Wake Forest™
School of Medicine

Omics Biomarkers & Liquid Chromatography/Mass Spectrometry for Clinical Pathology/Chemistry & TDM

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President-Elect, *AACC*

AACC and Chicago Pathology Society, March 11, 2013

Disclosures

- SAMHSA/DHHS – Grant for Oral fluid sample validity testing
- SAMHSA/DHHS – Drug Testing Advisory Board member
- Preferred Pain Management & Spine Care – Serving as Wake Forest Consulting Lab Director
- Biopharmaceutical Technology Center Institute (Promega) – Speaker honorarium
- ThermoFisher – Grants for immunosuppressant immunodiagnostics, and chemistry and toxicology app by Mass Spectrometry

5 MASS SPEC NOBEL PRIZE LAUREATES

Joseph John Thomson
1906 Nobel Prize for Physics
"in recognition of the great merits of his theoretical and experimental investigations on the conduction of electricity by gases"

Francis William Aston
1922 Nobel Prize for Chemistry
"for his discovery, by means of his mass spectrograph, of isotopes, in a large number of non-radioactive elements, and for his enunciation of the whole-number rule"

Wolfgang Paul
1989 Nobel Prize for Physics
"for the development of the ion trap technique"

John Bennet Fenn
2002 Nobel Prize for Chemistry
"for the development of soft desorption ionisation methods (ESI) for mass spectrometric analyses of biological macromolecules"

Koichi Tanaka
2002 Nobel Prize for Chemistry
"for the development of soft desorption ionisation methods (MALDI) for mass spectrometric analyses of biological macromolecules"

Source: S. Kuzdzal

References and resources

Journals

- Clinical Chemistry
- Therapeutic Drug Monitoring
- J Analytical Toxicology
- J Chromatography B
- CAP Today and Clin Chem News

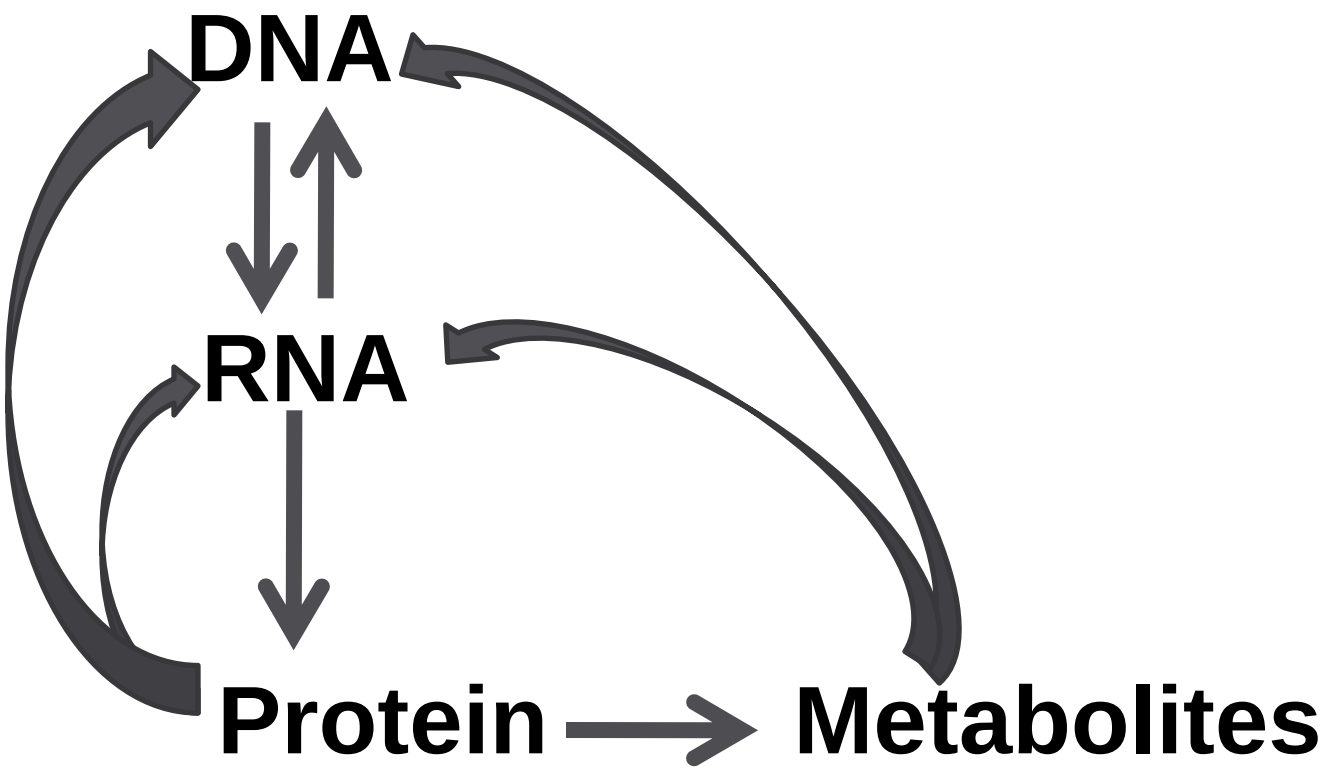
Meetings

- ICTDMCT 2011 Stuttgart
- DTAB meetings/SAMHSA/DHHS 2011-2013
- MSACL-MBDxA 2013 San Diego
- AACC MS meetings - 2012 Chicago and 2013 St. Louis
- AAFS, Feb. 2013, DC, and SOFT

Objectives

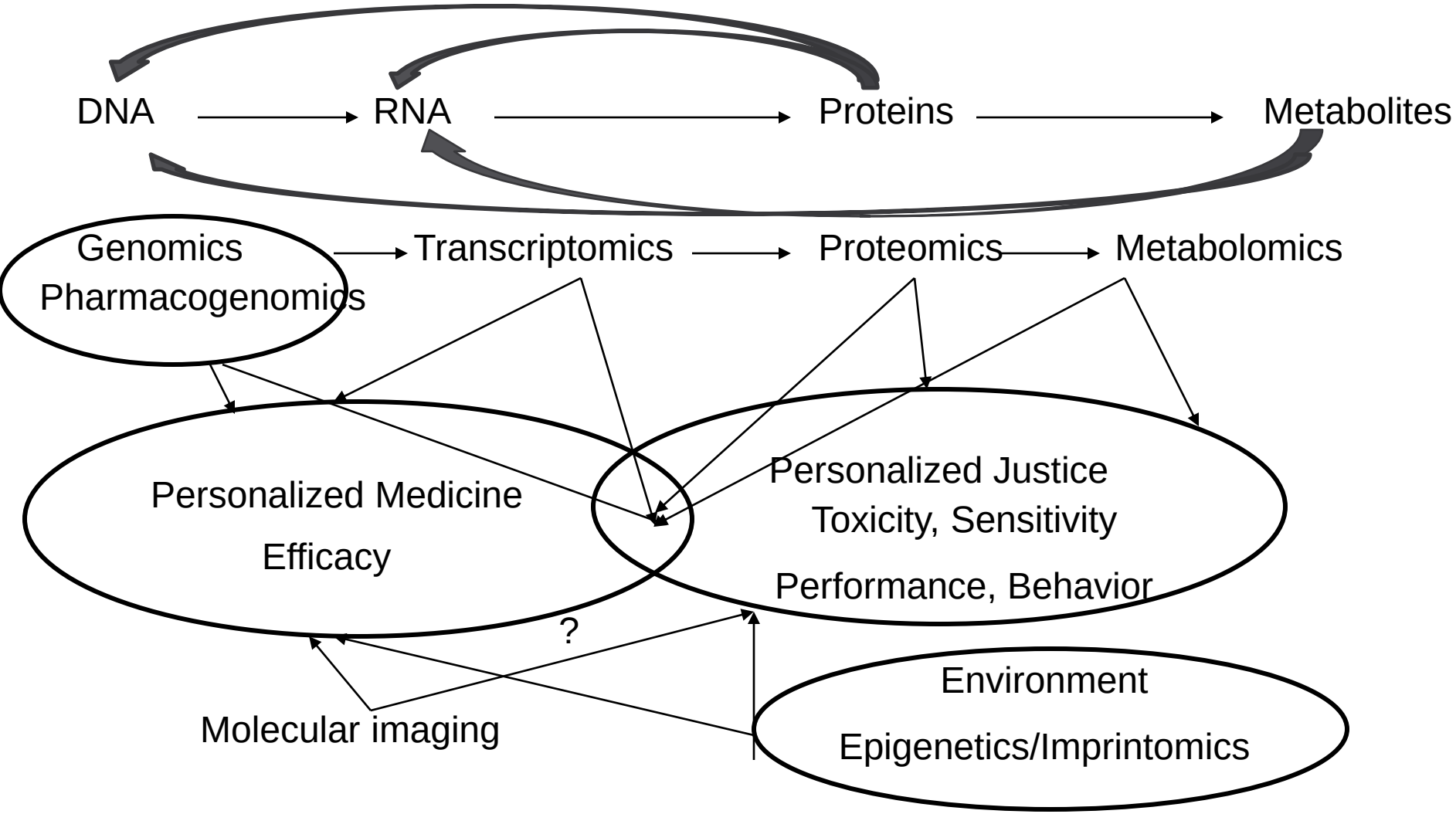
1. Update on the central dogma of molecular biology, pharmacogenomics and metabolomics., the 2012 IOM report on developing omics biomarkers and integration for Personalized Medicine and emerging Personalized Justice
2. Basic, Scientific, and Technological advances of LC-MS/MS, and MSACL 2013
3. Selected clinical pathology/chemistry and toxicology applications

Central Dogma

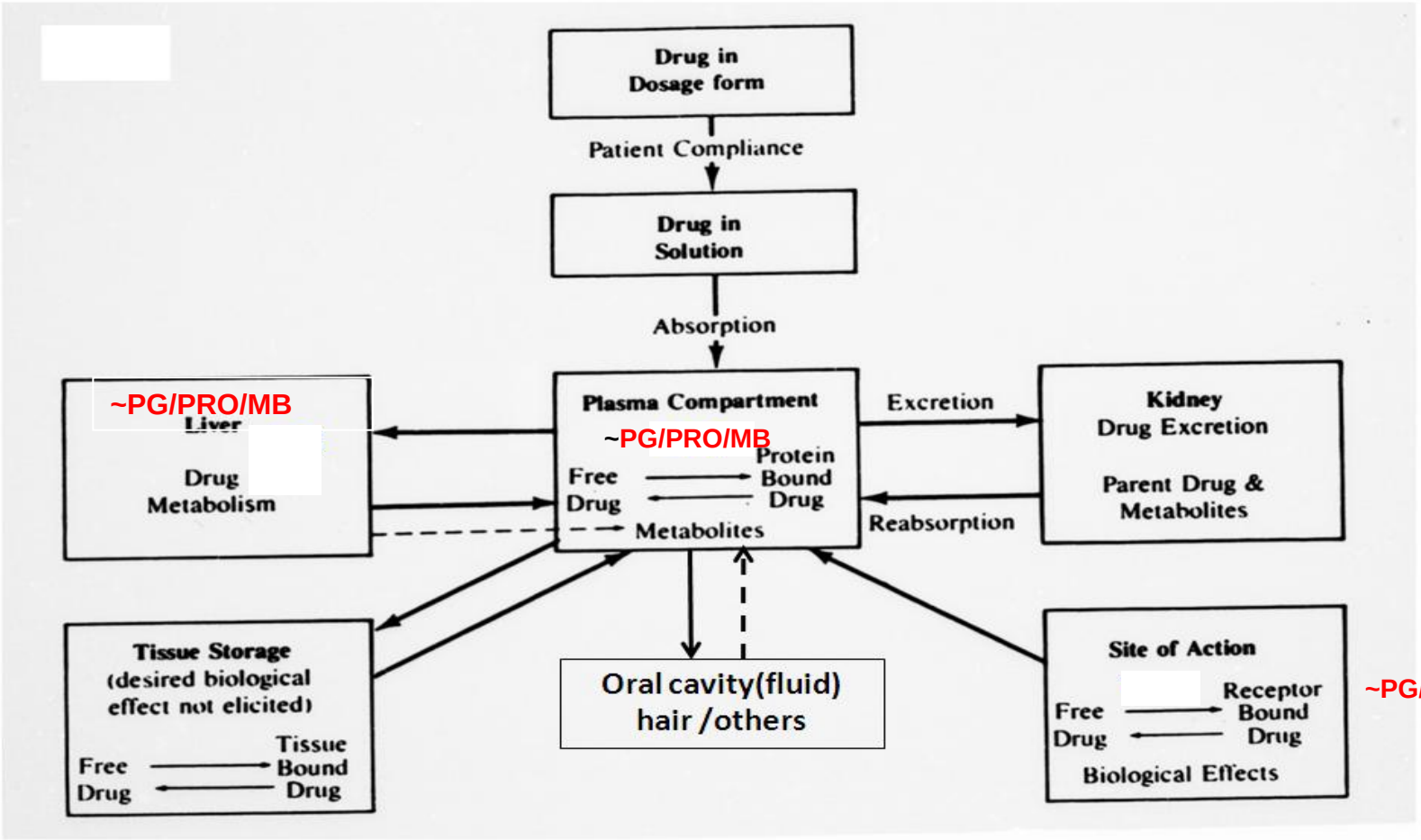


G. A. Hawkins, WFU, 2.26.11

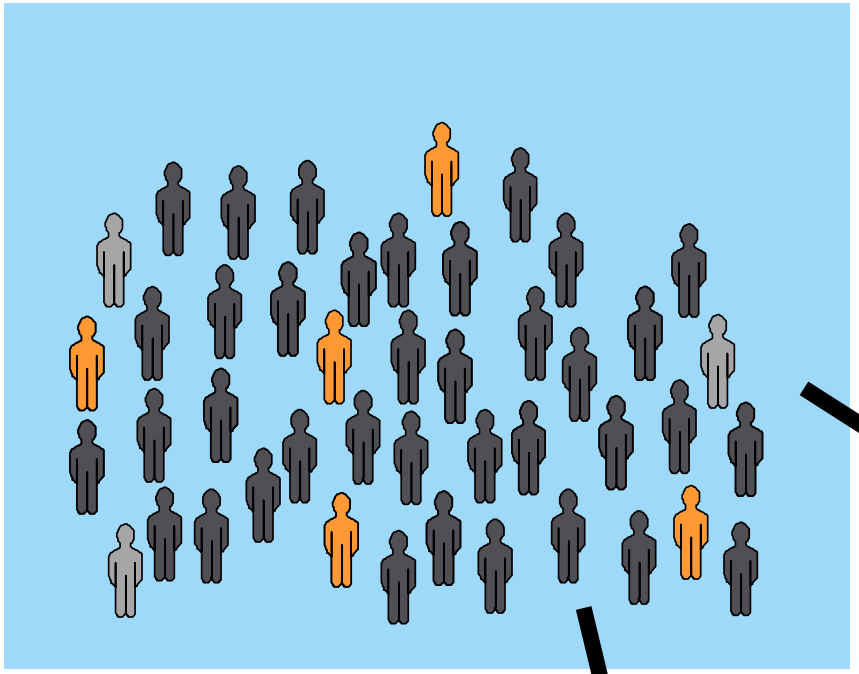
Proposed Interrelationship of Molecular biology/biomarkers, Personalized Medicine and Personalized Justice, Wong 2011



ADME ~ PG/PRO/MB



Pharmacogenomics PG Proteomics PR Metabolomics MB~ Drug Efficacy & Behavior



All patients with same diagnosis – pain, addiction

PG/PR/MB

1

Remove, DUID / WUID

non-responders and toxic responders

PG/PR/MB

PG/PR/MB

2

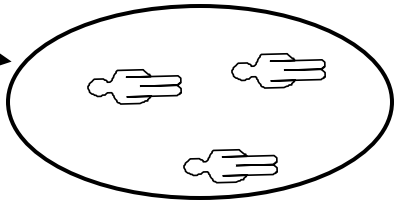
Treat , DUID WUID

Responders and Patients Not Predisposed to Toxicity

PG/PR/MB

3 Death, DUID/WUID

Drug Induced/related



Evans and McLeod, modified by Wong 2013

Personalized Medicine, PM

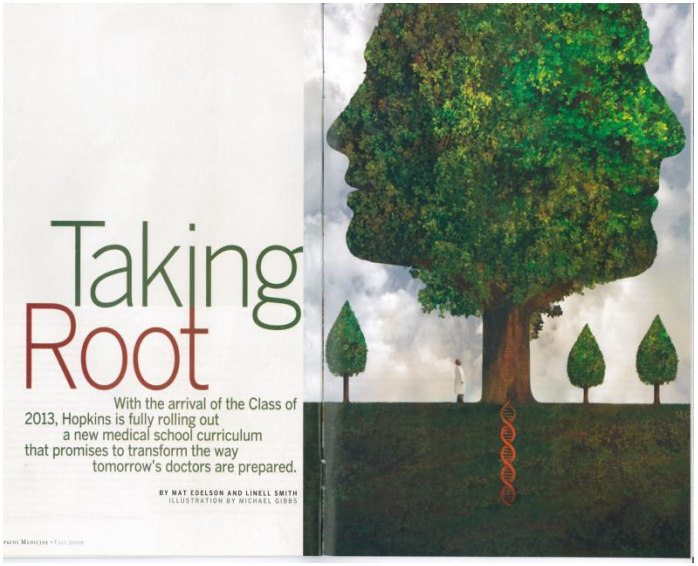
AACC PMAG (Chair – Valdes)

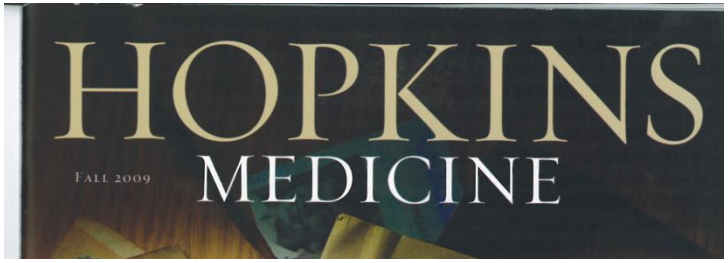
“PM is the integration and application of an individual’s unique healthcare information to predict, prevent, diagnose and treat disease as differentiated from traditional medical practice supported by population-based information.

