Stemina BIOMARKER DISCOVERY

From Cell Culture to Patients

Discovering Biomarkers of Human Disease and Development Using Stem Cells and Metabolomics

Paul R. West, Ph.D.

Stemina Biomarker Discovery, Inc.
504 S. Rosa Rd., Suite 150, Madison WI 53719

Stemina
BIOMARKER DISCOVERY

From Cell Culture to Patients

November 2006: Incorporated in Wisconsin as C Corporation

May 2007: Business plan developed. 1st round fund raising begun

November 2007: Open facilities at UW Research Park in Madison

Elizabeth L. R. Donley, JD, MBA, MS
Chief Executive Officer

Gabriela G. Cezar, DVM, PhD
Chief Scientific Officer
Assistant Professor, U. of Wisconsin-Madison

Stemina Research Projects

Project	Cell Type used for Metabolomic Analyses	Application
DevTOX Developmental Toxicity Assay	Stem Cells (hESC)	Birth Defects, Autism Pharmaceutical teratogenicity Diagnostic Biomarkers
CardioTOX Screening Anti-Cancer Drugs for Cardiomyopathy & Toxicity	Cardiomyocytes	Cardio Profile Screening Drugs for Cardiotoxicity Hospital Treatment Monitoring Diagnostic Biomarkers
CSC Cancer drug efficacy	Brain Tumor Cells Cancer Stem Cells, Neural Cells	Cancer drug efficacy Cancer Treatment sensitivity Diagnostic biomarkers

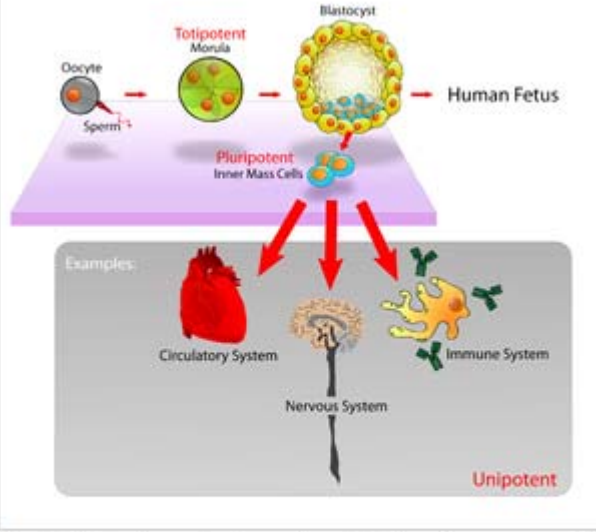
Stemina BIOMARKER DISCOVERY

External Service Projects

Client	Project
Mayo Clinic	*Metabolomics for Diabetes*
Dupont/Pioneer	*Metabolomics of corn plant stress*
Promega	*Stem Cell viability testing with analytical platform*
BMS	*Cardio Toxicity Testing*

Human Embryonic Stem Cells

- Stem cells are derived from the inner cell mass of an early stage embryo at the blastocyst stage (4–5 days post fertilization) (50–150 cells).
- Embryonic stem cells are pluripotent - they are able to differentiate into all any of the more than 220 cell types in the human adult body.
- When given no stimuli for differentiation, (i.e. when grown *in vitro*), ES cells maintain pluripotency through multiple cell divisions.



Pluripotent, embryonic stem cells originate as inner mass cells within a blastocyst. The stem cells can become any tissue in the body, excluding a placenta. Only the morula's cells are totipotent, able to become all tissues and a placenta.

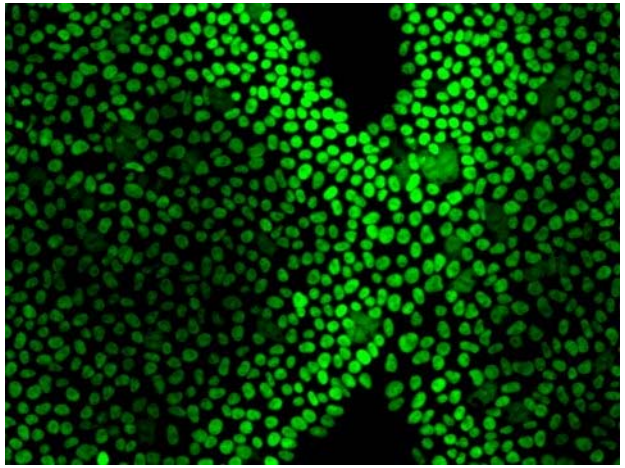
Human embryonic stem cells used for research are obtained from donated embryos that are leftover from in vitro fertilization.

Stemina BIOMARKER DISCOVERY

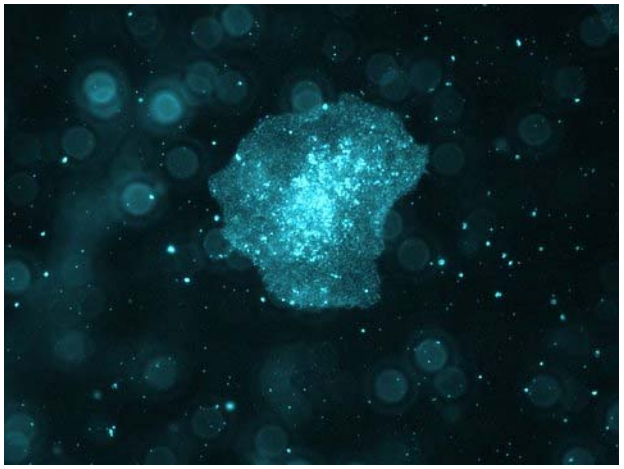
Human embryonic stem (hES) cells cultured at Stemina

Cultured in 6-well or 96-well plates

in mTeSR1 growth media on Matrigel



Oct-4 stained, H9 hES cells.
Oct-4 is a marker for pluripotency.



(We like to think that this colony is shaped like Wisconsin)

Stemina
BIOMARKER DISCOVERY

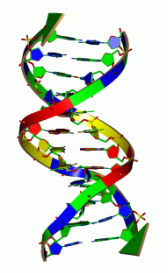
mTeSR Stem Cell Media Components

Many are non volatile “MS unfriendly” salts and buffers present at high concentrations.

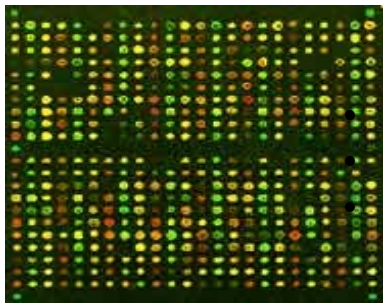
HEPES BUFFER	L-Glutamine	Palmitoleic acid
Glycine	L-Glutamic acid	Palmitic acid
2-mercaptoethanol	L-Methionine	Linolenic acid
Putrescine	L-Histidine	Linoleic Acid
L-Alanine	L-Phenylalanine	Oleic Acid
GABA	Pyridoxine HCl	Stearic acid
Choline chloride	L-Arginine	Thiamine HCl
L-Serine	L-Lysine	Arachidonic acid
L-Proline	Ascorbic acid	Glutathione
L-Valine	Inositol	Phenol Red
L-Threonine	L-Tyrosine	Riboflavin
L-Cysteine	L-Tryptophan	Cholesterol
Niacinamide	Lipoic Acid	Folic acid
Pipecolic acid	Pantothenic acid salt	alpha tocopherol acetate
L-Isoleucine	Myristic acid	Vitamin B12
L-Asparagine	L-Cystine	pluronic F-68
L-Aspartic acid	Thymidine	Tween 80
Hypoxanthine	Biotin	NaCl

Metabolomics

Genomics



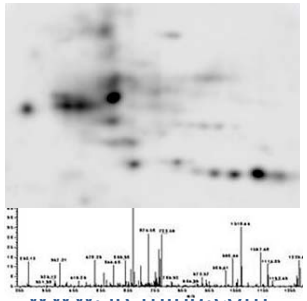
- Genetic Maps
- Regulatory Sequences
- Gene Prediction
- Polymorphisms



Transcriptomics

- Gene Expression
- Exon-Intron model
- Sense-antisense

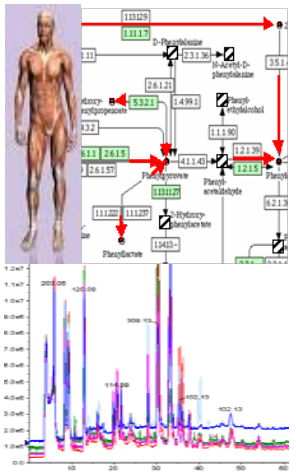
Proteomics



- Protein ID
- Isoforms
- Post-translation mods
- Interactions

- Metabolomics investigates the biochemical small molecule metabolites in an organism.
- Often, the organism is insulted or injured then changes to the small molecules are measured.

Metabolomics is closest to phenotype...



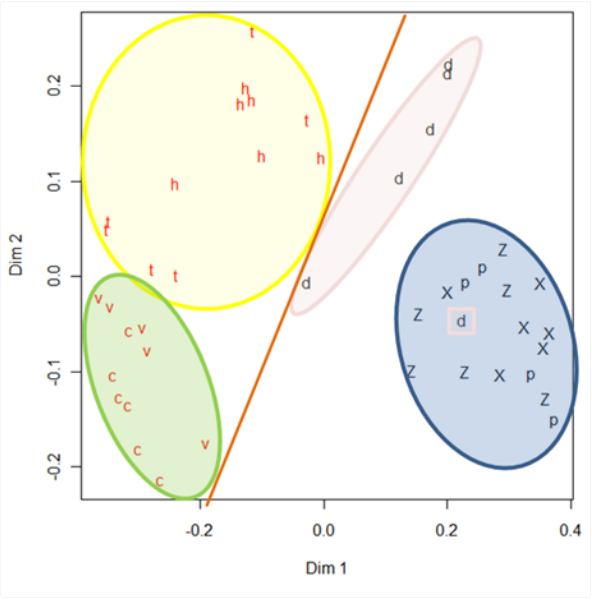
- Metabolites
- Functional pathways
- Biochemical Phenotype
- Subtle Phenotypes
- Data is usually acquired with LC-MS, GC-MS and/or NMR.
- High resolution TOF and QTOFMS analyzers provide accurate mass data, discerning between isobaric species and improving chemical formula determination.

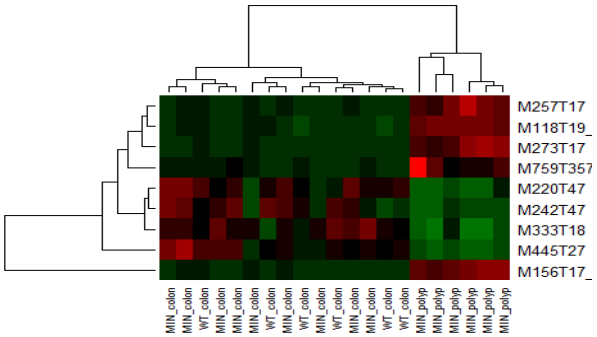
Stemina BIOMARKER DISCOVERY

from G. Cezar

Nontargeted Metabolite Profiling

- Measure Metabolic Changes Related to Sample
  - Not concerned with individual metabolites
  - Measuring pull metabolites have on sample grouping
  - Metabolites are scored by importance
- Chemometrics Analysis
  - Multivariate Statistical Methods
  - Clustering
  - Discriminate Analysis
  - Machine Learning Methods
    - Random Forest, Support Vector Machines
  - Identification of metabolites by VIP scores
  - Predictive Modeling
- Informatics
  - Mass Feature Annotation
    - MS-MS and spectral pattern matching
  - Pathway Placement and Enrichment





