinase target	Indication
Erlotinib (*Tarceva*; Genentech, OSI, Roche)	18 Nov 2004	EGFR	First-line monotherapy or maintenance therapy of locally advanced or metastatic NSCLC, locally advanced or metastatic pancreatic cancer
Gefitinib (*Iressa*; AZ)	5 May 2003 (restricted 2005, reapproved in Europe 2008)	EGFR	First-line monotherapy of EGFR mutation positive locally advanced or metastatic NSCLC
Lapatinib (*Tykerb*; GSK)	13 March 2007	EGFR (HER-1), HER-2	HER-2 positive breast cancer, in combination with capecitabine or letrozole

ErbB TKIs


R= CH3 Erlotinib
R = H Norerlotinib
HN
C
C
H
R
O
O
N
H3C
O
O
N

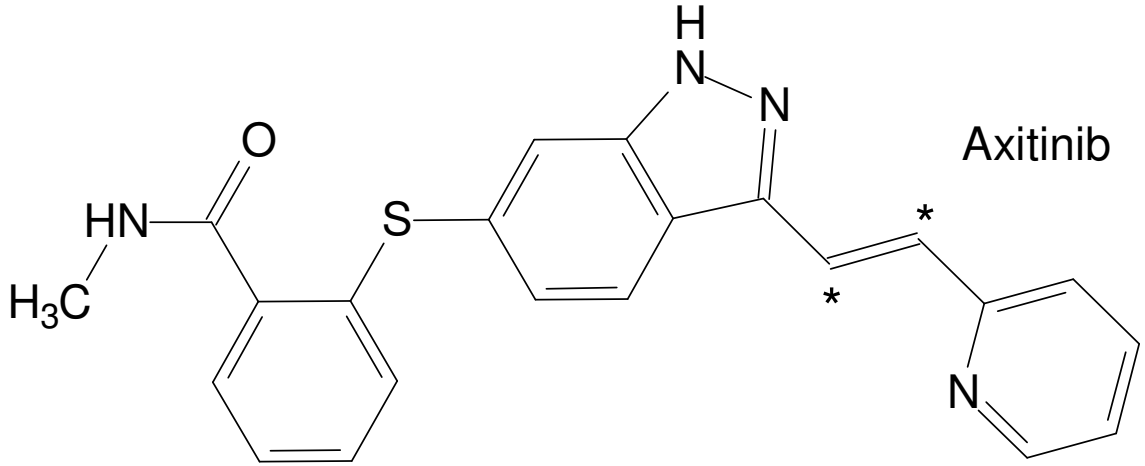

R = CH3 Gefitinib
R = H Norgefitinib
F
HN
Cl
O
N
O
N
R
O
N

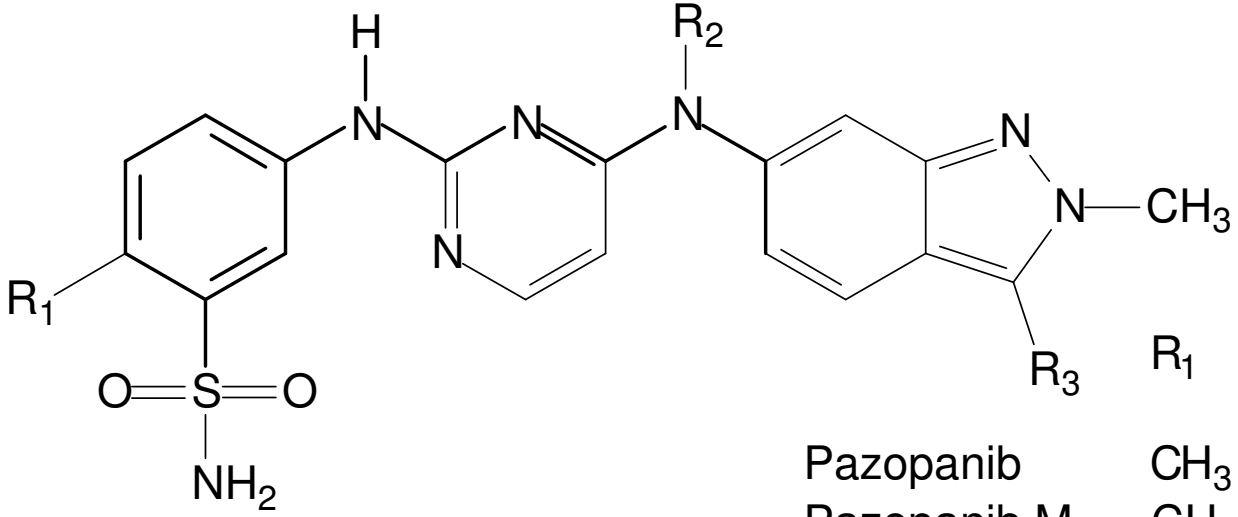

Lapatinib
F
Cl
O
O
S
O
HN
O
HN
N
N

VEGFR TKIs

Name	Registration date	Tyrosine kinase target	Indication
Axitinib (*Inlyta*; Pfizer)	27 Jan 2012	VEGFR -1, -2, -3, PDGFR, c-Kit	Advanced RCC
Pazopanib (*Votrient*; GSK)	19 Oct 2009	VEGFR-1, -2, -3, PDGFR-α, -β, c-Kit	Advanced RCC, advanced soft tissue sarcoma following prior chemotherapy
Sorafenib (*Nexavar*; Bayer)	20 Dec 2005	C-Raf, B-Raf, c-Kit, FLT3, VEGFR-1, -2, -3, PDGFR-β	Advanced RCC and unresectable HCC
Sunitinib (*Sutent*; Pfizer)	26 Jan 2007	PDGFR-α, -β, VEGFR-1, -2, -3, c-Kit, RET, CSF-1R, FLT3	Advanced RCC, GIST after progression on or intolerance to imatinib, progressive, unresectable locally-advanced or metastatic well-differentiated pNET
Vandetanib (*Caprelsa*; AZ)	06 April 2011	VEGFR, EGFR, RET	Medullary thyroid cancer

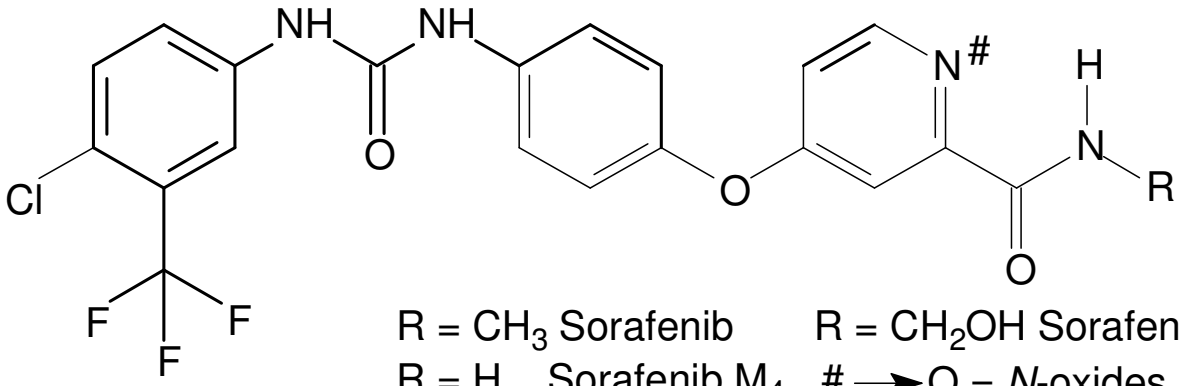
VEGFR TKIs - I



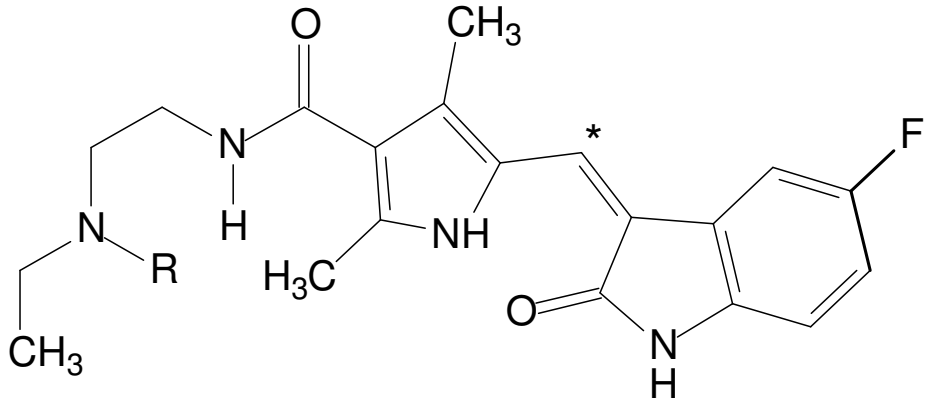


	R_1	R_2	R_3
Pazopanib	CH_3	CH_3	CH_3
Pazopanib M_{24}	CH_3	CH_3	CH_2OH
Pazopanib M_{26}	CH_2OH	CH_3	CH_3
Pazopanib M_{27}	CH_3	H	CH_3

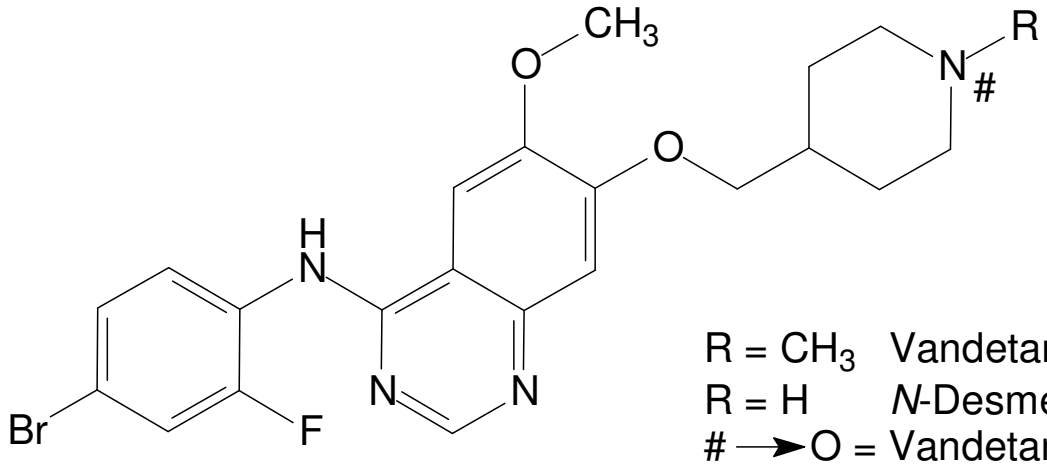
VEGFR TKIs - II



R = CH_3 Sorafenib R = CH_2OH Sorafenib M_3
R = H Sorafenib M_4 # → O = *N*-oxides



