Canadian Pharmacogenomics Network for Drug Safety



Prevention of Adverse Drug Reactions in Childhood by Identifying Predictive Genomic Markers: Use of Big (and Small) Data

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The Canadian Pharmacogenomics Network for Drug Safety has received financial support for its adverse drug reaction research from:

Canada Foundation for Innovation (CFI), Canadian Institutes of Health Research, Genome Canada , Genome British Columbia and the Provincial Health Services Authority. POPi has also received support by the University of British Columbia, Child & Family Research Institute (Vancouver), Health Canada, Michael Smith Foundation for Health Research, Eli Lilly Canada (unrestricted), Janssen Ortho Canada (unrestricted) Pfizer Canada (unrestricted) and Dynacare Next.

All industry funding was a partnership requirement of federal peer-reviewed Genome Canada research applications.

There are no patents or patents-pending for any of this work anywhere in the world.

Big Clinical Data Challenges

- Population Health Data is great, but drug outcomes remain a limitation
  - Particularly for quantifiable outcome data on specific outcomes (e.g., degree of cardiotoxicity induced by anthracyclines)
  - If such data can be linked, which data?
    - Pediatric echocardiography is done at baseline and throughout therapy
    - Test results bounce around
      - measurement error?
      - Measured too close to anthracycline dose?

ADR Case Definitions

- Critical *a priori* need
- CTCAE definitions are rarely quantitative enough to use without modification
- Definition develops as data are collected and plan for analysis is refined
- Modifications to case definition are always needed over time as more data become available and more research is published

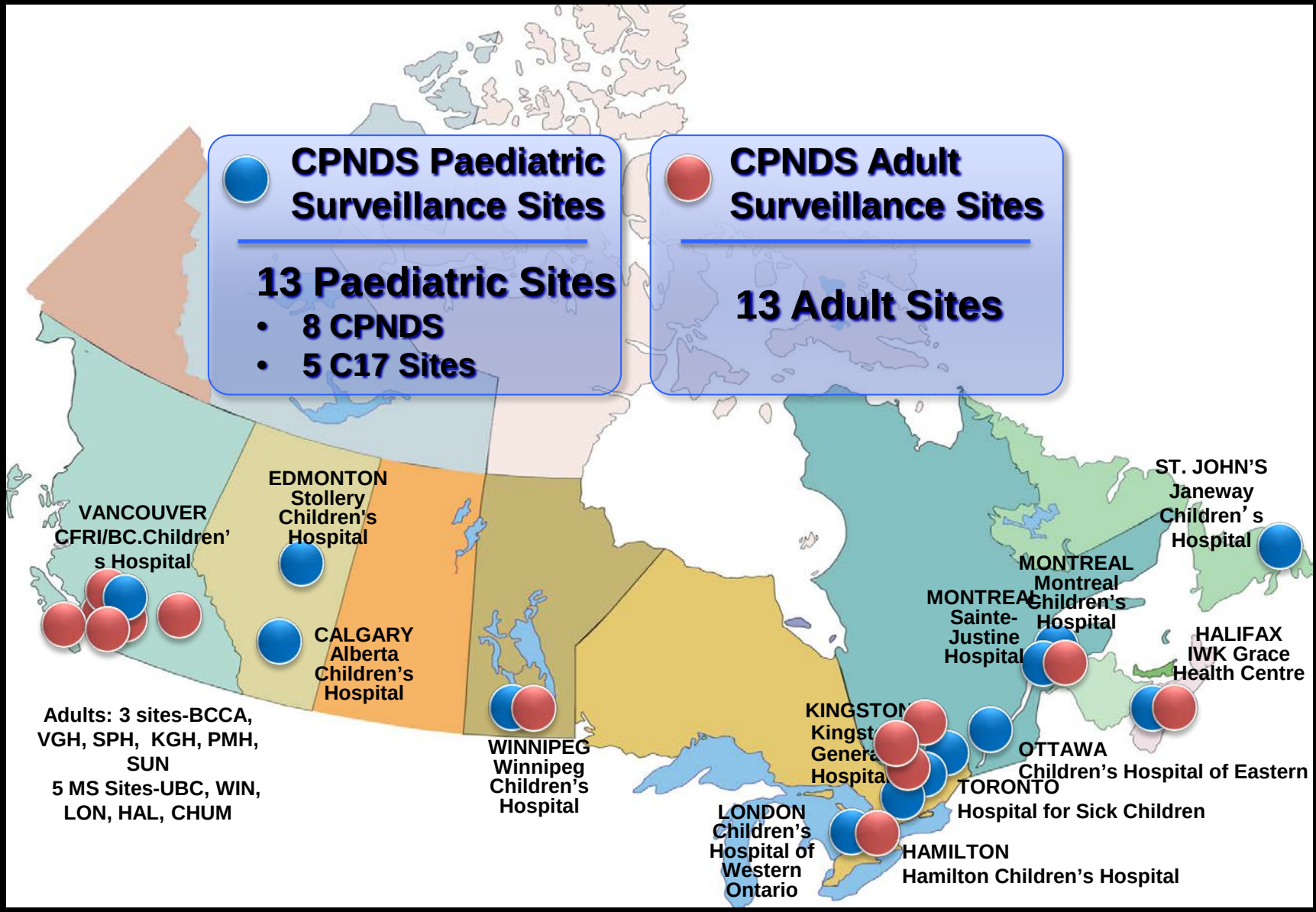
Pharmacoepidemiology Big Data Methods

- Good at describing and dealing with limitations in the data
- Another approach is to go into the clinical data itself and define how best to address limitations
  - Sometimes best approach is to collect more data prospectively such that temporal relation between drug and outcome is better understood
  - Required data can be hidden in the clinical record where it is not expected

Canadian Pharmacogenomics Network for Drug Safety (CPNDS)

- Established & co-founded in 2004 by Bruce Carleton first as GATC, then CPNDS
- Pan-Canadian network with clinical surveillance and research personnel located at 13 pediatric and 13 adult hospitals and clinics across Canada
- Collects detailed information on ADRs from medical records and patients/families, other sources
- Purpose-built to find high-association pharmacogenomic biomarkers, create innovative tools (pharmacogenomic tests) to predict the likelihood of ADR risk and implement drug-safety solution strategies

CPNDS Network in Canada



How are Targeted ADRs identified?