From Stem Cell Culture to Metabolites

Treat (dose) cells to cause stress/injury metabolomic response

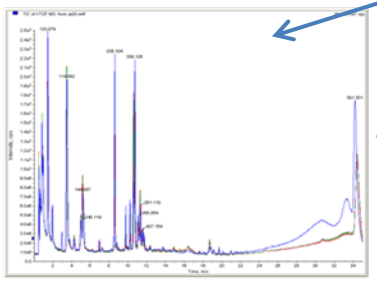
Small Molecule Preparation

Extracted Metabolites < 10 kDa

LC-MS analysis

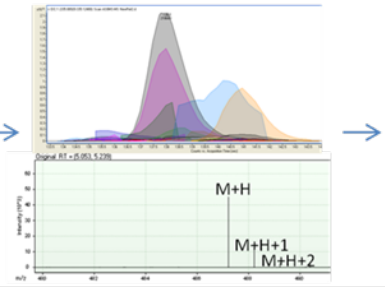
HILIC chromatography

High Mass Accuracy QTOF

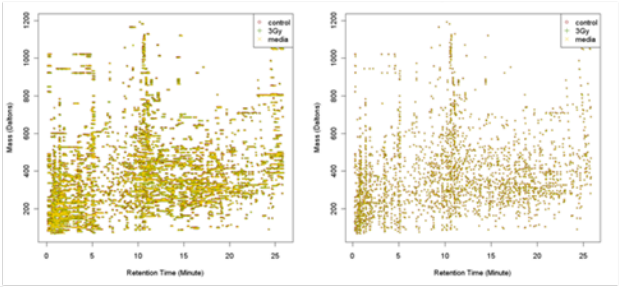


Data Acquisition

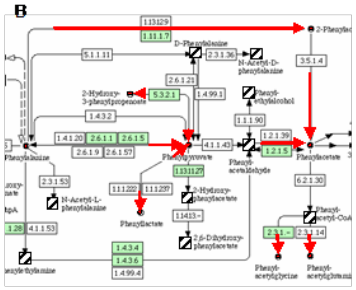
Centroid Deisotope



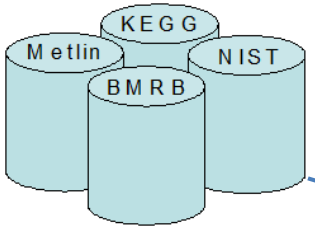
Generate mass features



Data Binning and Alignment



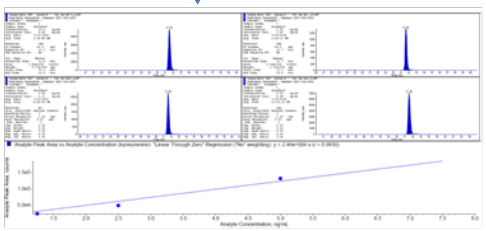
Functional Pathway Annotation



Bioinformatics

Chemoinformatics

Feature Annotation



Statistical Analysis

Log2(Abundances) = Treatment + Error

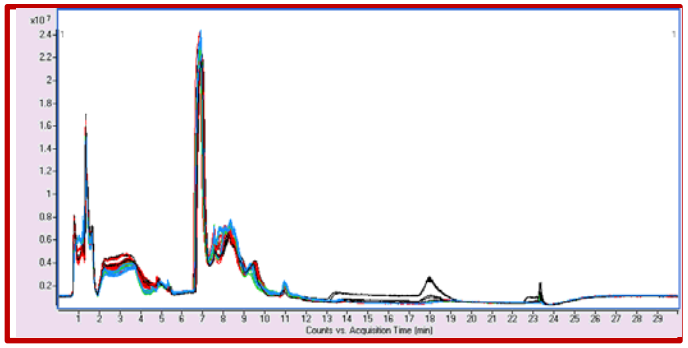
Stemina Metabolomics – LC-MS Instrumentation

(2) Agilent 6520A QTOF LC-MS Systems

- 1200 Rapid Resolution HPLC with 96-well plate chilled autosampler
- Dual ESI Source
- Mass reference solution delivered by Isocratic Pump @ 2 mL/min split 100:1
- Positive and negative ion modes for all samples

Stemina BIOMARKER DISCOVERY

Stemina Metabolomics – Typical LC-MS Conditions



HILIC gradient chromatography:

Column: Phenomenex HILIC; 100 x 3mm; 5mm

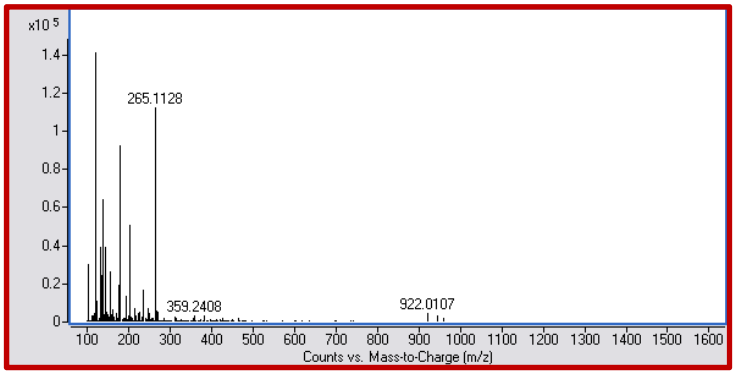
Solvent A: 0.1% Formic Acid in Water

Solvent B: 0.1% Formic Acid in ACN

- QTOF MS:

Scan range: *m/z* 70-1700 @ 3 Hz

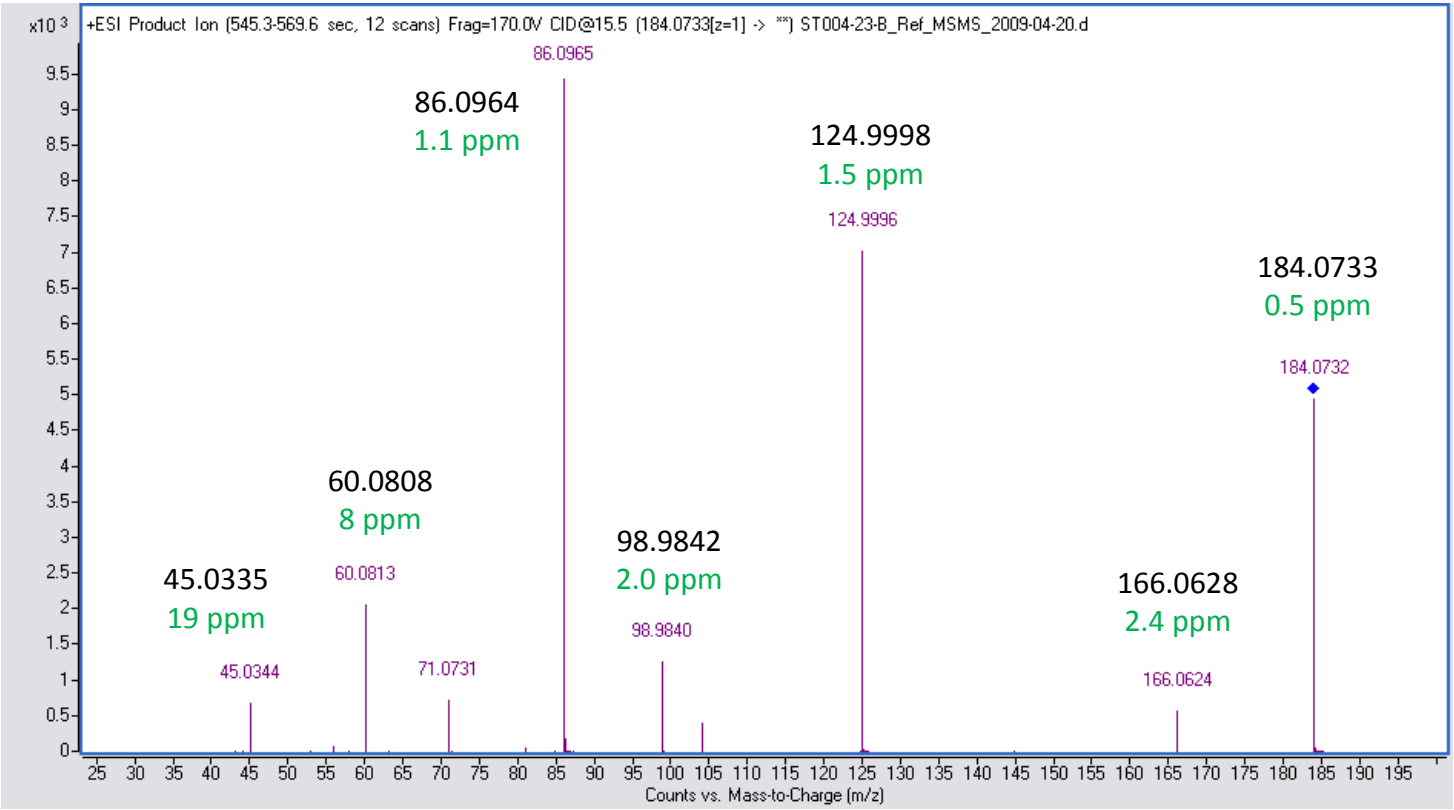
- ~ 5 ppm mass accuracy (MS)
- < 20 ppm mass accuracy (MS-MS)
- 2 GHz Extended dynamic range acquisition
- 5 orders of magnitude dynamic range