From the perspective of the practice of lab med, PM is the application of high-resolution analytical tools to obtain biochemical information that is characteristically unique to an individual and the interpretation of laboratory data into patient-specific actionable information for use by clinicians and other healthcare providers. “

Personalized Medicine with 5Rs Personalized Laboratory Medicine

Right patient/target, Right Diagnosis (including translational PGx/P RO/MB Biomarkers), Right treatment/drug/target, Right dose, Right time*



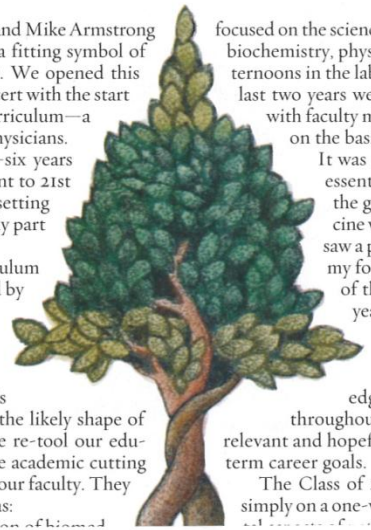


POST-OP

A Bold Leap into the Future

Personalized medicine is key to the new Genes to Society curriculum.

BY DEAN/CEO EDWARD D. MILLER, MD



The impressive glass façade of the Anne and Mike Armstrong Medical Education Building stands as a fitting symbol of the transformation taking place within. We opened this state-of-the-art building this fall in concert with the start of Hopkins' new Genes to Society curriculum—a totally different approach to training physicians.

I believe our curriculum overhaul—six years in the making—could prove as important to 21st century medicine as Hopkins' trend-setting medical education model was in the early part of the 20th century.

We have literally turned the curriculum on its head to meet the challenges posed by a genetics-based future, which were so accurately predicted by Hopkins Professor Emeritus Barton Childs.

Starting in 2003, I sat down with Vice Dean for Education David Nichols for a series of in-depth discussions on the likely shape of 21st-century medicine. How should we re-tool our education program to keep Hopkins on the academic cutting edge? David presented that question to our faculty. They wrestled with hundreds of issues, such as:

- How do you incorporate the explosion of biomed

focused on the sciences. We had morning lectures on anatomy, biochemistry, physiology and microbiology, followed by afternoons in the lab with lots of hands-on experiments. The last two years were the exact opposite: Clinical rotations with faculty mentors, an externship and nearly nothing on the basic sciences.

It was as though we had to endure the dull but essential book learning before we could get to the good stuff. These critical aspects of medicine weren't connected for us. So when I finally saw a patient with severe rheumatoid arthritis in my fourth year, there was no one to remind me of the immunology I had learned in my first year to help me make an informed diagnosis.

That won't happen in Hopkins' new curriculum, where the intermingling of fundamental scientific knowledge and clinical practice follows students throughout. This should make basic science more relevant and hopefully more integral to the students' long-term career goals.

The Class of 2013 will follow patients over time, not simply on a one-visit basis. Public health and environmen-

Evolution of Translational Omics: Lessons Learned and the Path Forward

http://books.nap.edu/openbook.php?record_id=13297&page=1 March 23, 2012

Summary

Omics as Molecular disciplines – genomics, transcriptomics, proteomics & metabolomics

Different from other medical technologies

- Regulatory oversight – LDT in academic center?
- Biological rationale – definition difficult
- Complex
- High expectations

Development & evaluations 2 stages:

- Discovery and test validation
- Clinical utilities – "*The candidate omics-based test from the research laboratory is then transferred to a CLIA- certified clinical laboratory for development of the clinical testing methods followed by analytical validation and clinical/biological validation*."

AACC - Omics & Mass Spec

1. NCI/NIH MOU – Proteomics
2. 2012 Omics conference (*Molecular Pathology, Translational Science, Proteomics (& Metabolomics 2014), and Clin. & Diag. Immunology Divisions) on IOM report, paper pending
3. 2013 MBDxA* with MSACL – ***Integration***
4. 2013 AACC annual meeting – OF symposium, " developed in co-operation " AAFS, *(NIDA/NIH, SOFT and SAMHSA/DHHS-pending)*
5. Personalized Medicine Division 2012/3
6. MS conferences: 2012 Chicago & 2013 St. Louis
7. MS survey
8. MS certificate program

Top 10 PGx tests (+ 1!!?) CLN 2005, May

Poll conducted during TDM Renaissance 2004 in Baltimore

Abbreviation	Name and Function
1. CYP 2D6	Cytochrome P 450 (CYP) 2D6, Phase I drug metabolizing enzyme
2. TPMT	Thiopurine S-methyltransferase, Phase II drug metabolizing enzyme
3. CYP 2C9	CYP 2C9, Phase I drug metabolizing enzyme
4. CYP 2C19	CYP2C19, Phase I drug metabolizing enzyme
5. NAT	N-acetyltransferase, Phase II drug metabolizing enzyme
6. CYP 3A5	CYP 3A5, Phase I drug metabolizing enzyme
7. UGT1A1	Urindine diphosphate glucuronosyltransferase 1A1, Phase II drug metabolizing enzyme
8. MDR1	Multidrug resistance (P-glycoprotein), drug protein transporter
9. CYP 2B6	CYP 2B6, Phase I drug metabolizing enzyme
10. MTHFR	Methylenetetrahydrofolate(CH_2THF) reductase, converts CH_2THF to 5-methyltetrahydrofolate
11. VKORC1	Vit. K epoxide reductase complex protein 1 - mediates vit. K reduction

Pharmacogenomics, PGx

- Scientific and clinical discipline uses genetic information to predict the efficacy and toxicity of a drug, and to identify responders and non-responders

- 2nd Translational Genomic Medicine App (low hanging fruit, after cancer) – Green, Opening Plenary Lecture, 2012 AACC Annual meeting

Building the case for PGx testing

January 2013

Feature Story

William Check, PhD

Mammals have a striking range of gestation periods, from the 12 days and 31 days of the opossum and rabbit to the 266 days and 360 days of the human and whale. Laboratory tests, too, take shorter or longer amounts of time to be delivered into routine clinical practice, with pharmacogenomics beginning to look like the elephant—more than 600 days' gestation—of laboratory testing. Our first major discussion of this topic was in 2005, and the clinical pathology world had been "expecting" its arrival for some time before that.

Perhaps it's finally time to hang the stork sign on the laboratory door. In a plenary session at the Association for Molecular Pathology 2012 Annual Meeting on Genomic Medicine, Michael Laposata, MD, PhD, the Edward and Nancy Fody professor of pathology and a professor of medicine at Vanderbilt University School of Medicine, spoke on "Making the Case for Pharmacogenomics Testing: Integration into a Healthcare System." In his talk, Dr. Laposata, who is also pathologist-in-chief at Vanderbilt University Hospital, described Vanderbilt's pharmacogenomics program, called PREDICT—Pharmacogenomic Resource for Enhanced Decisions in Care and Treatment.

As Dr. Laposata presented it, the case for pharmacogenomics is self-evident and seemingly incontrovertible. "Would it not be great," he asked attendees, "if we could select the right antihypertensive or the right antidepressant or the right antiplatelet agent immediately—rather than using the trial and error method and having a poor patient outcome until the drug that works is identified?" PREDICT seeks to achieve this goal for four analytes: warfarin, clopidogrel, simvastatin, and azathioprine. Tamoxifen, abacavir, and tacrolimus are in the works for 2013.

U.S. Department of Health and Human Services
NIH News
National Institutes of Health

NIH Office of the Director (OD)

NIH Common Fund

For Immediate Release
Wednesday, September 19, 2012

Contact:
Edmond Byrnes, DPCPSI/OSC (Common Fund)
301-451-6869

NIH Office of Communications
301-496-5787

NIH announces new program in metabolomics

Awards given to support research centers in an emerging field of research

The National Institutes of Health will invest $14.3 million this year, potentially investing more than $51.4 million over five years, to accelerate an emerging field of biomedical research known as metabolomics. Metabolomics is the study of small molecules called metabolites, found within cells and biological systems. Metabolites are produced or consumed in the chemical reactions that take place in the body to sustain life. The awards are supported by the NIH Common Fund.

The sum of all metabolites at any given moment — the metabolome — is a form of chemical readout of the state of health of the cell or body, and provides a wealth of information about nutrition, infection, health, and disease status. Metabolomics technologies have the potential to measure hundreds to thousands of unique metabolites, which can change as the result of disease, environmental exposures, or nutrition. In a clinical setting, metabolomics technologies can be powerful tools for diagnosis and disease follow-up. In basic research, these technologies will transform the ability of investigators to define the mechanisms underlying disease and to develop new strategies for treatment.

The NIH Common Fund is taking a comprehensive approach to increasing the research capacity in metabolomics by funding a variety of initiatives in this area, including training, technology development, standards synthesis, and data

Metabolomics

- Metabolome - sum of all metabolites at any given moment
  - A form of chemical readout of the state of health of the cell or body
  - Information about nutrition, infection, health, and disease status
  - Primary and secondary metabolites
  - Endogenous and exogenous (plants, drugs and environment) metabolites
- Metabolomics technologies - GC/MS, HPLC, CE, LC-MS/MS, MALDI-TOF, and NMR
- 3 Regional Comprehensive Metabolomics Resource Cores – Univ. of MI, UC Davis and RTI
- Metabolomics Research and Training:

1. Technology Development to Enable Large Scale Metabolomics Analyses (R01 RFA-RM-11-019)
2. Development of Courses or Workshops in Metabolomics (R25 RFA-RM-11-018)
   Mentored Research Scientist Development Award in Metabolomics (K01 RFA-RM-11-017)
3. Administrative Supplements for Collaborative Activities to Promote Metabolomics Research (NOT-RM-11-024)

Personalized Justice

Judicial proceedings

"Transjudicial" (*not translational*) omic biomarkers - genetic, proteomic and metabolomic

Possible contributions to adverse toxicity/ hypersensitivity/behavior/outcome

Assessing possible drug toxicity in DUID or WUID.

From personalized medicine to personalized justice: the promises of translational pharmacogenomics in the justice system

"Personalized justice complements personalized medicine and the overlapping practice of translational medicine, which hold that individual differences are caused primarily by genetic and environmental factors."

Steven HY Wong†, Christopher Happy, Dan Blinka, Susan Gock, Jeffrey M Jentzen, Joseph Donald, Howard Coleman, Saeed A Jortani, Yolande Lucire, Cynthia L Morris-Kukoski,

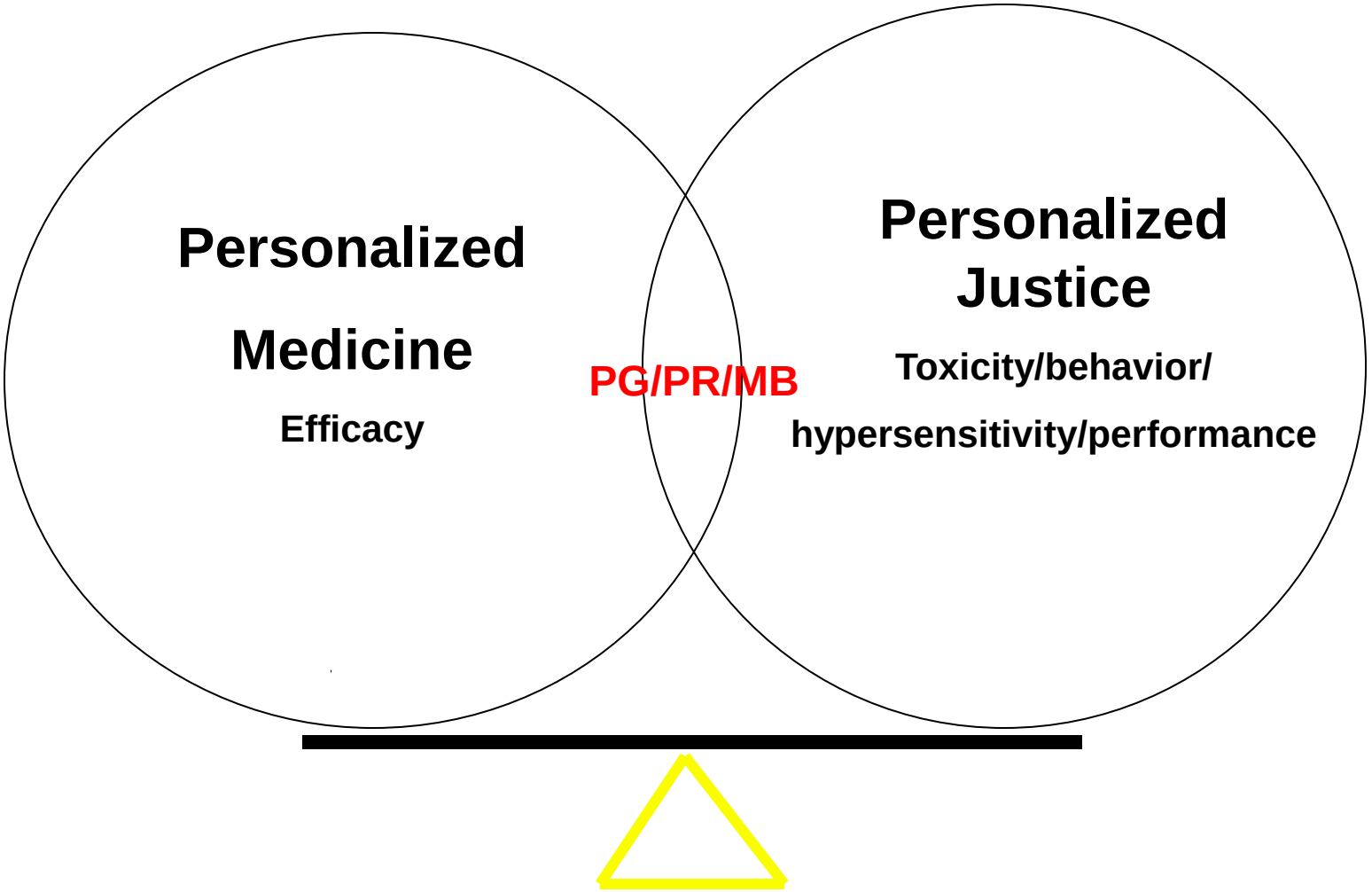
abled by pharmacogenomics (PGx), molecu- imaging and other molecular biomarkers, rsonalized medicine (PM) promises to opti- ze therapy while minimizing side effects. It y also dramatically impact the justice system ways we are only beginning to understand. Personalized medicine has already entered the rricula of well-regarded medical schools such that of Johns Hopkins University (MD, USA) but law schools offer no analog. Although nical acceptance of PM has proved slow even th US FDA support [2,10]. PM's legal ramifica-

obsessive–compulsive disorder, and Tourette's syndrome, was treated with methylphenidate, clonidine and fluoxetine [10]. Over a 10-month period, he developed gastrointestinal toxicity, incoordination and disorientation, and seizures. He died from a cardiac arrest. Postmortem toxicology showed high fluoxetine and norfluoxetine concentrations, and PGx revealed a poor CYP2D6 metabolizer, resulting in fluoxetine accumulation and toxicity. Subsequently, the boy's parent was absolved from involvement in fluoxetine intoxication

Content

- Evidence – fluoxetine and warfarin
- Legal framework - *"Daubert standard" by the federal courts,* judges serve as gatekeepers, only "reliable" science admitted
- Forensic pathology perspectives (Handbook)
- Methodology and Hypersensitivity
- Drugs – alcohol, warfarin, antidepressants and pain management
- Suggest PJ working group – "gatekeeper" against "junk science", education, criteria and others

Inevitable Social Balance?
Check and balance



Objectives

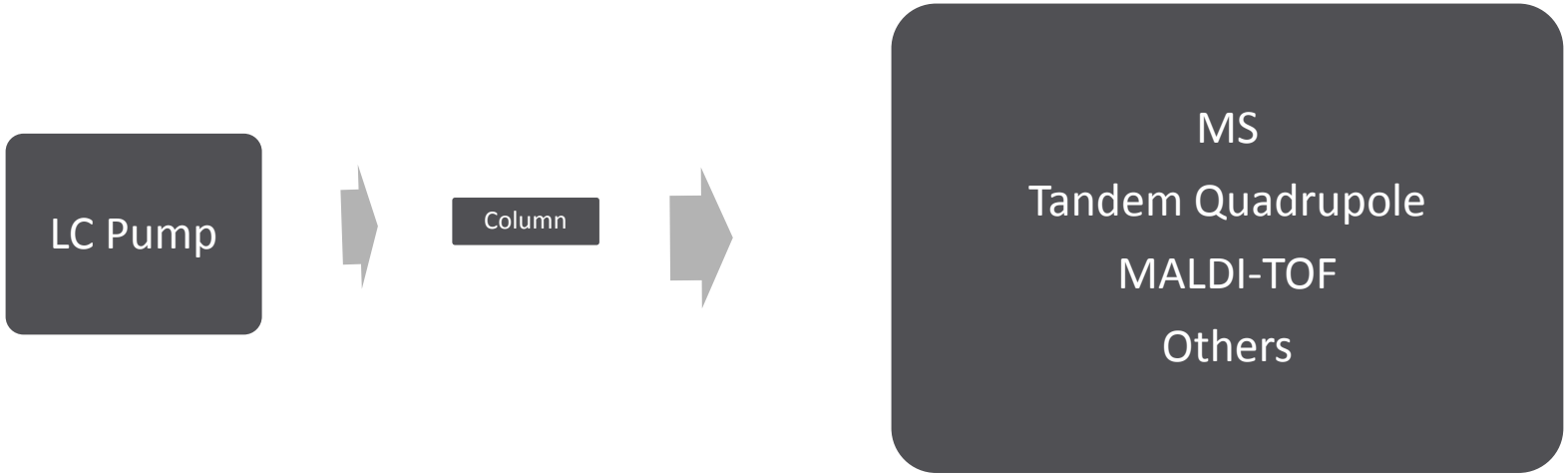
1. Update on the central dogma of molecular biology, pharmacogenomics and metabolomics, the 2012 IOM report on developing omics biomarkers and integration for Personalized Medicine and emerging Personalized Justice
2. Basic, scientific, and technological advances of LC-MS/MS, and MSACL 2013
3. Selected clinical pathology/chemistry and toxicology applications

LC-MS/MS

- Price is Right?
- Increased Capability
- Increased Sensitivity
- Specificity
- Chemical Ionization
  - APCI
  - Electrospray

LC-MS/MS

or LC-MS/MS?



