Reverse Translational Studies to Understand Drug-Induced Toxicity

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Adverse Drug Events Are Significant Clinical Problems

~4.5 million adverse drug event-related visits to clinic or ER/yr

Attrition Due to Clinical Safety Impedes New Drug Development

A Major Goal of the Precision Medicine Initiative: Reduce Therapeutic Adverse Events

• Precision Medicine (2015)
  • Tailoring of medical treatment to individual characteristics such as lifestyle, environmental and biological uniqueness (i.e., genome, microbiome, etc.)

• Goal
  • Focusing therapeutic interventions on those who would benefit
  • **Sparing expense and adverse events for those who will not**

• Driver
  • Advances in technology

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[Image: All of Us - The future of health begins with you]
Reverse Translation of Adverse Drug Events

Bedside to Bench

Bench to Bedside

Computational Analysis

Lab Analyses
Motivation

• Improved patient outcomes

• Increased understanding of molecular mechanisms for drug toxicities
  
  o Targeted therapies to treat/prevent toxicity

  o Screening in drug development
Two Tales of Reverse Translation: From Genomics towards Mechanism

- Chemotherapy-induced peripheral neuropathy
  - GWAS
  - iPSC-Induced sensory neuron studies

- Bevacizumab-induced hypertension
  - Exome sequencing
  - Cell-based studies
Sensory Peripheral Neuropathy is a Serious and Common Adverse Event
Chemotherapy-Induced Sensory Peripheral Neuropathy (CIPN)

- Affects 30-40% of cancer patients
  - Platinum agents, **Taxanes, Epothilones, Eribulin**, Vincristine, Bortezomib, Thalidomide, Lenalidomide
- One of the most common reasons that cancer patients stop treatment early
- Affects quality of life
- No effective drug therapies to prevent CIPN
- Largely managed with physical therapy, massage, acupuncture
Despite Decades of Animal Studies There are No Effective Strategies for Prevention of CIPN

<table>
<thead>
<tr>
<th>Strategy</th>
<th>ASCO Recommendation</th>
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<tbody>
<tr>
<td></td>
<td>Strong Against</td>
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<tr>
<td></td>
<td>Moderate Against</td>
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<td>Neuroprotectants</td>
<td>Acetyl-L-carnitine</td>
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<td>Diethyldithiocarbamate</td>
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<td>Amifostine</td>
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<td>Leukemia Inhibitory Factor</td>
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<td>ACTH analogs</td>
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<td>Glutamine/Glutamate</td>
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<td>Neurotransmitter Release</td>
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<tr>
<td>Channel activity</td>
<td>Ca²⁺/Mg²⁺</td>
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<td>Antioxidants</td>
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<tr>
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<td>Vitamin E</td>
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<td>Retinoic Acid</td>
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<td>N-acetylcysteine</td>
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<td>ω3 Fatty Acids</td>
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And Only Moderate Evidence for Effective Treatment of CIPN

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<thead>
<tr>
<th>Strategy</th>
<th>ASCO Recommendation</th>
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<tr>
<td></td>
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<td>Neurotransmitter Release</td>
<td>Duloxetine</td>
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<tr>
<td>Channel Activity</td>
<td>Lamotrigine</td>
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Paclitaxel-Induced Sensory Peripheral Neuropathy

- Risk factors include:
  - high single dose
  - high cumulative dose
  - treatment with other neurotoxic drugs
  - other conditions which cause neuropathy (e.g., diabetes, alcoholism, HIV)

- Genetic Variability?
Most associations had small effect sizes and did not consistently replicate in additional studies.
Association of CYP2C8*3 and ABCB1 -129A>G with Paclitaxel-Induced Neuropathy

Grade 2+
HR = 1.95 (1.06 – 3.58)
P = 0.031


Extreme phenotyping
73 cases, 46 controls
HR = 0.12
P = 0.03

## CALGB/Alliance Microtubule Targeting Agent Breast Cancer Studies with Pharmacogenetic Companions

<table>
<thead>
<tr>
<th>Study</th>
<th>Drugs</th>
<th>PG Samples</th>
<th>Phenotypes</th>
<th>Genotyping</th>
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<td>40101/60202</td>
<td>Paclitaxel, Adriamycin, Cyclophosphamide</td>
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<td>Peripheral neuropathy, Neutropenia, Ovarian suppression, Cardiotoxicity</td>
<td>GWAS, targeted resequencing</td>
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<td>40502/60704</td>
<td>Paclitaxel, Nab-paclitaxel, Ixabepilone, Bevacizumab</td>
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<td>Peripheral neuropathy, Response, Hypertension</td>
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<td>40601/60701</td>
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<td>Response, Peripheral neuropathy, Cardiotoxicity</td>
<td>Replication for exome sequencing</td>
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CALGB 40101 – 2 X 2 Factorial Design
Adjuvant Therapy for Women with Breast Cancer with 0-3 + Nodes