Stemina BIOMARKER DISCOVERY

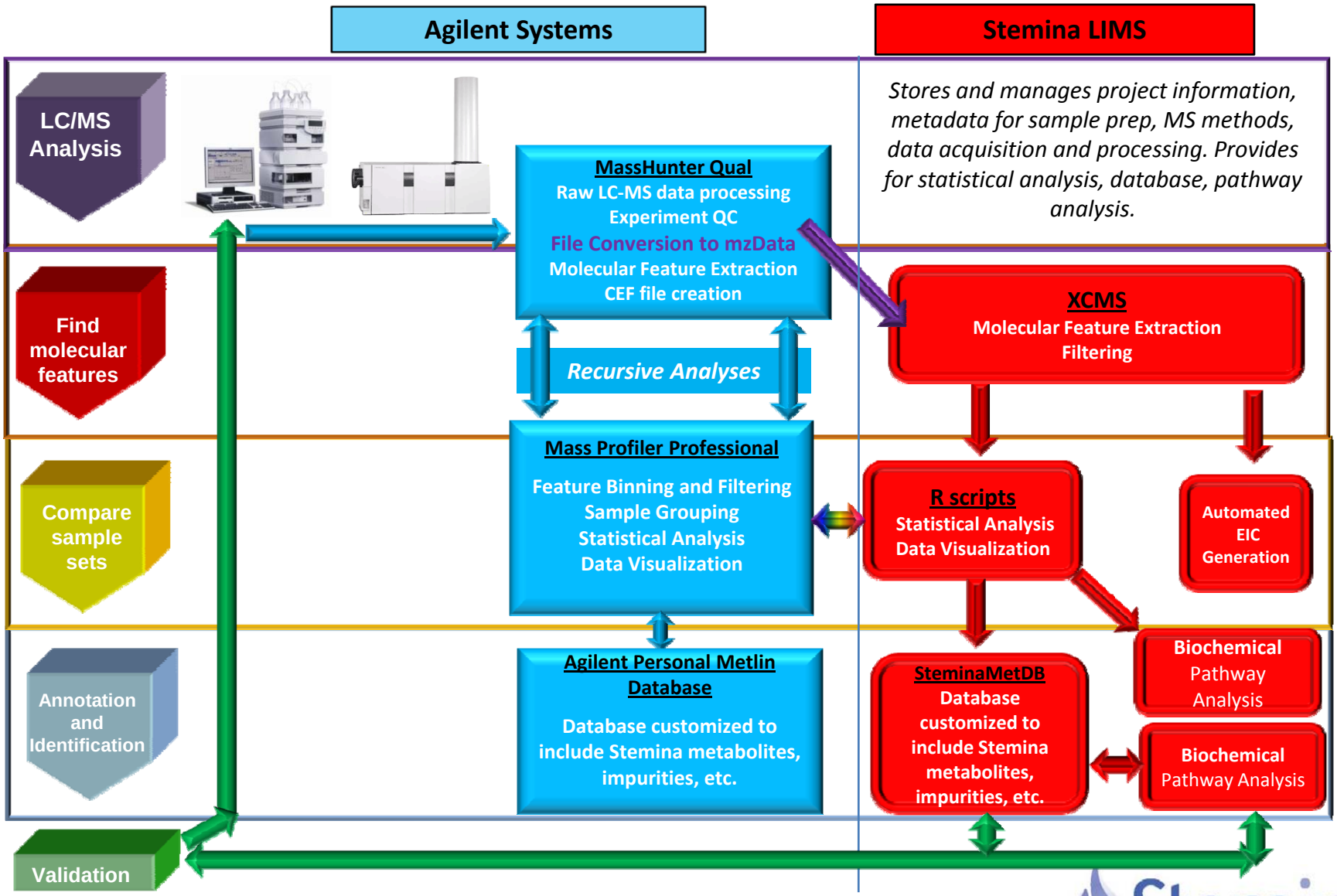
Metabolomics Methods

An illustration of QTOF MS-MS product-ion mass accuracy



Stemina BIOMARKER DISCOVERY

Stemina Metabolomics Data Workflow



Stemina BIOMARKER DISCOVERY

Molecular Feature Extraction

Agilent MassHunter Qual or XCMS

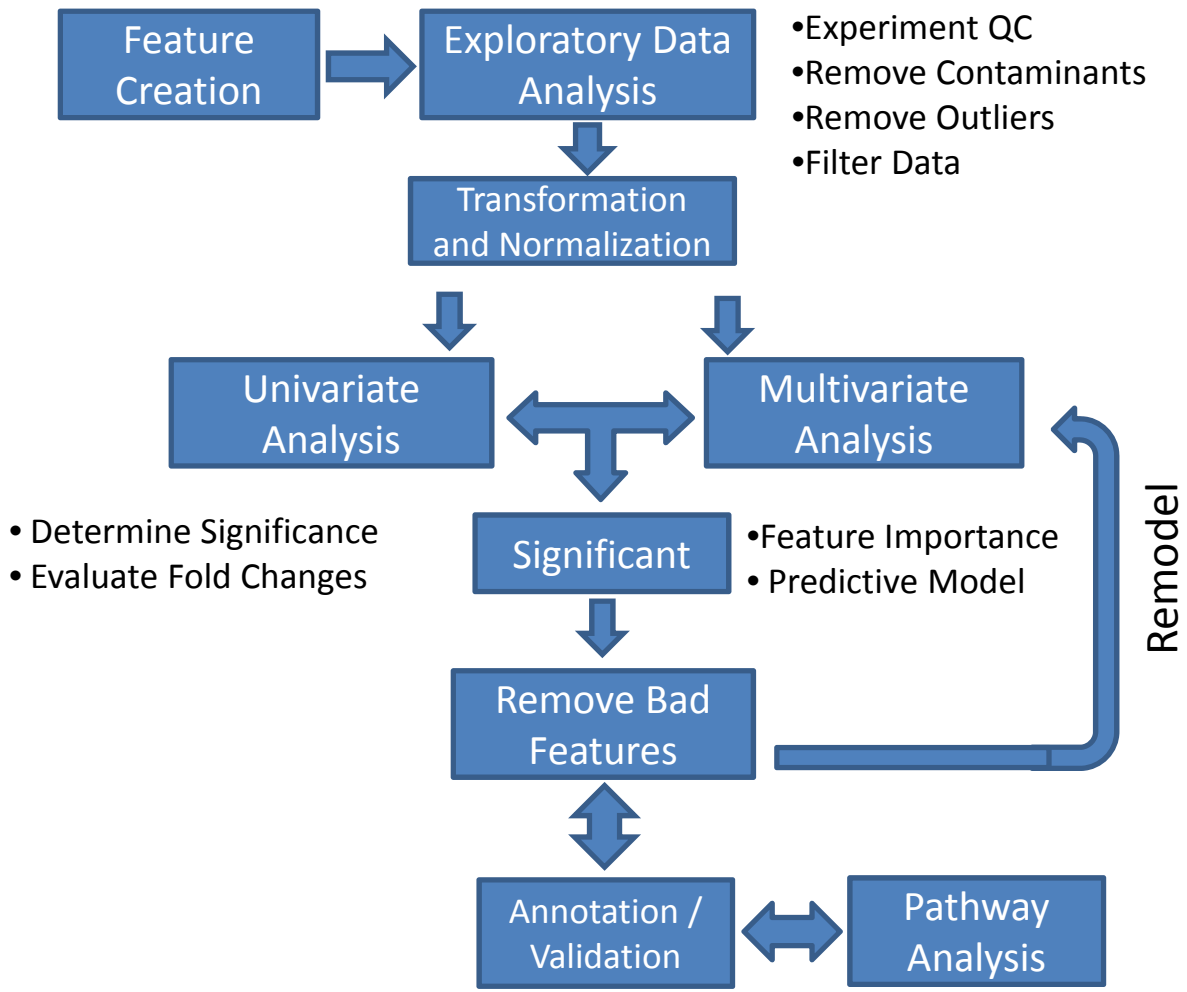
Molecular Feature: a discrete molecular entity defined by the combination of retention time, mass and response in an LC/MS analysis.

Molecular Feature Extraction is basically automated mass spectral data interpretation.
It makes the assumption that there are many compounds ("features") detected across an LC/MS run and attempts to automatically process the mass spectra to find these "features". Depending on the settings in the software, 0 to >20,000 compounds can be found, and many of these can be false positives (not real compounds, just chemical noise/background), so it is important to use the optimal filter settings in the software.

The software uses characteristics in a mass spectrum to discern different features:

1. The mass accuracy of the instrument is sufficient to measure the *m/z* values for a compound to within a small ppm error range.
2. Each detected compound shows several peaks in a mass spectrum including:
   Protonated Molecular ion $(M+H)^+$ and its isotope peaks
   Other adduct ions such as $(M+Na)^+$, $(M+K)^+$.
3. A feature elutes for a limited period of time (limited chromatographic peak width).

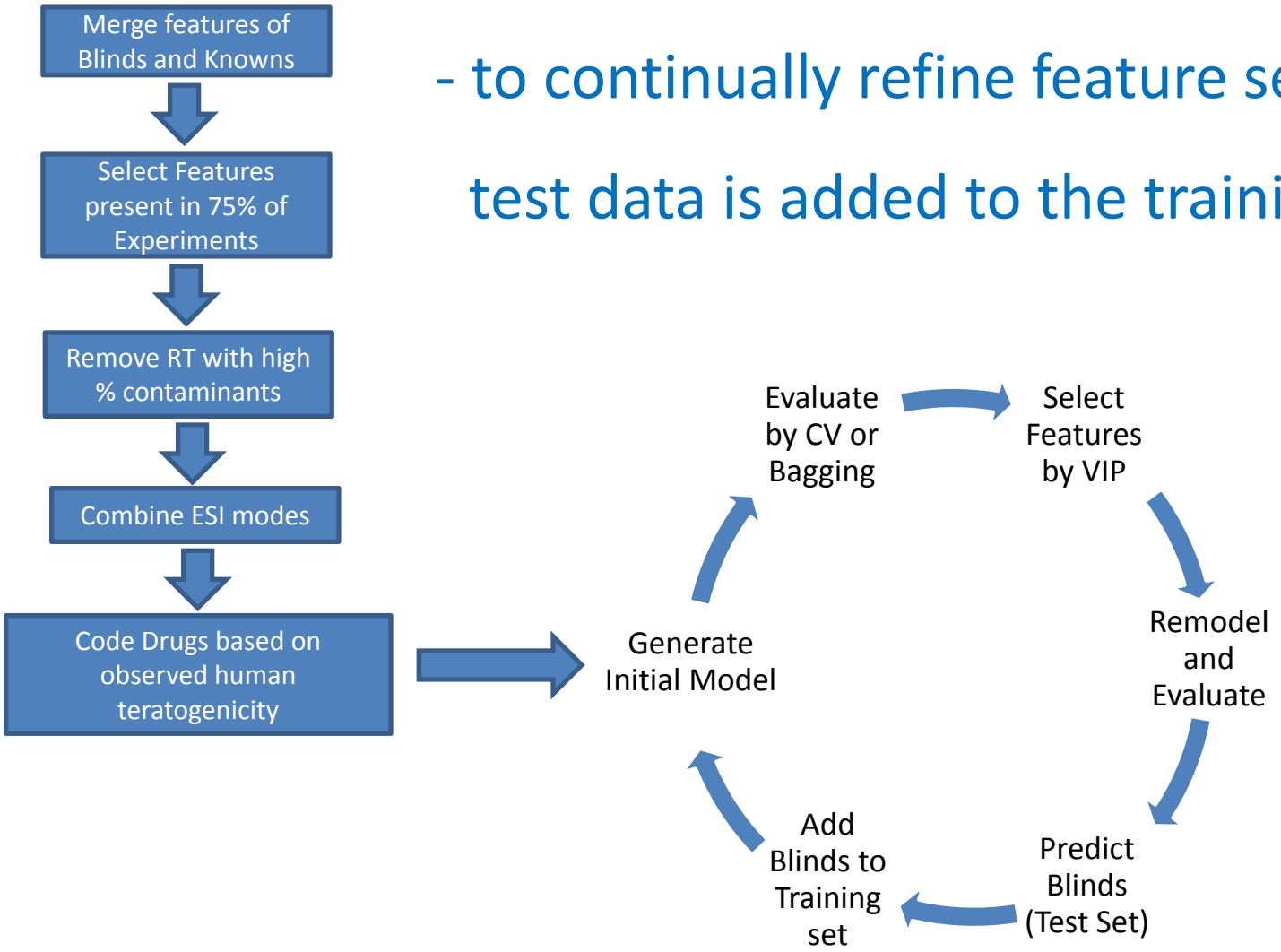
General Metabolomics Data Analysis Process



Stemina BIOMARKER DISCOVERY

Iterative modeling process

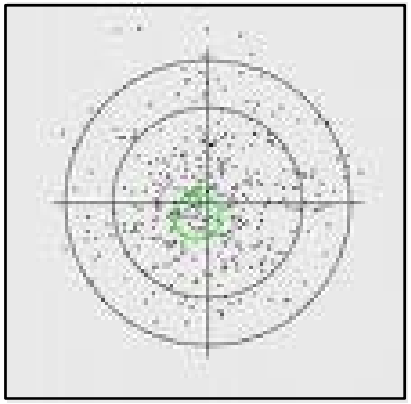
- to continually refine feature set as new test data is added to the training set.



Stemina BIOMARKER DISCOVERY

Metabolite Identification/Validation

Non-targeted Metabolomics (shotgun)



1. Determine accurate mass of feature.
2. Eliminate unrealistic formulae.
3. Search a metabolite database.
   - Agilent METLIN Personal Database
   - Metabolite AMRT Database
4. Search a chemical structure database.
   - Chemspider
   - Google!
5. Acquire and interpret an MS/MS spectra
6. Purchase reference standards, obtain MS and MSMS and compare.
7. Purify the compound and analyze by NMR with mass spectral data support
   - Mass directed purification systems

Targeted Metabolomics

1. Perform analysis focused on limited number of compounds.
2. Quantitative (standards used)
3. MRM on QQQ
4. Can use fast flow injection

Data Processing - Methods

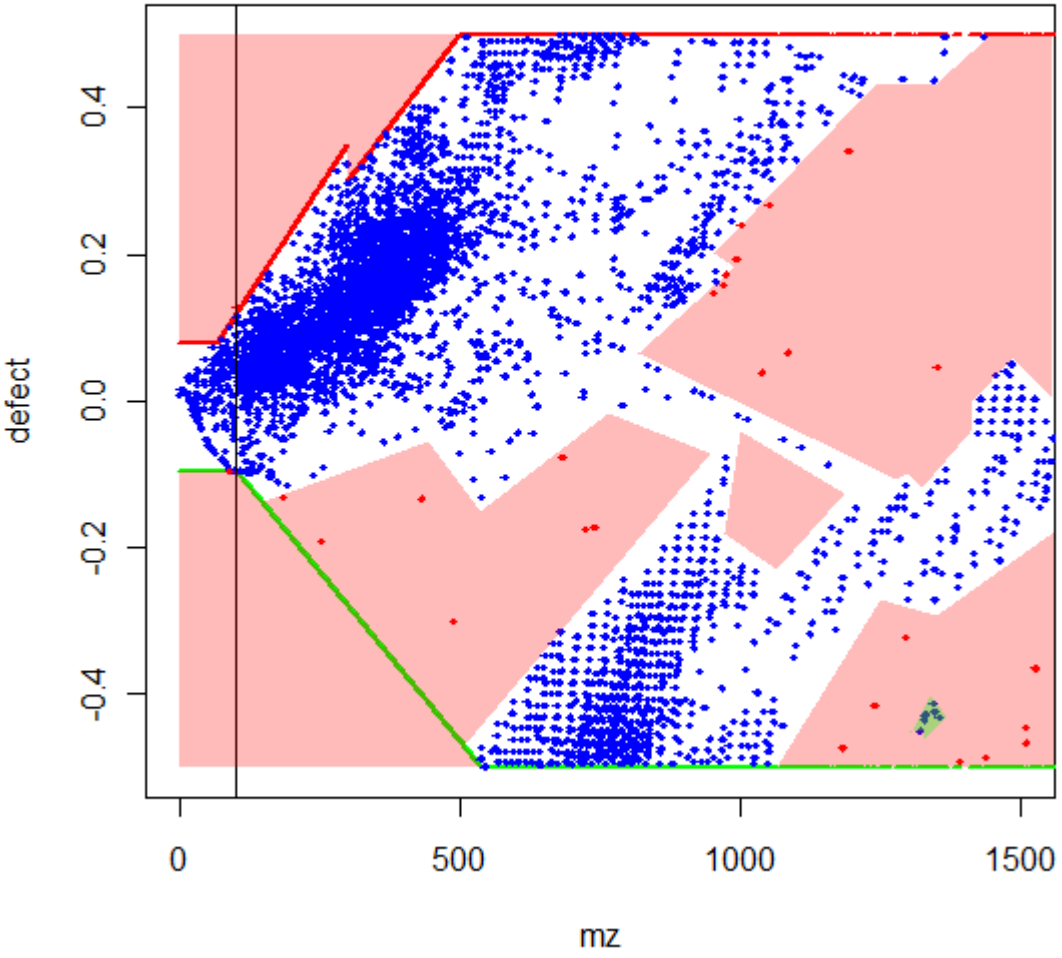
Stemina's Mass Defect Filter

- Mass Defects of all human metabolites present in the Stemina Metabolite DB were used to create a filter mask to reduce the presence of undesirable features in the final metabolomics dataset.
- Features from a DevTox metabolomics dataset can be filtered by the mask to remove any features that are not likely related to known endogenous metabolites based on mass defects.

Data Processing - Methods

Stemina's Mass Defect Filter

Mass Defect vs *m/z*



Each data point represents a known human metabolite from public and Stemina databases.

A mask was then constructed to filter out regions on the graph where few known metabolites reside.