- Targeted surveillance for ADRs of interest to member institutions and Network Executive Steering Committee
- Standardized case definitions
- Complete data; clinician surveillors are paid by the Network but work under contract to the Network at local sites

CPNDS ACTIVE Surveillance

- Responsive to local needs
- No local funding, despite my efforts and the alarming number of ADRs of clinical relevance
- Best way to determine ADR causation is to witness it or find temporal relations that can be further explored (e.g., ECGs before/after drug administration in two unlabeled populations receiving ondansetron)

Surveillance Tools

- Clinical Characterization System Development: Case Definitions
  - serious skin rashes (SJS/TEN, HSS) – data collection form
  - nephrotoxicity (cisplatin)
  - pancreatitis
  - thrombosis
  - hepatotoxicity (valproic acid)
- Clinical Characterization Quality Assurance
- Site quarterly reporting
- Training Logs: Site visitation and training

Canadian Pharmacogenomics Network for Drug Safety

Standardized data collection

Rash

Morphology:
- Typical targets ☐
- Raised atypical targets ☐
- Flat atypical targets ☐
- Macules with/without blisters ☐
- Erythema ☐
- Other ☐

Description:

% BSA affected:

% BSA skin detachment:

Duration of eruption:

Photographs: Yes ☐ No ☐

Mucous membrane involvement Yes ☐ No ☐

Number of sites affected:

Location:

Fever Yes ☐ No ☐

Peak temperature:

Time of onset:

Lymphadenopathy Yes ☐ No ☐

Number of sites affected:

Location:

Diagnostics

Blood count: Yes ☐ No ☐
Result:

Liver function test Yes ☐ No ☐
Result:

Renal function test Yes ☐ No ☐
Result:

Dermatology consult Yes ☐ No ☐
Result:

Skin biopsy Yes ☐ No ☐
Result:

Other: Result:

Other organ manifestations

Lung: Yes ☐ No ☐
Description:

CNS: Yes ☐ No ☐
Description:

Heart: Yes ☐ No ☐
Description:

Muscle: Yes ☐ No ☐
Description:

GI tract: Yes ☐ No ☐
Description:

Thyroid: Yes ☐ No ☐
Description:

Infections/Virus reactivation

HIV Yes ☐ No ☐ Not assessed ☐

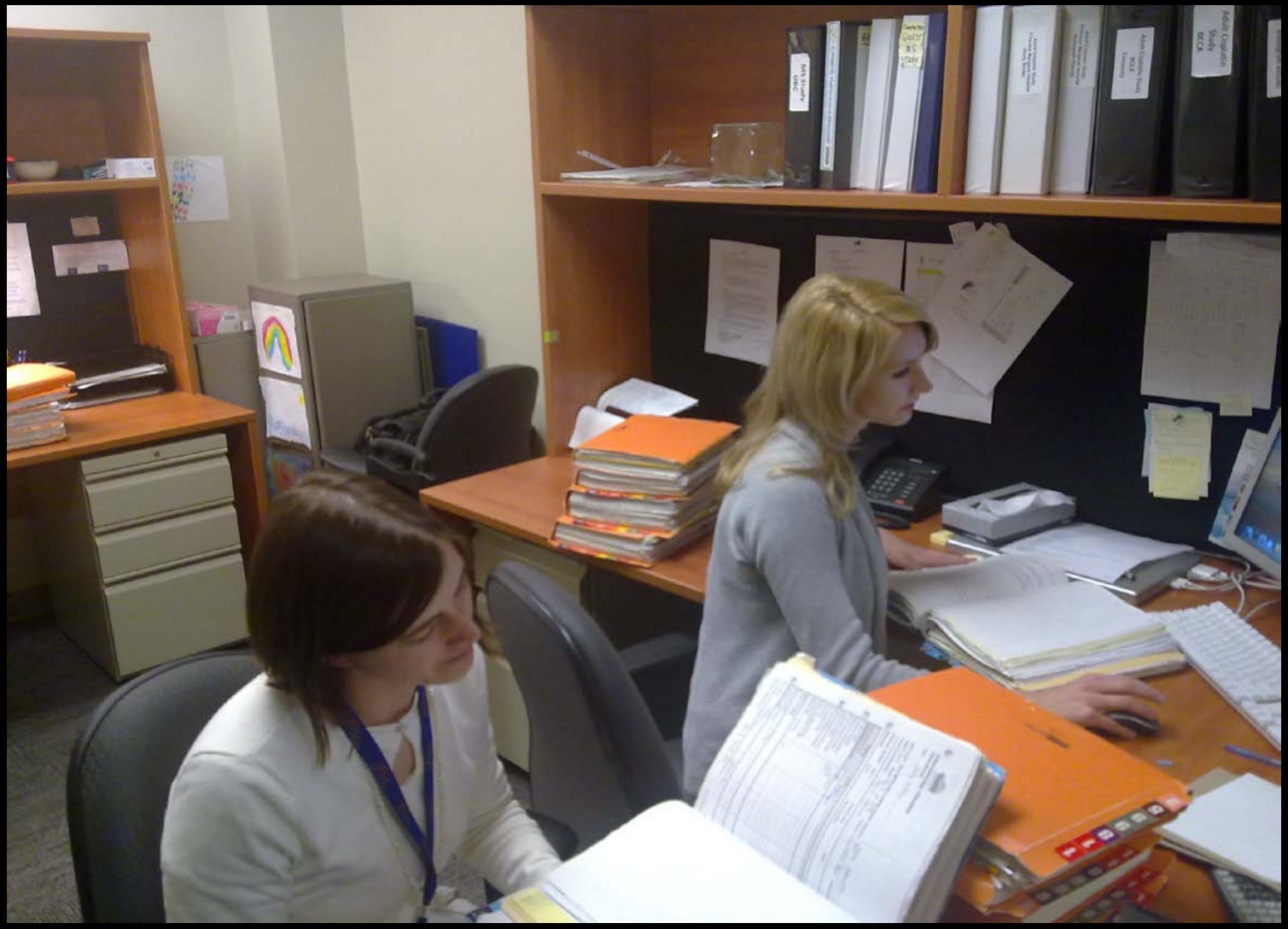
HHV-6 Yes ☐ No ☐ Not assessed ☐

Mycoplasma pneumoniae Yes ☐ No ☐ Not assessed ☐

Other:

Canadian Pharmacogenomics Network for Drug Safety

Could take 4-5 hours, or up to 4-5 days to complete clinical characterizations



DNA Information

	Sample collected	Collection method ?	Date sent to CMMT ?	Courier tracking/bill of lading #?
Patient		○ Blood ◉ Saliva ○ Buccal Swab	21/02/2012 21-Feb-2012	
Mother	◉ Yes ○ No	○ Blood ◉ Saliva ○ Buccal Swab	21/02/2012 21-Feb-2012	
Father	○ Yes ◉ No	○ Blood ○ Saliva ○ Buccal Swab	DD-MM-YYYY	

Patient Information

1.1 Date of birth **25-05-1998** 25-May-1998 Age at time of enrolment **13.7** years

1.2 Height inches **130.8** cm Body Surface Area **0.93** m^2

1.3 Weight lbs **24** kg

1.4 Country of Ancestry ?

Patient **Ire/Germ/Eng**

Mother **Ireland/German** Maternal grandmother **Ireland** Maternal grandfather **Germany**

Father **Germany/Englan** Paternal grandmother **Germany** Paternal grandfather **England**

1.5 Sex ◉ Male ○ Female ○ Unknown

Notes

Diagnosed with high risk T-cell acute lymphoblastic leukemia in December 2006
Protocol AALL0434, Arm C (December 2006 to September 2008)
Modified Protocol 0232 (September to November 2008)
Protocol BMT ASCT0431 (December 2008 to January 2009)

Vincristine given: Total cumulative dose: 51mg/m2
Anthracyclines given: Total cumulative dose: 275mg/m2
Radiation given: Total body radiation, 1200cGy (December 2008)
Cranial radiation, 1200cGy (September 2007)

6.1 Generic Name **tobramycin**

Dose ? **35-40 mg q8h** Total daily dose ?