LC columns and technology

- Reversed phase – C18, based on hydrophobic interaction

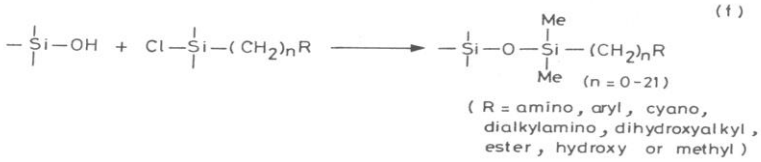
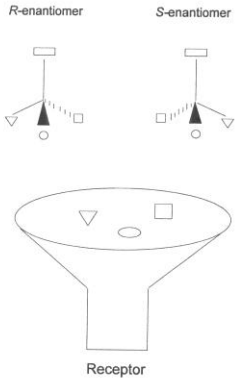
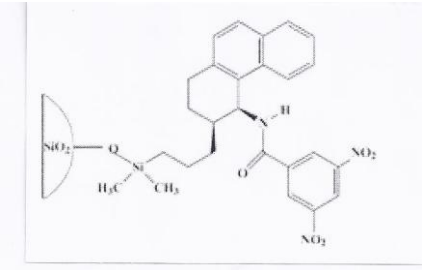


Fig. 6.15 — Preparation of bonded phases.

- Ion exchange
- Normal phase - silica
- Size exclusion and protein columns
- Chiral separation



(R,R) – Whelk - 01 chiral column for S-warfarin Chiral phase analysis of warfarin enantiomers in patient plasma in relation to CYP 2C9 genotype. Henne et al. J Chromat B. 1998;710:143-8.



- Multidimensional, multimodal, column switching
- Towards submicron, UPLC, nanoflow and others

Current and Future Applications of Mass Spectrometry to the Clinical Laboratory

Frederick G. Strathmann, PhD,[1] *and Andrew N. Hoofnagle, MD, PhD*[1,2]

Key Words: Mass spectrometry; Liquid chromatography-tandem mass spectrometry; Electrospray ionization; Atmospheric pressure

Table 1
Characteristics of Selected Mass Analyzers

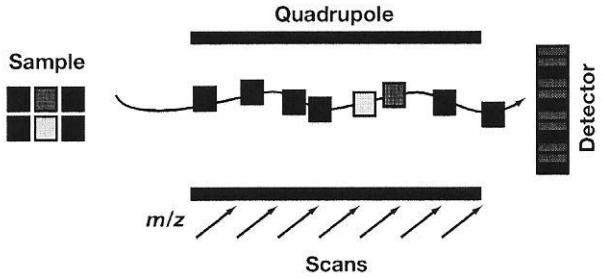
	Time of Flight	Quadrupole
Ion introduction	Pulsed	Continuous
Mode of separation	Velocity and flight time	Flight path stabilization
Sensitivity	High	Very high
Mass accuracy	High	Very high
Percentage of ion transmission	High	Low
Range of mass/charge ratio	Unlimited	Limited
Dynamic range	Limited	Wide

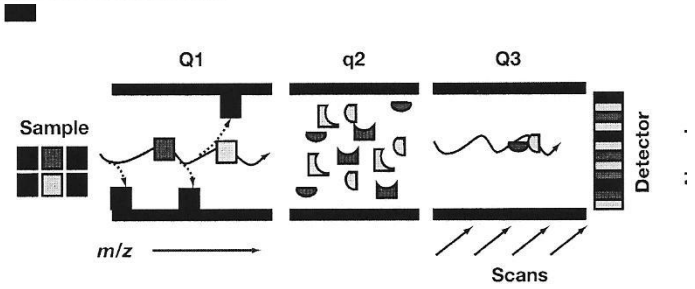
Quadrupole

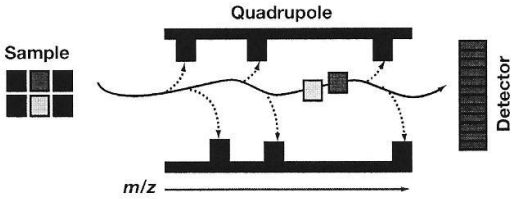
Table 2
Methods of Analysis

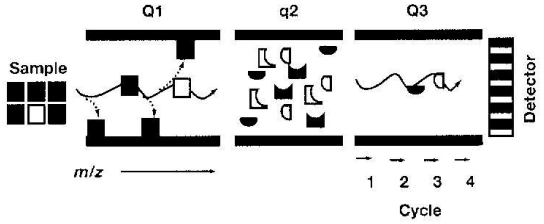
	MS	SIM	MS/MS	MRM
Quadrupoles	1	1	3	3
Specificity	Low	High	High	Very high
Selectivity	Low	High	Low	Very high
Data complexity	High	Low	High	Low
Ions detected	Precursor ions	Precursor ions	Fragments	Fragments
Application	Comprehensive drug screening	Very long chain fatty acid analysis	Metabolomics	Drug confirmation testing

MRM, multiple reaction monitoring; MS, mass spectrometry; MS/MS, tandem MS; SIM, selected ion monitoring.

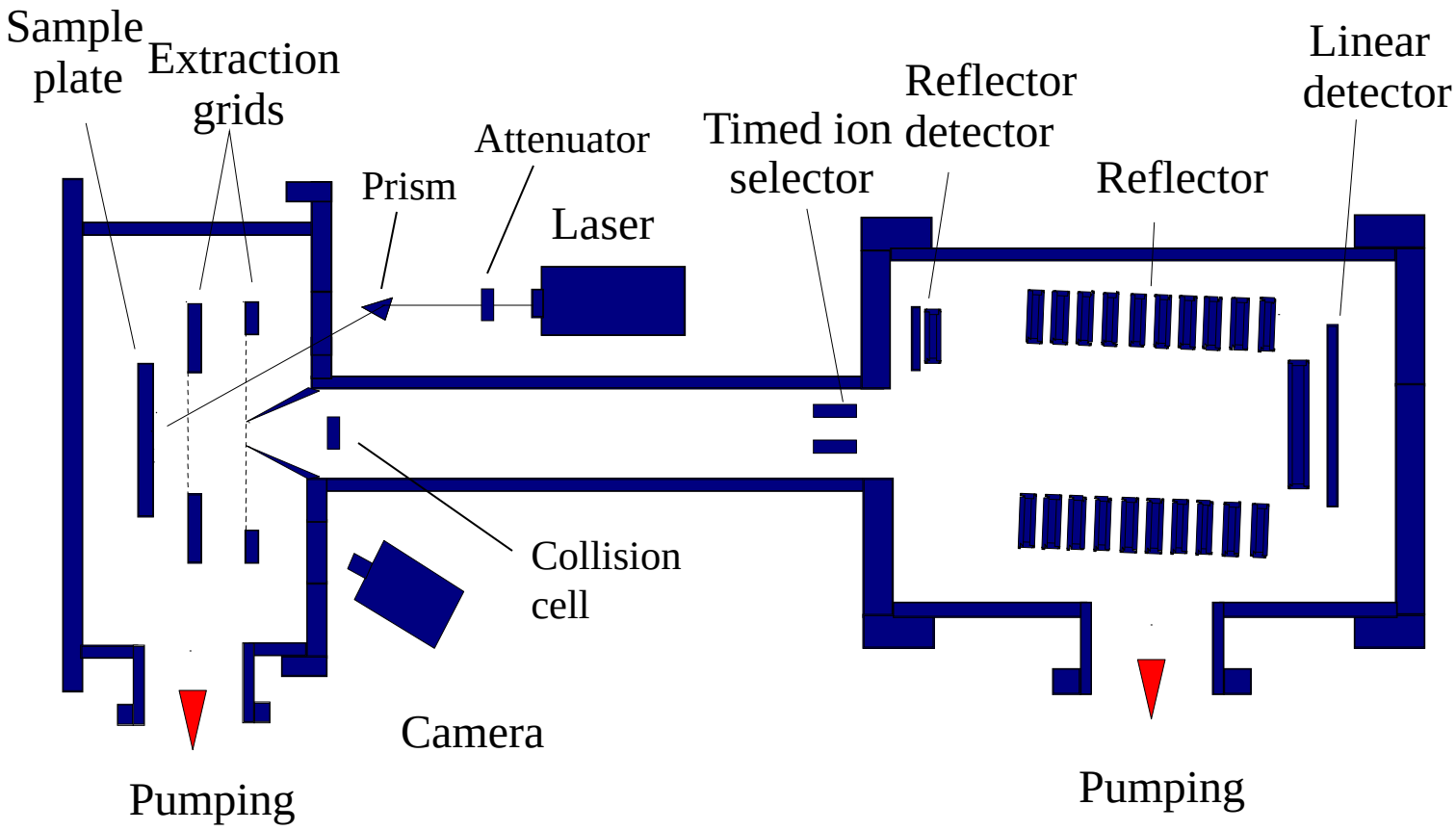






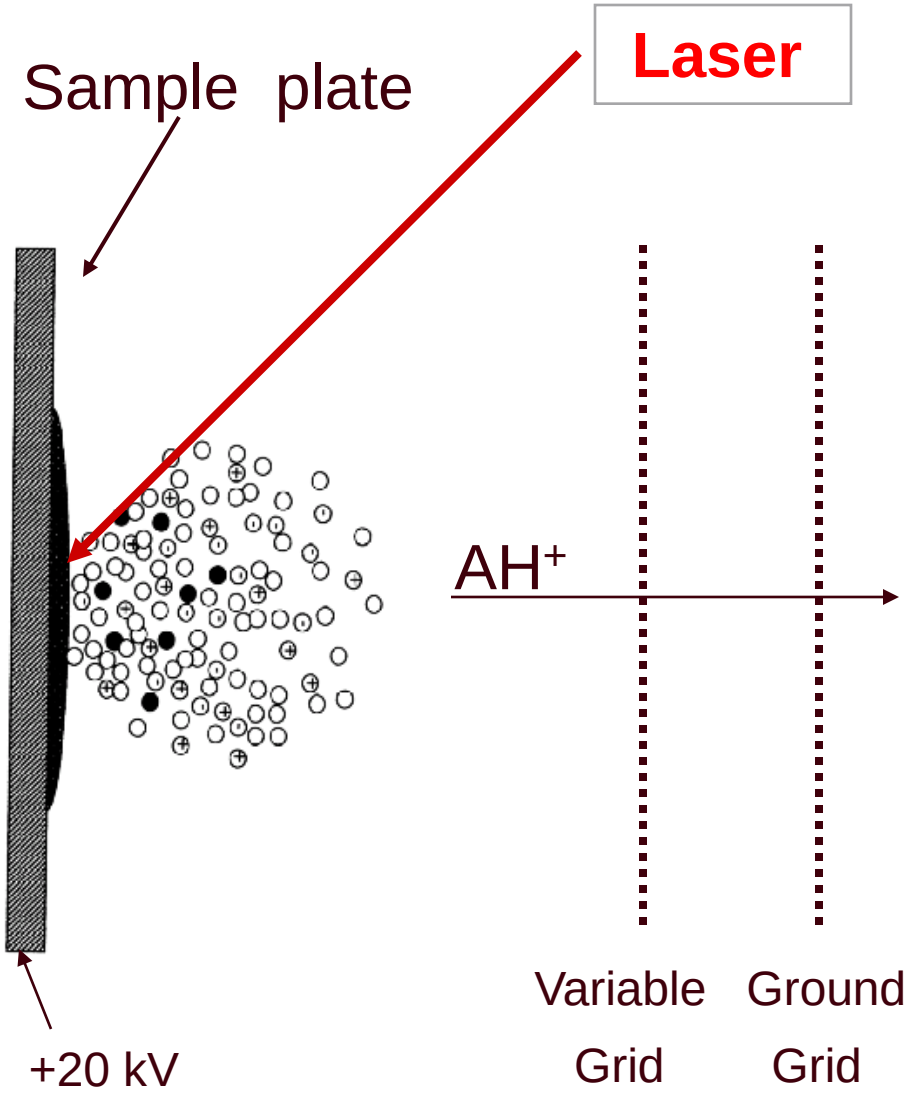


Voyager-DE STR MALDI TOF



Introduction to MALDI-TOF, Sander Mass Spectrometry User's Group
UC San Francisco, May 20, 2003

MALDI: Matrix Assisted Laser Desorption Ionization



1. Sample (A) is mixed with excess matrix (M) and dried on a MALDI plate.
2. Laser flash ionizes matrix molecules.
3. Sample molecules are ionized by proton transfer from matrix:

   $MH^+ + A \rightarrow M + AH^+$.

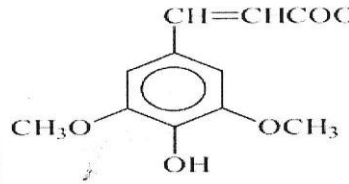
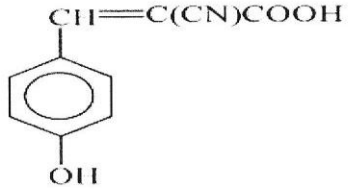
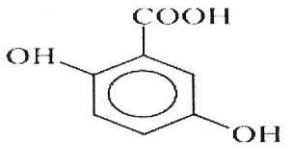
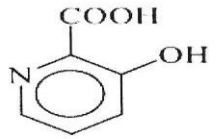
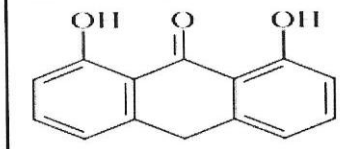
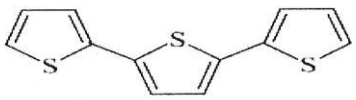
Sander Mass Spectrometry User's Group

Common matrices in MALDI-TOF

Protein Facility

Iowa State University

Common Matrices in MALDI-TOF

Matrix	MW (Da)	Applications preparation	Initial velocity (m/sec) [1,2]	Characteristic monoisotopic matrix ions (in Da)
 3,5-Dimethoxy-4-hydroxy-cinnamic acid (Sinapinic Acid) SA	224.07	Peptides Proteins 10 mg/mL in 70% ACN/water, 0.1% TFA	350	225.076 224.068 207.066 431.134
 α-Cyano-4-hydroxycinnamic acid (ACH)	189.04	Peptides Proteins Saturated solution in 70% ACN/water, 0.1% TFA	300	164.047 195.050 172.040 379.093 212.032 294.076
 2,5-Dihyroxybenzoic acid (DHB)	154.03	Peptides Carbohydrates Small molecules 10 mg/mL in 70% ACN/water, 0.1% TFA	500	155.034 154.027 137.024 273.040
 3-Hydroxypicolinic acid (3HPA)	139.03	Oligonucleotides 9:1 dilution of matrix:diammonium citrate matrix: 50 mg/mL in 50% ACN/water Diammonium citrate: 50 mg/mL in water	550	96.045 140.035 279.062 235.072 234.064 233.056 191.082 189.066
 Dithranol	226.06	Polymers 10 mg/mL in THF Add silver triflouroacetate to minimize Na+ and K+ adduct formation		225.055 226.063 227.071 211.076
 2,2':5',2"-Terthiophene	248.39	Polymers 10 mg/mL in THF, mix 10:1 with analyte		

ACN-acetonitrile

Objectives

1. Update on the central dogma of molecular biology, pharmacogenomics and metabolomics, the 2012 IOM report on developing omics biomarkers and integration for Personalized Medicine and emerging Personalized Justice
2. Basic, Scientific, and Technological advances of LC-MS/MS, and MSACL 2013
3. Selected clinical pathology/chemistry and toxicology applications

The Association for
Mass Spectrometry:
Applications to the Clinical Lab

MSACL . MBDxA 2013

The 5th Annual Conference & Exhibits of MSACL
with
The 1st Annual Conference of MBDxA

Final Program

Sheraton Hotel & Marina
San Diego, CA USA
February 9-13, 2013

Selected MSACL Plenary Lectures

1. Novel Pathogens by electrospray ionization MS – PCR for SNP and others, and a large no. of variations by nucleic acid MS - Cantor
2. Blood-borne, respiratory, enteric and tick-borne disease using Mass Tag PCR – amplification followed by separation and release of mass tags by UV radiation, tag then analyzed by MS – Tokarz
3. Orbitrap MS history, “Valley of death” development, high resolution and accuracy - Makarov

Scientific sessions - Proteomics and Metabolomics

- Disease markers e.g Alzheimer's disease
- Endocrine standardization
- ICP-MS
- Metabolomics
- Microbiology
- New advances
- Newborn screening
- Pain management
- Proteomics
- Sample preparation
- Small molecule analytes
- Tissue imaging and analysis
- Toxicology and DAU – oral fluid
- Vitamin D

ARUP and a west coast academic center(*) Mass Spectrometry Test List

Testing areas include, among others:

- Thyroglobulin
- Androstenedione
- Fractionated bile acids
- DHEA
- Nicotine and metabolites
- Dexamethasone
- Estrogens for men and children as well as free estrogen by equilibrium dialysis
- Testosterone for females and children

* 25-hydroxy vitamin D2 and D3
- Free testosterone by equilibrium dialysis/LC-tandem mass spectrometry
* Cortisol and cortisone
- Catecholamines fractionated by LC-MS/MS
- Cortisol and cortisol/cortisone, urine free, by LC-MS/MS
- Cyclosporine A and cyclosporine A C2 by tandem mass spectrometry
- Metanephrines

- Methylmalonic acid
- Sirolimus, tacrolimus, and everolimus by tandem mass spectrometry
- Thyroxine, free, by equilibrium dialysis/HPLC-tandem mass spectrometry
- Drug screening by TOF mass spectrometry
- Bacteria identification by mass spectrometry
- Expanded newborn screening

* Leflunomide, MPA & MPA-G

Metabolomics MSACL

- Basic on metabolite structure characterization, accurate mass determination and principal components analysis – Siuzdak and Patti
- Polycystic ovarian syndrome by profiling steroids with LCMSMS, identified increased androsterone-sulfate – Cruthfield (NIH)
- Predictive biomarkers of L-Asparaginase response in ovarian cancer, identified by untargeted metabolomics LCMS in OVCAR-8 cell line, changes in two polyamines, putrescine and N-actylputrescine – Sliva
- Monitoring human metabolic individuality by using highly "Personalized" LS-MS – Deelder
- Urinary metabolomics for colorectal cancer ? (Mouse model) – Manna

Vitamin D epimers

C-3 epimers can account for a significant proportion of total circulating 25-hydroxyvitamin D in infants, complicating accurate measurement and interpretation of vitamin D status. Singh et al., *J Clin Endocrinol Metab* 2006;91:3055-61.