Stratification

Pre/Post Menopausal

ER/PgR

AC q2 wk

60 mg/m²

600 mg/m²

4 cycles – 8 wk

6 cycles – 12 wk

Paclitaxel q2 wk

175 mg/m²

4 cycles – 8 wk

6 cycles – 12 wk

AC = doxorubicin/cyclophosphamide

Total Accrual = 3871
Accrual to Paclitaxel 4 cycles = 1005
Accrual to Paclitaxel 6 cycles = 648

CALGB 40101: Paclitaxel-Induced Sensory Peripheral Neuropathy is Dose Dependent

Overall incidence of ≥ Grade 2 sensory peripheral neuropathy was 24%
- 17% in 4 cycle arm
- 33% in 6 cycle arm
Common FGD4 and EPHA5 SNPs Associated with Onset of Sensory Peripheral Neuropathy

N = 855

FGD4 - rs10771973

MAF = 31%

HR 1.57 (1.30-1.91)

p = 2.6 x 10^{-6}


EPHA5 - rs7349683

MAF = 36%

HR 1.63 (1.34-1.98)

p = 9.6 x 10^{-7}
Replication of FGD4 Association in Europeans and African Americans

Europeans

HR = 1.72 (1.06-2.80)  
\( p = 0.013 \)

African Americans

HR = 1.93 (1.13-3.28)  
\( p = 6.7 \times 10^{-3} \)

EPHA Receptor Associations Have Been Replicated by Others


EPHA6 rs301927
OR 1.29 (1.07-1.55)  
P = 0.008
Targeted Resequencing in Tails of CALGB 40101
Neuropathy Distribution

Chhibber, Li and Ho unpublished
Targeted Resequencing Regions

Chhibber, Li and Ho unpublished
DNM1L Synonymous Variant Enriched in High Risk Group

rs148634653

MAF (%)

High Risk | Low Risk | ExAC

Chhibber, Li and Ho unpublished
Heritability Captured by SNPs in GO Axonogenesis and Axonogenesis Children Sets

Genes in Overlap of Axon Regulation and Extension

- Implicated in pain pathways
- Involved in regeneration after peripheral nerve injury
- Variants identified as risk factors for HIV and diabetic peripheral neuropathy
- Widely tested as a potential treatment for peripheral neuropathy; reduced activity in mice causes sensory neuropathy

Gene + binding partner can modulate neuropathic pain and repair sensory neurons

Mutations associated with a form of hereditary neuropathy (giant axonal)

Associated with hyperalgesia in sensory neurons

Implicated in pain pathways

WNT signaling implicated in several mouse models of neuropathic pain

Upregulated after nerve injury

Upregulated after nerve injury
CALGB 40502: Randomized Phase III Trial of Paclitaxel, Nab-Paclitaxel, or Ixabepilone with Bevacizumab for Locally Recurrent or Metastatic Breast Cancer

Stratification
Taxane as adjuvant therapy
ER/PgR

Accrual to Paclitaxel = 275
Accrual to Nab-Paclitaxel = 267
Accrual to Ixabepilone = 241
635 Consented for PG Companion

Paclitaxel
90 mg/m² IV qw
Bevacizumab
10 mg/kg q2w

Nab-paclitaxel
150 mg/m² IV qw
Bevacizumab
10 mg/kg q2w

Ixabepilone
16 mg/m² IV qw
Bevacizumab
10 mg/kg q2w

Meta Analysis of CALGB 40101 and 40502

Typed & Imputed Genotypes

Cox PH analysis
- Adjust for age
- Phenotype: cumulative dose to 2+ SPN

Effect size per SNP

Meta Analysis
- Inverse Variance Weighting

Kat Chua – see poster PI-051
Meta Analysis Identified Novel Genetic Markers of Microtubule Targeting Agent-Induced Peripheral Neuropathy

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
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<th>CALGB 40502</th>
<th>Meta-Analysis</th>
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<td>P</td>
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SNP Downstream of S1PR1 is Associated with Microtubule Targeting Agent-Induced Neuropathy

Kat Chua – see poster PI-051
# Novel Genes Associated with Paclitaxel-Induced Peripheral Neuropathy

<table>
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<td>RhoGEF for Cdc42, cell shape</td>
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<td>EPHA4/5/6/8</td>
<td>Receptor tyrosine kinase, axon guidance</td>
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<td>ARHG EF10</td>
<td>RhoGEF, slow nerve conduction velocity</td>
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<td>PRX</td>
<td>Myelin maintenance</td>
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<td>SBF2</td>
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<td>FCAMR</td>
<td>Immune function</td>
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</table>