Dose/kg ? Combination Product ? ○Yes ◉No (If "Yes" press: +) **Switch**

Route used ○Oral ◉IV ○IM ○SC ○Other:

Therapy Dates: ? from **09/11/2009** 09-Nov-2009 to **11/02/2010** 11-Feb-2010 Duration **94 days**

Indication ?

Brand Name ? Manufacturer ?

Therapeutic Class ? **antibiotic-aminoglycoside**

Notes ? **Intermittent: 09/11/09-11/11/09, 11/12/09-21/12/09, 27/01/10-11/02/10**

6.1 Generic Name **vancomycin**

Dose ? **150-200 mg q6-8h** Total daily dose ?

Dose/kg ? Combination Product ? ○Yes ◉No (If "Yes" press: +) **Switch**

Route used ○Oral ◉IV ○IM ○SC ○Other:

Therapy Dates: ? from **29/01/2010** 29-Jan-2010 to **13/08/2010** 13-Aug-2010 Duration **196 days**

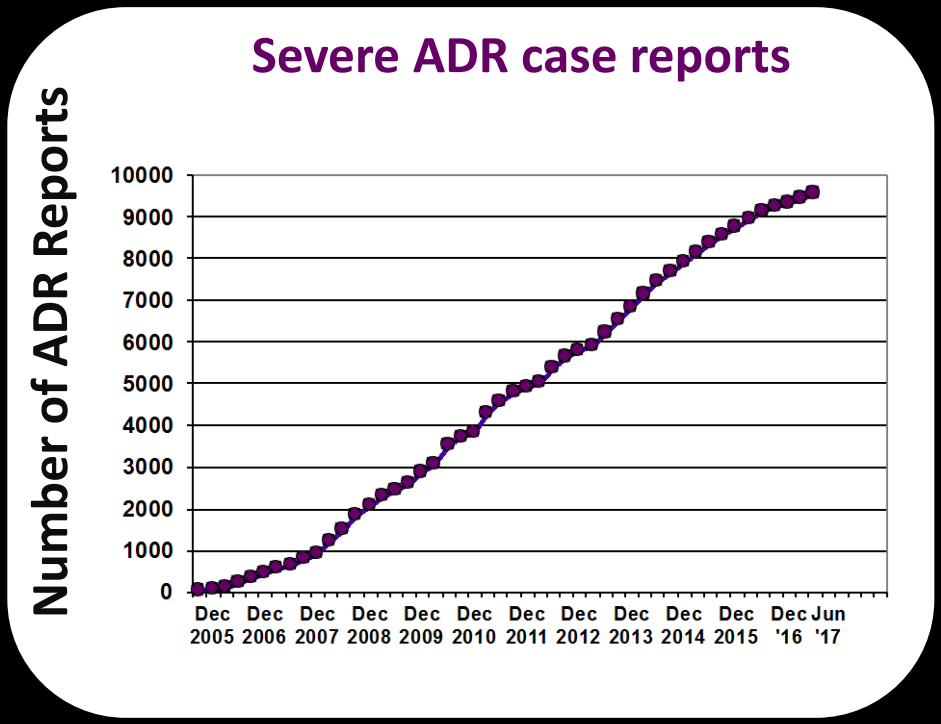
Indication ?

Brand Name ? Manufacturer ?

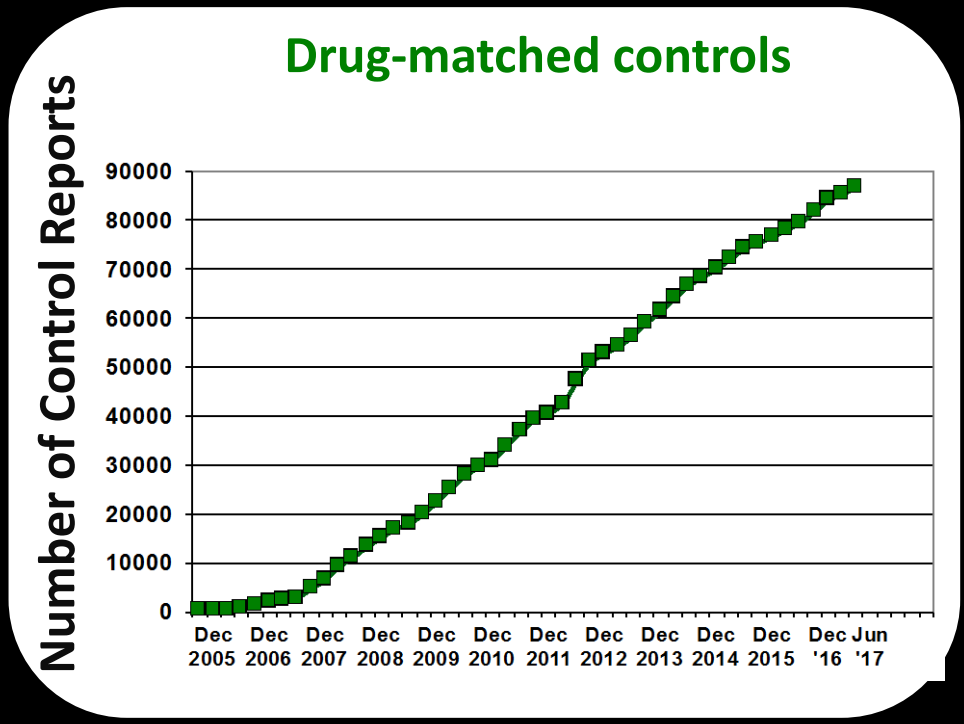
Therapeutic Class ? **antibiotic**

Notes ? **Intermittent: 29/01/10-10/02/10, 21/04/10-23/04/10, 18/05/10-22/05/10, 11/08/10 -13/08/10**