- LC-MS/MS and various age groups
- Significant concentrations of C-3 epimers of 25OHD(2) or 25OHD(3) are commonly found in infants, overestimation --. children less than 1 yr --assay that allows accurate detection --of C-3 epimers."

The C-3 Epimer of 25-Hydroxyvitamin D3 Is Present in Adult Serum Lensmeyer, Poquette, Wiebe, Binkley. *J Clin Endocrinol Metab 2012;97: 0000–0000, 2012*

- C-3 epimer was detected in 212 of 214 (99%) of samples-- 1 to 93 ng/ml for 25(OH)D3 and 0.1 to 23.7 ng/ml for 3-epi-25(OH)D3--relative amounts of epimer to25(OH)D3 --0 to 25.5% (mean 4.75%).
- 3-Epi-25(OH)D3 in majority,-- generally low, --impact of 3-epi-25(OH)D3 on the various 25-hydroxyvitamin D assays

Microbiology

Triple play in lab's MALDI-TOF efforts

CAP TODAY

January 2013

Feature Story

Karen Titus

When James Musser, MD, PhD, and colleagues at Houston's The Methodist Hospital submitted a study for publication this fall (to "one of the prestigious weeklies based in a northeastern part of the country," says Dr. Musser), they were prepared to answer questions from reviewers.

The study tackled rapid pathogen identification and antimicrobial stewardship—topics near and dear to the hearts of microbiology laboratories, infectious disease specialists, pharmacists, and anyone scared silly by the book *Rising Plague*. In an era when antibiotics seem to be applied as liberally as sheep dip, looking at ways to use them more judiciously would merit national discussion.

Dr. James Musser and Dr. Katherine Perez showed how patient care improves and money is saved when MALDI-TOF mass spec, susceptibility testing, and antimicrobial stewardship are linked. "You get a bit of a sticker shock at first," Dr. Musser says, "but the downstream cost savings are tremendous."

Microbiology from MSACL

Bacterial, fungal, mold and others

Detection of functional antibiotic resistance using MALDI-TOF MS – Shi

Anti. Resistance and virulence factors by LCMSMS in SRM for 127 peptides from 17 staphylococcal proteins, id of species or sub-species, MRSA screening and toxin producing strains – Charrier

Clinical comprehensive data base and simple procedure for molds ID using MALDI-TOF – Lau

PGx, PRO and MB applications for TDM , clinical and forensic toxicology

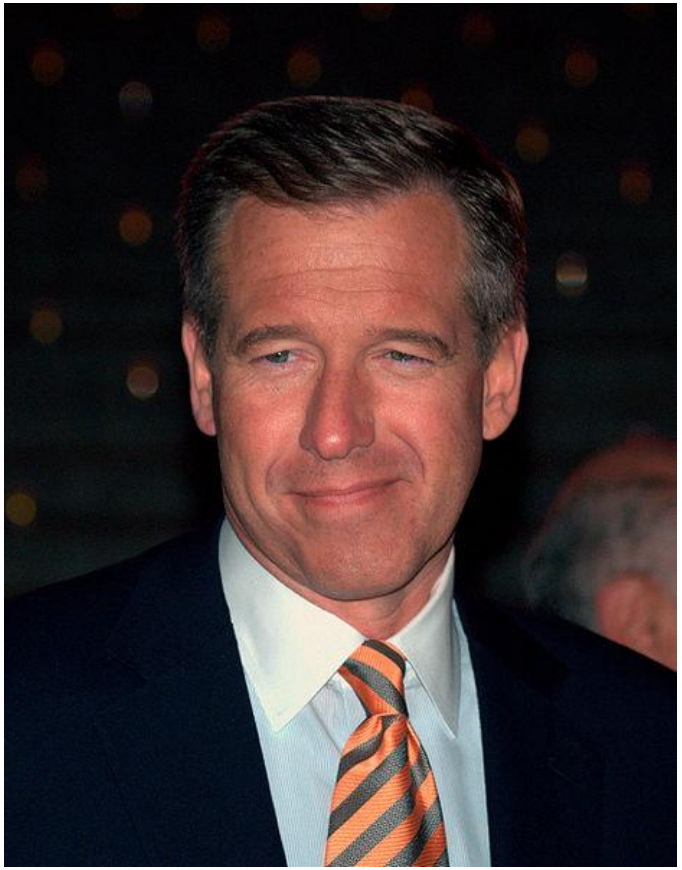
1. Plavix
2. Methadone
3. Propofol
4. Clinical and forensic toxicology - Oral fluid
5. Immunosuppressant

FDA labeling & PGx

http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm

Drug	Enzyme	Goal	Year	Status
6-MP	TPMT	Safety	2003	Complete
Azathiopurine	TPMT	Safety	2003	Complete
Atomoxetine	CYP2D6	Safety	2004	Complete
Irinotecan	UGT1A1	Safety	2004	Complete
Warfarin	CYP2C9, VKCOR1	Safety	2007	Complete
Abacavir	HLA-B*5701	Safety	2007	Complete
Carbamazepine	HLA-B*1502	Safety	2007	Complete
Phenytoin /fosphenytoin	HLA-B*1502	Safety	2008	Complete
Cetuximab Panitumumab	K-RAS	Efficacy	2008	Complete
Clopidogrel (Plavix) (No. 2)	CYP 2C19	Efficacy	3/12/ 2010	Complete

NBC Nightly News, March, 12, 2010



Plavix

(No. 2!! and PGx & MB)

FDA black box warning

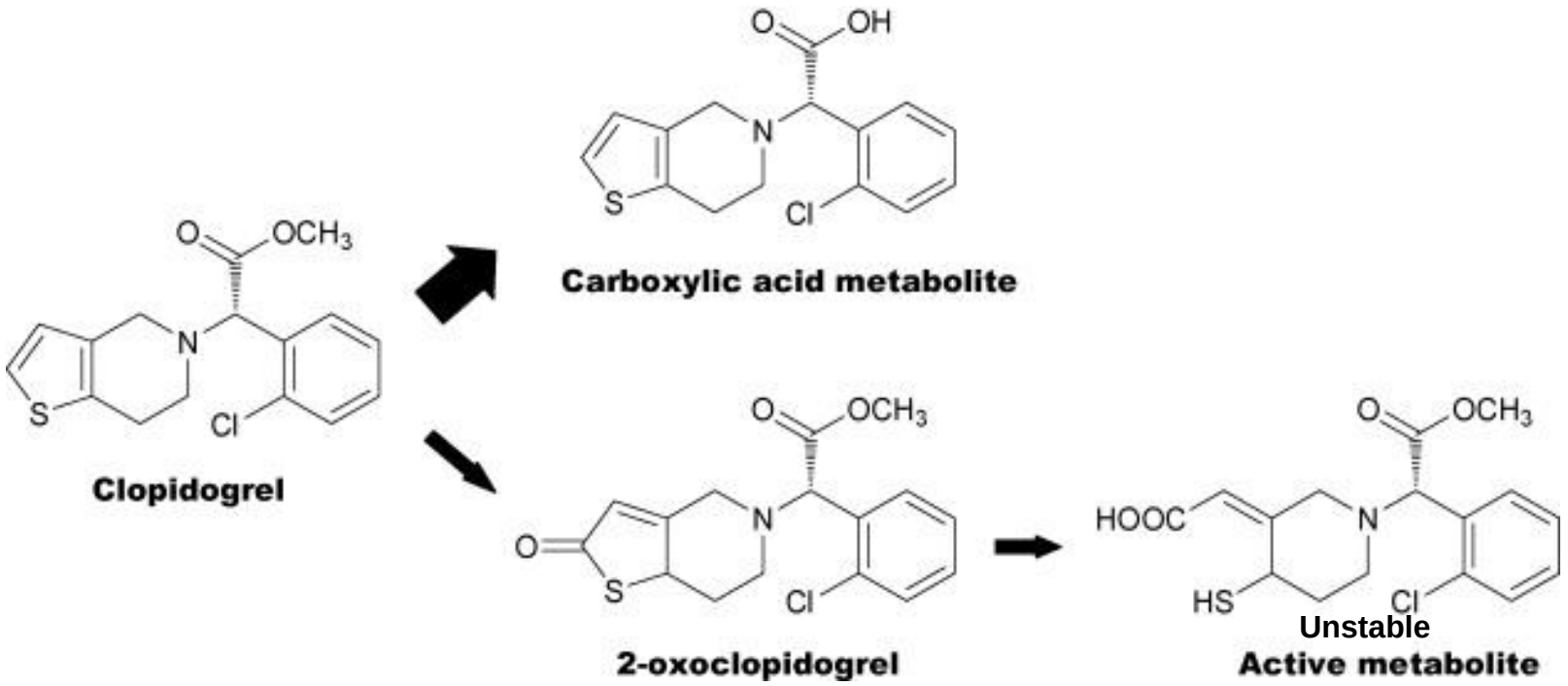
Might not work for some people due to genetics

~~~~~~

Plavix (prodrug) - CYP 2C19 to active metabolite
~~~~~~

Quantitative determination of clopidogrel active metabolite in human plasma by LC-MS/MS

Takahashi et al. J Pharma Biomed Anal 2008;48:1219 - 1224



Metabolic pathways of clopidogrel

http://dx.doi.org/10.1016/j.jpba.2008.08.020

Derivatization of clopidogrel active metabolite (AM) with 2-bromo-3'-methoxyacetophenone (MPB) and the chemical structures of the derivatized AM of clopidogrel (MP-AM) and its analog internal standard (IS) compound.

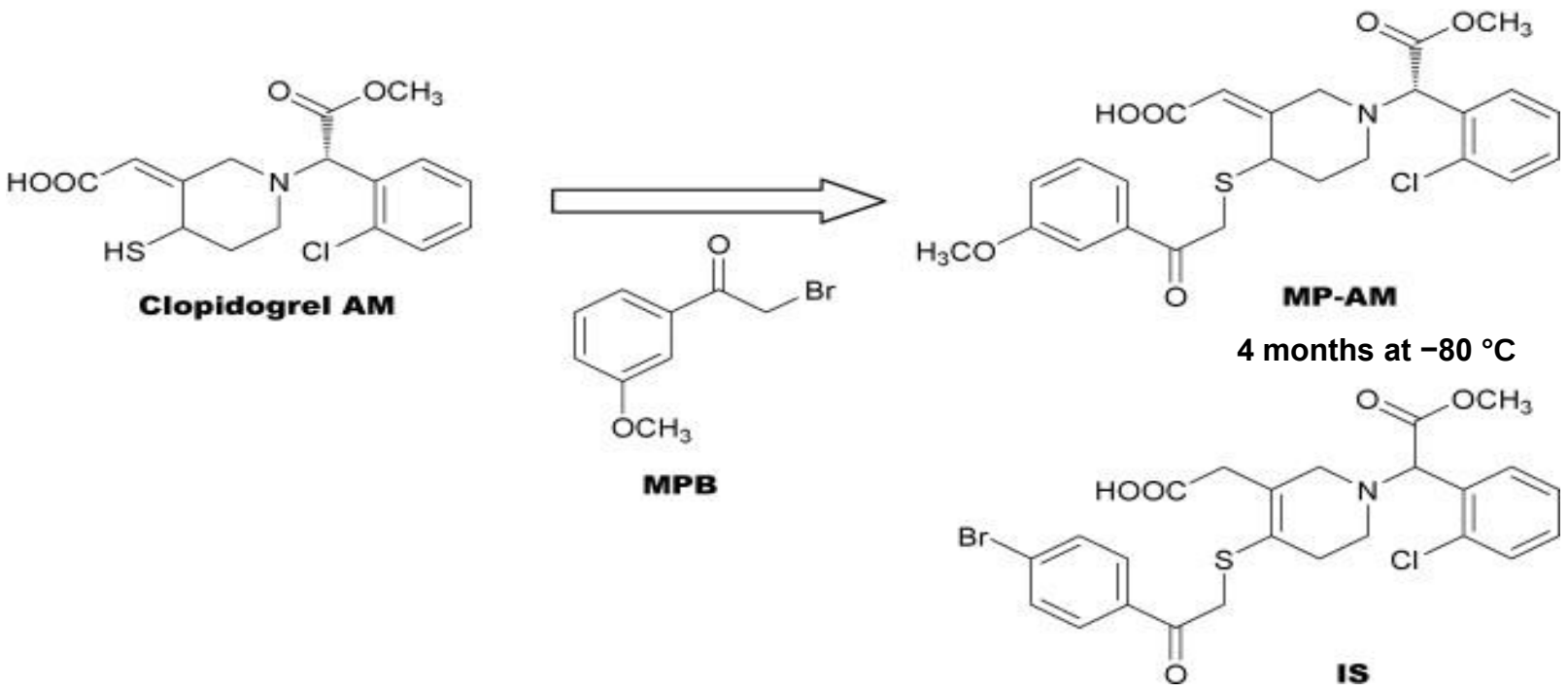


Fig. 4 Product ion spectra of the derivatized active metabolite of clopidogrel (MP-AM) and of the internal standard (IS).

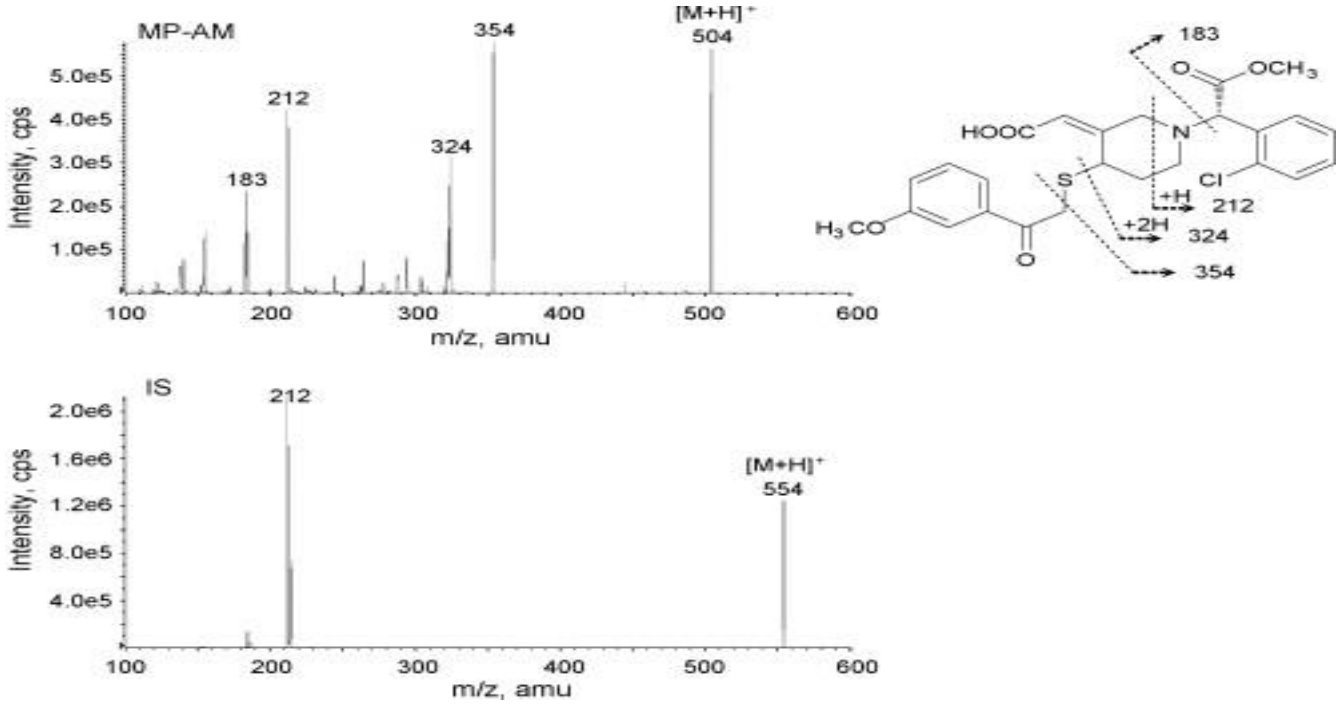
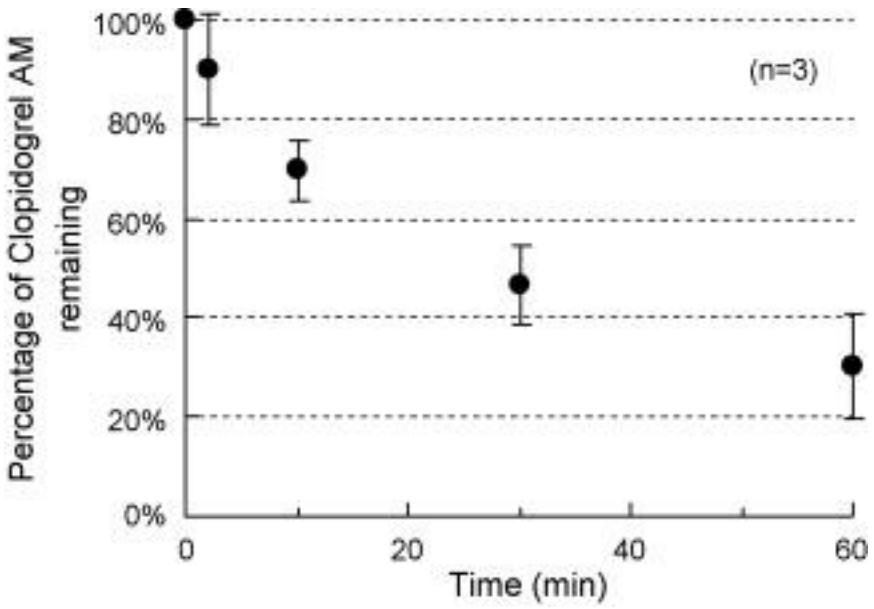


Fig. 3 Stability of clopidogrel active metabolite (AM) in human plasma at 37°C.