R = C_2H_5 Sunitinib
R = H Norsunitinib

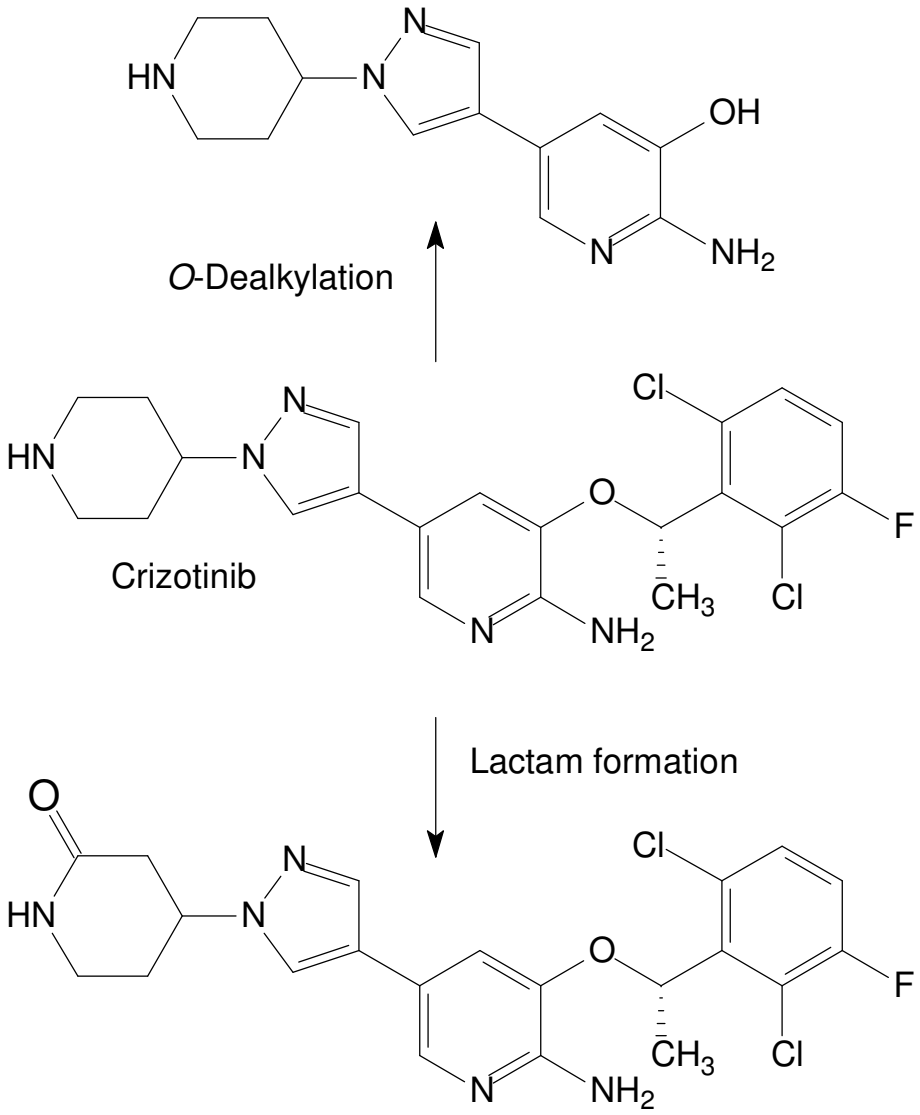


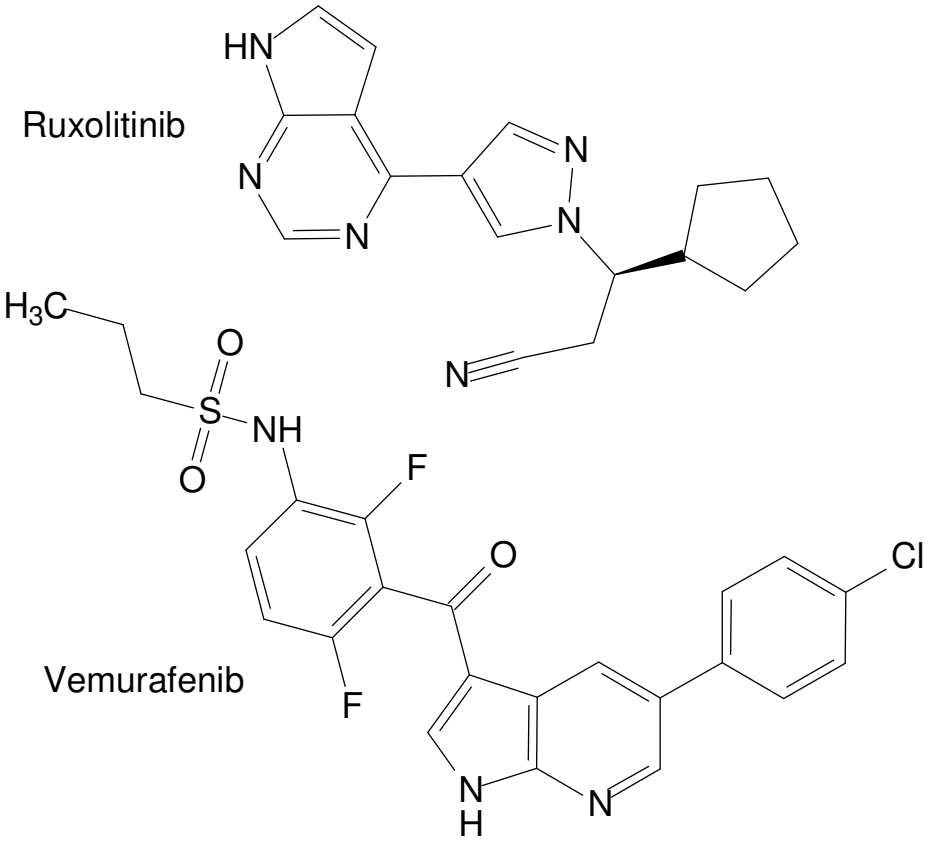
R = CH_3 Vandetanib
R = H *N*-Desmethylvandetanib
# → O = Vandetanib *N*-oxide

Crizotinib, Ruxolitinib, Vemurafenib

Name	Registration date	Tyrosine kinase target	Indication
Crizotinib (*Xalkori*; Pfizer)	26 Aug 2011	ALK, HGFR, cMet	ALK-positive locally advanced or metastatic NSCLC
Ruxolitinib (*Jakafi*; Incyte, Novartis)	16 Nov 2011	JAK 1,-2	Intermediate and high risk MF
Vemurafenib (*Zelboraf*; Roche, Daiichi Sankyo)	17 Aug 2011	B-Raf	$BRAF^{V600E}$ mutation positive metastatic or unresectable melanoma

Crizotinib, Ruxolitinib, Vemurafenib





TDM of TKIs: Pk Aspects

- Mostly metabolised by CYP3A4
  - Activity subject to large inter-individual variability
- Drug-drug interactions
  - Induction: Anticonvulsants, St John's Wort
  - Inhibition: Azole antifungals, grapefruit juice
- Concomitant food intake on bioavailability
  - Nilotinib, lapatinib
- Resistance
  - GIST patients showed 33 % increase in imatinib clearance over a year: 42 % decrease in systemic exposure
- Dose-dependent toxicity
  - Liver function, skin rash, hypertension, thyroid dysfunction

TDM of TKIs

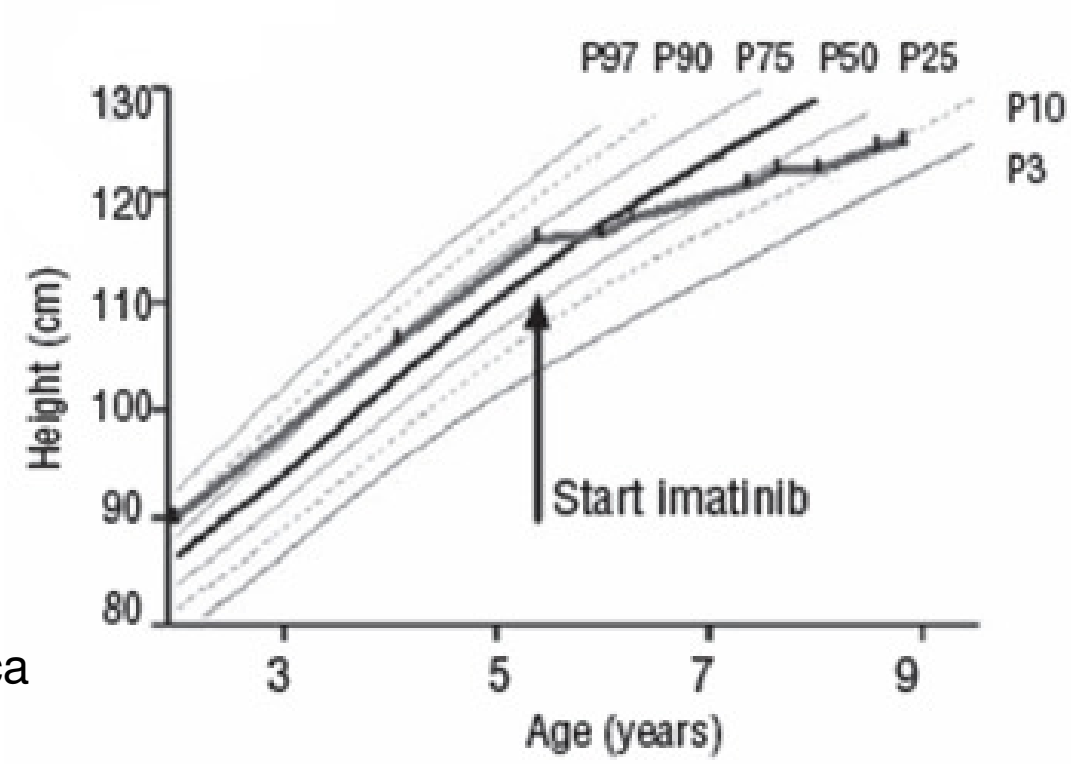
Possible indication	Rationale
Assessing AEs	Some AEs non-specific (e.g. anorexia, lethargy, nausea, vomiting): may not be AEs, or may simply reflect the disease the TKI being given to treat
Diagnosis of dietary or drug-drug interactions	Dietary and drug-drug interactions may affect exposure: could either increase the risk of AEs, or compromise response
Dose adjustment	Response not immediate hence guidance on magnitude of dose adjustment, e.g. to minimize incidence of AEs yet not compromise response, may be helpful