Recruitment of ADR cases and drug-matched controls in Canada



9,537
ADR case reports

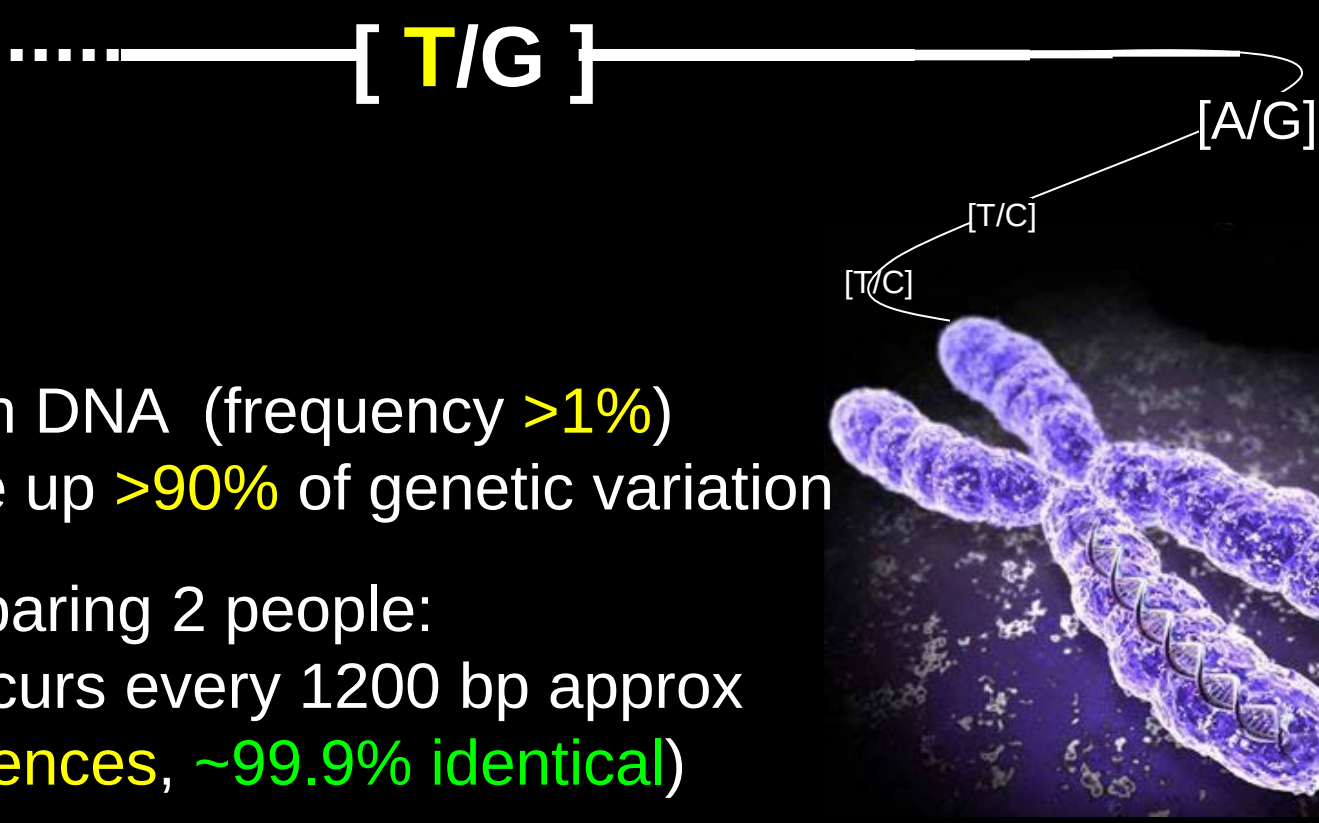


86,818
Drug-matched controls

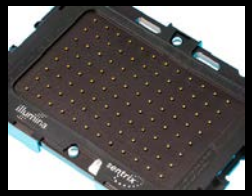
Human Genome: ~3 billion nucleotides.
Typed out 1 per mm = 3,000 km long

Human Genome: ~3 billion nucleotides.
Typed out 1 per mm = 3,000 km long x 2 copies

Single Nucleotide Polymorphisms (SNP)



- Variations in DNA (frequency >1%)
- SNPs make up >90% of genetic variation
- When comparing 2 people:
  1 SNP occurs every 1200 bp approx
  (= 5 differences, ~99.9% identical)
- More than 15 Million known SNPs
- SNPs can alter the amino acid sequence of the encoded protein as well as alter RNA splicing and transcription
- New technology can test > 24 million SNPs per day



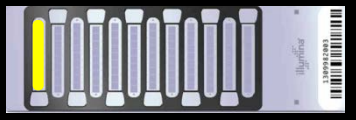
Illumina Sentrix™ Array Matrix

ADME/Tox Genes SNP Arrays

Gene Classification	Examples
Phase I Metabolizing Enzymes	CYP1A1, CYP2B6, ALDH2
Phase II Metabolizing Enzymes	UGT2B7, GSTM1, NAT1, COMT
Receptors / Drug Targets	VDR, PPARG, CETP
Transporters	ABCB1, ABCC1, ABCC2
Transcription factors	HNF4A, STAT3, NR1I2
Immunity	HLA variants
Ion Channels	SCN5A, KCNH2, KCNQ1
Others	EPHX1, FMO1, PTGS1

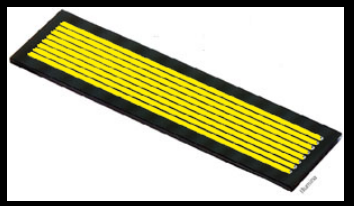
Versions:

Initial: 2k ADME SNP panel *(220 genes)*



Phase II: 4.6k ADME *(300 genes)*
or 1.2M genome-wide scan

Current: 10k ADME & 2.5-5M+ arrays
Exome and genome sequencing



CPNDS Biomarker Discovery Strategy

1. Identify children with ADRs & matched controls

2. Collect DNA samples (blood/ saliva)

3. Detailed patient clinical character-ization

4. Screen genetic variants

5. Replication

ADR cases

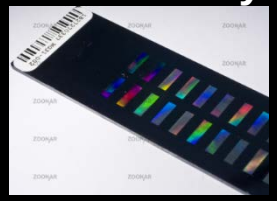
Matched controls

Patient blood/ saliva

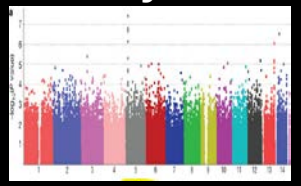
Patient charts

Clinical data

Custom ADME Array



Statistical Analyses



ADR cases & controls

Assay DNA samples

Statistical Analyses

What Data are Missing?

- A lot
  - QoL impacts, longitudinal outcomes
  - Especially in pediatrics
    - Outcomes should be measured in yrs, not months
- Systems Pharmacology is needed
  - Networks of interactions
    - Drug-protein, protein-protein, cell signaling
    - Physiological (at cellular, tissue, organ and whole body levels)
- Even bigger data are needed!

If the Purpose of Surveillance is to Improve Patient Care…

- Buy-in from clinicians is critical for quantity AND quality of data submitted
- Surveillors need to know HOW the data are being used to improve reporting details
- Detailed reporting can fill in missing gaps from epidemiological databases
- Active surveillance can help confirm epidemiological findings such that practice change is more likely to occur

Small Data Solutions for Big Data

- Active surveillance both retrospective and prospective to ensure proper granularity of data is captured
- Directed by relevant public health needs

- These two things address data limitations
  - *Get whatever data you desire or need*

Case Report

- A previously healthy 10-year-old child presented with neuroblastoma to B.C. Children's Hospital
- Began doxorubicin chemotherapy
- Prior to last cycle of treatment, child became unwell during a routine CT scan at BC Children's Hospital
  - Intubated and rushed to ICU
  - **Developed serious cardiac dysfunction, virtually no cardiac output**
  - **Child placed on extracorporeal membrane oxygenation (ECMO)** ***(heart-lung machine)***
  - **Child received a heart transplant**
  - **First transplanted heart rejected**
  - **Child received a second heart transplant**
- Child is currently cancer remission