Increased use of Heroin and Treatments

Higher purity ~~ the user

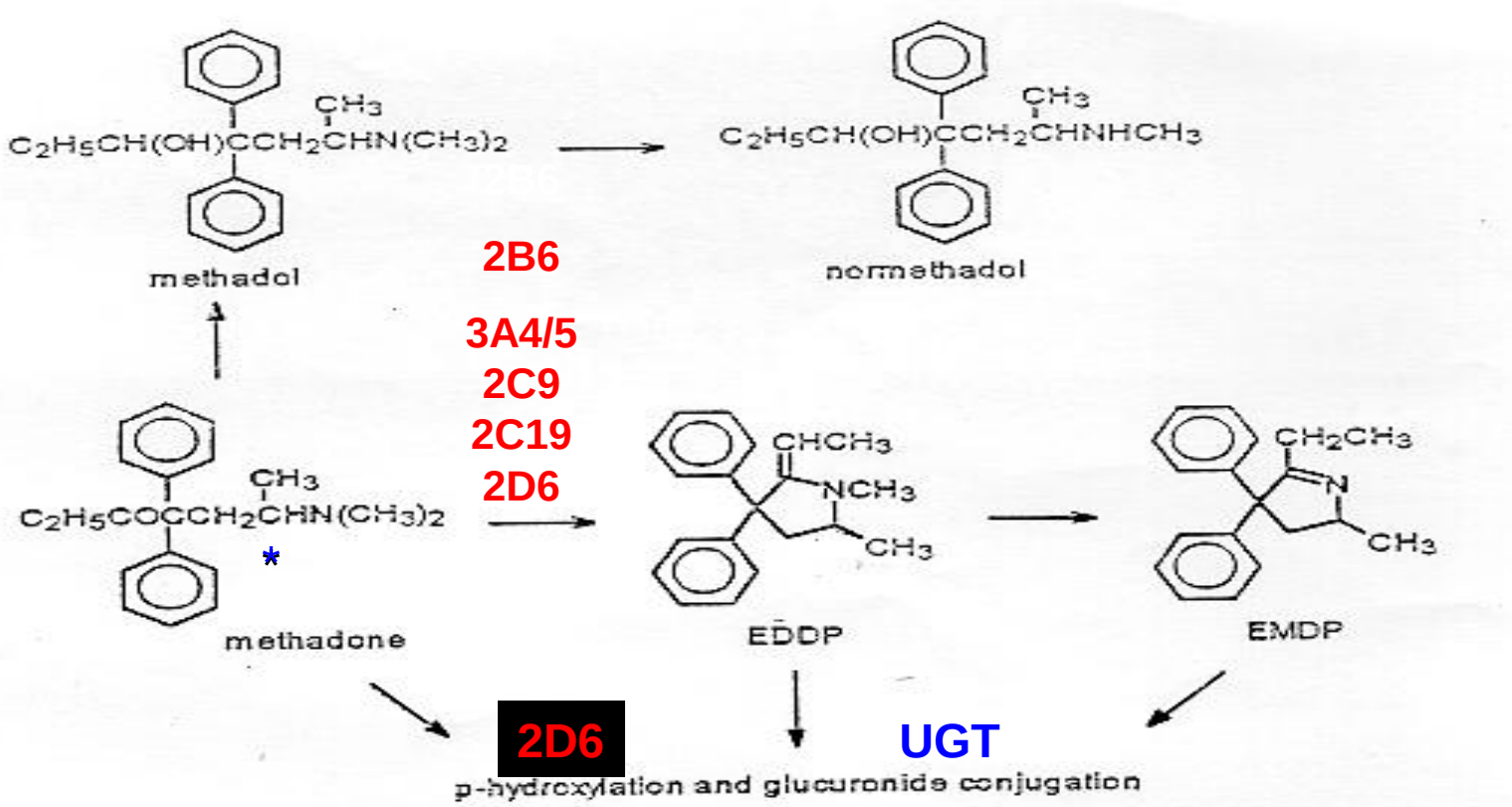
- Ability to snort
  - No risk of AIDS
  - 4 hour "High" @ $10 - $20 for each dose
  - Not an Aggressive "High"
- Ability to smoke

Treatment – Methadone, Buprenorphine, (Prescribed Heroin in Switzerland!!)

Methadone – Chiral pharmacology

- Methadone activity is almost solely to the drug itself rather than the metabolites
- Half life is variable 15-55 hrs
- R Methadone active form is 25-50 times more active than S
- However – CYP 2B6 poor metabolizer and S-methadone $\longrightarrow$ cardiotoxicity

Methadone Metabolism (2003-4)



*Asymmetric carbon

Moody.SOFT WS 2003

Winecker, Clin For Tox News(AACC), June 2003

Methadone Therapeutic and Toxic levels

Chronic administration:

100-200 mg daily 0.83 mg/L (0.57-1.06), 24hrs 0.46 mg/L

$t_{1/2}$ = 25 hrs

Lethal Concentrations:

	Blood	Brain	Liver	Bile	Kidney
Avg	1.0	1.0	3.8	7.5	2.9
Range	0.4-1.8	0.5-1.4	1.8-7.5	2.9-18.0	1.1-6.0

Case - Methadone & antidepressants

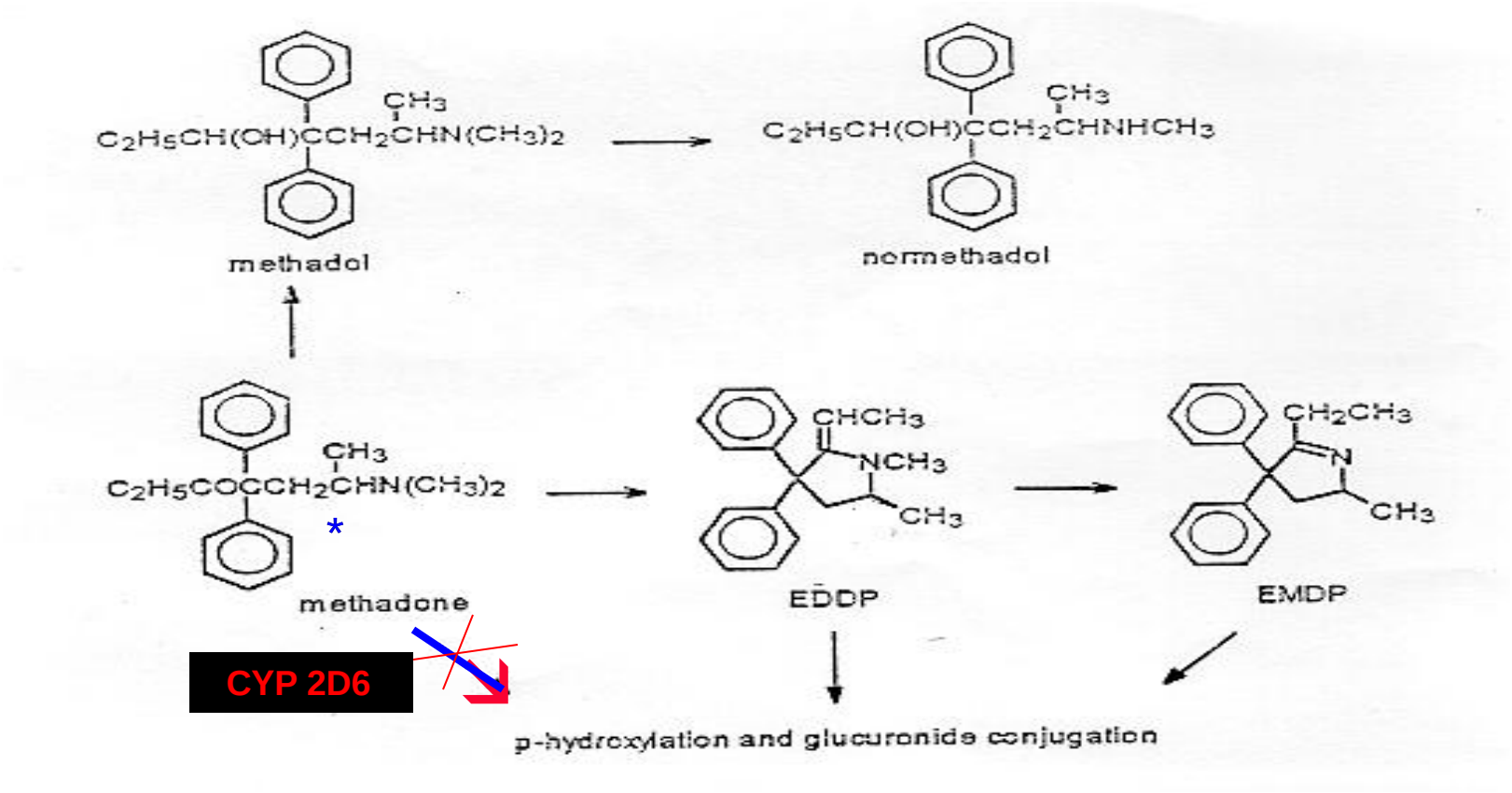
- 41, female, pregnant, history of heart murmur, arthritis, avid drug and alcohol abuser, alive at 2030, found by husband not breathing the following morning at 1130

- Med. - methadone, synthroid, fluoxetine hydroxyzine, amitriptyline and albuterol

Case findings

- Autopsy
  - Rheumatic heart disease
  - Dilated left ventricle
  - No internal trauma
- Molecular Autopsy - Pharmacogenetics
  - *CYP2D6*3* and **5* - WT
  - *CYP2D6*4* - HM, deficient enzyme activity, poor metabolizer

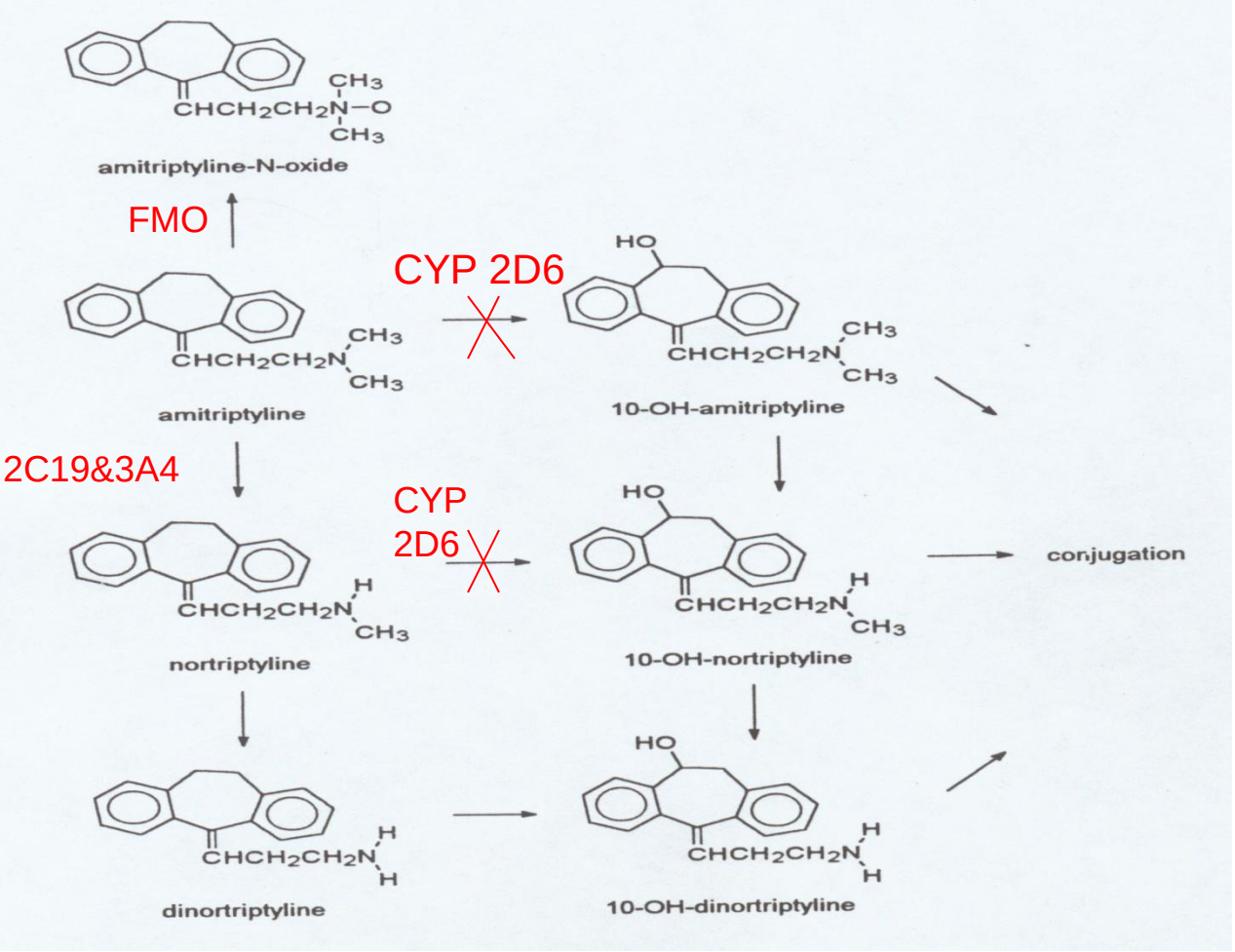
Methadone Metabolism (2000)



CYP450 : 3A4, 2D6 (→ p-hydroxylation), 1A2

* Asymmetric carbon

Amitriptyline metabolism



Tox findings by GC/MS

Drugs	Samples	Conc. µg/L	Fatal conc. µg/L
Amitriptyline	IB	1.5	2.7 – 4.7
Nortriptyline	IB	2.2	0.5 – 1.7
Methadone	IB	0.7	0.4 – 1.8
Diazepam	IB	0.19	30
Nordiazepam	IB	0.13	4

Death certifications

- Cause of death : MDO - Amitriptyline, methadone and diazepam
  - OSC - drug and alcohol abuse, rheumatic heart disease
- Manner of death: Accident

OPRM1 & CYP2B6 gene variants as risk factors in methadone-related deaths Bunten Ph.D. Thesis, 2010

Clin Parmacol Ther. 2010;88:383-9

- Methadone fatal poisonings
- Association between CYP2B6 and μ-opioid receptor (OPRM1) gene variations and apparent susceptibility to methadone poisoning.
- 40 Genomic DNA
- Genotype CYP2B6*4,*9, and *6 alleles and the OPRM1 A118G variant
- CYP2B6 *4,*9, and *6 alleles were found to be associated with higher postmortem methadone concentrations in blood (P < or = 0.05).
- OPRM1 A118G was also associated with higher postmortem methadone concentrations in blood but not to a level of statistical significance (P = 0.39).
- OPRM1 118GA was associated with higher postmortem benzodiazepine concentrations (P = 0.04), a finding not associated with morphine-related deaths.
- Risk of a methadone-related fatality during treatment may be evaluated in part by screening for CYP2B6*6 and A118G.

.

THE ROLE OF CYP3A4 AND CYP2D6 POLYMORPHISM IN UNEXPECTED METHADONE FATALITY – Lauren Waugh, Ph.D. Thesis 2010

- *CYP 3A4* and *2D6*
- 228 deaths – 136 methadone only, and 92 methadone/benzo
- Genotyped 12 SNPs
- *CYP 2D6* – NS from caucasian
- ***CYP3A4 (rs224280 and re2740574)* enriched in OD – may be associated with PM**
- No SNP – increased methadone or methadone/EDDP ratio
- **Combination of SNPs – collectively may increase methadone fatality.**

UNDERSTANDING AROMATASE: A MECHANISTIC BASIS FOR DRUG INTERACTIONS AND NEW INHIBITORS

Wenjie Lu Thesis, Jan. 2012

- Methadone: A Substrate and Mechanism-Based Inhibitor of CYP19 (Aromatase)
- Aromatase as a catalyst for methadone metabolism and as a mediator of the effects of tamoxifen by demonstrating that a number of its metabolites can act as aromatase inhibitors.
- A new mechanistic framework for the design of novel aromatase inhibitors that can be used in breast cancer

Categories
U.S. news
World news
Politics
Business
Sports
Entertainment
Michael Jackson
Gossip
Celebrities
Television
Reality TV
Movies
Music
Arts, books, more

Coroner rules Jackson's death a homicide

Court document reveals singer had lethal levels of propofol in system

BREAKING NEWS

msnbc.com news services
updated 3:44 p.m. CT, Mon., Aug 24, 2009

HOUSTON - The Los Angeles County coroner ruled Michael Jackson's death a homicide, a law enforcement official told the Associated Press on Monday. The official spoke on condition of anonymity because the findings have not been publicly released. A spokesperson for the coroner's office told Access Hollywood that the office had no comment on the AP report.

Dateline NBC's Josh Mankiewicz confirmed that Murray is the target of a manslaughter probe.