TDM of TKIs (ctd)

High-risk patients	Children/adolescents susceptible to AEs (e.g. effects on growth) that do not apply in adults, and that may be dose related. The elderly and those with impaired hepatic and/or renal function, or who have had GI surgery, may be at especial risk of under- or over-dosage
Minimising under-dosing	Unlike cytotoxics, under-dosage with TKIs not easily assessed by absence of AEs. Under-dosage may be associated with treatment failure and may encourage formation of resistant clones
Monitoring adherence	TKIs associated with a variety of GI and other AEs, which may contribute to poor adherence/ treatment failure. Self-medicating to excess also possible, and may be associated with an increased incidence of AEs

Imatinib in Children/Adolescents

- Little data available
- Dose of 260–340 mg/m^2 results in similar exposure to the standard adult dose (400 mg)
  - 300 mg/m^2 orally once daily (max. dose 400 mg)
- But adverse effects
  - Growth retardation



Schmid *et al.* Haematologica 2009; 94: 1177-1179

	Target range (mg/L)	Possible indication for metabolite measurement
Axitinib[1]	0.01-0.1	Axitinib sulfoxide may indicate metabolic capacity or drug-drug interactions
Crizotinib	> 0.2 [2]	*O*-Desalkylcrizotinib may indicate metabolic capacity or drug-drug interactions
Dasatinib	0.01-0.1	M4 and M5 active, but at low concentrations
Erlotinib	> 0.5	Norerlotinib pharmacologically active
Gefitinib	> 0.2	Norgefitinib may indicate metabolic capacity or drug-drug interactions
Imatinib	> 1 (CML & GIST)	Norimatinib may indicate metabolic capacity or drug-drug interactions
Lapatinib	> 0.5 [3]	Metabolite-induced hepatotoxicty may require the metabolites to be quantified

[1] Light-dependent *trans-/cis-* isomerism, therefore total analyte concentration reported
[2] Mean concentration attained from patients prescribed 250 mg twice-daily
[3] Mean concentration in patients prescribed 1200 mg once-daily

	Target range (mg/L)	Possible indication for metabolite measurement
Nilotinib	> 0.6 [1]	**-**
Pazopanib	> 20	Metabolites may indicate metabolic capacity or drug-drug interactions
Ruxolitinib	Not known	Two metabolites may display activity
Sorafenib	> 3 [2]	Sorafenib *N*-oxide may indicate metabolic capacity or drug-drug interactions
Sunitinib*	> 0.05 (sunitinib + norsunitinib)	Norsunitinib active and considered in target range
Vandetanib	> 0.4 [3]	*N*-Desmethylvandetanib active metabolite
Vemurafenib	> 40 [4]	Two metabolites may display activity

[1] C_{min} concentration applicable to quartile 1 from cytogenetic response
[2] Concentration attained in patients where dose has been increased due to poor response
[3] Minimum concentration attained in patients prescribed 300 mg/d
[4] Concentration attained in patients prescribed 960 mg 2 x daily

TurboFlow & TDM

- Reduced sample preparation time
  - Need either large dilution of plasma, or protein precipitation
  - Direct injection of diluted sample
- Reduced matrix effects
- Less risk of operator error
- Multiplexing
  - High throughput, reduced turnround time
- Easy to add new compounds/metabolites as reference compounds become available

Anal Bioanal Chem
DOI 10.1007/s00216-012-5970-2

ORIGINAL PAPER

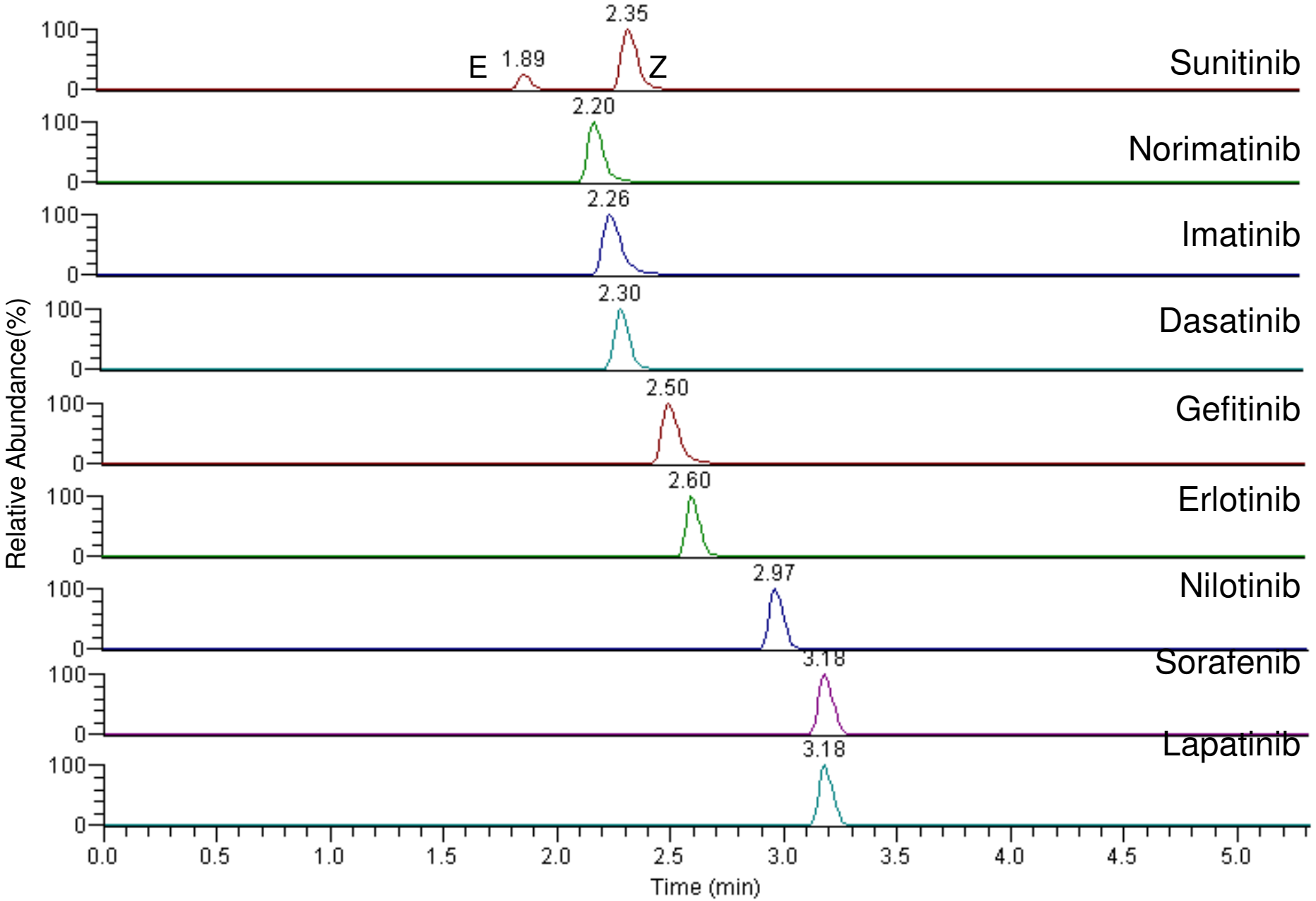
An automated method for the measurement of a range of tyrosine kinase inhibitors in human plasma or serum using turbulent flow liquid chromatography–tandem mass spectrometry

L. Couchman · M. Birch · R. Ireland · A. Corrigan · S. Wickramasinghe · D. Josephs · J. Spicer · R. J. Flanagan

Analytical and Bioanalytical Chemistry 2012; 403: 1685-95

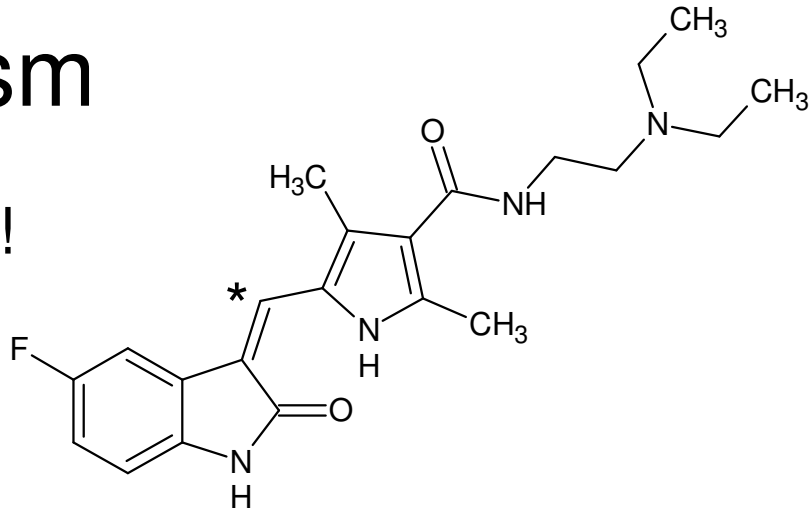
Turboflow LC-MS/MS of TKIs

- 50 µL sample + 150 µL acetonitrile containing imatinib-D_8, gefitinib-D_8, sunitinib-D_{10}, and nilotinib-$^{13}C_2{}^{15}N_2$
- Vortex-mix, centrifuge
- Inject 100 µL supernatant: Cyclone TurboFlow column
- Transfer analytes: 3 µm Hypersil GOLD analytical column, gradient elution
- Detection: APCI, +ve mode
- SRM: 2 transitions per analyte
- Total analysis time: 7 min