Anthracycline-induced Cardiotoxicity

- Most important risk factor is high cumulative dose
- However there is no absolute safe dose
- Large inter-individual variability suggests genetic susceptibility

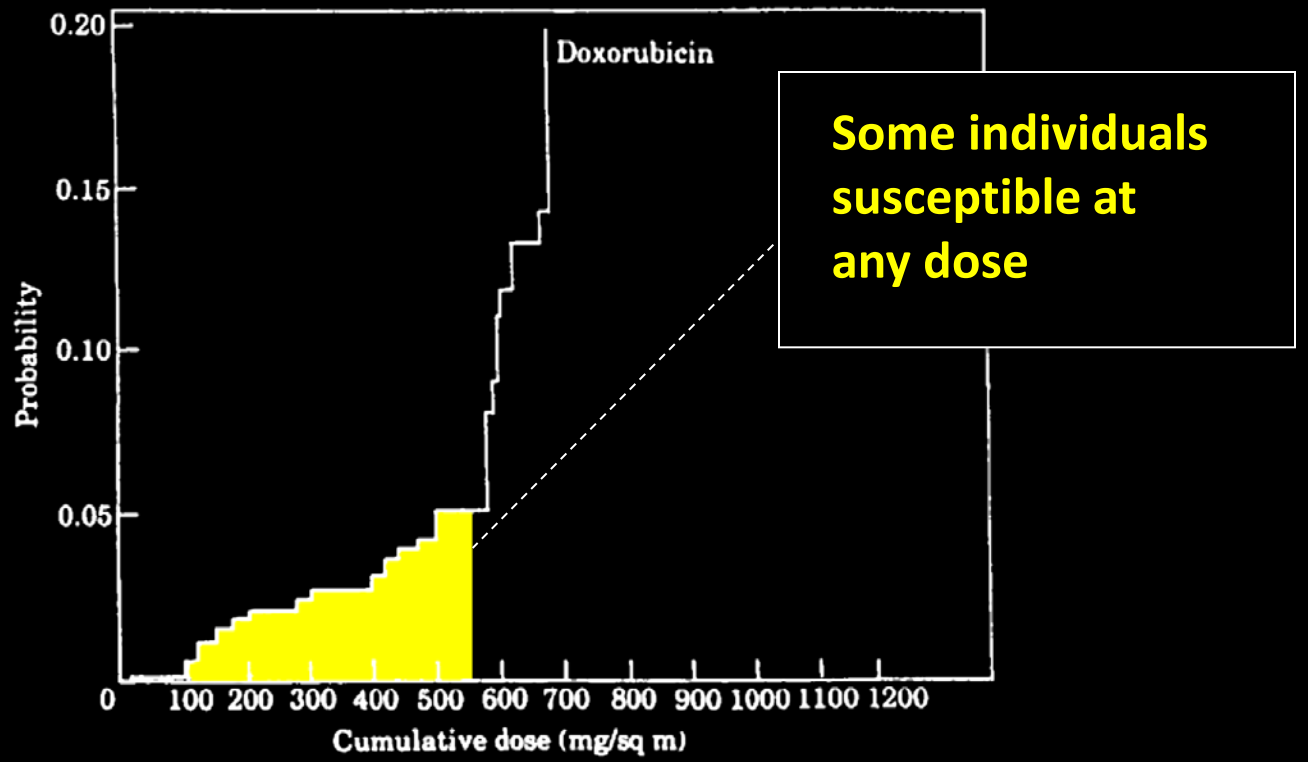


Figure adopted from: Launchbury & Habboubi. *Cancer Treat Rev*. 1993;19(3):197-228

Wouters et al. *Br J Haematol.* 2005;131(5):561-78
Lipshultz et al. *Heart*. 2008;94(4):525-33

Classification of Anthracycline-Cardiotoxicity

Controls n=266

- **No cardiotoxicity, SF ≥30%, ≥5yr follow-up**

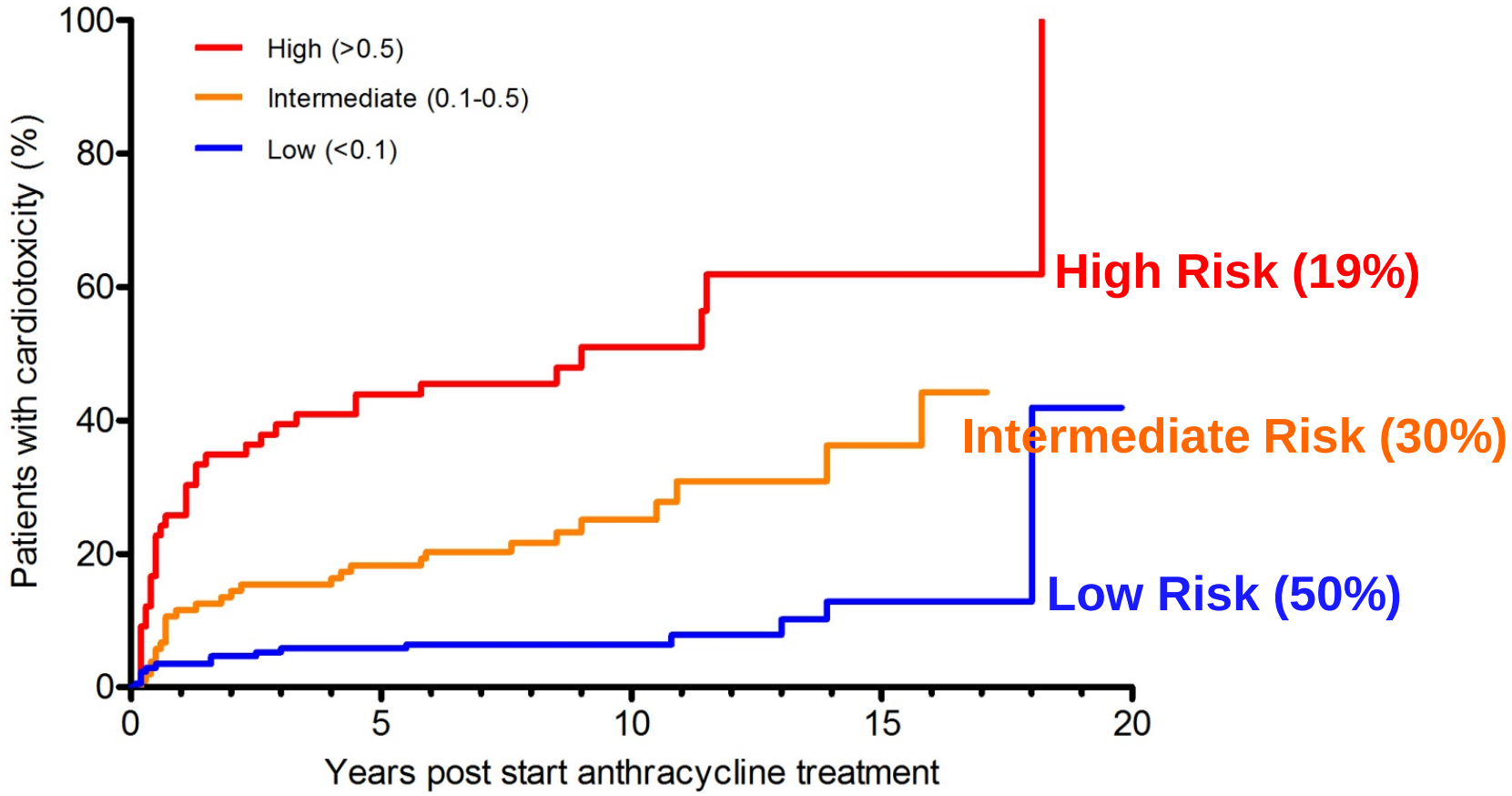
- **Grade 1 toxicity:**
  - Shortening fraction 27-30% or
  - Resting ejection fraction 50-60%