Meanwhile, a search warrant affidavit revealed

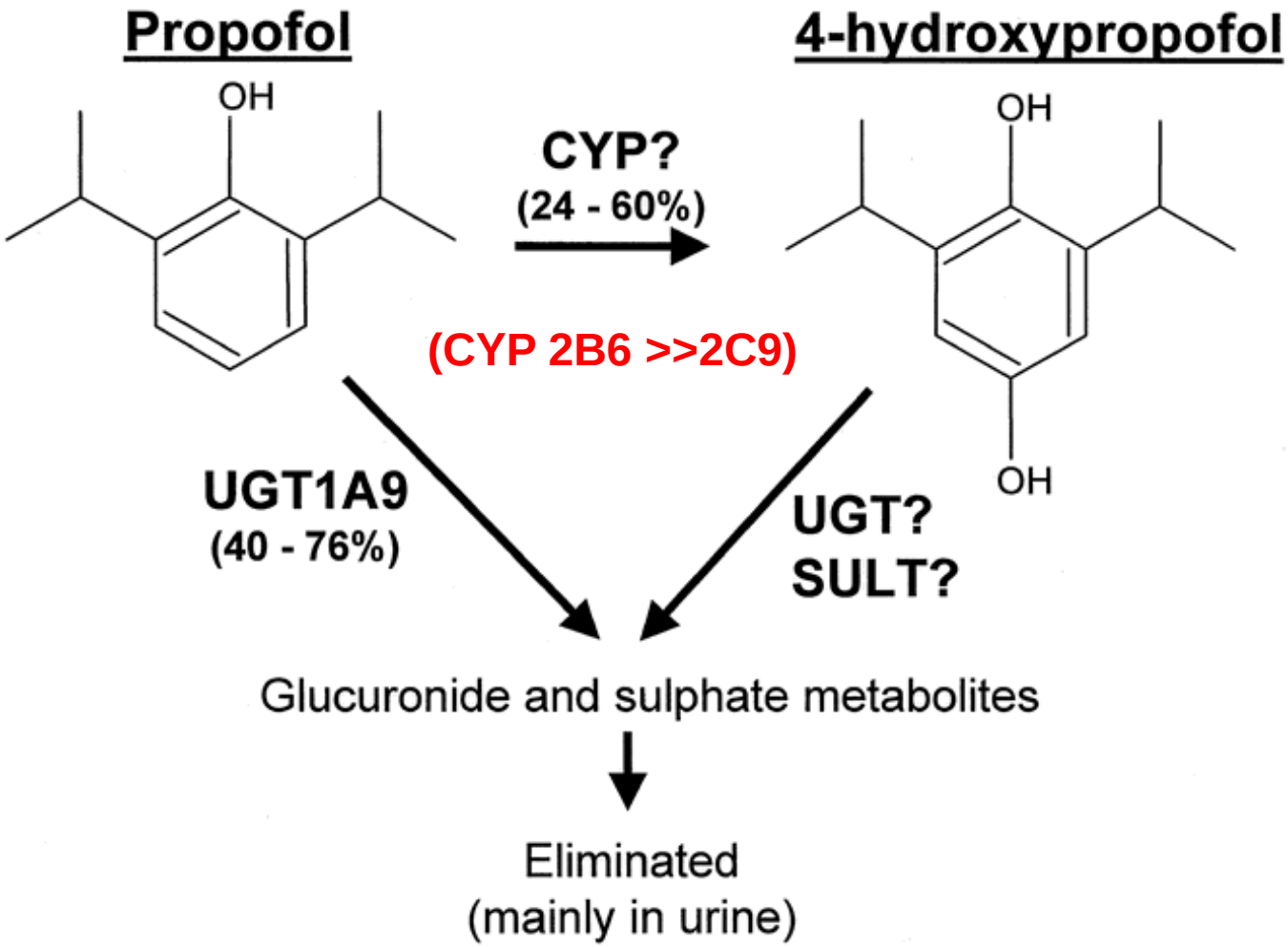
Video

Launch

Coroner: Jackson's death a homicide
NEW Aug. 24: The Los Angeles coroner rules Michael

Propofol metabolism and PGx

Cytochrome P-450 2B6 Is Responsible for Interindividual Variability of Propofol Hydroxylation by Human Liver Microsomes. Court, Duan, Hesse, Venkatakrishnan, Greenblatt, .Anesthesiology. 2001, 94(1), 110-119



Grey’ s Anatomy

Patient suddenly woke up in surgery! Run some tests?

Episode, 22 , April 22, 2010, ABC

Run some tests for propofol!?

May include?

1. Metabolomics LC-MS/MS – toxicity/efficacy and metabolic ratio

2. Genotype CYP 2B6 for URM phenotpye

Grey’ s Anatomy

Patient suddenly woke up in surgery!

URM phenotype!!

Episode, 22 , April 22, 2010, ABC

Designer drugs – Spice, Bath Salts, Bubbles, 'Zombie', Legal Highs - Loop Hole

65th Anniversary **WORKSHOPS** 1948 - 2013

Pre-Registration Required

#19 Developments in Emerging and Designer Drug Markets 2013

Tuesday, February 19 **8:30 a.m. - 5:00 p.m.** **6.5 CE Hours**

Program Description: The goals of this workshop are to provide updates on the rapidly changing field of designer drugs and their analogs, and to present epidemiological information, trends reports from forensic laboratories, novel testing methodologies, and new analytical resources for criminalistics and toxicology laboratories.

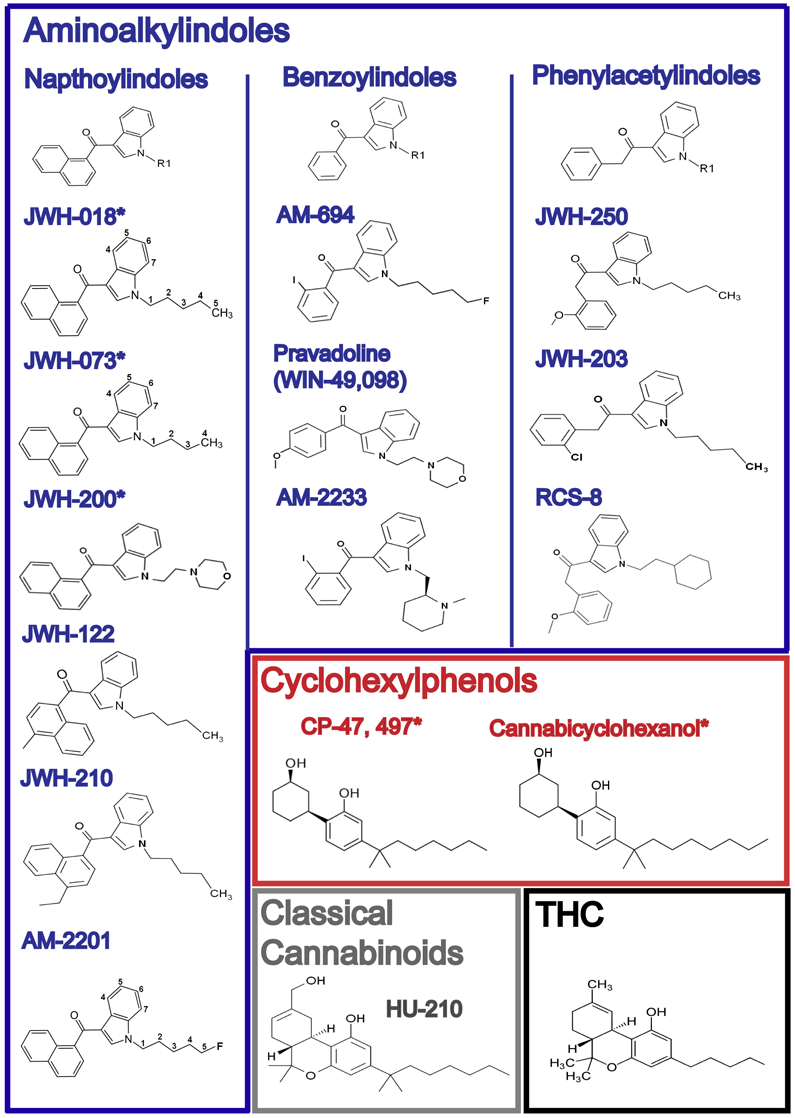
Program:

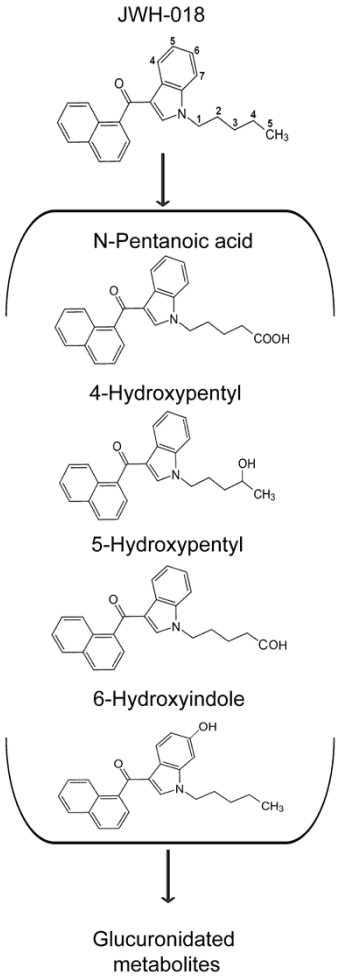
8:30 a.m. - 8:35 a.m.	Introduction and Welcome *Jeri D. Ropero-Miller, PhD*
8:35 a.m. - 9:15 a.m.	Insight Into Emerging Drugs on the U.S. Market Through Solid Dosage and Biological Monitoring *Barry K. Logan, PhD*
9:15 a.m. - 9:55 a.m.	NFLIS Update on Designer Drug Trends in the United States and DEA Guidelines for Controlling Cannabimimetic Agents *DeMia P. Pressley, MS*

Program cont.

9:55 a.m. - 10:40 a.m.	Designer Drug Trends in the European Union — Is the Future Already Here? *Roumen Sedefov, MD*
10:40 a.m. - 11:10 a.m.	Break
11:10 a.m. - 11:50 a.m.	The Inaudible Barking Dog: U.S. Policy Implications of "Legal Highs" *Kabrena E. Rodda, PhD*
11:50 a.m. - 12:30 p.m.	Assessment of Testing Approaches for Large Scale Designer Drug Testing *Marilyn A. Huestis, PhD*
12:30 p.m. - 1:30 p.m.	Lunch
1:30 p.m. - 2:10 p.m.	Pharmacology of "Bath Salts" and Related Designer Drugs *Michael Baumann, PhD*
2:10 p.m. - 3:10 p.m.	Elucidation of Metabolic Pathways for Emerging Cannabinoid Agonists *Jeffrey Moran, PhD*
3:10 p.m. - 3:40 p.m.	Break
3:40 p.m. - 4:20 p.m.	Designer Drugs - A Killer Among Us *Jeri D. Ropero-Miller, PhD*
4:20 p.m. - 5:00 p.m.	Online Database Resource for the Identification of Novel and Emerging Drugs *Peter R. Stout, PhD*

Synthetic Cannabinoids -The Challenges of Testing for Designer Drugs
Crews, CLN, 2013. Feb.

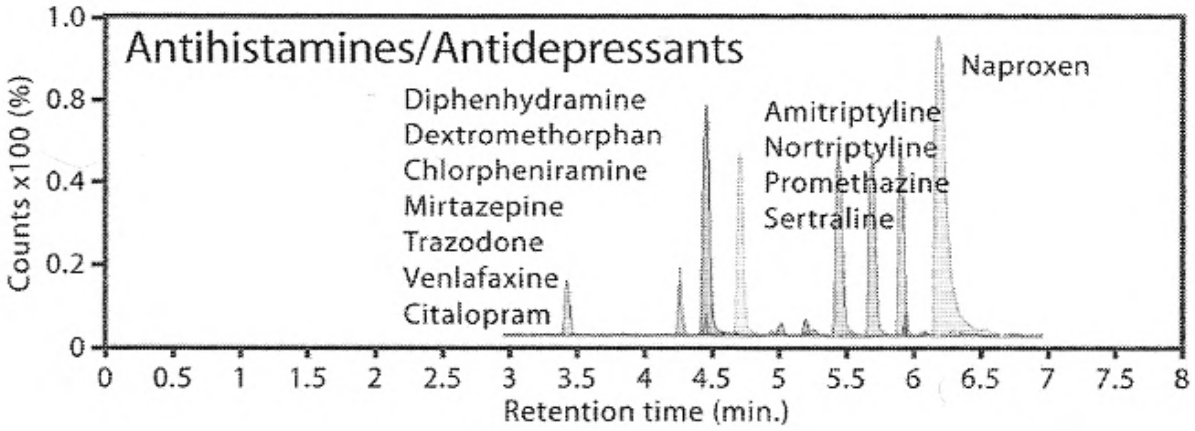


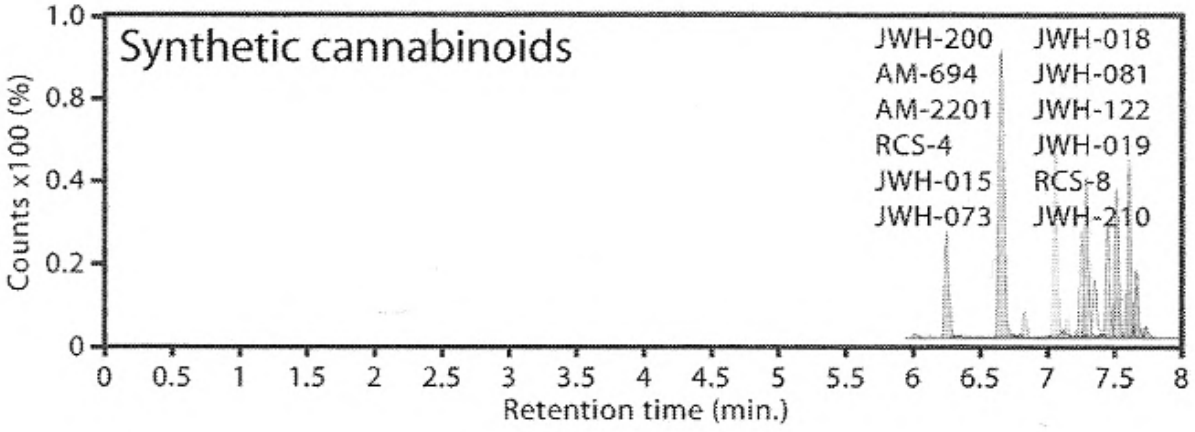


Validation of LC-TOF-MS screening for drugs, metabolites, and collateral compounds in forensic toxicology specimens

Guale F, -- Mozayani A. JAT, 2013;37:17-24.

- LC-TOF-MS – gentle electrospray ionization, accurate mass, and retention data for ID
- Automated SPE, 13 m gradient, resolve isobaric compounds within 15 ppm, and <10 S separation
- Blood, urine postmortem, DUID, DFSA
- Stimulants, benzo., opiates, muscle relaxants, hypnotics, antihistaines, antideprssants, newer synthetic drugs - “designer” “loop holes“ “Spice/K2 “ cannabinoids, and cathinone ” bath salt”


Antihistamines/Antidepressants
Diphenhydramine
Dextromethorphan
Chlorpheniramine
Mirtazepine
Trazodone
Venlafaxine
Citalopram
Amitriptyline
Nortriptyline
Promethazine
Sertraline
Naproxen
Counts x100 (%)
Retention time (min.)


Synthetic cannabinoids
JWH-200
AM-694
AM-2201
RCS-4
JWH-015
JWH-073
JWH-018
JWH-081
JWH-122
JWH-019
RCS-8
JWH-210
Counts x100 (%)
Retention time (min.)

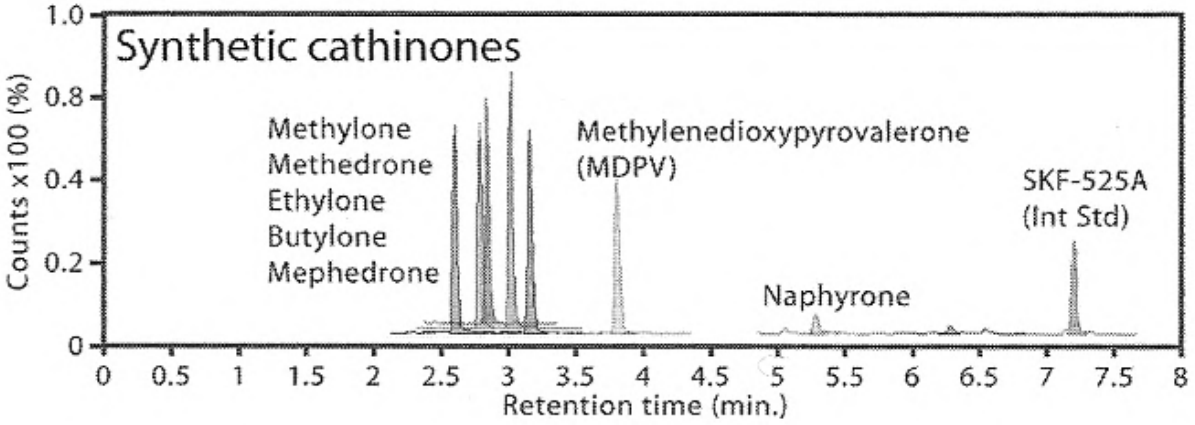
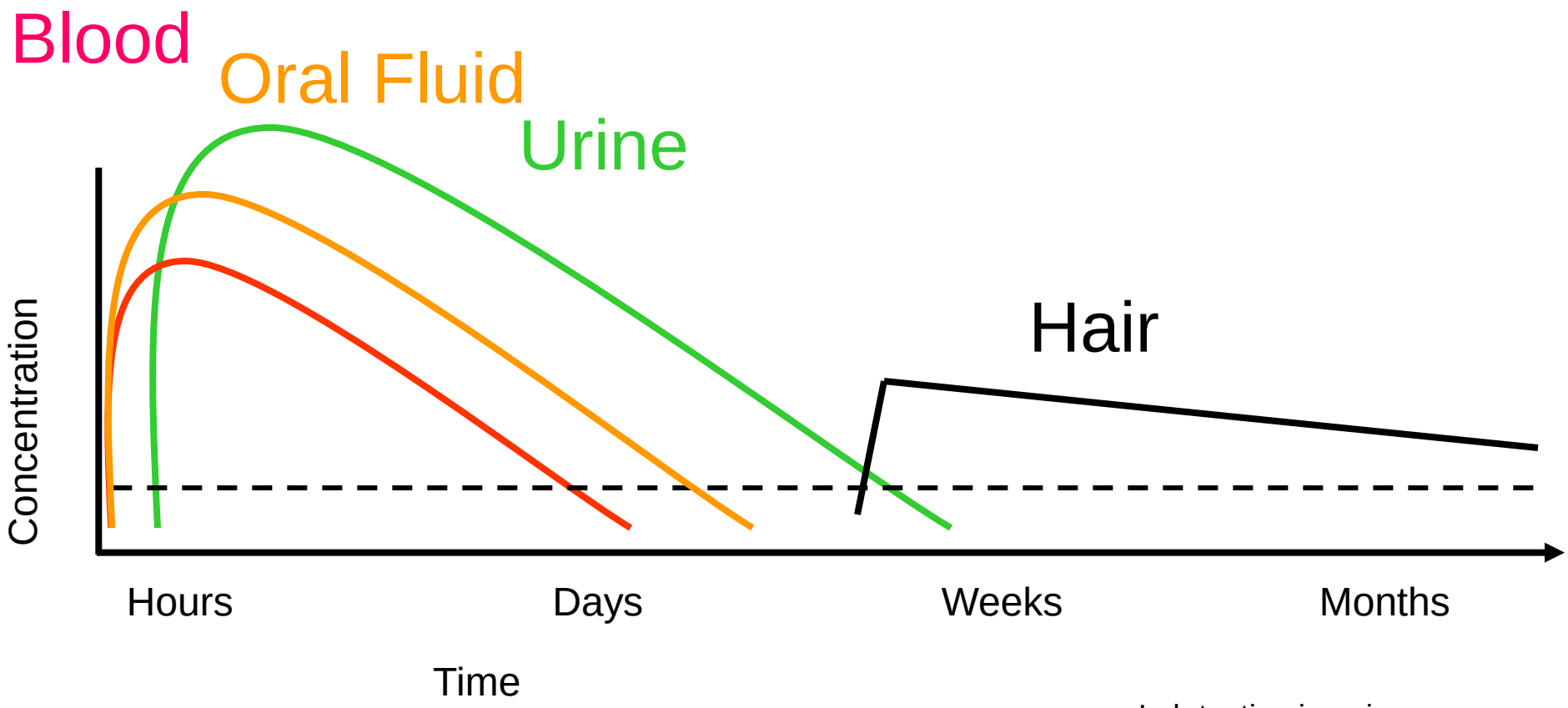

Synthetic cathinones
Methylone
Methedrone
Ethylone
Butylone
Mephedrone
Methylenedioxypyrovalerone
(MDPV)
Naphyrone
SKF-525A
(Int Std)
Counts x100 (%)
Retention time (min.)