Regions Filtered out

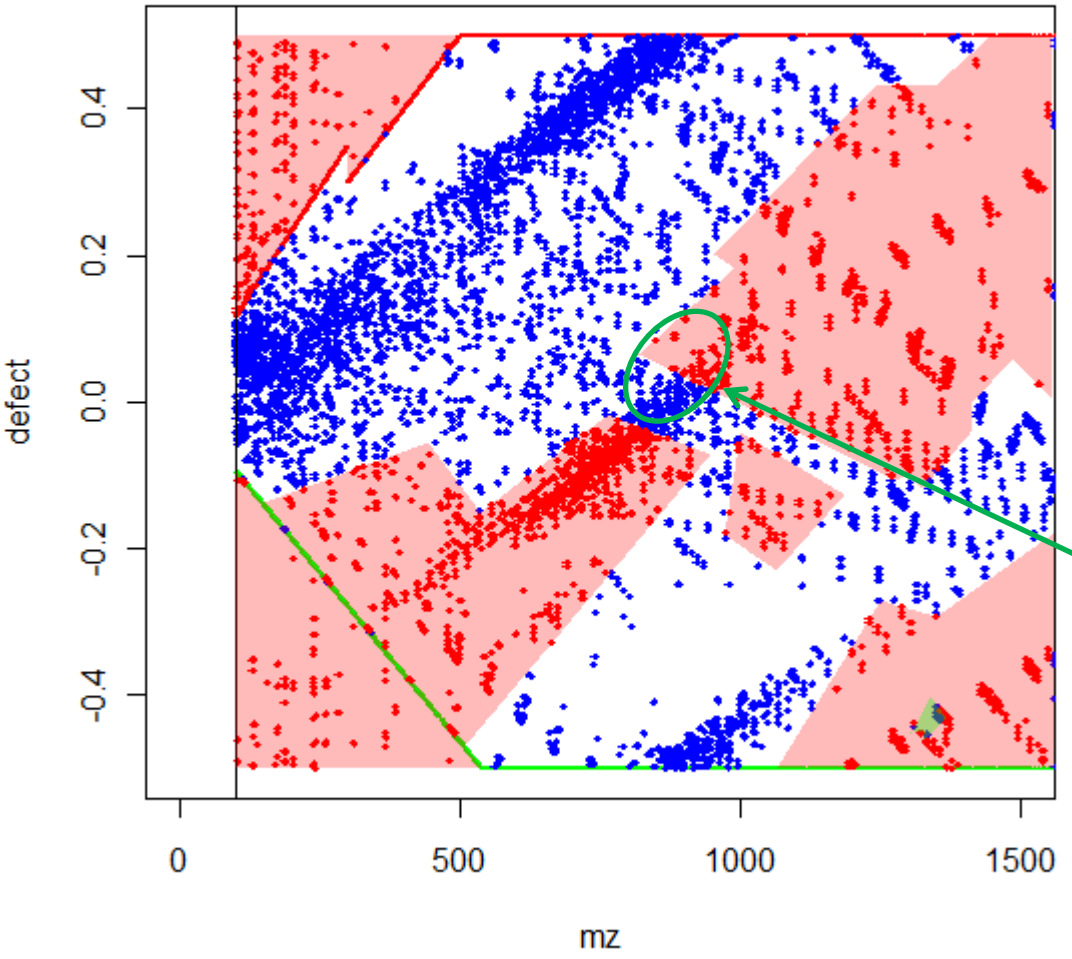
- Features that pass filter
- Features lost

(27 masses, 31 metabolites)

Stemina BIOMARKER DISCOVERY

Data Processing - Methods

Stemina's Mass Defect Filter



5000 Feature DevTox Dataset

Each data point represents a mass feature from a set of experiments.

Actual Filter Results

Regions Filtered out

- Features that pass filter
- Features filtered out

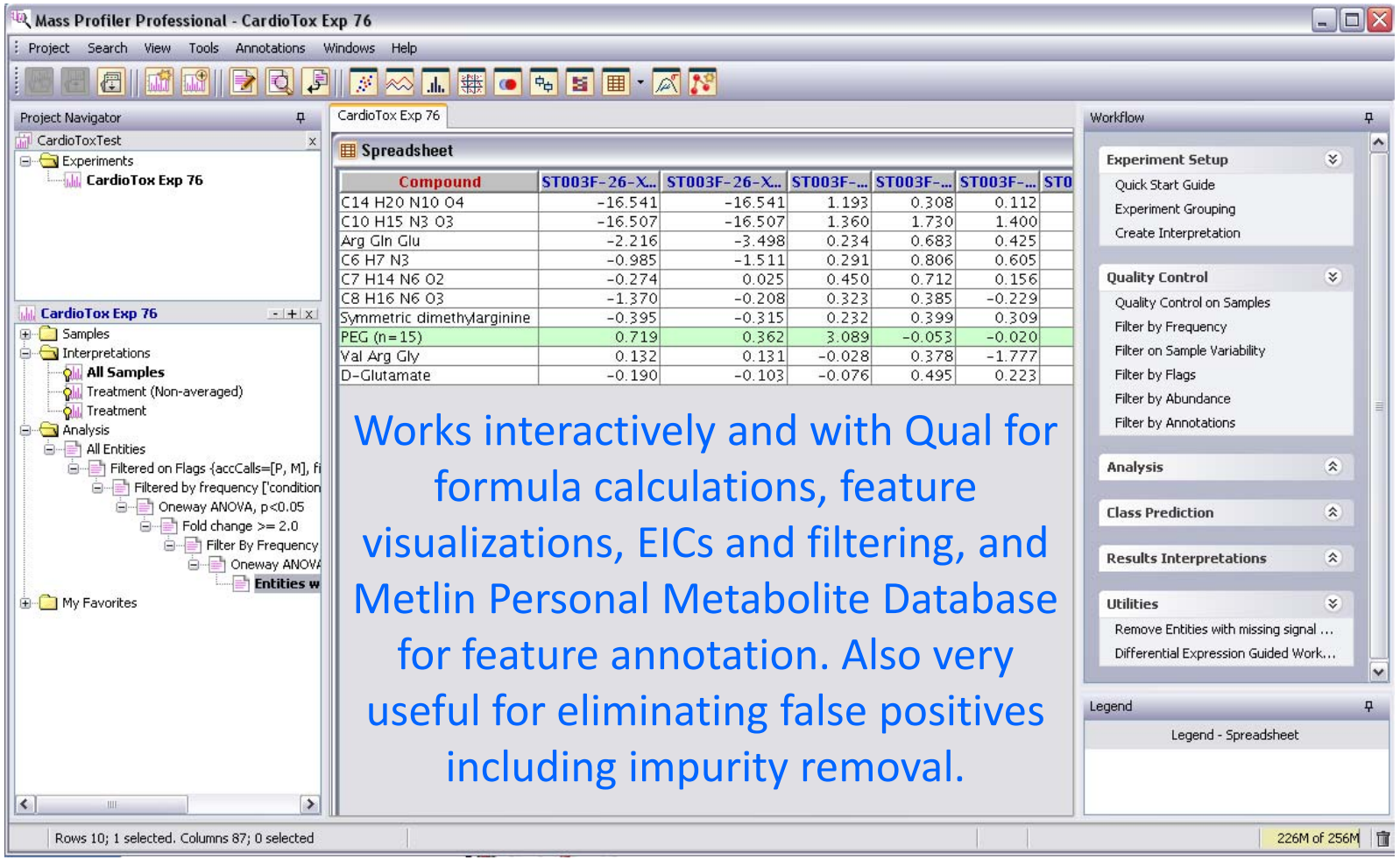
Probable noise. Need to add this region to filter

Stemina BIOMARKER DISCOVERY

Data Processing - Methods

Agilent Mass Profiler Professional

Recursive data analysis

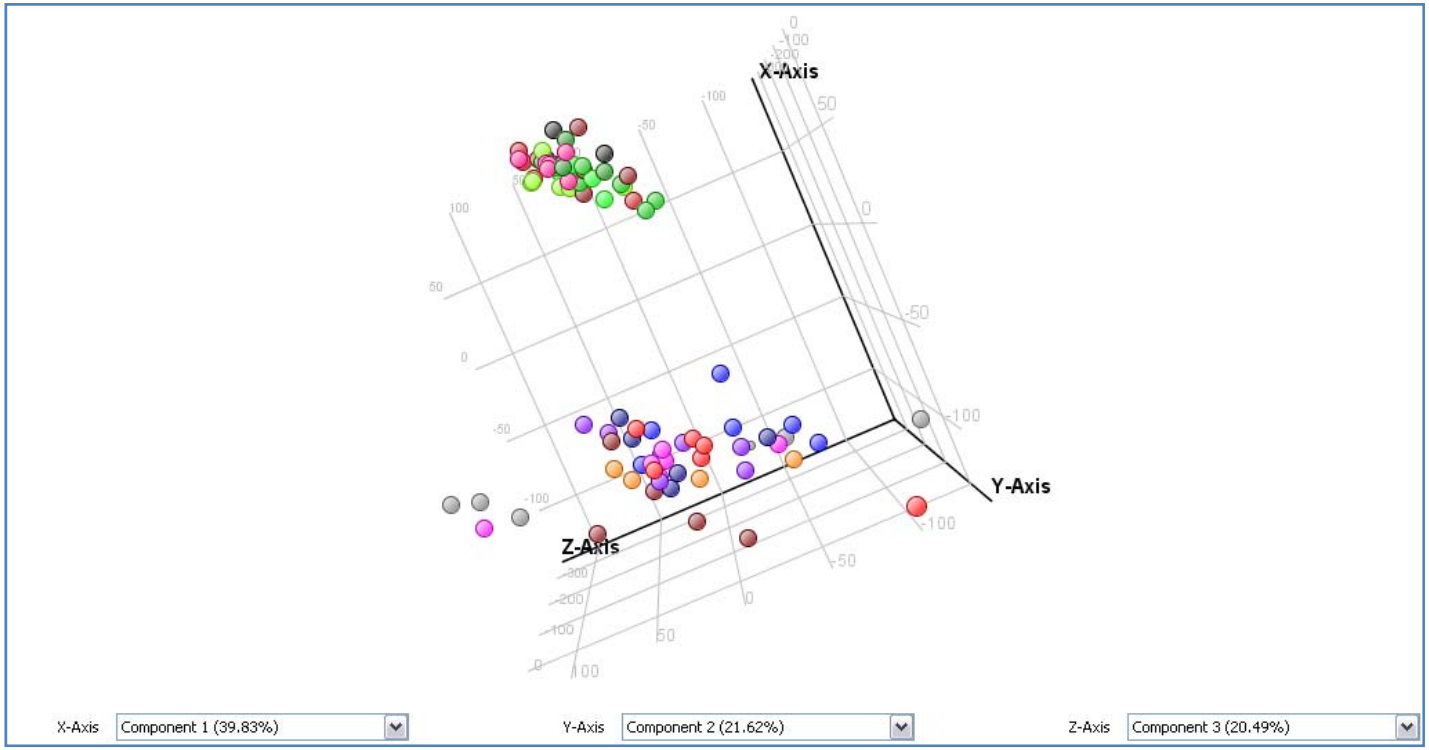


Stemina BIOMARKER DISCOVERY

Data Processing - Methods

Agilent Mass Profiler Professional
Statistical analyses and data visualization

PCA Plot

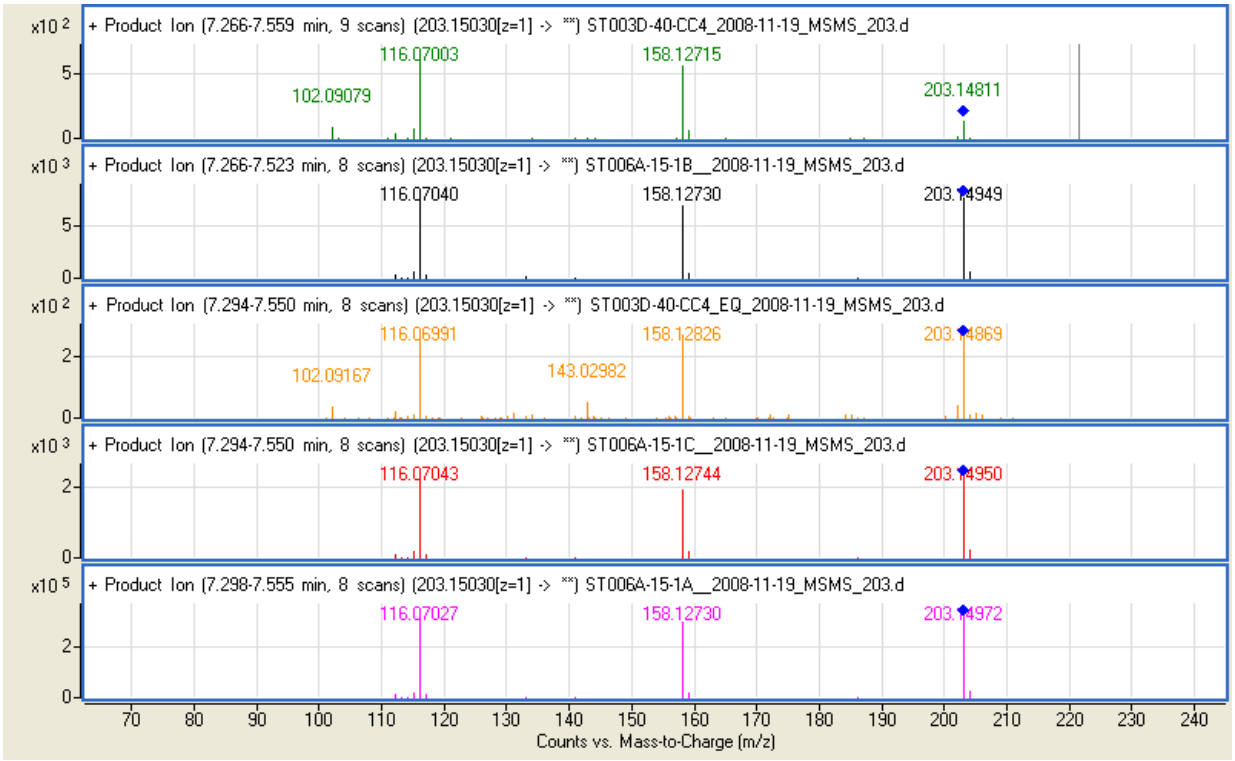


Stemina BIOMARKER DISCOVERY

Metabolomics Methods

Validation : Chemical Structure Confirmation by MS-MS

After a molecular feature of interest is annotated and a structure is proposed, **LC-QTOF MS-MS accurate mass product ion spectra** are obtained under identical conditions for both a reference standard and the feature of interest in the sample. The retention time and MS-MS spectra are compared and matched.