ADR Cases n=78

- **Grade 2 toxicity: Moderate to severe cardiotoxicity**
  - Shortening fraction < 15% or Shortening fraction 15-26%
  - or resting ejection fraction 40-50%
- **Grade 3 toxicity: Symptomatic congestive heart failure**
  - Shortening fraction < 15% or
  - Resting ejection fraction < 40%
- **Grade 4 toxicity: Congestive heart failure requiring heart transplant or ventricular assist device**
  - Resting ejection fraction < 20%

Modified National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 *With modified Grade 1 from 24-30% SF to 27-30% SF*

SLC28A3 + *UGT1A6* + Clinical Variables for Risk Prediction of Anthracycline Cardiotoxicity



Cdn Cohorts

ROC: AUC (SNPs + Clinical) = 0.76

1ST GWAS of Anthracycline Cardiotoxicity uncovers *RARG*

Stage 1& 2 – Discovery & Replication, European Patients

Canada 280 patients → The Netherlands 96 patients → Combined 376 patients

Gene	Variant	O.R.	P-value	O.R.	P-value	O.R.	P-value
RARG	rs2229774	6.0	4.1×10^{-8}	4.1	0.0043	4.9	1.2×10^{-9}

Stage 3 – Replication, Worldwide:

(N = 80; 19 cases, 61 controls)

Variant	O.R.	-value
rs2229774	> 6	0.00012

	Africans 11 patients		Hispanics 23 patients		First Nations 15 patients		East Asians 31 patients	
Variant	O.R.	P-value	O.R.	P-value	O.R.	P-value	O.R.	P-value
rs2229774	9.5	0.026	12.3	0.052	9.9	0.012	5.9	0.085

Novel Biomarker in Adult Patients

Adult Cancer Patients from BCCA, VGH and SPH
N = 73 patients: 41 cases and 32 drug-matched controls

Gene	Variant	O.R.	P-value
RARG	rs2229774	11.0	0.0064

Genetic Biomarker					Logistic Regression Analysis (Additive Model)			
					Without Covariates		Adjusting for Dose	
Gene	Variant	Function	MAF Cases	MAF Controls	P	Odds Ratio (95%CI)	P	Odds Ratio (95%CI)
RARG	rs2229774	NON-SYN CODING (S427L)	0.073	0	0.0067	$1.5 \times 10^{+16}$	0.0064	$1.7 \times 10^{+16}$

Manuscript in Preparation

nature genetics

A coding variant in *RARG* confers susceptibility to anthracycline-induced cardiotoxicity in childhood cancer

Folefac Aminkeng[1,2,13], Amit P Bhavsar[2,3,13], Henk Visscher[1,4], Shahrad R Rassekh[2,5], Yuliang Li[2,3], Jenny W Lee[2], Liam R Brunham[6], Huib N Caron[7], Elvira C van Dalen[7], Leontien C Kremer[7], Helena J van der Pal[7,8], Ursula Amstutz[2,3,12], Michael J Rieder[9], Daniel Bernstein[10], Bruce C Carleton[2,3,11,14], Michael R Hayden[1,2,6,14], Colin J D Ross[1–3,11,14] & The Canadian Pharmacogenomics Network for Drug Safety Consortium[15]

Aminkeng et al., *In Press*, 2015

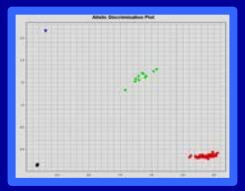
Personalized Medicine Program (PMP):

Implementation of a Pharmacogenomic ADR Prevention Program in British Columbia

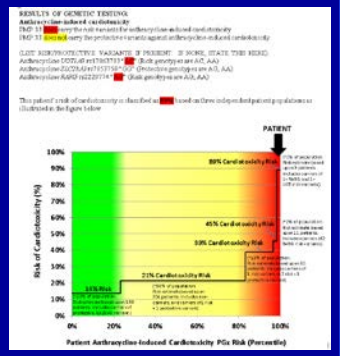
- ADRs: Cisplatin-induced ototoxicity
  Anthracycline-induced cardiotoxicity
- Sites: BC Children's Hospital, BCCA, and VGH

CPGs Prepared → **Tests Developed** → **Patients Tested** → **Results Delivered** → **Ongoing Follow-up**