Table I Continued

Name	Formula	RT (min)	Mass	Mass (Tgt)	Difference (ppm)	Score	Area	Isobaric/isomer
C) Synthetic Cannabinoid Spice Compounds and Synthetic Cathinone Bath Salts								
Synthetic cannabinoids								
RCS-4	$C_{21}H_{23}NO_2$	7.29	321.17395	321.17288	3.34	83	4670598	
JWH-073	$C_{23}H_{21}NO$	7.33	327.16322	327.16231	2.76	92	6744872	JWH-015
JWH-019	$C_{25}H_{25}NO$	7.64	355.19398	355.19361	1.03	90	8203283	JWH-007, JWH-122
JWH-122	$C_{25}H_{25}NO$	7.64	355.19399	355.19361	1.04	98	8180911	JWH-007, JWH-019
AM-2201	$C_{24}H_{22}FNO$	7.10	359.16917	359.16854	1.75	97	8283323	
JWH-210	$C_{26}H_{27}NO$	7.77	369.20959	369.20926	0.87	86	467841	JWH-020
JWH-081	$C_{25}H_{25}NO_2$	7.55	371.18892	371.18853	1.05	99	6697934	JWH-018-6-MeO
RCS-8	$C_{25}H_{29}NO_2$	7.69	375.22051	375.21983	1.82	99	3012613	
JWH-200	$C_{25}H_{24}N_2O_2$	6.69	384.18418	384.18378	1.04	93	16634958	
JWH-251	$C_{22}H_{25}NO$	7.43	319.19418	319.19361	1.78	75	7669899	
JWH-015	$C_{23}H_{21}NO$	7.23	327.16331	327.16231	3.05	94	10038079	JWH-073
JWH-250	$C_{22}H_{25}NO_2$	7.33	335.18924	335.18853	2.13	88	8064283	JWH-201, JWH-302
JWH-203	$C_{21}H_{22}ClNO$	7.42	339.13989	339.13899	2.64	86	220661	
JWH-022	$C_{24}H_{21}NO$	7.35	339.16313	339.16231	2.40	78	563953	
JWH-018	$C_{24}H_{23}NO$	7.46	341.18006	341.17796	6.13	64	122556	JWH-016
JWH-016	$C_{24}H_{23}NO$	7.42	341.17868	341.17796	2.09	83	727863	JWH-018
JWH-007	$C_{25}H_{25}NO$	7.56	355.19409	355.19361	1.35	97	2276986	JWH-019, JWH-122
JWH-018-6-MeO	$C_{25}H_{25}NO_2$	7.47	371.18949	371.18853	2.58	84	179250	JWH-081
Pravadoline WIN-48098	$C_{23}H_{26}N_2O_3$	6.58	378.19627	378.19434	5.10	43	137908	
JWH-098	$C_{26}H_{27}NO_2$	7.61	385.20562	385.20418	3.75	61	89646	
AM-1241	$C_{22}H_{22}IN_3O_3$	5.45	503.07024	503.07058	−0.68	83	863232	
AM-694	$C_{20}H_{19}FINO$	6.87	435.04974	435.04954	0.46	99	1099238	
Synthetic cathinones								
Mephedrone	$C_{11}H_{15}NO$	3.15	177.11515	177.11536	−1.22	63	116712	Phenmetrazine, AMMI
Methedrone	$C_{11}H_{15}NO_2$	2.84	193.11062	193.11028	1.76	54	296298	MDMA, 5-methyl-MDA
4′-Methyl-α-pyrrolidino-propiophenone (MPPP)	$C_{14}H_{19}NO$	3.42	217.14606	217.14666	−2.80	93	352269	
Butylone	$C_{12}H_{15}NO_3$	3.01	221.10554	221.10519	1.57	79	433487	Ethylone, metaxalone
Ethylone	$C_{12}H_{15}NO_3$	2.78	221.10605	221.10519	3.89	74	268832	Butylone, metaxalone
Methylenedioxy-pyrovalerone (MDPV)	$C_{16}H_{21}NO_3$	3.80	275.15267	275.15214	1.92	91	564883	
Methylone	$C_{11}H_{13}NO_3$	2.61	207.09019	207.08954	−3.12	94	1233166	
Pentedrone	$C_{12}H_{17}NO$	3.57	191.13125	191.13101	−1.27	72	9596955	Phendimetrazine, 4-MEC, DMMC
αpyrrolidino-propiophenone (α-PPP)	$C_{13}H_{17}NO$	2.78	203.13121	203.13101	−0.97	94	7569091	
Naphyrone	$C_{19}H_{23}NO$	5.28	281.17842	281.17796	1.63	91	1244472	

Drug Testing Profiles



Lab testing in pain management: Approach & issue. Caplan /Cone. SAMHSA/DTAB, Aug. 08

Oral Fluid Testing:

Promises and Pitfalls

Moderator: Marilyn A. Huestis

Experts: Alain Verstraete,Tai C. Kwong, Jorg Morland,

Michael J. Vincent, and Raphael de la Torre

Clinical Chemistry 57:6:805–810 (2011) Q&A

Recommended Oral Fluid Analytes & Cutoffs for Workplace Drug Testing

Marilyn A. Huestis
Chief, Chemistry & Drug Metabolism, IRP
National Institute on Drug Abuse, NIH

Drug Testing Advisory Board January 27, 2011

Potential Advantages

- Unique information
- Less invasive collection under direct observation
- Less opportunity for adulteration
- No specialized facilities or same sex collector needed
- Presence of parent drug
- Lower disease risk
- Detection window cutoff dependent

Huestis

Guidelines for Oral Fluid

Version 001, March 2011

Foreword

The following guidelines for oral fluid were adapted from the draft United Kingdom Guidelines for Legally Defensible Workplace Drug and Alcohol Testing. The European Workplace Drug Testing Society (EWDTS) acknowledges the substantive work completed by the steering group members of the United Kingdom Workplace Drug Testing Forum (UKWDTF).

The EWDTS recommends that all European laboratories that undertake legally defensible workplace drug testing should use these guidelines as a template for accreditation, in addition to meeting the general requirements of the international standard ISO/IEC 17025.

Contents

Drug Testing Advisory Board (DTAB) Meeting
Center for Substance Abuse Prevention (CSAP)
Substance Abuse and Mental Health Services Administration (SAMHSA)

Sugarloaf Conference Room
One Choke Cherry Road, Rockville, MD

Agenda

January 31, 2012

Open Session (9:00 am – 3:00 pm EST)

9:00 Call to Order

Janine Denis Cook, Ph.D., DABCC, FACB
Designated Federal Official, DTAB
Division of Workplace Programs (DWP)
CSAP, SAMHSA

Welcome and Opening Remarks

Janine Denis Cook, Ph.D., DABCC, FACB
Acting Chair, DTAB

CAPT Carol Rest-Mincberg, M.P.H.
Director
DWP, CSAP, SAMHSA

David K. Mineta, M.S.W.
Deputy Director of Demand Reduction
Office of Demand Reduction
White House Office of National Drug Control Policy

Update: Proposed Recommendations

Pamela J. Hyde, J.D.
Administrator
SAMHSA

9:30 Synthetic Opioids

Janine Denis Cook, Ph.D., DABCC, FACB
Designated Federal Official, DTAB

10:15 Synthetic Opioid Metabolism

James A. Bourland, Ph.D., DABFT
Scientific Director
Alere Toxicology

11:00 Laboratory Data - Synthetic Opioids

Barbara J. Rowland, B.S., MT, M.P.A.
Director, Laboratory Operations and Responsible Person
Quest Diagnostics, Inc.

11:30 Pain Management Data - Synthetic Opioids

Edward J. Cone, Ph.D., FTCB
Adjunct Professor
The Johns Hopkins University

12:15 Lunch Break

1:30 MRO-Verified Data - Synthetic Opioids

J. Michael Walsh, Ph.D.
President
The Walsh Group, P.A

2:00 MRO Review - Synthetic Opioids

Nicholas M. Lomangino, M.D.
Medical Review Officer
Federal Aviation Administration
U.S. Department of Transportation

2:30 Public Comments

3:00 Adjourn Open Session

Closed Session (3:00 pm – 5:00 pm EST)

3:00 Call to Order

3:00 Review of the Draft of the Proposed Revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs to Include Oral Fluid as an Alternate Specimen

Janine Denis Cook, Ph.D., DABCC, FACB
Acting Chair, DTAB

5:00 Adjourn

Round-the-clock oral 20 mg THC up to 120 mg/day for 8 days

- 440 oral fluid specimens collected with the Quantisal device & by expectoration
- Cannabinoids after self-administration of smoked cannabis
- Single-dose oral pharmacokinetics
- Drug disposition during continuous dosing
- Duration of cannabinoid detection during monitored abstinence
- OF/plasma ratios

Huestis

THC, 11-OH-THC & THCCOOH Analysis in Oral Fluid

- Solid phase extraction
  - 1st eluent 3 mL Hex/EtOAc/Acetone (60:20:30) for THC, CBD, CBN, 11-OH-THC
  - 2nd eluent 3 mL Hex/EtOAc/HOAc (75:25:2.5) for THCCOOH
- Two dimensional GCMS with cryofocusing
  - 1st eluent electron impact for THC & 11-OH-THC with LOQ of 0.5 ng/mL
  - 2nd eluent negative chemical ionization for THCCOOH with LOQ of 7.5 pg/mL

Huestis

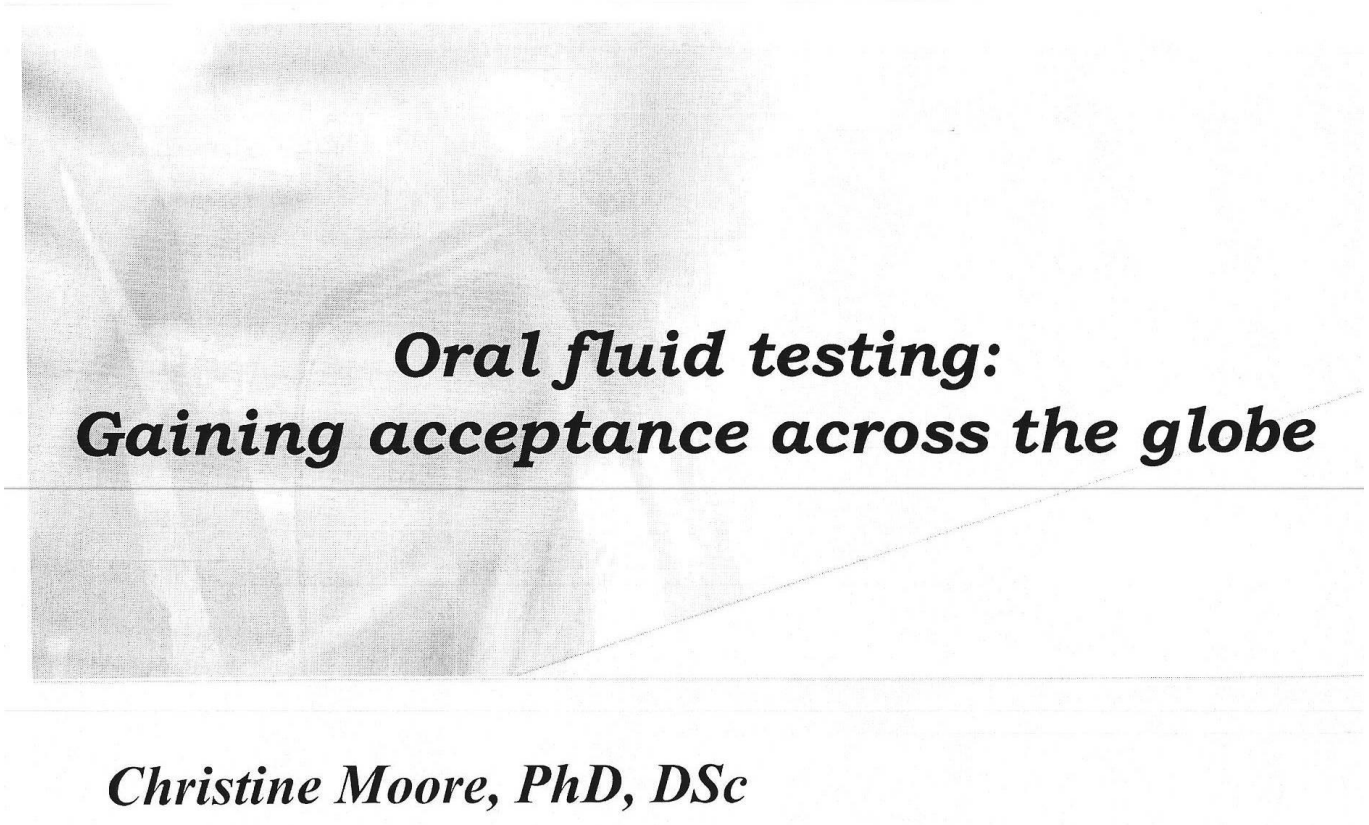
Cannabinoids in Oral Fluid

n=440	THC ng/mL	CBD ng/mL	CBN ng/mL	11-OH-THC ng/mL	THCCOOH pg/mL
LOQ	0.5	0.5	1	0.5	7.5
Positive	91	3	8	0	432
% Positive	20.7%	0.7%	1.8%	-	98.2%
Median	1.7	1.4	2.3	-	84.5
Cmax	481.9	2.1	13.0	-	1117.9

Huestis

Cannabinoids in oral fluid following passive exposure to marijiuana smoke

Moore C, et al. For Sci Intern. 2011;212:227-30



Experimental design

- IRB approved
- Two locations:“Coffee shops” (16X23X11ft and 6.5X23X10 ft) in Groningen, Holland
- Oral fluid specimens collected outside the shop where marijuana use was on-going
- Collected two samples with Quantisal™ devices at each time point

1. Prior to exposure; 20 min; 40 min; 60 min
2. 2 hrs; 3 hrs
3. Subjects left shop
4. Final sample: 12 -22 hrs after entering shop
5. In addition:
    - one collection pad at location #1; two pads at location #2 were opened, left on table in the shop throughout the experiment

Analytical procedures

- *After collection, specimens shipped to test facility*
- *All specimens analyzed using ELISA: 4ng/mL*
- *All specimens analyzed using GC/MS:*
  - *THC (0.5ng/mL)*
  - *Cannabidiol (CBD; 1ng/mL)*
  - *Cannabinol (CBN; 0.5ng/mL)*
- *All specimens analyzed using GC-GC/MS*
  - *THC-COOH (5pg/mL)*

Passive exposure

- THC is absorbed by drug-free individuals under realistic conditions when exposed to marijuana smoke
- After only 2 hrs of exposure some individuals showed THC concentrations above proposed screening and confirmatory cut-offs
- The metabolite, THC-COOH, NOT detected in any of the specimens
- THC-COOH: appears to be a bio-marker for active marijuana use as opposed to THC which may be present due to passive exposure

“Is THCCOOH a useful determinant for passive inhalation in oral fluid THC testing?” Moore JAT, 2012;36:358.