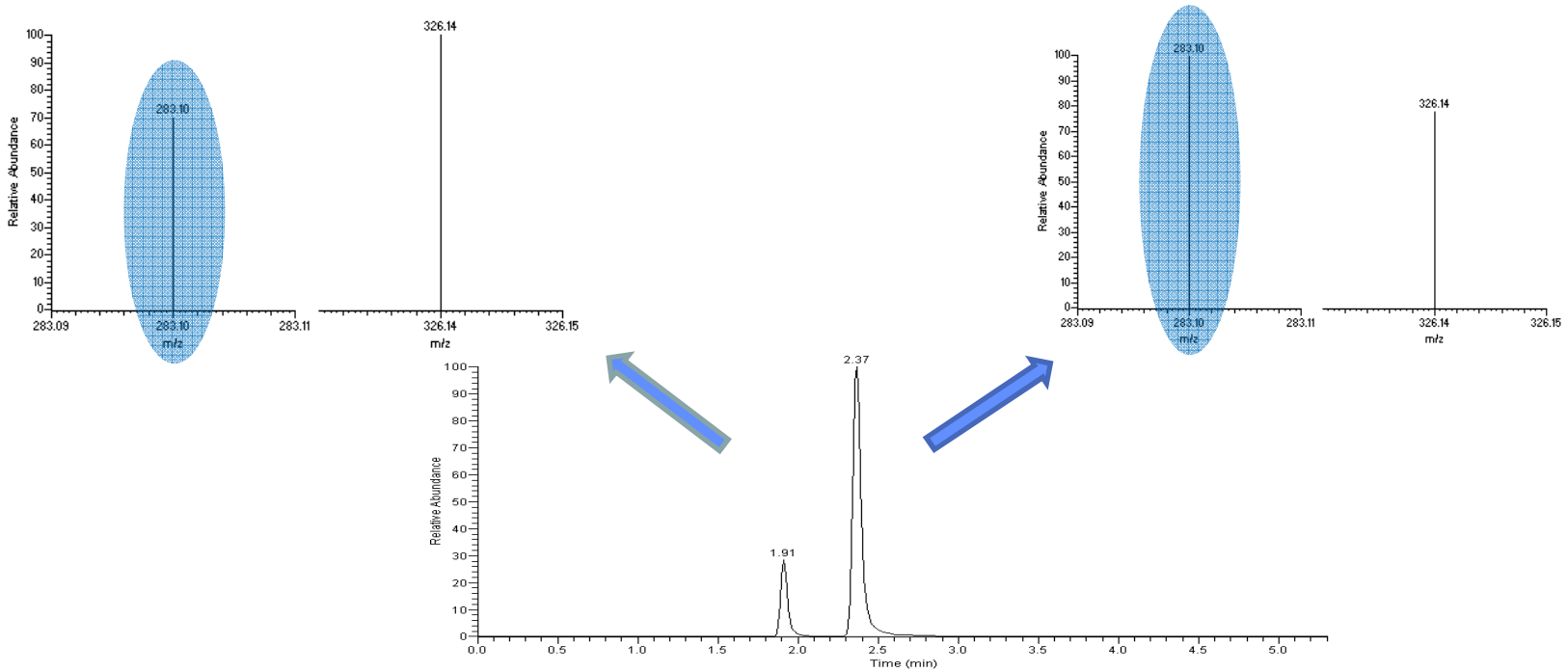

Relative Abundance(%)
100
0
2.35
E
1.89
Z
Sunitinib
2.20
Norimatinib
2.26
Imatinib
2.30
Dasatinib
2.50
Gefitinib
2.60
Erlotinib
2.97
Nilotinib
3.18
Sorafenib
3.18
Lapatinib
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Time (min)

Sunitinib Photoisomerism

Use LC-MS rather than LC-MS/MS!



SRM data (*m/z* 399 – 283, 326)

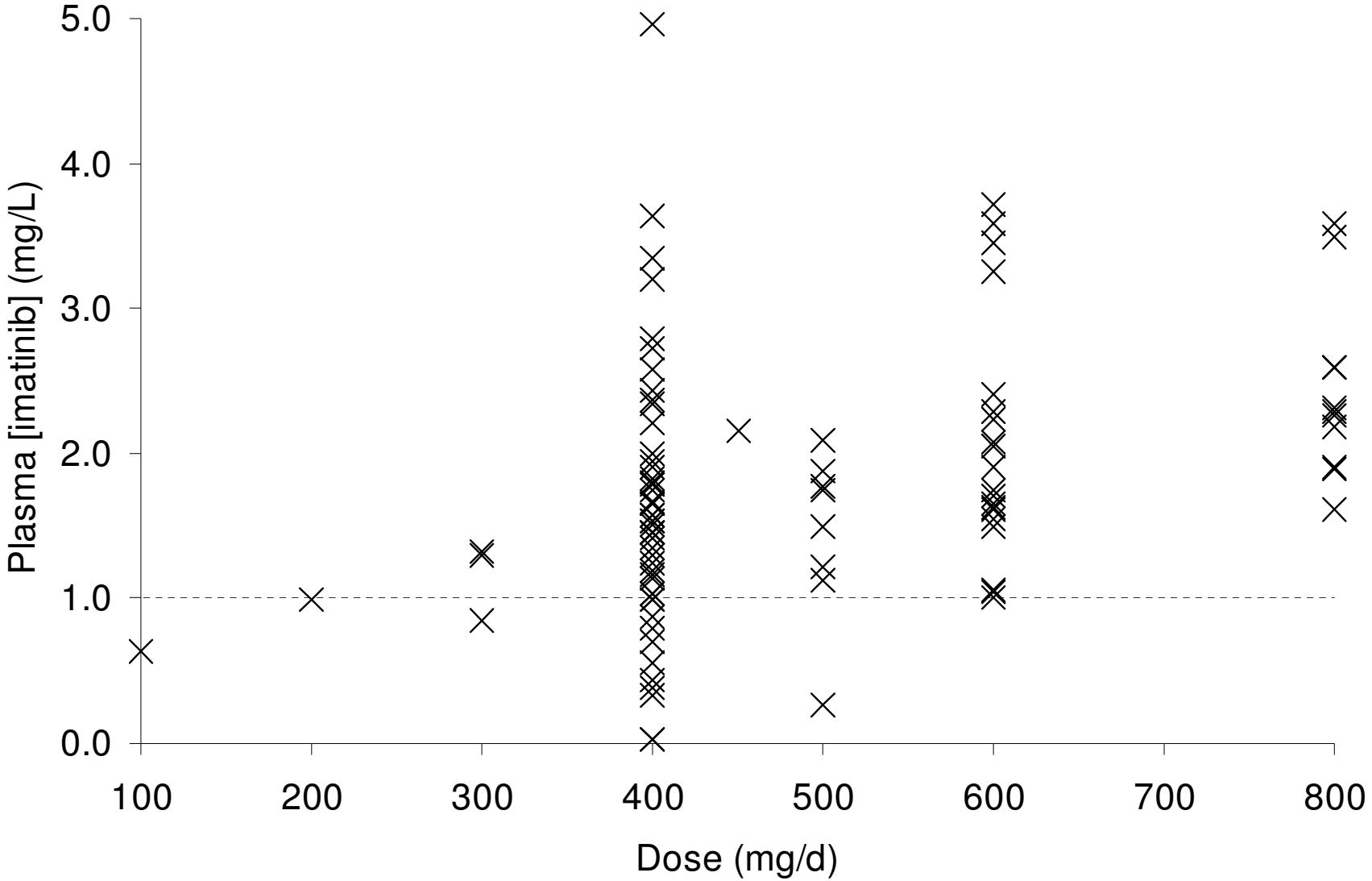


Assay Characteristics

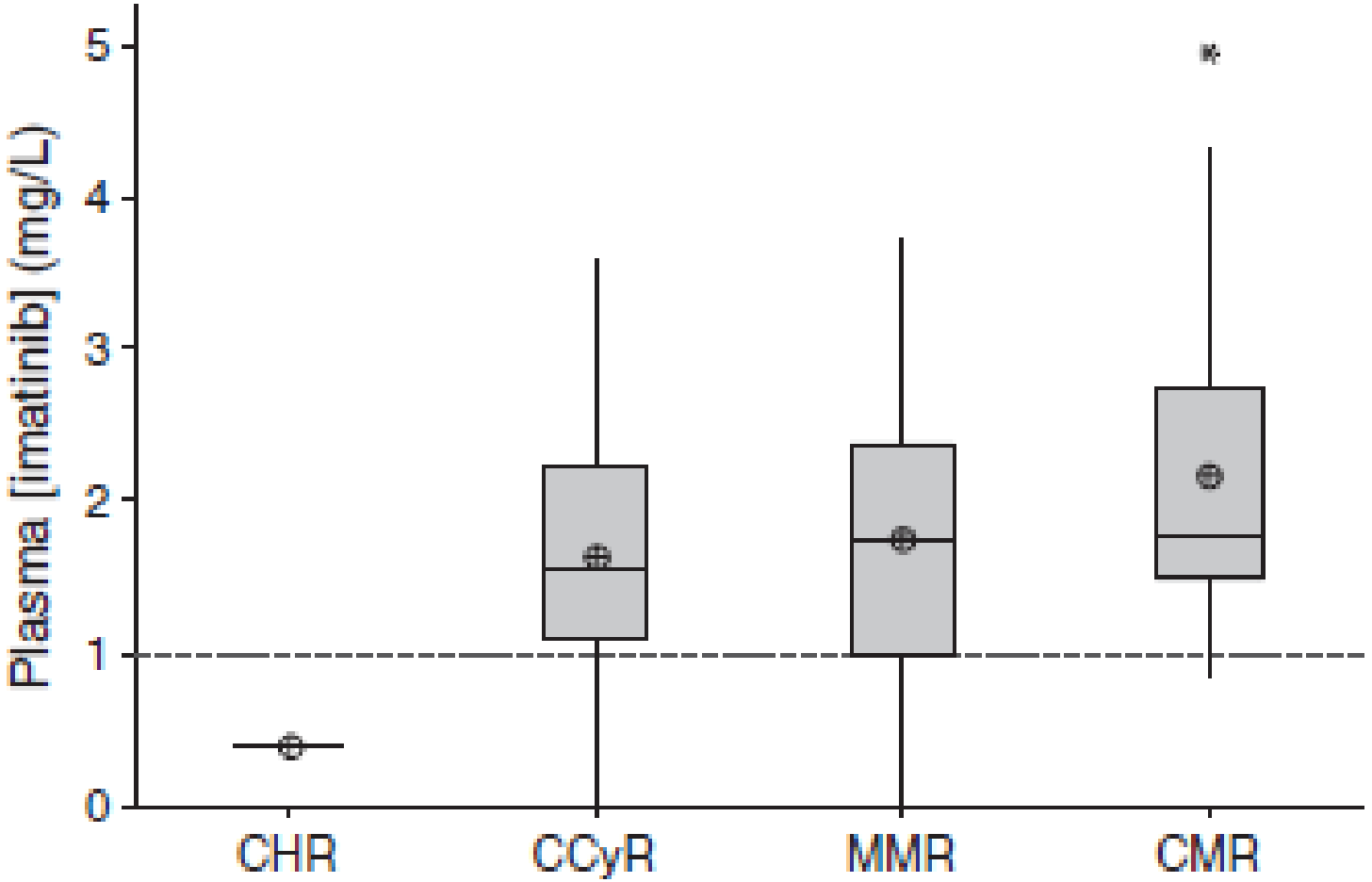
Precision	< 11 %
Accuracy	89–117 %
Recovery	> 80 %
	(Lapatinib – 65 %)