Frabin/FGD4 Induces Cdc42-mediated Filopidia/Lamellipodia Formation

EphrinA5 Expression is Attenuated in a Mouse Model of Peripheral Nerve Injury

Inhibition of S1PR1 Attenuates Paclitaxel-Induced Neuropathic Pain

Mechano-hyperalgesia

- V-V
- P-V
- P-W146 (0.2 nmol/d)
- P-W146 (0.7 nmol/d)
- P-W146 (2.2 nmol/d)
- V-W146
- P-W140 (2.2 nmol/d)

NF-κB, p38, ERK, TNFα, IL-1β

Days post first paclitaxel dose

0 25 50 75 100 125

PWT (g)
**Human iPSC-Derived Sensory Neurons**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Neurocrest</th>
<th>Neuroectoderm</th>
<th>Sensory Neuron</th>
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<tbody>
<tr>
<td>PAX6 (day 4)</td>
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<td>SOX10/TUBB3 (day 12)</td>
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<td>BRN3A/TUBB3 (day 12)</td>
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<td>Peripherin (day 24)</td>
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<td>TRPV1 (day 25)</td>
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<td>(day 50)</td>
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</table>

Tore Stage
Chenling Xiong
Annie Altman
Human iPSC-Derived Sensory Neurons Have Expected Channel and Receptor Activity

Control

1 µM Capsaicin

25 mM KCl
Human iPSC-Derived Sensory Neurons are Resistant to Paclitaxel

![Graph showing cytotoxicity relative to control for Paclitaxel at 72 hrs with and without Verapamil.]
Pacitaxel Affects Neurite Networks in iPSC-Derived Sensory Neurons

Tore Stage
Chenling Xiong
Annie Altman
Pacitaxel Causes Mitochondrial Aggregation in Human iPSC-Derived Sensory Neurons

Tore Stage
Chenling Xiong
Annie Altman
Hypothesis: Paclitaxel-Induced EPHA5 and FGD4 Signaling Modulates Rho/Rac/Cdc42 GTPase Activity and Actinomysin Contractility
Conclusions

• Genome-wide studies identified variants in several genes involved in neuron function that are associated with microtubule targeting agent-induced sensory peripheral neuropathy

• Genes involved in axonogenesis contribute significantly to the heritability of this adverse event

• Human iPSC-derived sensory neurons are a robust model for understanding the molecular basis of this toxicity
Current Focus

**Replication and discovery of new risk variants and genes**
- Exome sequencing of 622 samples from CALGB 40502 to discover new risk variants and genes - Including regulatory regions of candidate genes
- Replication in BioVU and other cohorts
- Meta-analysis with other NCI cooperative groups

**Mechanistic studies of genetic findings**
- CRISPR being used to understand gene function and to identify causal variants
- FDA library screen underway
- iPSC-derived Schwann cells
- Mouse studies
Two Tales of Reverse Translation: From Genomics towards Mechanism

- Chemotherapy-induced peripheral neuropathy
  - GWAS
  - iPSC-Induced sensory neuron studies
- Bevacizumab-induced hypertension
  - Exome sequencing
  - Cell-based studies
# Bevacizumab-Induced Hypertension

<table>
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<tr>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
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</thead>
<tbody>
<tr>
<td>Prehypertension</td>
<td>Stage 1 Hypertension</td>
<td>Stage 2 Hypertension</td>
<td>Life-threatening</td>
<td>Death</td>
</tr>
<tr>
<td>120 mmHg 80 mmHg</td>
<td>140 mmHg 100 mmHg</td>
<td>160 mmHg</td>
<td>Neurologic deficit Hypertensive crisis</td>
<td></td>
</tr>
</tbody>
</table>

- **5 - 18% Incidence**
- **0.5 - 1% Incidence**

Hypertension severity levels:

- **Grade 1**: Prehypertension
- **Grade 2**: Stage 1 Hypertension (monotherapy indicated)
- **Grade 3**: Stage 2 Hypertension (>1 drug indicated)
- **Grade 4**: Life-threatening
- **Grade 5**: Death

Blood pressure levels:

- **120 mmHg 80 mmHg** (Grade 1)
- **140 mmHg 100 mmHg** (Grade 2)
- **160 mmHg** (Grade 3)
- **Neurologic deficit Hypertensive crisis** (Grade 4)
- **Death** (Grade 5)
Inhibition of VEGF Signaling Predicted to Disrupt Vascular Tone
Candidate Gene Studies Identified VEGFA/VEGFR2 Variants Associated With Bevacizumab-Induced Hypertension

VEGFA

- PROMOTER
- 5'-UTR: rs699947, rs833601, rs2010963
- CODING
- 3'-UTR: rs3025039

KDR (VEGFR2)