Results in 8 hrs

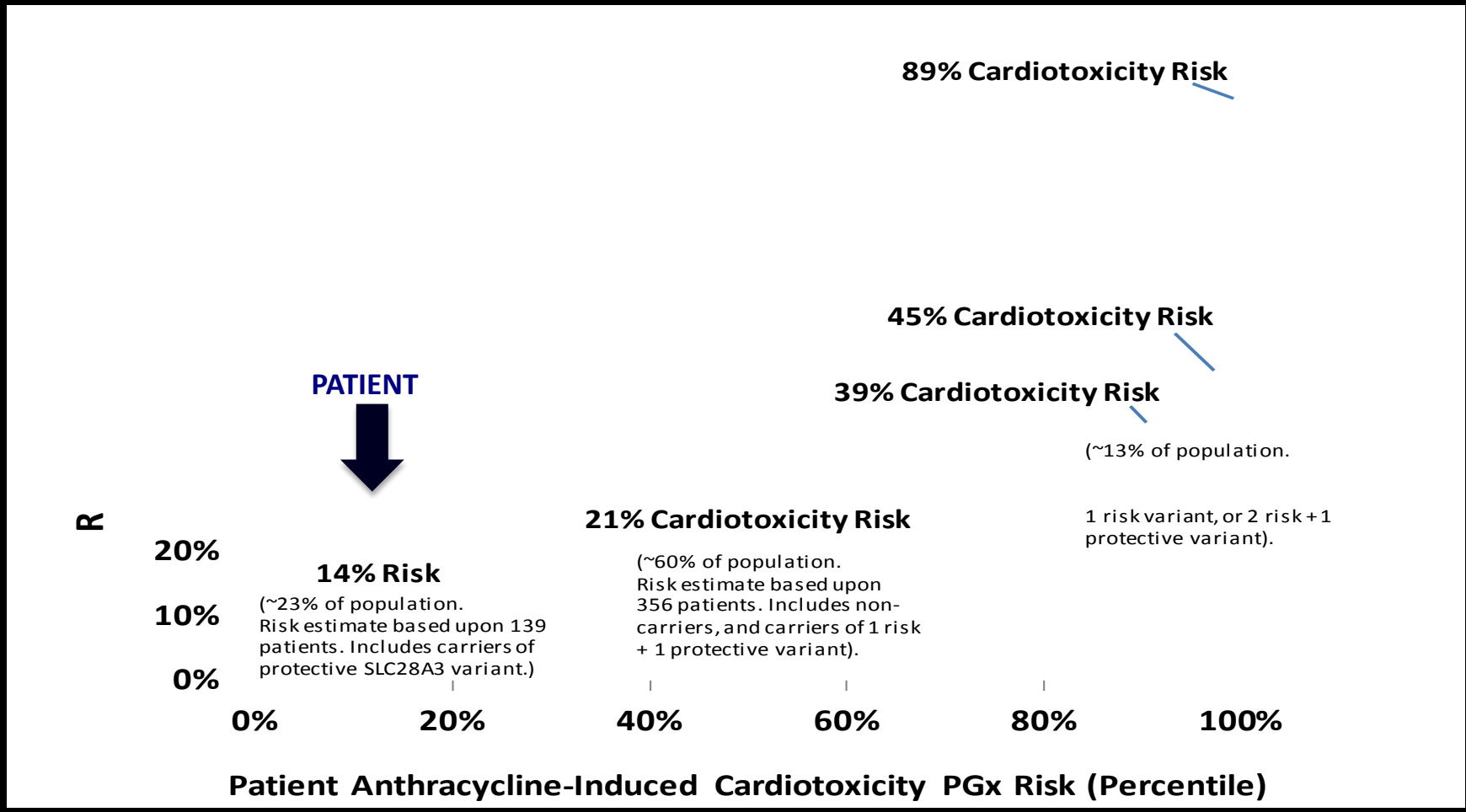


BCCH, VGH, BCCA



- Education
- Interviews
- Workshops
- Focus Groups
- Cost-effectiveness

Pediatric Anthracycline Cardiotoxicity Risk Prediction Tool



Potential Clinical Options for Personalized Anthracycline Therapy

Depending on risk prediction, clinician could take different actions:

Low Risk

- Echocardiogram follow-up as usual

Intermediate Risk

- Intensify echocardiogram follow-up

  e.g. patients in rural centres often miss appointments

High Risk

- Alternative medication or dose
- Add cardioprotectant (e.g. dexrazoxane)
- Start treatment with ACE-inhibitors or beta-blockers to prevent further damage

Functional Validation of Pharmacogenetic Biomarkers

PLOS ONE

RESEARCH ARTICLE

Pharmacogenetic variants in *TPMT* alter cellular responses to cisplatin in inner ear cell lines

Amit P. Bhavsar[1,2¤], Erandika P. Gunaretnam[1,2,3], Yuling Li[2,3], Jafar S. Hasbullah[2,4], Bruce C. Carleton[2,3], Colin J. D. Ross[1,2*]

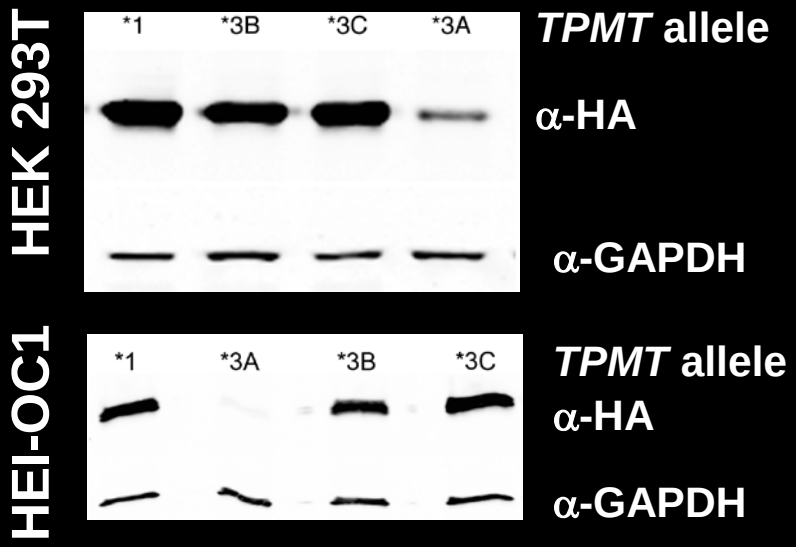
Aim: Explore the impact of pharmacogenetic variants in *TPMT* on cellular responses to cisplatin

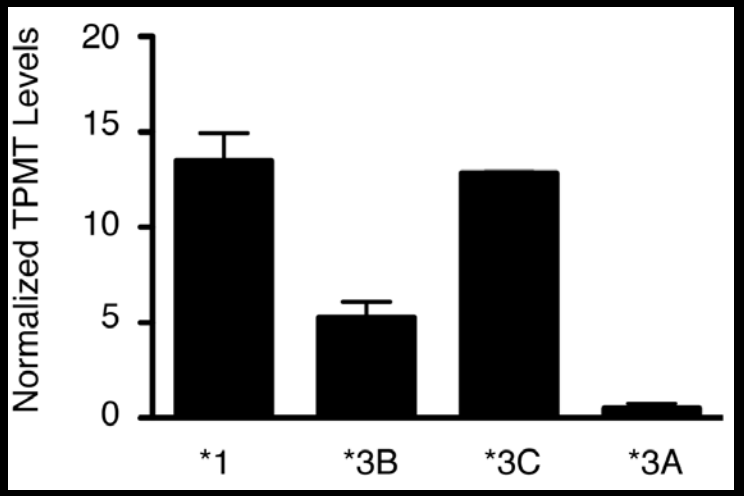
Approach:

1. Express *TPMT* variants in murine inner ear cell lines (HEI-OC1 and UB/OC-1)

2. Monitor the impact of *TPMT* variants on cisplatin response in these cell lines by measuring:
   - Cytotoxicity (MTT assay)
   - Activation of a sensitive cisplatin-response gene (*TLR4*)

Results: TPMT variants expressed in cells, and as expected, TPMT*3A is unstable in cell culture





Western blot of HA-epitope tagged TPMT constructs:

*3B (Ala154Thr)

*3C (Tyr240Cys)