- No ion suppression/enhancement
- All analytes good stability (except lapatinib: decrease after 24 h at room temperature, and after freeze-thaw)

Plasma Imatinib vs. Dose



Plasma Imatinib vs. Response



CHR, complete haematological response, N = 1; CCyR, complete cytogenetic response, N = 42; MMR, major molecular response, N = 37; CMR, complete molecular response, N = 23

blood

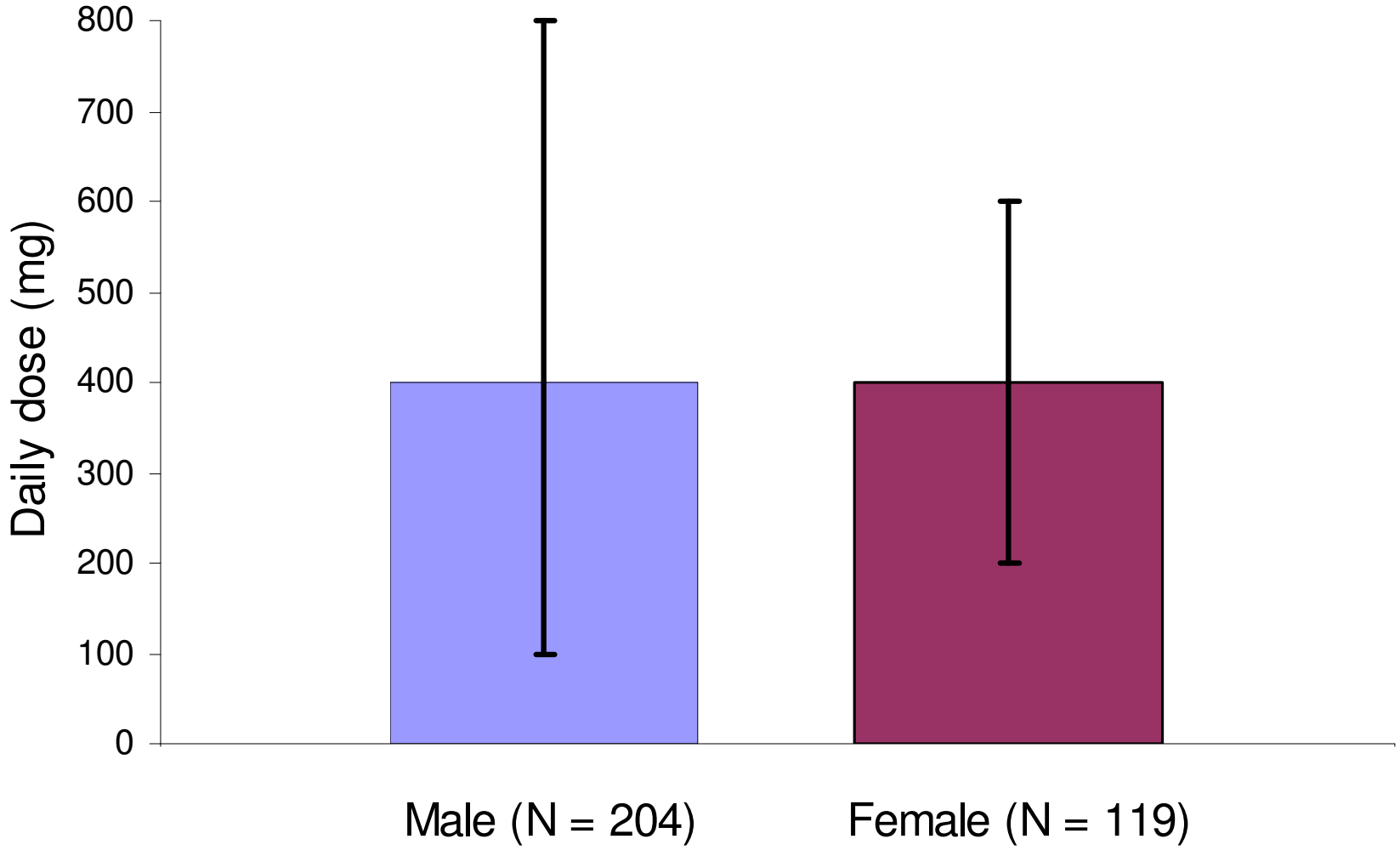
2013 121: 3818-3824
Prepublished online March 20, 2013;
doi:10.1182/blood-2012-10-462291

Early molecular response and female sex strongly predict stable undetectable *BCR-ABL1*, the criteria for imatinib discontinuation in patients with CML

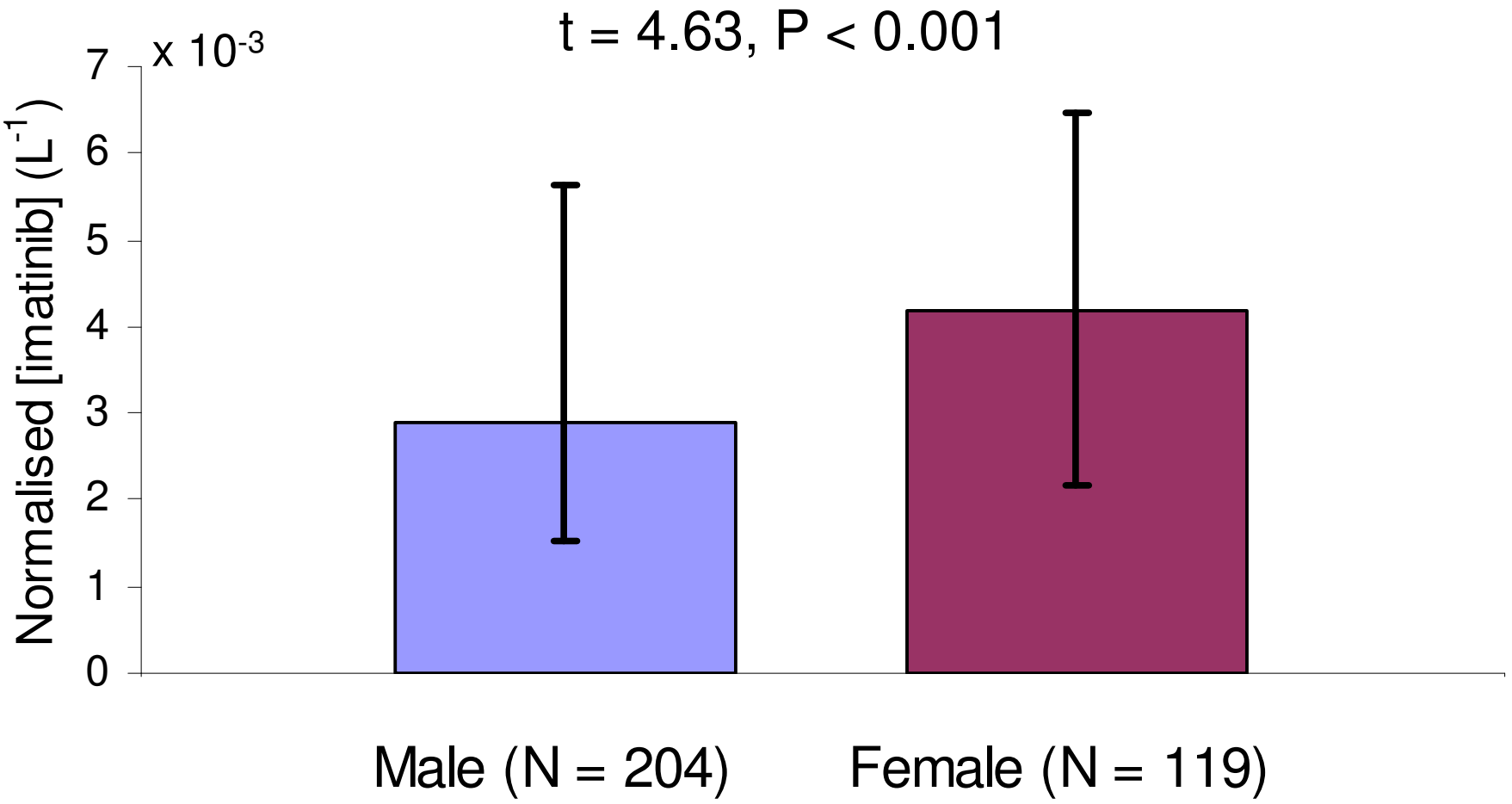
Susan Branford, David T. Yeung, David M. Ross, Jodi A. Prime, Chani R. Field, Haley K. Altamura, Alexandra L. Yeoman, Jasmina Georgievski, Bronte A. Jamison, Stuart Phillis, Brad Sullivan, Nancy E. Briggs, Mark Hertzberg, John F. Seymour, John Reynolds and Timothy P. Hughes

- The only independent predictors were female sex (54 % *vs* 27 %; P = 0.02) & the 3-month *BCR-ABL1* (P < 0.001)
- Pharmacokinetic differences might be relevant:
  - Correlation between pre-dose plasma imatinib and response described
  - Imatinib concentrations up to 30% higher in women

Imatinib: Dose (median, range)
(Patients: 86 male, 62 female)