Sample

Reference Standard

Sample

Reference Standard

Reference Standard

Stemina BIOMARKER DISCOVERY

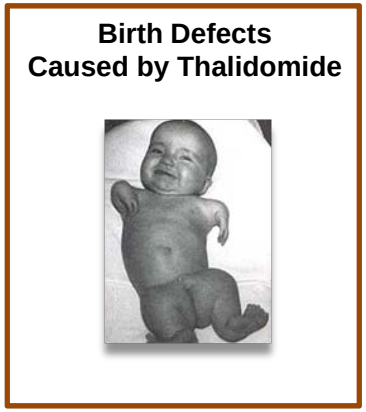
Predicting Human Developmental Toxicity Using Human Embryonic Stem Cells and Metabolomics

“DevTox Project”

Stemina BIOMARKER DISCOVERY

Stemina's Developmental Toxicity Project

- Birth defects occur in 6% of births nationally.
- Teratogens cause 5-10% of these birth defects.
- Animal models used predict developmental toxicity of pharmaceuticals are ~ 50% accurate.
- Stemina uses both human embryonic stem (hES) cells and LC-MS metabolomics to detect measurable modulation of specific secreted metabolites as a result of treatment of the cells with teratogens. These metabolites will serve as biomarkers of developmental toxicity.



Stemina
BIOMARKER DISCOVERY

DevTox Project (6-well)

Test Compound Set

Stemina Classification	Drug	FDA Classification
Non-Teratogens	Ascorbic Acid	A
	Isoniazid	C
	Penicillin G	B
	Saccharin	A
	Folic Acid	A
	Levothyroxine	A
	Retinol (blind 1)	A
	Doxylamine (blind 2)	A
	Thiamine (blind 8)	A
	Aspirin	C
	Caffeine	B
	Diphenhydramine	B
	Indomethacin*	B
Teratogens	Dexamethasone *	C
	Diphenylhydantoin	D
	Methotrexate	X
	5-Fluorouracil	D
	Busulfan	D
	Cytosine Arabinoside	D
	Hydroxyurea	D
	Retinoic Acid	X
	Thalidomide	X
	Valproic Acid	D
	Amiodarone (blind 3)	D
	Rifampicin (blind 4)	C
	Carbamazepine (blind 5)	C
	Accutane (blind 6)	X
	Cyclophosphamide (blind 7)	D

FDA PREGNANCY CATEGORIES

The FDA-assigned pregnancy categories as used in the Drug Formulary are as follows:

Category A
Adequate and well-controlled studies have failed to demonstrate a risk to the fetus in the first trimester of pregnancy (and there is no evidence of risk in later trimesters).

Category B
Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.

Category C
Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.

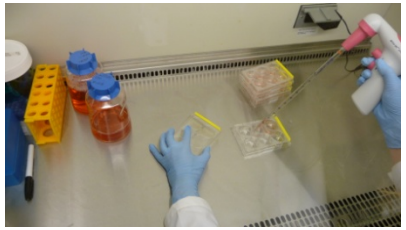
Category D
There is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience or studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.

Category X
Studies in animals or humans have demonstrated fetal abnormalities and/or there is positive evidence of human fetal risk based on adverse reaction data from investigational or marketing experience, and the risks involved in use of the drug in pregnant women clearly outweigh potential benefits.

DevTox Project (6-well) – 6-Well Method

Dosing, sample prep and data acquisition

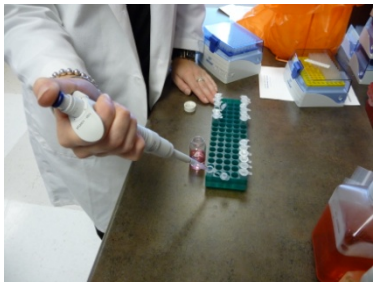
4 days



3 hours

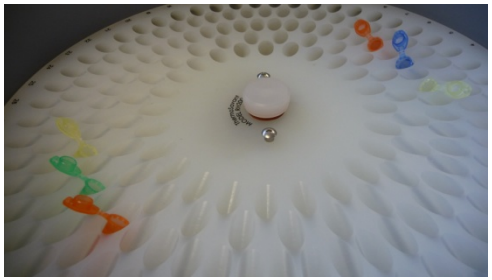
Stem cells were dosed for 4 days with 18 drugs of known teratogenicity then spent media was removed and bioactivity was quenched with acetonitrile.

Proteins were removed by centrifugation with a 3 Kda Centricon filter



Each sample was dissolved in acetonitrile…

Overnight



…then evaporated to dryness in a SpeedVac overnight, then dissolved in 0.1% formic acid before LC/MS analysis.

2 Days for (+) & (-)

LC/MS data was then acquired on the QTOF.

Total Time: approx 1 week

Stemina BIOMARKER DISCOVERY

DevTox Project (6-well) - Methods

HILIC LC-MS

HILIC LC-MS methods were developed that tolerate high salt concentration and were optimized to provide a good compromise for the separation of both hydrophilic and hydrophobic compounds.

Retention times are very reproducible. Maximum variation is about 12 sec.

Column: Phenomenex Luna HILIC; 100 x 3mm; 5µm
Solvent A 0.1% Formic Acid in Water
Solvent B 0.1% Formic Acid in ACN

Original Method (for 6-well)
30 minute run time

Time	%ACN	Flow	Divert Valve
0.0	95	0.5	MS
1.5	95	0.5	MS
16.0	60	0.5	MS
17.0	5	0.5	MS
21.0	5	0.5	MS
22.0	95	0.5	MS
30.0	95	1	MS

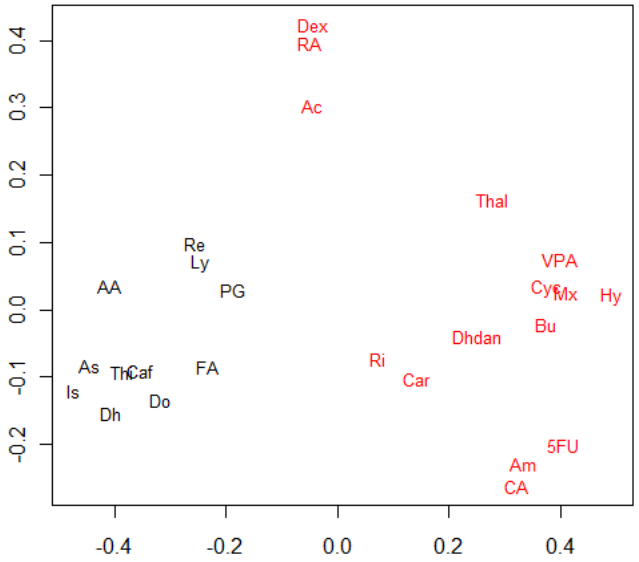
Stemina BIOMARKER DISCOVERY

DevTox Project (6-well) – Results

Teratogenicity Model was 87.5% Predictive

Blinded Experiments	Actual	Predicted
Blind 1 (Retinol)	Non	Non
Blind 2 (Doxylamine)	Non	Non
Blind 3 (Amiodarone)	Ter	Ter
Blind 4 (Rifampicin)	Ter	Ter
Blind 5 (Carbamazepine)	Ter	Ter
Blind 6 (Accutane)	Ter	Non
Blind 7 (Cyclophosphamide)	Ter	Ter
Blind 8 (Thiamine)	Non	Non

7/8 predicted correctly



- Predictive model developed using Random Forest model and feature selection

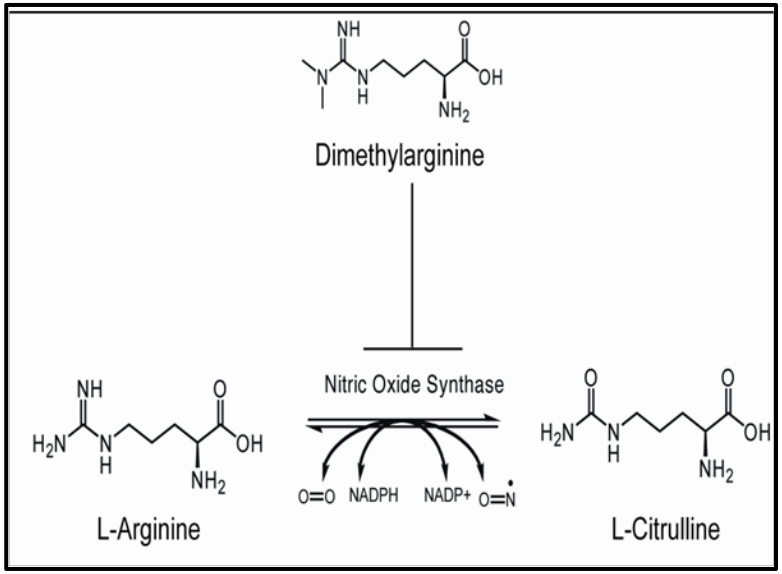
DevTox Project (6-well) - Results

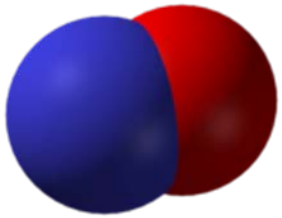
- The biologically active molecule nitric oxide (NO) is formed by the conversion of arginine to citrulline, with the release of NO. NO has multiple cellular molecular targets. It influences the activity of transcription factors, modulates upstream signaling cascades, mRNA stability and translation, and processes the primary gene products. In the brain, many processes are linked to NO.

High levels of nitric oxide (NO) block the process of NT closure in the chick embryo

Unraveling Mechanism

ADMA inhibits NOS





Journal of Neurochemistry, 2006, 96, 247–253 doi:10.1111/j.1471-4159.2005.03542

Neural tube closure depends on nitric oxide synthase activity

Amir Nachmany, Veronica Gold, Asaf Tsur, Dan Arad and Miguel Weil

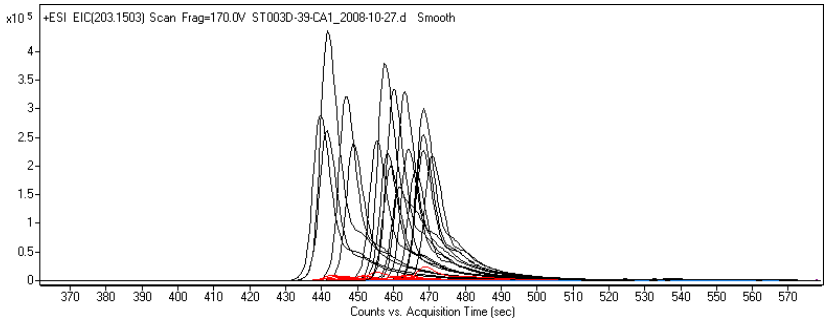
Department of Cell Research and Immunology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

Stemina BIOMARKER DISCOVERY

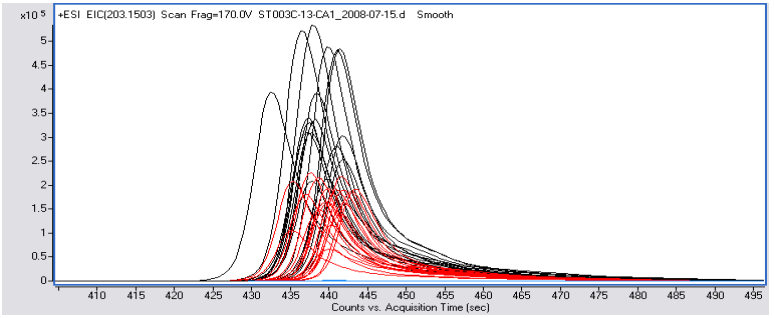
DevTox Project (6-well) - Results

Asymmetric Dimethylarginine (ADMA) Extracted Ion Chromatograms (EICs)

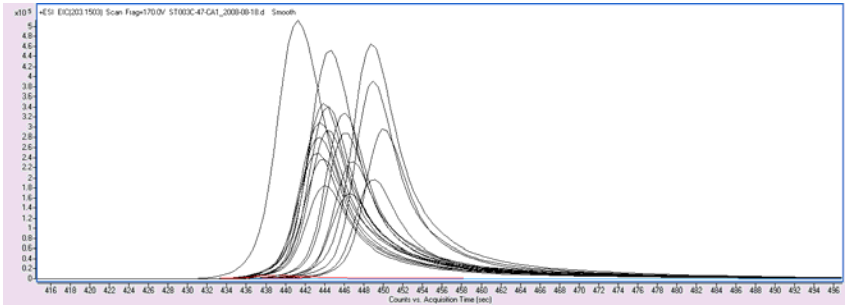
For strong teratogens: dosed shows lower levels than controls.