- Acute and intense passive inhalation
- THC detectable from 3 to 22 hr – may be longer
- THCCOOH in OF – indicative of marijuana use
- Screening for THC, and in questionable cases, MRO request THCCOOH

Toxicology MSACL 2013

THC-A in oral fluid using GCGCMS (Multidimensional, Dean Switch and cyrofocusing), and nano-flow LC-MS/MS to low pg/mL - Huestis

Synthetic cannabinoids urine analysis by LC-MS/MS showed a major shift – McMullin

Designer drugs “Bath Salt” by TOF MS – Gerona

Proposed screening cut-off concentrations (ng/mL)

Drug class	EWDTS	SAMHSA	Comments
Amphetamines	*40*	*50*	(120)
Cannabinoids	*10*	*4*	(3)
Cocaine	*30*	*20*	(15)
Opiates (morphine)	*40*	*40**	*(30)* **Includes synthetic opioids: oxycodone and hydrocodone*
6-acetylmorphine	*4*	*4*	
Phencyclidine		*10*	(3)
Methadone	*50*		
Buprenorphine	*5*		
Propoxyphene	*40*		
Benzodiazepines	*10*		

Proposed confirmation cut-off concentrations (ng/mL)

Drug class	EWDTS	SAMHSA	Comments
Amphetamines	30	50	(120) AMP, METH, MDMA, MDA, MDEA
Cannabinoids	2	2 (0.02*)	(1.5) THC *THC-COOH for SAMHSA ?
Cocaine	8	8	(6)
Opiates (morphine)	40**	40*	(30) ** Includes dihydrocodeine *Includes synthetic opioids: Oxycodone, oxymorphone, hydromorphone, hydrocodone
6-acetylmorphine	4	4	
Phencyclidine		10	(3)
Methadone	20		
Buprenorphine	5		
Propoxyphene	40		
Benzodiazepines	10		Temazepam, Oxazepam, Nordiazepam

SAMHSA Urine Screening & confirmation cutoffs

Analytes	Screening, ng/mL	Confirmation, ng/mL
Amphetamines	**500**	**250**
Amphetamine		
Methamphetamine		
MDMA, MDA, MDEA		
Cannabinoids	**50**	
THC-COOH		**15**
Cocaine metabolites	**150**	
Benzoylecgonine		**150**
Opiates	**2000**	
Codeine		**2000**
Morphine		**2000**
6-MAM	**10**	**10**
Phencyclidine	**25**	**25**

Immunosuppressants

- Cyclosporine
- Tacrolimus
- Rapamycin
- Mycophenolic Acid
- Everolimus

  Kidney, liver

2012 Immunosuppressant CAP survey
CsA and MPA

		No. Labs	Mean	S.D.	C.V.	Median	Low Value	High Value
CS-01	Abbott Architect *i* System	204	83.47	11.49	13.8	83.1	50.2	117.0
	Abbott AxSYM	6	-	-	-	71.0	63.0	78.5
	CEDIA Plus	51	71.95	13.30	18.5	71.7	46.0	103.8
	LC-MS-MS	42	83.32	8.88	10.7	82.8	62.0	99.0
	Mass Spectrometry	27	84.13	6.15	7.3	83.0	74.0	98.0
	Roche COBAS Integra	14	74.21	8.19	11.0	75.0	60.7	87.5
	Siemens Diag. ADVIA Centaur, XP	21	72.26	7.95	11.0	76.0	50.7	82.4
	Siemens Diagnostics Dimension	72	81.41	8.29	10.2	81.3	58.8	105.0
	Siemens Diag. Dimension Vista	20	84.50	7.96	9.4	86.0	69.0	102.0
	Syva EMIT 2000	11	73.83	10.99	14.9	74.2	52.0	93.0
	All Results	472	80.79	11.28	14.0	81.0	46.0	117.0

		No. Labs	Mean	S.D.	C.V.	Median	Low Value	High Value
MPA-01	HPLC	10	2.57	0.41	16.1	2.6	1.8	3.3
	LC-MS-MS	9	-	-	-	2.7	2.3	3.1
	Mass Spectrometry	8	-	-	-	2.5	2.0	2.7
	Roche Hitachi/cobas c	5	-	-	-	2.6	2.6	2.7
	Syva EMIT 2000	6	-	-	-	2.7	2.6	2.9
	All Results	47	2.57	0.28	10.7	2.6	1.8	3.3

2012 Immunosuppressant CAP survey
Tacrolimus & Sirolimus

		No. Labs	Mean	S.D.	C.V.	Median	Low Value	High Value
CS-01	Abbott Architect *i* System	242	4.63	0.30	6.5	4.6	3.9	5.5
	CEDIA	15	2.67	1.03	38.6	2.5	0.9	4.2
	LC-MS-MS	42	4.64	0.51	11.1	4.7	3.5	5.8
	Mass Spectrometry	29	4.90	0.65	13.2	5.0	3.8	6.6
	Siemens Diagnostics Dimension	94	4.27	0.80	18.7	4.4	2.5	6.1
	Syva EMIT 2000	16	5.40	0.95	17.5	5.3	3.0	6.6
	Waters MassTrak	5	-	-	-	5.0	4.4	5.2
	All Results	446	4.53	0.69	15.3	4.6	0.9	6.6

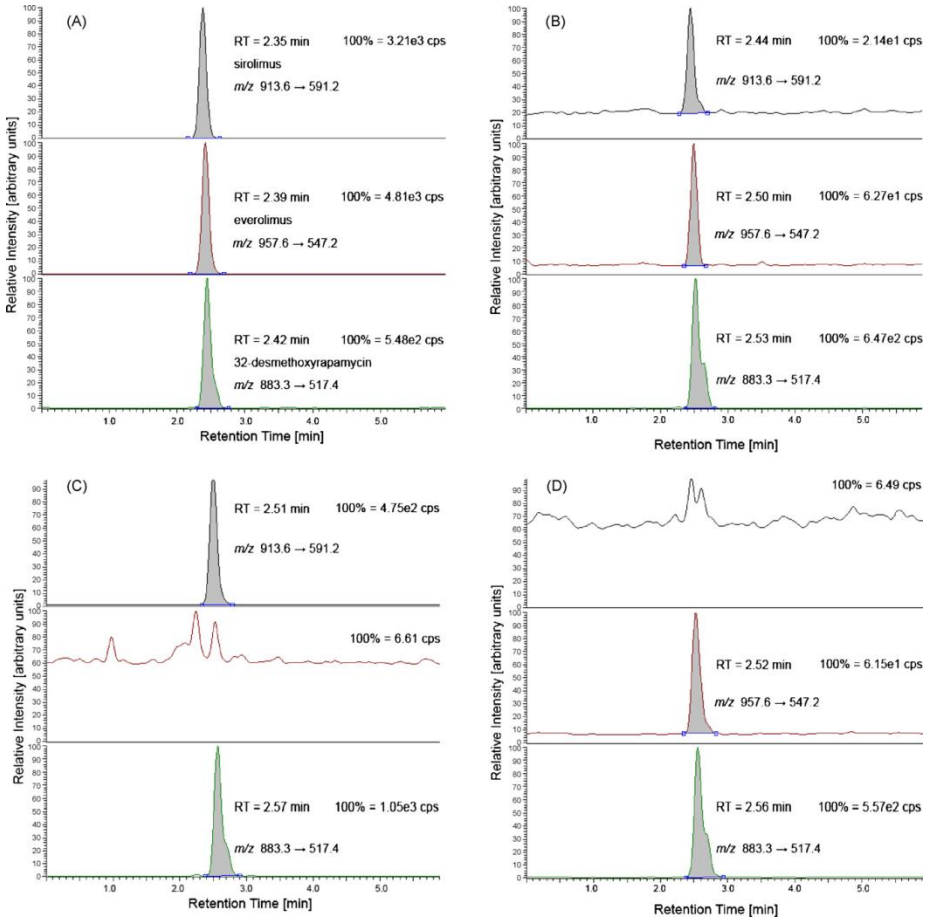
		No. Labs	Mean	S.D.	C.V.	Median	Low Value	High Value
CS-01	Abbott Architect *i* System	143	4.46	0.39	8.8	4.4	3.4	5.6
	LC-MS-MS	40	4.73	0.74	15.7	4.8	3.4	6.7
	Mass Spectrometry	31	4.93	0.73	14.8	5.0	3.9	6.4
	Siemens Diagnostics Dimension	37	3.33	0.60	18.1	3.2	2.3	4.9
	All Results	254	4.40	0.72	16.4	4.4	2.3	6.7

Sensitive quantification of sirolimus and everolimus by LC–MS/MS with online sample cleanup

Daniel M. Mueller, Katharina M. Rentsch J Chromat. B 2010;8781007-1012

- A fast, simple and sensitive high-throughput procedure using online extraction with turbulent flow chromatography
- 200 μL whole blood mixed with IS - 23-desmethoxyrapamycin) & ppt agent
- 50 μL of the supernatant was extracted using turbulent flow chromatography and RPLC
- APCI negative ionization
- Calibration range (2.2–43.7 ng/mL) or everolimus and 2.9–51.2 ng/mL for sirolimus).
- LOQ 0.5 ng/mL for both compounds
- Free of matrix effects

4.1 ng/mL sirolimus



0.8 ng/mL everolimus

Immunosuppressants PGx (→ Methadone?)

- A New Functional *CYP3A4* Intron 6 Polymorphism Significantly Affects Tacrolimus Pharmacokinetics in Kidney Transplant Recipients. van Schaik. Clin Chem 2011
- *CYP3A5*3* – top SNP and *CYP3A4*22 (*rs35599367C>T) *,* allelic frequency ~5%. PGx of Immunosuppression. Hesslelink, 24th Int. Congress of The Transplantation Society, Berlin. 7.17.12

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- QMS immunoassay by DXc, AU680, Indiko – comparable., preliminary - stored samples conc. lower than LC-MS/MS? – Johnson-Davis & Wong
~~~~~~~~~~~~~~~~~~

Characterization of renal allograft rejection by urinary proteomic analysis

Clarke et al. Ann Surg. 2003;237:660-5.

- 32 renal transplant patients
- 17 with acute rejection
- 15 no rejection
- Confirmed by kidney biopsy
- Urine triplicate by SELDI MS

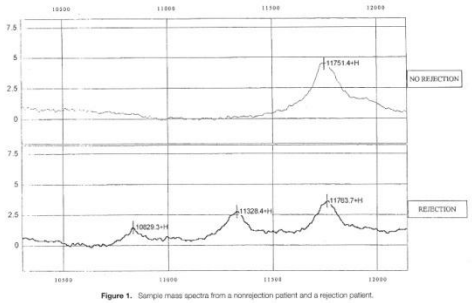
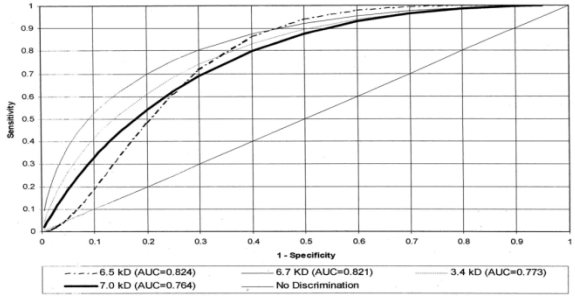


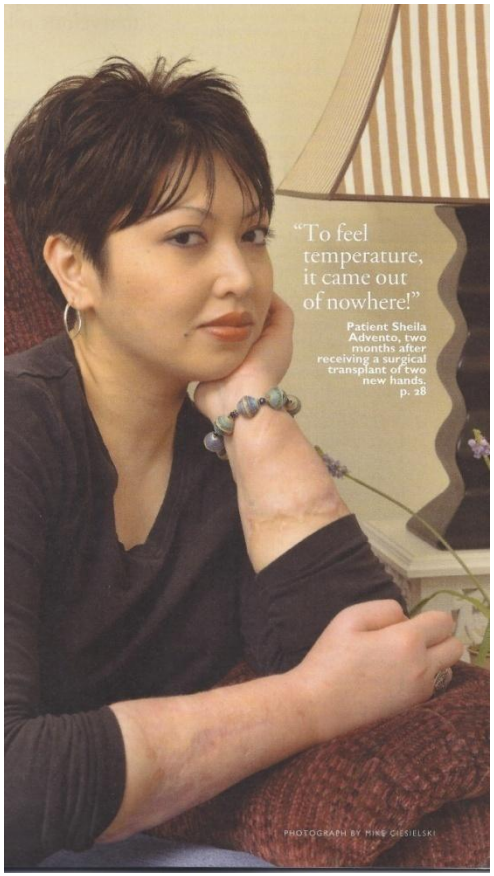
Figure 1. Sample mass spectra from a nonrejection patient and a rejection patient.

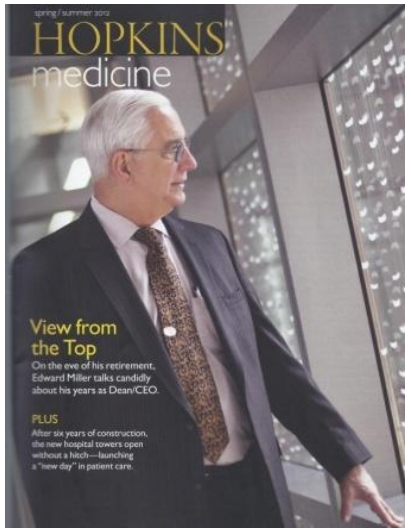
- Spectra – bioinformatics with ProPeak and CART algorithms
- ROC



- Biomarkers panel – enhanced diagnostic performance

Hand transplants "*Within Grasp*" !!





"We are hoping that we can do away with all immune suppressive medication in the future. Or maybe we'll get to the point where people just take medication for a finite period."
—W.P. Andrew Lee

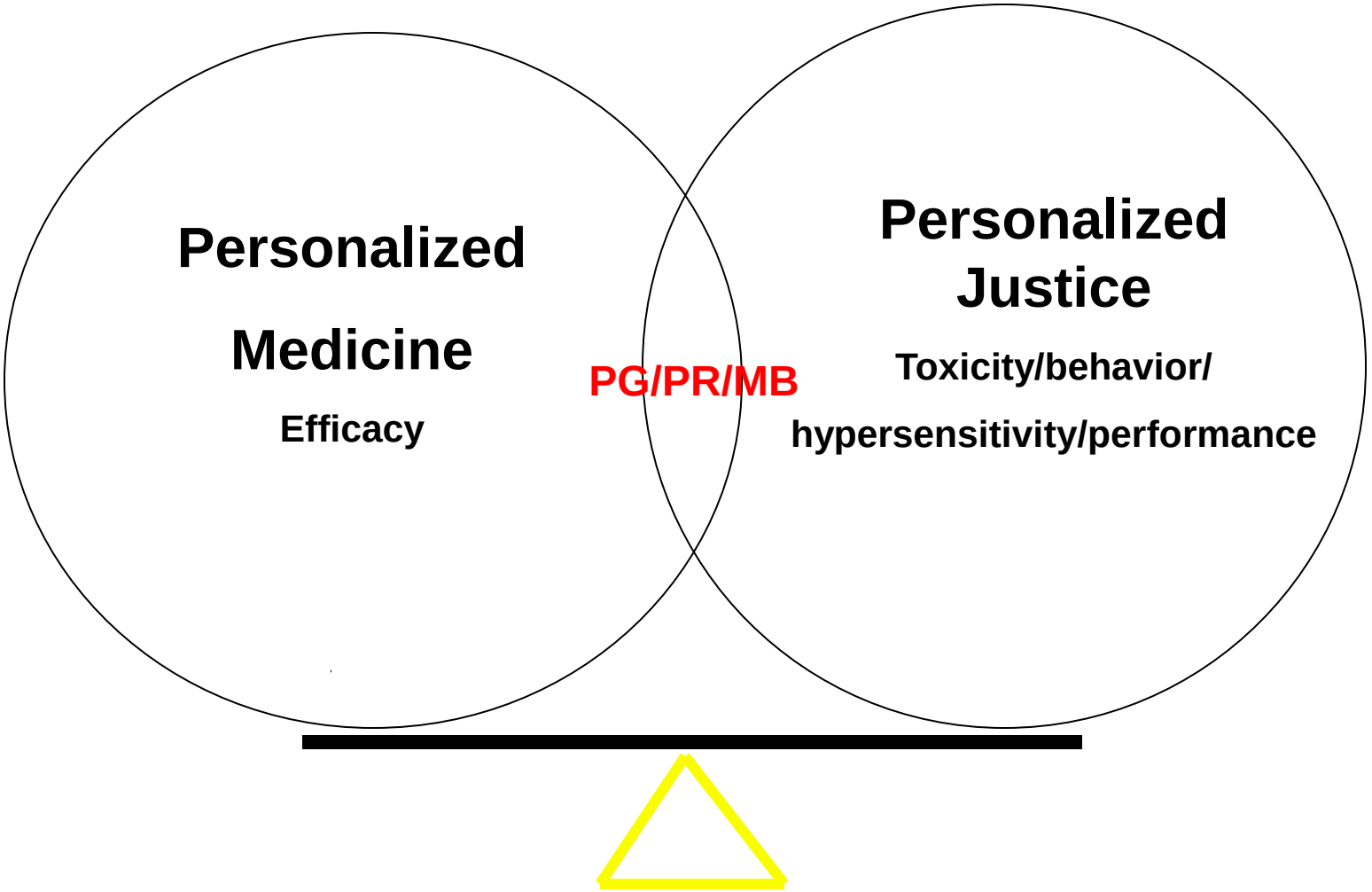
(Trlple immunosuppressants cocktail may include - Tac/MMF/steroid)

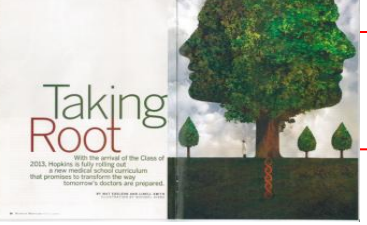
Brendan Marrocco, Jan. 18, 2013

1. “ replaced Marrocco's right arm, his entire left forearm
2. Transplanted bone marrow from donor ---using fewer anti-rejection drugs
3. Lee said: "He will take only one anti-rejection medication instead of the usual triple-drug cocktail.”

Sources: blog.usa.gov/post/.../double-**arm**-**transplant**-helps-iraqi-vet &
http://www.army.mil/article/95523
Double_arm_transplant_helps_Soldier_who_lost_limbs_in_Iraq/

Inevitable Social Balance?
Check and balance

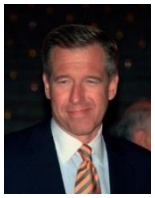




Conclusions

I. Omics

- Med (law) Sch & Clinician education/awareness/acceptance
- Public awareness – gaining ?!



- Reimbursements? (*Encouraging sign*)
- Still emerging?!! – collection of genes
  - Warfarin
  - Methadone – *CYP 3A4 (2 +1SNPs), CYP3A5*3,CYP2B6, OPRM1 , CYP19!!??*
- Pain management – PGx for dosing?
- Personalized Medicine ~ Personalized Justice?

Conclusions

2. MS – expanding role

- Monitoring biologics/biosimilars, pharmacodynamics biomarkers
- Poor id for polymicrobials by MALDI-TOF(~ DNA identity testing for >1 blood sources)-limitation & opportunity
- Alternate approaches for screening & automation, accurate mass for toxicology and microbiology
- OF complementary to Urine for Workplace Forensic? THCCOOH biomarker?

3. Omics and MS

- Regulatory & Outreach – FDA, SAMHSA and others
- Complementary/enabling for clinical pathology LDT?
- Bioinformatics – data base harmonization