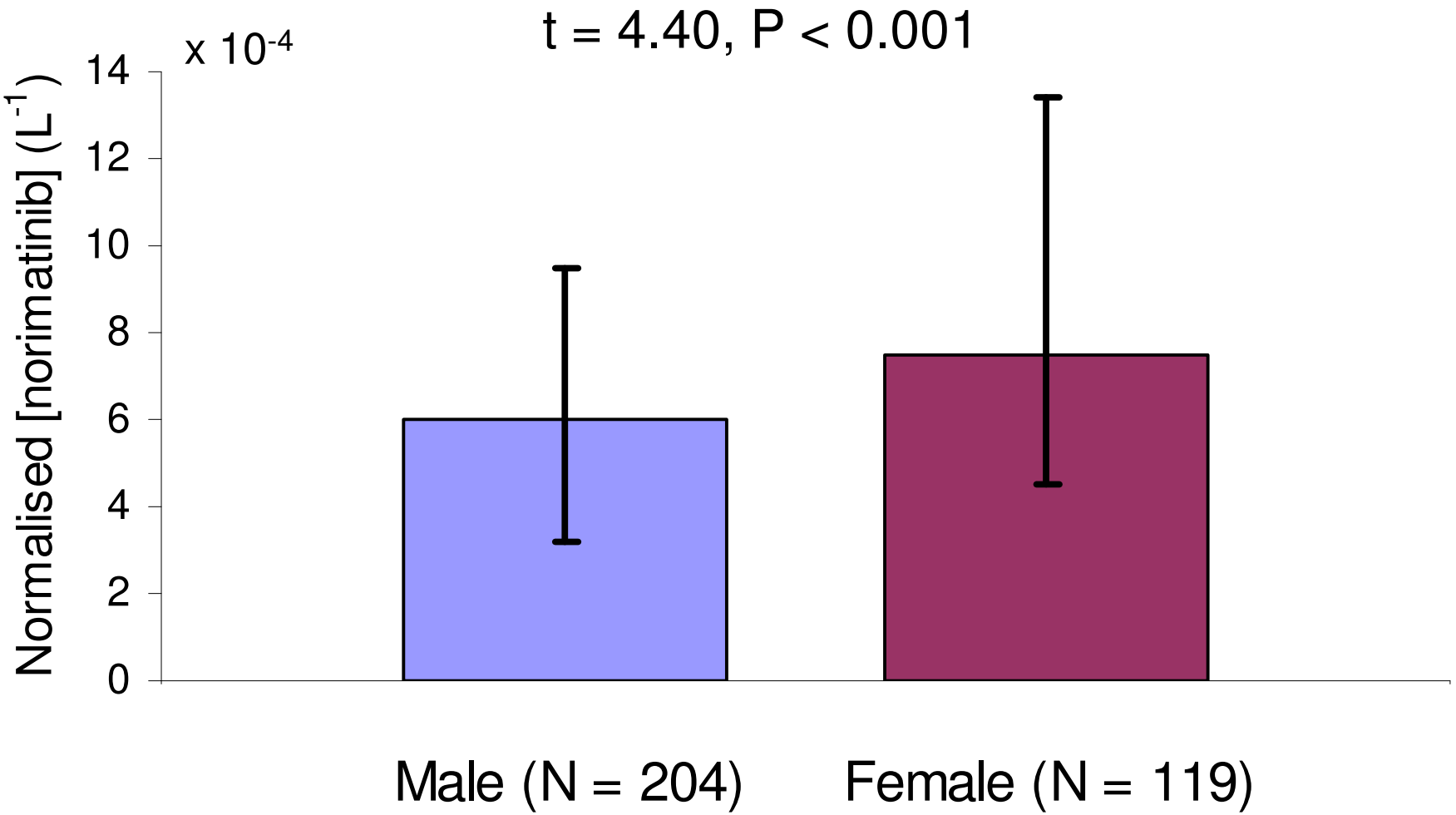


Dose-normalized Plasma Imatinib
(median, 10–90th percentiles)



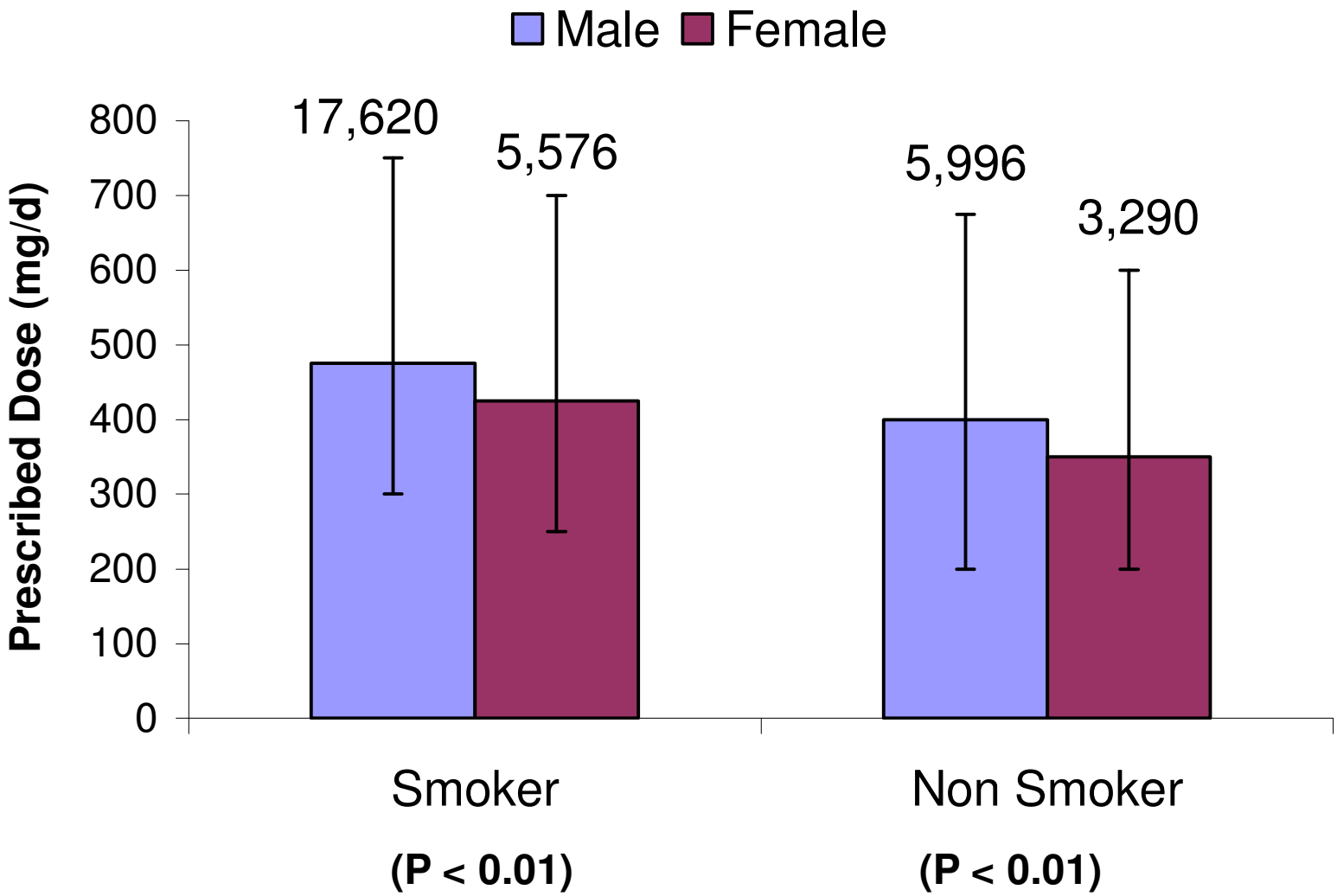
Dose-normalized Plasma Norimatinib

(median, 10–90th percentiles)



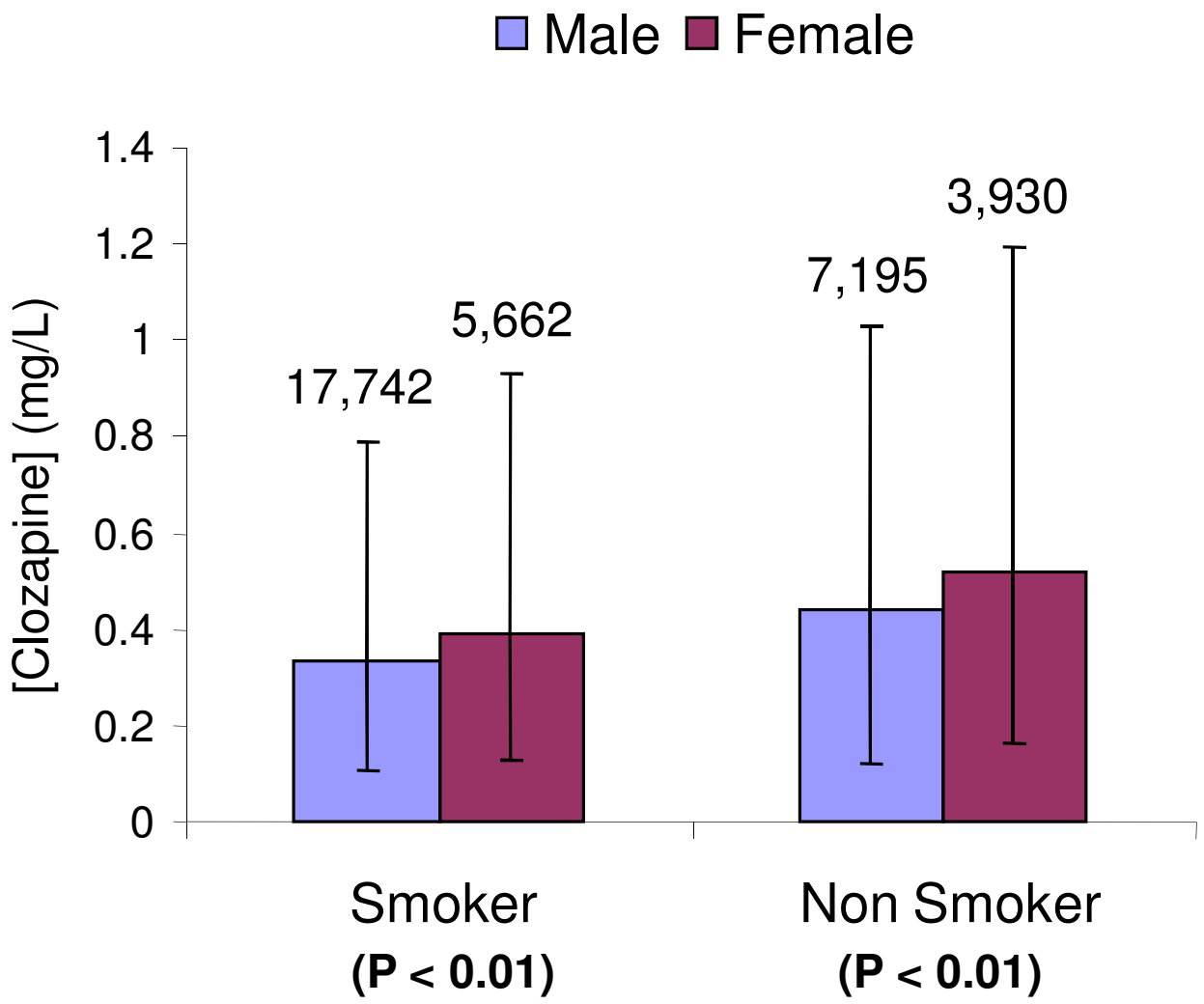
Clozapine 1993-2003: Dose

(Median, 10–90th percentile, N = 32,082)