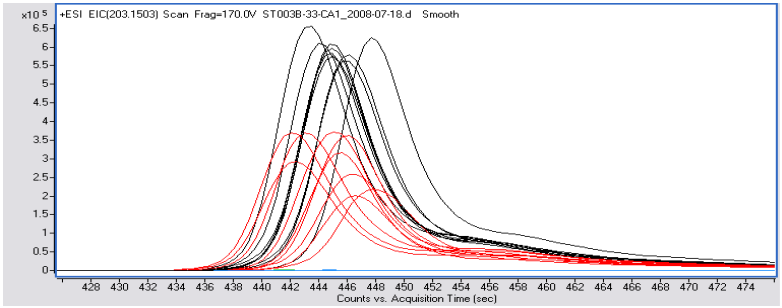
Cytosine Arabinoside



Hydroxyurea



5-Fluoruracil



Valproic Acid

Stemina BIOMARKER DISCOVERY

DevTox Project (6-well) - Results

Fold Change Ratios of Arginine/ADMA are indicators of teratogenicity

- EICs for these compounds were integrated for all datafiles
- Fold changes of the resulting areas for controls vs. dosed were compared

Stemina Classification	Compound	Arg fold change / ADMA fold change	Prediction
Non-Teratogens	Ascorbic Acid	1.28	Ter
	Aspirin	1.07	Non
	Caffeine	1.33	Ter
	Doxylamine (Blind 2)	0.97	Non
	Isoniazid	0.94	Non
	Levothyroxine	1.03	Non
	Penicillin G	0.96	Non
	Folic Acid	1.08	Non
	Retinol (Blind 1)	1.03	Non
	Thiamine (Blind 8)	1.00	Non
Teratogens	5-Fluorouracil	43.93	Ter
	Methotrexate	2.54	Ter
	Accutane (Blind 6)	0.55	Ter
	Amiodarone (Blind 3)	1.64	Ter
	Busulfan	1.12	Ter
	Carbamazepine (Blind 5)	1.12	Ter
	Cyclophosphamide (Blind 7)	1.56	Ter
	Cytosine Arabinoside	67.01	Ter
	Hydroxyurea	2.52	Ter
	Retinoic Acid	0.48	Ter
	Rifampicin (Blind 4)	0.81	Ter
	Thalidomide	0.85	Ter
	Valproic Acid	2.11	Ter

Non-teratogens show smaller fold change ratios (0.9 to 1.1)

Teratogens show larger fold change ratios (<0.9 and >1.1)

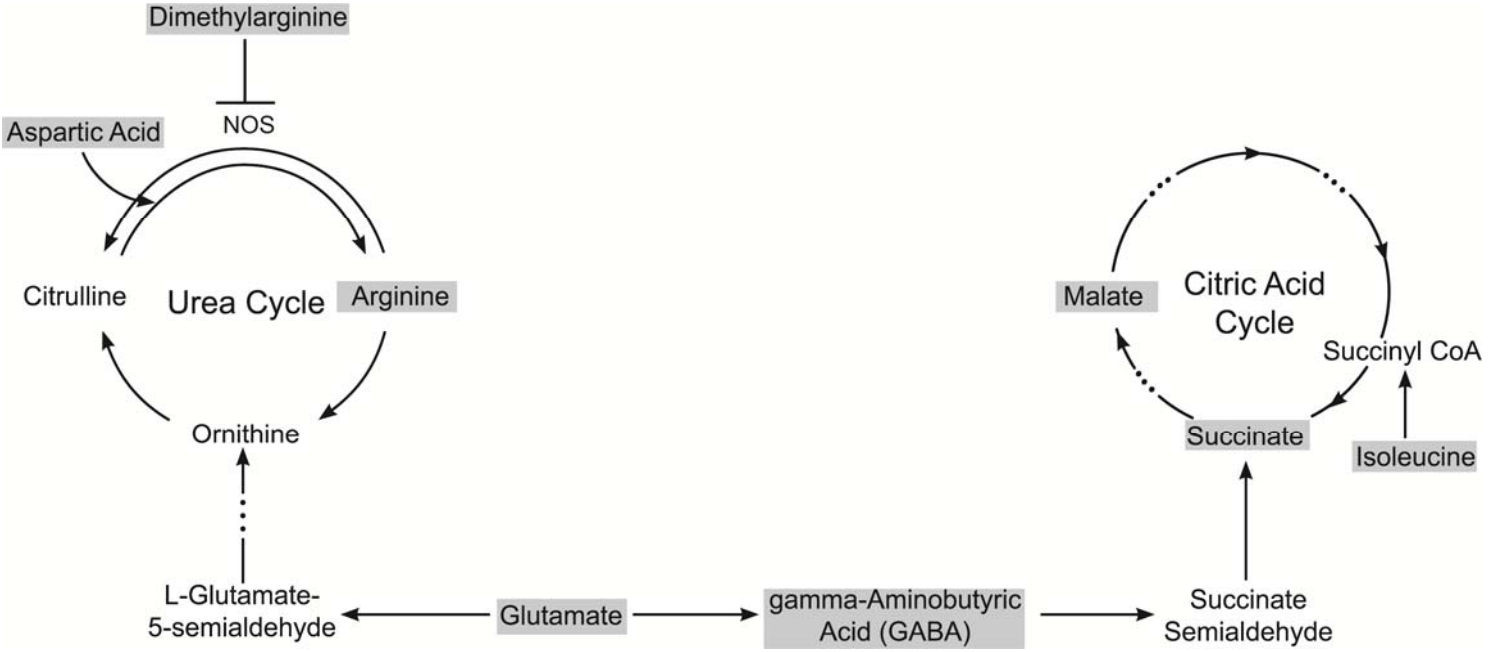
- No false negatives for teratogenicity
- Only ascorbic acid and caffeine are false positives

Stemina BIOMARKER DISCOVERY

DevTox Project (6-well) - Results

Validated Biomarkers and

Possible Related Pathways of Developmental Toxicity



Stemina BIOMARKER DISCOVERY

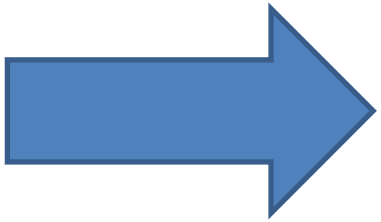
DevTox Project (96-well) - Methods

Development of High Throughput Metabolomics System

Funded by NSF Grant

From 6- to 96-well format for Higher Throughput:

- hES cell culture
- Protein Precipitation and Filtering
- Speed Vac concentration
- MS Data Analysis

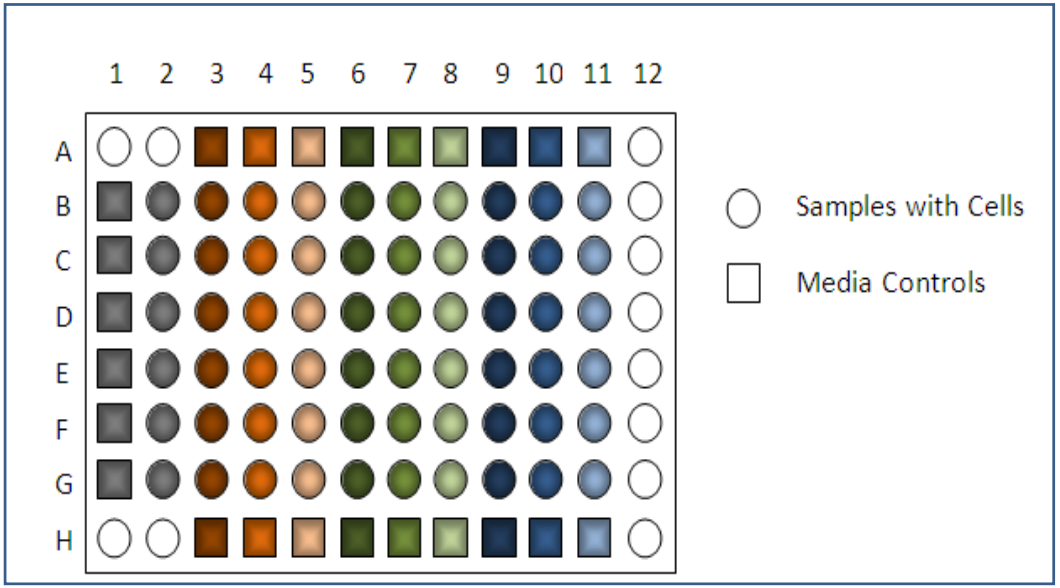


Stemina BIOMARKER DISCOVERY

DevTox Project - 96-well Method

96 Well Dosing Experimental Design

- Three compounds per plate
- Dosed at circulating dose, 10x above, and 10x below
- Control, dosed, media control and dosed media
- 6 replicates per dose with 3 doses allow for visualization of fold changes over a broader range



Symbol	Samples	Sample Name
	Media Controls	ST003F-95-1B-G
	Controls	ST003F-95-2B-G
	VPA Dosed Media Control	ST003F-95-3A/3H
	Valproic Acid ([1.66 mg/mL])	ST003F-95-3B-G
	VPA Dosed Media Control	ST003F-95-4A/4H
	Valproic Acid ([0.166 mg/mL])	ST003F-95-4B-G
	VPA Dosed Media Control	ST003F-95-5A/5H
	Valproic Acid ([0.0166 mg/mL])	ST003F-95-5B-G
	Cytosine Arabinoside Dosed Media Control	ST003F-95-6A/6H
	Cytosine Arabinoside ([2.19 ug/mL])	ST003F-95-6B-G
	Cytosine Arabinoside Dosed Media Control	ST003F-95-7A/7H
	Cytosine Arabinoside ([0.219 ug/mL])	ST003F-95-7B-G
	Cytosine Arabinoside Dosed Media Control	ST003F-95-8A/8H
	Cytosine Arabinoside ([0.0219 ug/mL])	ST003F-95-8B-G
	Doxylamine Dosed Media Control	ST003F-95-9A/9H
	Doxylamine ([1.03 ug/mL])	ST003F-95-9B-G
	Doxylamine Dosed Media Control	ST003F-95-10A/10H
	Doxylamine ([0.103ug/mL])	ST003F-95-10B-G
	Doxylamine Dosed Media Control	ST003F-95-11A/11H
	Doxylamine ([0.0103 ug/mL])	ST003F-95-11B-G

DevTox Project - 96-well Method

6-well vs. 96-well Culture and Dosing Summary

Procedure	6-well	96-well
hES cell culture	3 x 10^6 cells/well in 2.5 ml media	2.5 x 10^5 cells/well in 200 ul media
	2 day wait time prior to dosing	1 day wait time prior to dosing
	H9 cell line	H1, H7, H9 cell lines
Post-Dose Cell Analyses	(none)	Cell Viability
		Differentiation
Sample Preparation	3 KDa MWCO	10 KDa MWCO
	Separate filtration column per sample	96-well filter plate
Dosing/Prep Throughput	2 drugs in 1 week	54 drugs in 1 week

DevTox Project - 96-well Method

6 well Analysis Vs 96 Well Cell Culture and Sampling

(Every Cell Matters)

- 6 Well sample analysis- 3 x 10e6 cells/well in 2.5 ml media. Process a portion of the spent media to produce an effective final volume of:

  54,000 cells/injection on column.

- 96 Well sample analysis – 2.5 x 10e5 cells/well in 200 ul media. Process the entire volume of spent media to produce an effective final volume of:

  ~115,000 cells/injection on column.

- ~ 2 fold increase in overall sensitivity.

Stemina BIOMARKER DISCOVERY

DevTox Project - Methods

HILIC LC-MS

HILIC LC-MS methods were developed and optimized to provide a good compromise for the separation of both hydrophilic and hydrophobic compounds.

Retention times are very reproducible. Maximum variation is about 12 sec.

Column: Phenomenex Luna HILIC; 100 x 3mm; 5µm
Solvent A 0.1% Formic Acid in Water
Solvent B 0.1% Formic Acid in ACN

New 96-well Method
22 minute run time

Time	%ACN	Flow	Divert Valve
0.0	95	0.5	Waste ("Junk Dump")
0.5	95	0.5	MS
1.5	95	0.5	MS
12.0	69.7	0.5	MS
13.0	5	0.5	MS
17.0	5	0.5	MS
18.0	95	1	Waste
22.0	95	1	Waste

Stemina BIOMARKER DISCOVERY

DevTox Project - 96-well Method

HILIC LC-MS Method Improvements

1. Divert first 30 seconds of LC eluent to waste to reduce salts and other non-volatiles in MS.

   Two- to three-fold increase in sensitivity

2. Change the injection solvent from 0.1% Formic acid to 50:50 ACN /0.1% formic acid

   Better sample solubility

   Improved chromatographic peak shape

   Increased recovery of more hydrophobic metabolites

3. Shorten the run time from 30 to 22 minutes

   - End the gradient after the analytes of interest elute
   - Double the flow rate during the equilibration step

   Maintains the 'history' of the chromatography.