- PROMOTER
- 5'-UTR
- CODING
- 3'-UTR: rs1870377 (H472Q)

No validation

Exome Sequencing of Extreme Phenotypes

All genes: Exonic regions (64 Mb)

+ 181 Candidate genes: UTRs, introns, ±50 kb upstream/downstream (22 Mb)

VEGF signaling
Nitric oxide signaling
Hypertension
Endothelial cell biology
Published association hits

Megan Li Clin Cancer Res in press
CALGB 80405

**Phase III: Colorectal Cancer Treatment**

**Arm A**
Bevacizumab + FOLFOX/FOLFIRI

**Arm B**
Cetuximab + FOLFOX/FOLFIRI

No HTN: 75%
Grade 1-2: 20%
Grade 3-4: 5%

PI Alan Venook

Megan Li Clin Cancer Res in press
Bevacizumab-Induced Hypertension Cases and Controls

CALGB 80405
Bev-treated arm
N = 899 (688 PG consented)

Cases:
≥ Grade 3 HTN in first 3 cycles
48
-13 no PG consent
-10 no DNA

Controls:
No HTN in first 6 cycles and ≥4 uninterrupted cycles with AE form coverage
299
-204 missing bev doses
-37 any grade HTN yr 1
-4 gaps in AE coverage
-1 missing trt form
-7 no DNA

Exploratory!

25 Cases
21 CA/4 AA

46 Controls
44 CA/2 AA

Megan Li Clin Cancer Res in press
Variant Association Testing

Total Variants
n = 327,184

Exome Wide
Restrict to deleterious (nonsynonymous, splice-site)
n = 39,981 variants in 12,653 genes

Candidate Gene
Restrict to candidate genes (coding + non-coding)
n = 92,886 variants in 174 genes

Single-variant association (PLINK/SEQ)

MAF ≥ 0.10
LD-pruned r^2 > 0.8
n = 8,254
n = 9,356

Megan Li Clin Cancer Res in press
## Candidate Gene SNP Analysis of Bevacizumab-Induced Hypertension

<table>
<thead>
<tr>
<th>rsid</th>
<th>Candidate Gene</th>
<th>Function</th>
<th>P</th>
<th>OR</th>
<th>Case genotypes (MAF)</th>
<th>Control genotypes (MAF)</th>
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<td>36/7/0 (0.08)</td>
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<td>rs73057960</td>
<td>NOSIP</td>
<td>intronic</td>
<td>0.004</td>
<td>18.9</td>
<td>11/7/1 (0.24)</td>
<td>40/2/1 (0.05)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Bonferroni-adjusted significance threshold: P = 5.3E-06

Megan Li Clin Cancer Res in press
Carriers of HSP90AB1 SNPs Have Higher Incidence of Bevacizumab-Induced Grade 3 Hypertension
SLC29A1-HSP90AB1 Intergenic Region

- rs6929249
- rs3734704
- rs6929249: r2=0.52
- rs834576
- rs9381299
rs9381299 eQTL: Increased SLC29A1 Expression in Monocytes

Data from Fairfax et al, Nat Genet (2012)
rs9381299 Carriers Have Higher Incidence of Grade 3+ Bevacizumab-Induced Hypertension in Replication Cohorts

CALGB 40502

ECOG 5103

Frequency of SBP > 160

4.7%
14.3%

7.0%
16.2%

Non-carriers
Carriers

23/331
16/99
ENT1 (SLC29A1) Regulates Adenosine Intra- and Extracellular Levels and Adenosine Receptor Signaling
VEGF Signaling but not Adenosine Signaling is Responsive to Bevacizumab Treatment in HUVECs

A

0.1 μM Ado

[Ca²⁺] (nM)

Vehicle Bevacizumab

10 μM Ado

Vehicle Bevacizumab

100 μM Ado

Vehicle Bevacizumab

B

0.1 μM Ado

Total nitrate and nitrite (μM)

Vehicle Bevacizumab

10 μM Ado

Vehicle Bevacizumab

100 μM Ado

Vehicle Bevacizumab
Inhibition of ENT1 Increases Adenosine Signaling in a Bevacizumab-Independent Manner
Overexpression of ENT1 Increases the Response of HUVECs to Bevacizumab
Hypothesis: Endothelial Cells are more Sensitive to VEGF Inhibition Under Conditions of Decreased Basal Adenosine Signaling
Conclusions and Current Focus

- Variation in adenosine signaling influences vascular response to bevacizumab treatment
- Whether variants in the SLC29A1-HSP90AB1 genomic region influence gene expression requires further study
- Variation in HSP90AB1 signaling may independently influence the risk of developing bevacizumab-induced hypertension
Reverse Translation of Adverse Drug Events

Bedside to Bench

Bench to Bedside

Computational Analysis

Lab Analyses
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