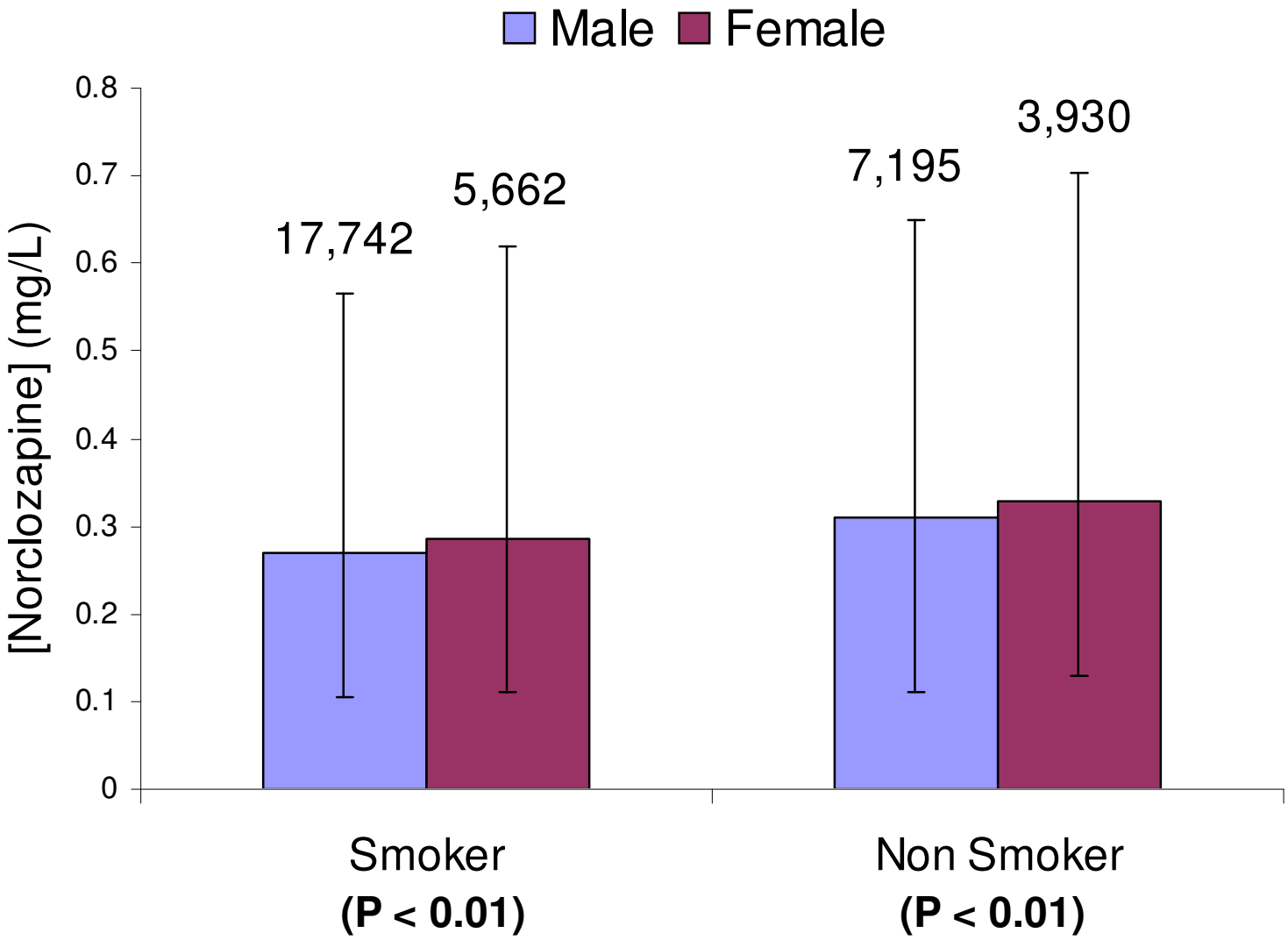
Clozapine 1993–2003: Plasma Clozapine

(Median, 10–90th percentile, N = 34,530)

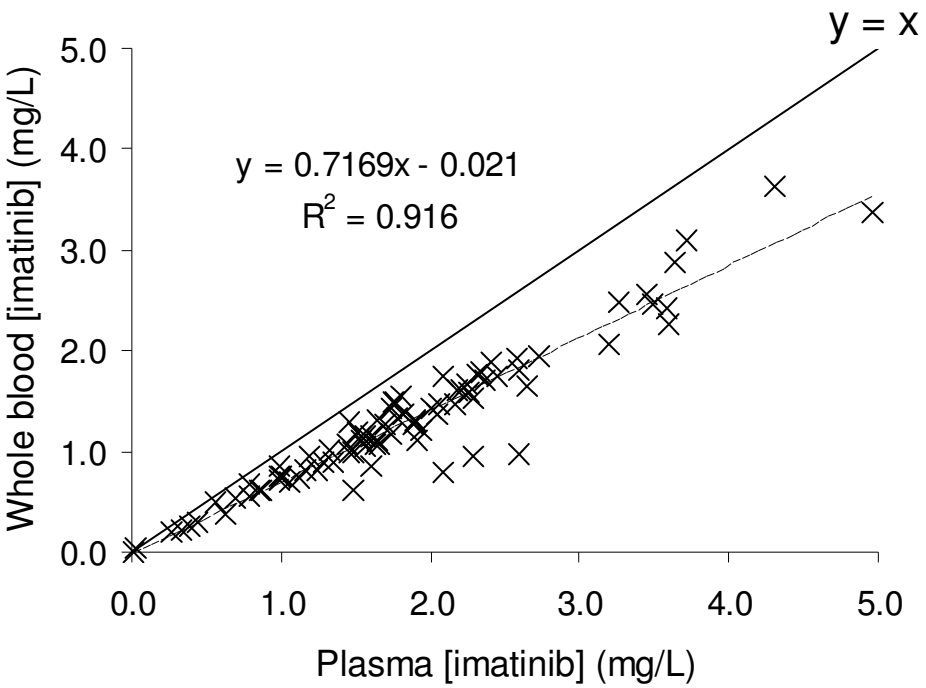


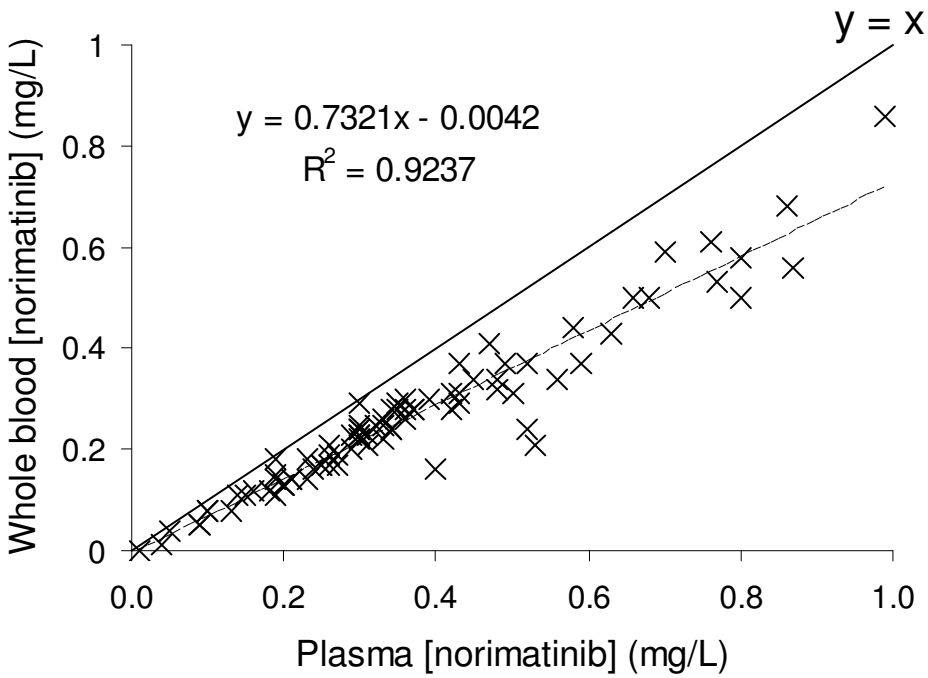
Clozapine 1993–2003: Norclozapine

(Median, 10–90th percentile, N = 34,530)