Stemina BIOMARKER DISCOVERY

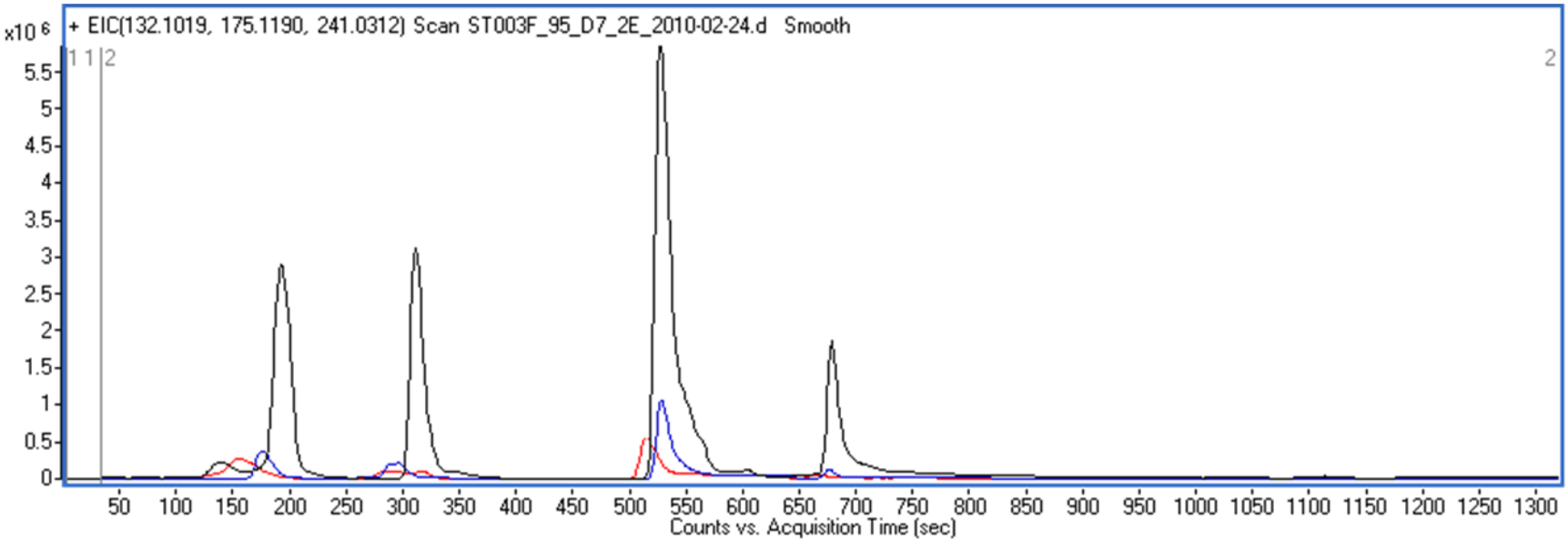
DevTox Project - 96-well Method

Sum Total of All Optimized Method Parameters

Over 10X improvement in sensitivity

Better chromatographic peak shape/resolution

Maintains similar analyte retention times



6 well filtration method with 0.1 % Formic injection solvent

6 well filtration method with 50:50 injection solvent

96 well filtration method with 50:50 injection solvent.

DevTox Project
SUMMARY

Stemina Drug Teratogenicity Model Screen

Model (6-well dosing) was 87.5% predictive.

Data for 96-well dosing is being analyzed and looks very promising.

Pharmaceutical Discovery/Development

Provides teratogenicity data early in the pipeline for “fail early fail often”

Should be used to “flag” possible teratogens

Can be employed simultaneously with animal testing

Publication in Press:

Predicting Human Developmental Toxicity of Pharmaceuticals Using Human Embryonic Stem Cells and Metabolomics; P. West, A. Weir, A. Smith, E.L.R. Donley and G. Cezar. Toxicology and Applied Pharmacology.

Stemina BIOMARKER DISCOVERY

“Predictive Efficacy Screening in Cancer Stem Cells Using Metabolomics”

NIH/NCI SBIR Phase I Contract in collaboration with:

John S. Kuo MD PhD

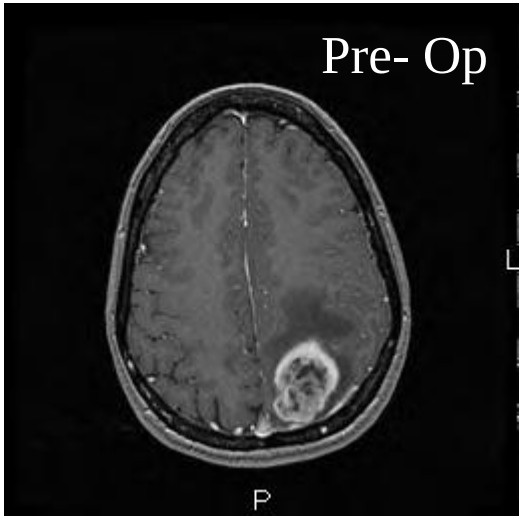
Dept of Neurological Surgery
Dept of Human Oncology
Paul P. Carbone Comprehensive Cancer Center
Stem Cell and Regenerative Medicine Center
School of Medicine and Public Health
University of Wisconsin-Madison

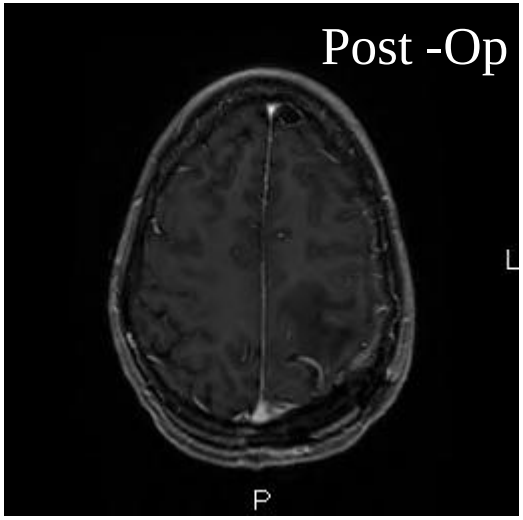
ASMS 2010 Wednesday Poster 343

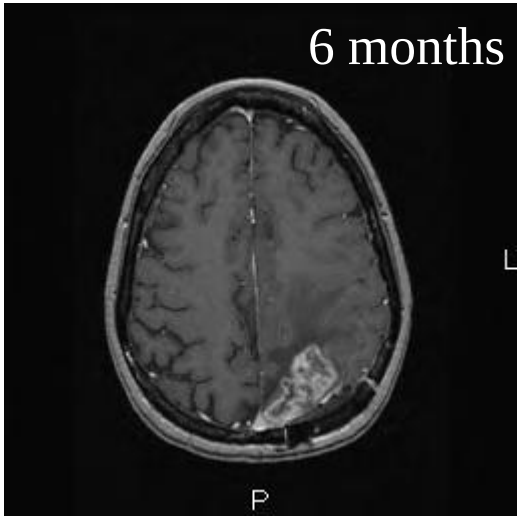
Cancer Stem Cell Metabolomics Project

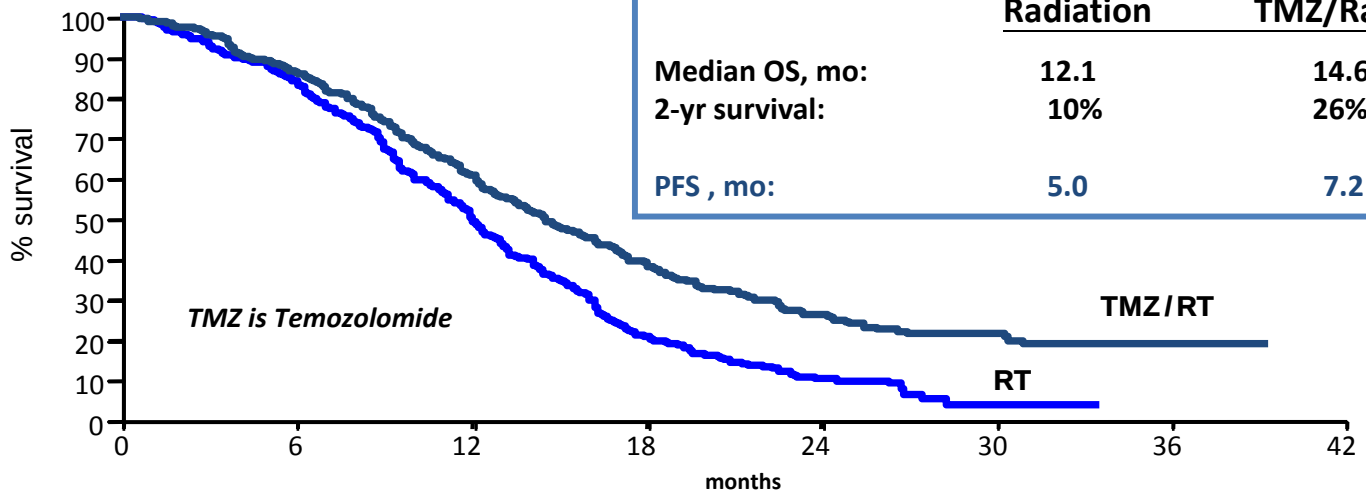
Glioblastoma Multiforme (GBM) rapidly recur after treatment

Despite advances in surgery, radiation, chemotherapy and imaging technologies









	Radiation	TMZ/Radiation
Median OS, mo:	12.1	14.6
2-yr survival:	10%	26%
PFS , mo:	5.0	7.2

THE UNIVERSITY of WISCONSIN MADISON

www.stemina.com

THE UNIVERSITY of WISCONSIN MADISON

Cancer Stem Cell Hypothesis

- **Cancer stem cells / tumor progenitor cells:**
  - A small subpopulation of cells within tumors
  - Display stem cell-like characteristics of self-renewal, enhanced proliferation, multipotent differentiation
  - Highly efficient in tumor initiation

- **GBM Cancer Stem Cells (CSCs):**
  - Originally identified in 2003
  - **Display enhanced therapeutic resistance to radiation and chemotherapy**

Stemina BIOMARKER DISCOVERY

Cancer Stem Cell Metabolomics Project
Objectives

- Investigate and discover metabolomic biomarkers that are specific to cancer stem cells (CSCs) to use as biomarkers of drug efficacy against CSCs leading to more effective therapies.

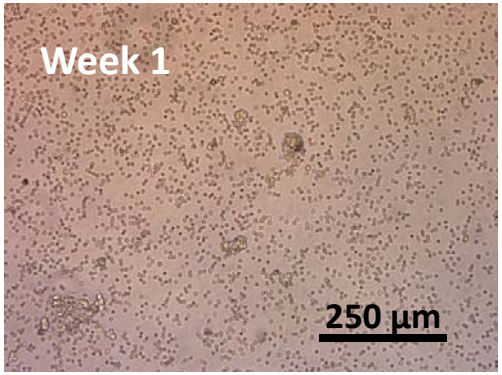
- The primary cellular targets were cancer stem cells derived from glioblastoma multiforme (GBM), brain tumor stem cells (BTSCs), as well as non-cancer stem cells derived from the same tumors.

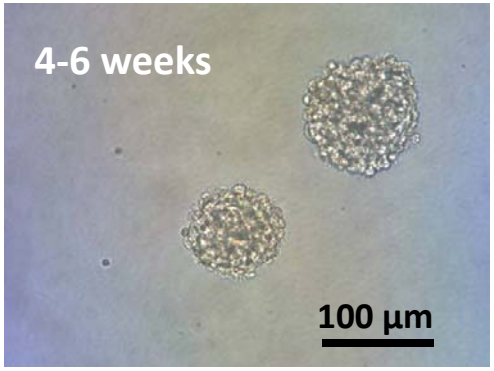
I dedicate my work on this project to the memory of Paul Geno, my grad school advisor who was stricken with GBM and passed away in 1997.

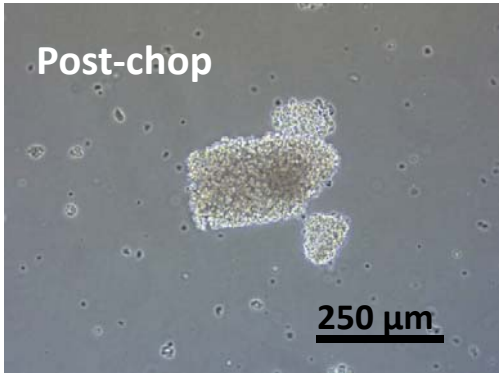
Stemina BIOMARKER DISCOVERY

Cancer Stem Cells Metabolomics Project
Methods

GBM Cancer Stem Cell isolation in Kuo Lab





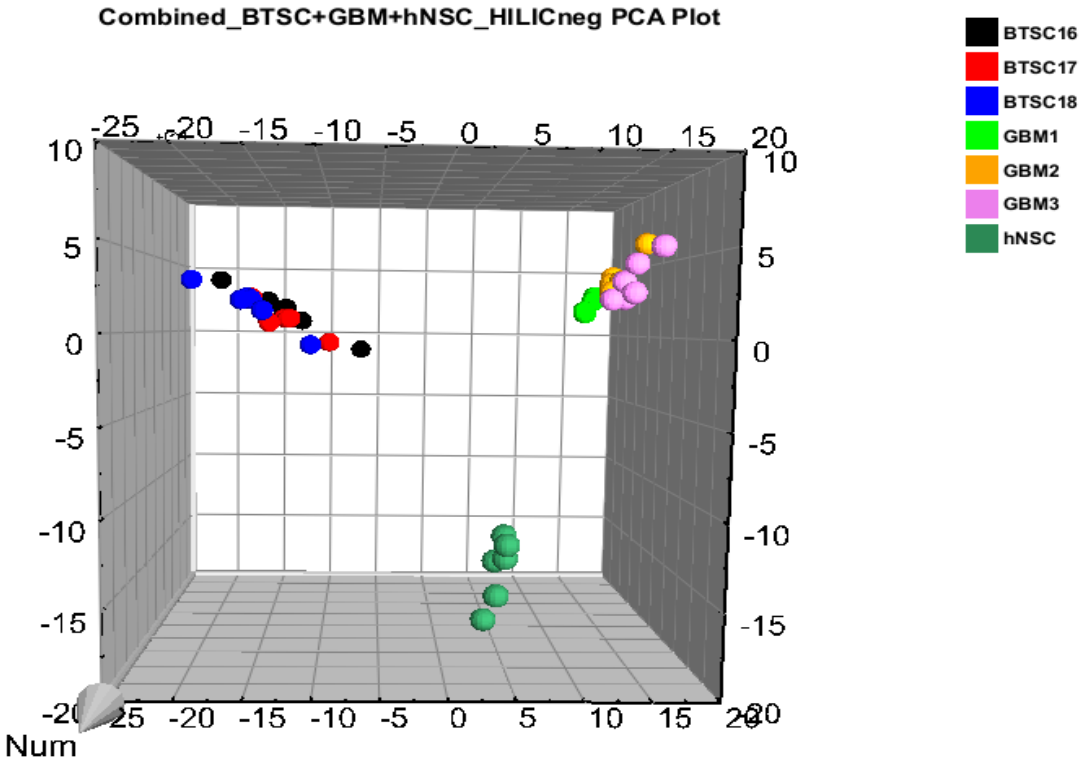


Cancer Stem Cells Metabolomics Project

Results

We have identified a unique signature of small molecule biomarkers in GBM CSC compared to differentiated GBM cells and normal neural stem cells.