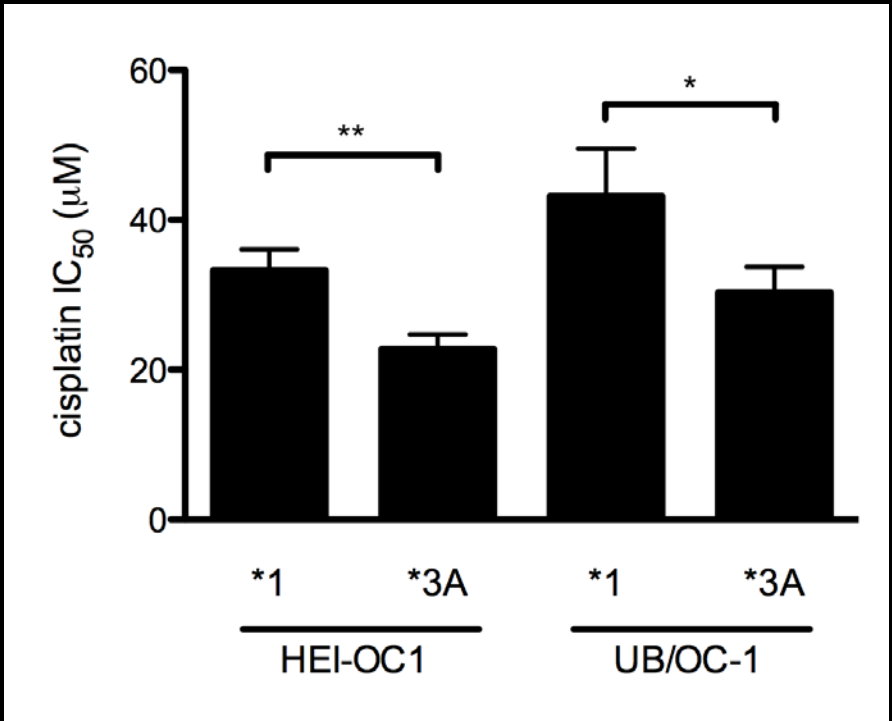
*3A (Ala154Thr, Tyr240Cys)

- TPMT*3A is especially unstable

Normalized protein expression

- Reduced protein levels of *3B and *3A

Results: TPMT*3A expression sensitizes cells to cisplatin cytotoxicity compared to *1 (wild-type)



		IC_{50} (95% CI)[a] (μM)	*P*	n	R^2
HEI-OC1	TPMT*1	33.27 (28.25 – 39.17)	**0.0022**	20	0.83
	TPMT*3A	22.79 (19.34 – 26.84)		20	0.82
UB/OC-1[b]	TPMT*1	43.18 (32.51 –57.35)	**0.0345**	12	0.86
	TPMT*3A	30.31 (24.33 – 37.75)		12	0.81

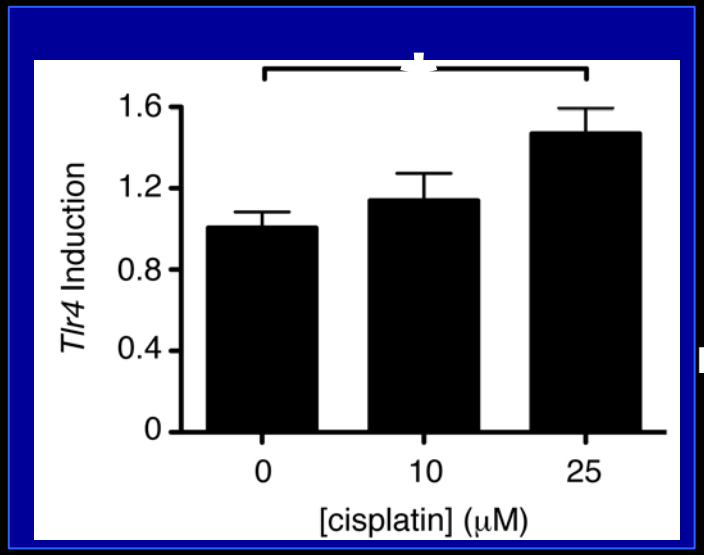
[a] 95% CI, 95% confidence interval

[b] endogenous *Tpmt* expression was silenced in these cells using siRNA

- **TPMT*3A-expressing cells have cellular phenotypes consistent with higher effective cisplatin concentrations**

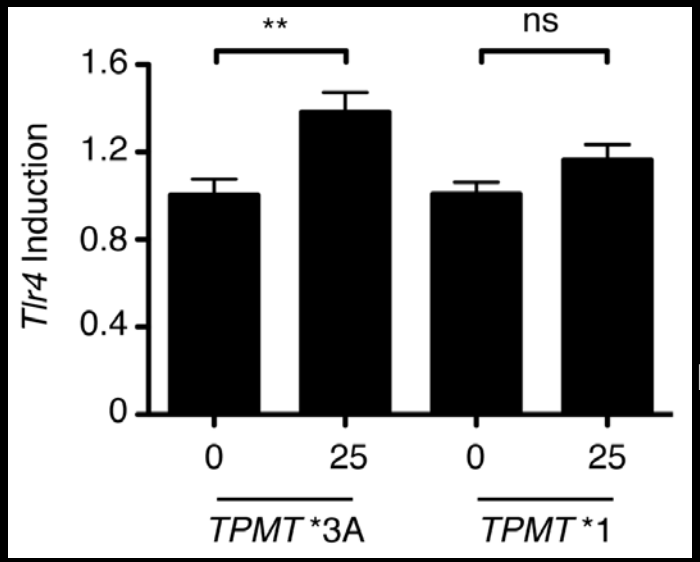
Results: TPMT *3A expressing cells exhibit a significantly greater response to cisplatin, as measured by TLR4, a sensitive marker of cisplatin-response

- TLR4 is a sensitive cisplatin biosensor:
  - *TLR4* expression is induced by increasing cisplatin concentrations



HEI-OC1 cells

- TPMT*3A-expressing cells exhibit significantly increased *TLR4*-response to cisplatin
  - Consistent with higher effective cisplatin concentrations in TPMT*3A expressing cells



HEI-OC1 cells

Cisplatin Functional Validation Summary

- **Multiple independent *in vitro* cisplatin phenotypes altered by genetic variations in *TPMT* gene**
- **Validates a cisplatin-TPMT drug-gene interaction**
- **Functionally validates the pharmacogenomic association between TPMT variants and cisplatin ototoxicity:**
  - ***TPMT**3A-expressing cells have cellular phenotypes consistent with higher effective cisplatin concentrations**
  - **Suggests TPMT is involved in cisplatin metabolism**
  - **We postulate that a nephrotoxic glutathione-derived cisplatin-thiol conjugate[1,2] could act as a TPMT substrate**

1. Townsend, D. M. *et al. J Am Soc Nephrol* 14, 1-10 (2003).
2. Zhang, L. & Hanigan, M. H. *J Pharmacol Exp Ther* 306, 988-994, (2003).


Patients & Clinicians
DISCOVERY
Clinical Characterization
Pharmacogenomics
Active Surveillance
Population Therapeutics
Pharmacoepi
VALIDATION
Evidence-based policy recommendations
Systematic Reviews
Functional Validation, PK
Replication
TRANSLATION
Knowledge Dissemination
Uptake in clinical practice
Technology Development
Communication strategies
CPGs
SUSTAINABILITY
Predictive testing
Industry partnerships
Commercialization
Health Economics
Regulatory Authorities
Training

Concerns for the Future

- National and international networks are needed
  - Particularly in childhood or rare diseases
- No real funding options for sustained funding of international networks
  - Need longitudinal Big Data for outcomes, particularly in childhood cancer where late effects of drugs are an increasing concern

Canadian Pharmacogenomics Network for Drug Safety

At the Child & Family Research Institute
Children's & Women's Health Centre of British Columbia
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