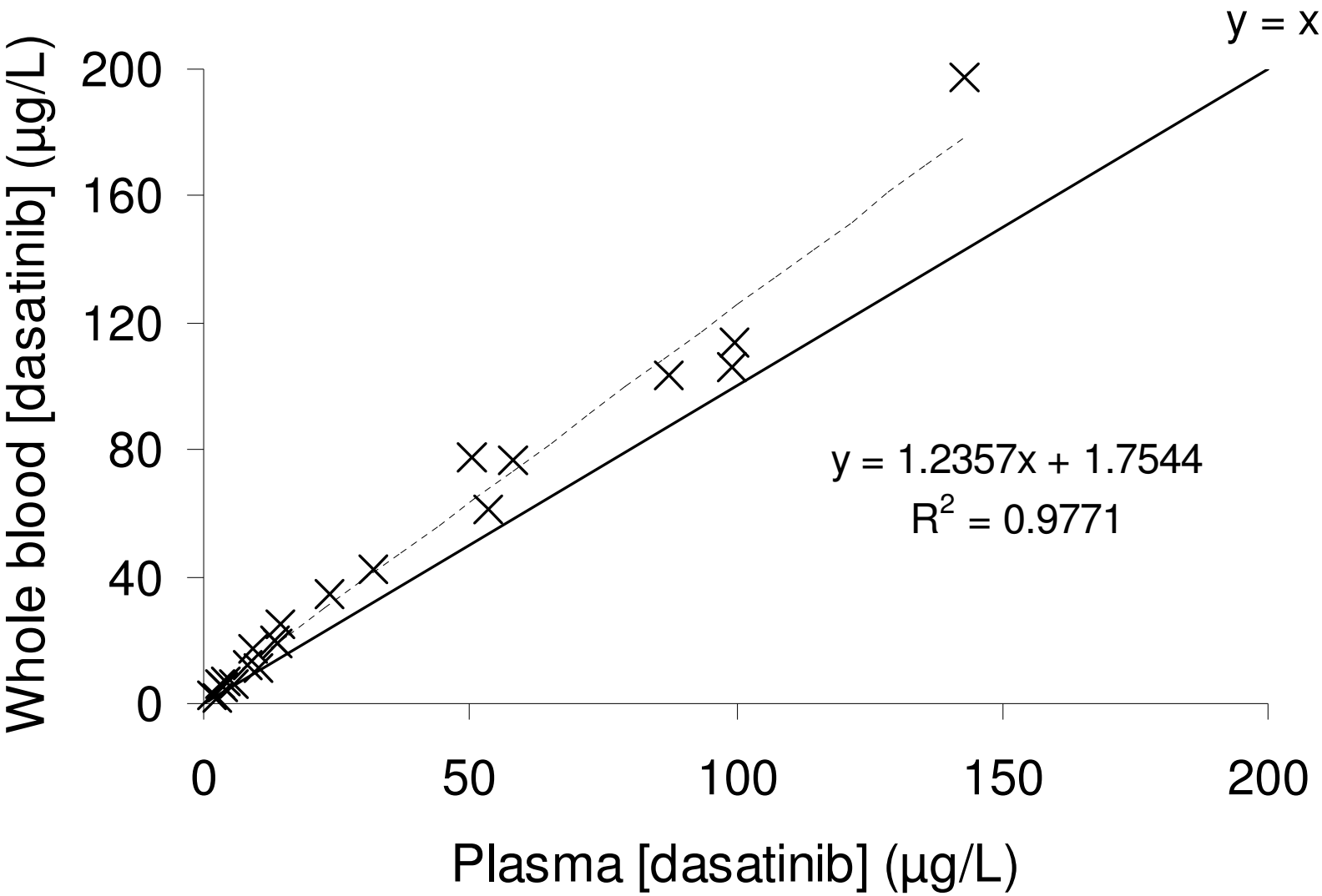


Plasma: Whole blood – Imatinib

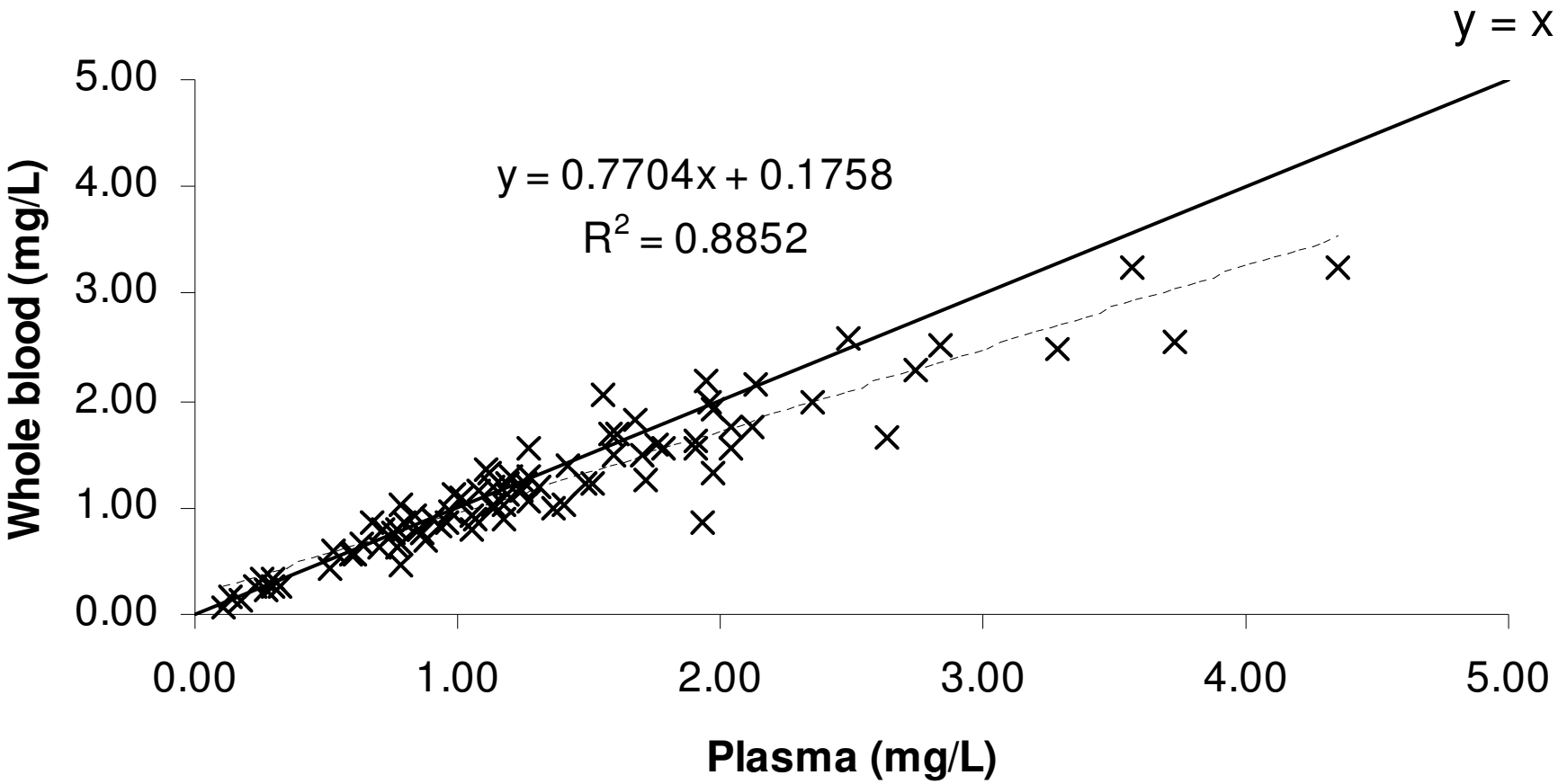




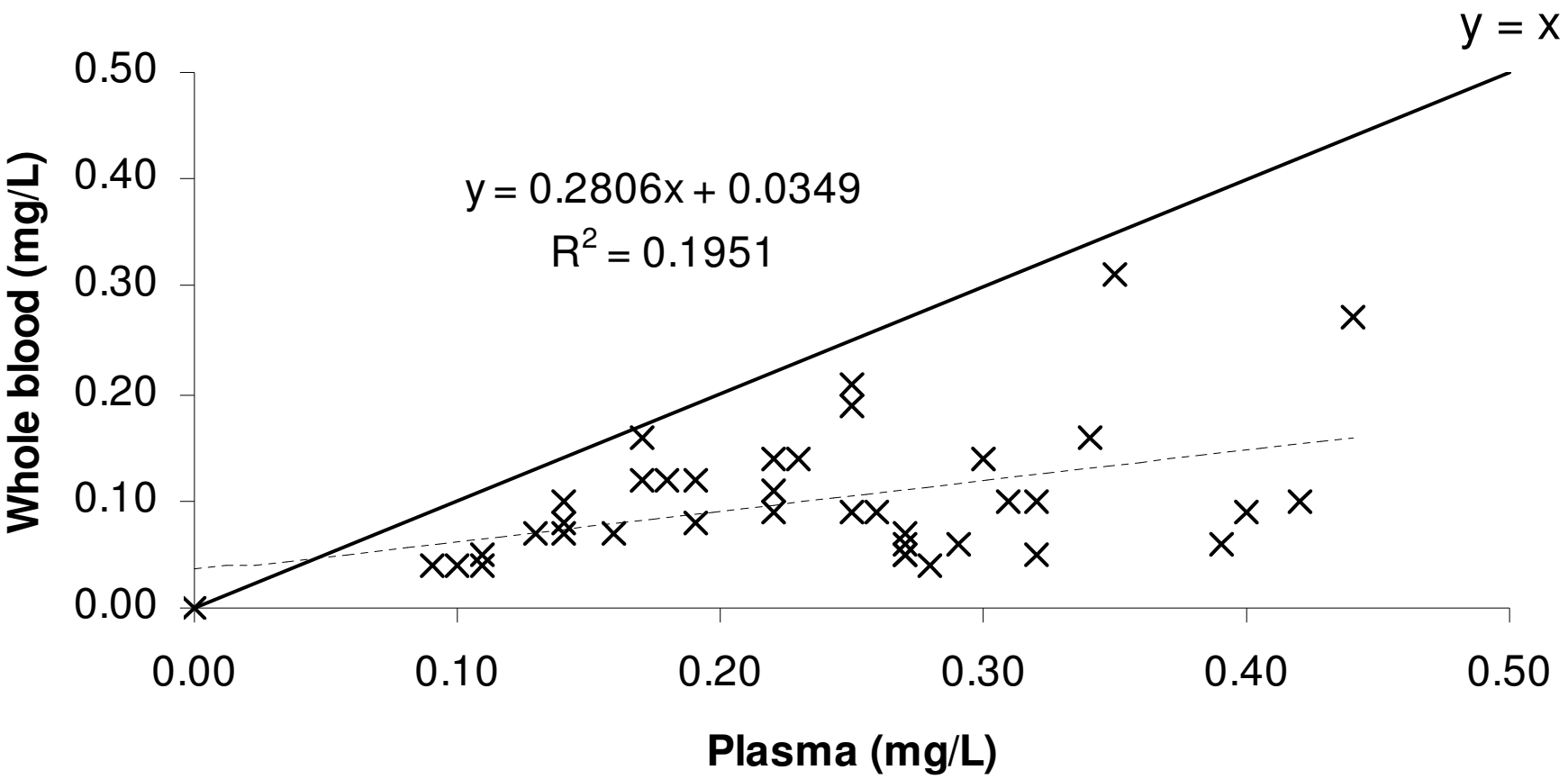
Plasma: Whole blood – Dasatinib



Plasma: Whole blood – Erlotinib



Plasma: Whole blood – Gefitinib



Conclusions

- TDM of TKIs may be beneficial in:
  - Assessing adherence
  - Ensuring adequate dosage (minimise selection of resistant clones)
  - Minimising the risk/severity of toxicity (improve adherence hence outcome)
  - Children/adolescents (maintain normal growth)
- Need assays with:
  - Minimal sample preparation
  - Fast turn-around time
  - Good accuracy, sensitivity, selectivity, measure plasma metabolites

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