The goal is to find sensitive, quantitative biomarkers to predict CSC-specific efficacy and treatment responses.



R

Stemina BIOMARKER DISCOVERY

“A Predictive Model of Cardiomyopathy Using Human Embryonic Stem Cell Derived Cardiac Precursors and Metabolomics”

“CardioTox Project”

ASMS 2010 Tuesday Poster 367

Stemina BIOMARKER DISCOVERY

CardioTox Project - Overview

- Cardiac safety is a leading cause of pharmaceutical (particularly anti-cancer drugs) attrition

- Stemina is combining hES cell derived cardiac precursors and metabolomics to uncover the metabolic signature of cardiomyopathy inducing drugs.

- hES derived cardiac precursors were exposed to a training set consisting of both chemotherapeutics known to induce cardiomyopathies and non-cardiotoxic pharmaceutical agents.

- The cardiotoxicity of the treatments was first evaluated by cell viability assays.

- Biomarkers of cardiotoxicity were identified using both univariate and multivariate analysis.

- Features were selected for predictive models using partial least squares discriminate analysis and random forests.

- A predictive metabolic signature of cardiomyopathy with the ability to differentiate cardiotoxic from non-cardiotoxic drug treatments in the training set of compounds was discovered and evaluated by unsupervised statistical analysis.

- We are currently developing an *in vitro* screen capable of predicting cardiomyopathy-inducing properties of pharmaceuticals.

Stemina BIOMARKER DISCOVERY

CardioTox Project - Methods

Drug Dosing and Timing				
Drug (Cardiotoxic) (Non-cardiotoxic)	Dose	Time in Culture Prior to Dose (hours)	Dose Time (hours)	Time in Culture Sample Point (hours)
Herceptin	7 ug/ml	48	24	72
Paclitaxel	15 uM	24	48	72
Doxorubicin	26 uM	48	24	72
Herceptin and Paclitaxel	7 ug/ml and 15uM	0	72*	72
Doxorubicin and Paclitaxel	26 uM and 15 uM	0	72^	72
Tamoxifen	15 uM	48	24	72
Valproate	1mM	24	48	72
Untreated			0	72

* sample was treated first with Herceptin for 24 hours, then media was replaced with media containing Paclitaxel for 48 hours

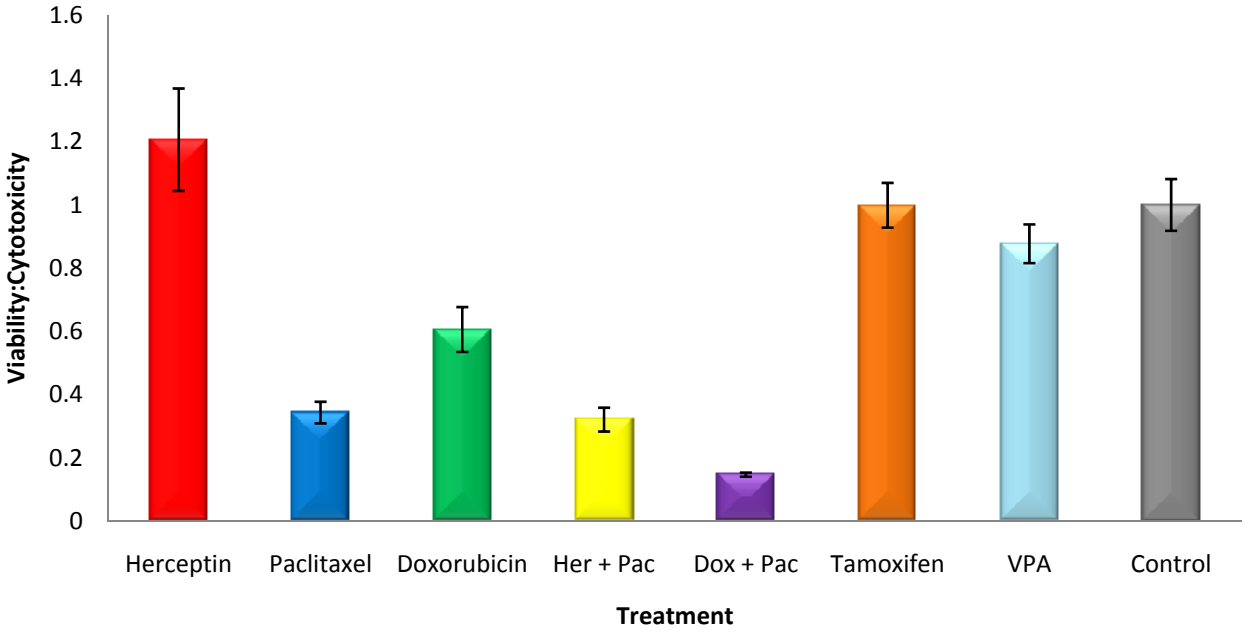
^ sample was treated first with Doxorubicin for 24 hours, then media was replaced with media containing Paclitaxel for 48 hours

Stemina BIOMARKER DISCOVERY

CardioTox Project - Results

Cell Viability Assays

The cell viability data suggest that treatment regimens selected for modeling cardiotoxicity are eliciting an appropriate toxic response in the cardiac precursors.

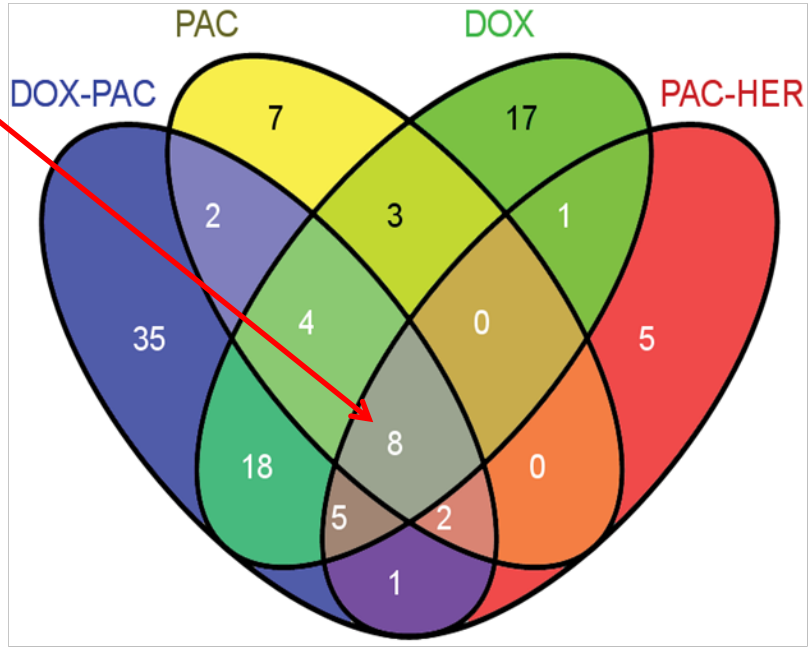


The MultiTox-Fluor kit (Promega) was used for viability/cytotoxicity testing. This kit uses a cell-permeant peptide which fluoresces green in live cells, and another peptide that measures dead protease activity that fluoresces red.

Stemina BIOMARKER DISCOVERY

CardioTox Project - Results

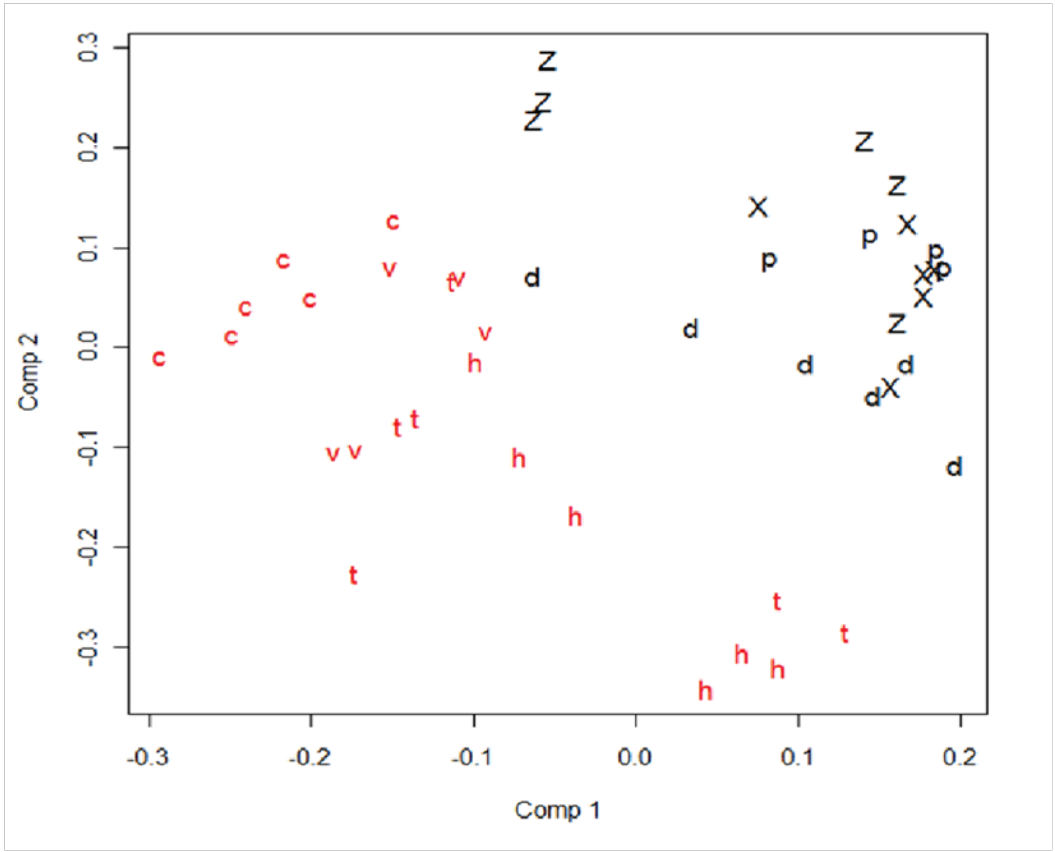
- 108 statistically significant mass features in human cardiac precursors by treatment.
- Eight features were common to strong cardiotoxicants.



Stemina BIOMARKER DISCOVERY

CardioTox Project - Results

PLS-DA scores plot of 2 principal components



From mass features selected based on VIP scores.

Cardiotoxic:
Z: herceptin-paclitaxel
X: doxorubicin-paclitaxel
d: doxorubicin
p: paclitaxel;

Non-cardiotoxic
c: control
h: herceptin
t: tamoxifen
v: valproate

Stemina BIOMARKER DISCOVERY

Thank You...

Collaborators

Agilent Technologies

Wisconsin
University of Wisconsin–Madison

California Stem Cell

Promega

CAAT Johns Hopkins University Center for Alternatives to Animal Testing

Funding

National Cancer Institute

NSF

Customers

Mayo Clinic

Pioneer
A DuPont Business

Bristol-Myers Squibb
Together we can prevail.®

SIGMA-ALDRICH®

Stemina
BIOMARKER DISCOVERY

Stemina BIOMARKER DISCOVERY

From Cell Culture to Patients

The Stemina Science Team

Paul R. West, PhD
Director of BioAnalytical Chemistry

Alan Smith, PhD
Senior Scientist - Bioinformatics

April M. Weir, MS
Senior Cell Biologist and Biochemist

Kevin Conard, BS
Scientist - BioAnalytical Chemistry

Burr Fontaine, MD, MS
Research Scientist - Bioinformatics

James T. Davis, BS
Laboratory Technician

Stemina BIOMARKER DISCOVERY

Acknowledgements

Agilent Technologies

Steve Fischer – Metabolomics

John Sausen – LC/MS sales and consultation

Rick Carberry –North America Sales

Cancer Stem Cell Project

"Predictive Efficacy Screening in Cancer Stem Cells Using Metabolomics"

Funding: NIH National Cancer Institute SBIR Contract Number: HHSN261200800024C

Collaboration: UW Madison Dr. John S. Kuo laboratory:

Paul Clark, Sasha Rackman and Priya Ezhilan.

DevTox Project

"SBIR Phase I: Metabolomics of Human Embryonic Stem Cells to Predict Teratogenicity: An Alternative Developmental Toxicity Model."

Funding: NSF Award Number: IIP-0945105