Canadian Pharmacogenomics Network for Drug Safety

Pharmacogenomic Prediction of Anthracycline-induced Cardiotoxicity in Childhood Cancer

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The University of British Columbia

CHILD & FAMILY RESEARCH INSTITUTE

Canada Foundation for Innovation
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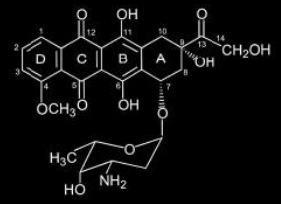
CIHR IRSC
Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

UBC

CMMT Centre for Molecular Medicine and Therapeutics

POPi Pharmaceutical Outcomes & Policy Innovations



Outline

- Background and Rationale
- *Aminkeng F et al, Nature Genetics 2015 Sep;47(9):1079-84*
- Importance of SNP & Variation Suite (SVS)

Background and Rationale

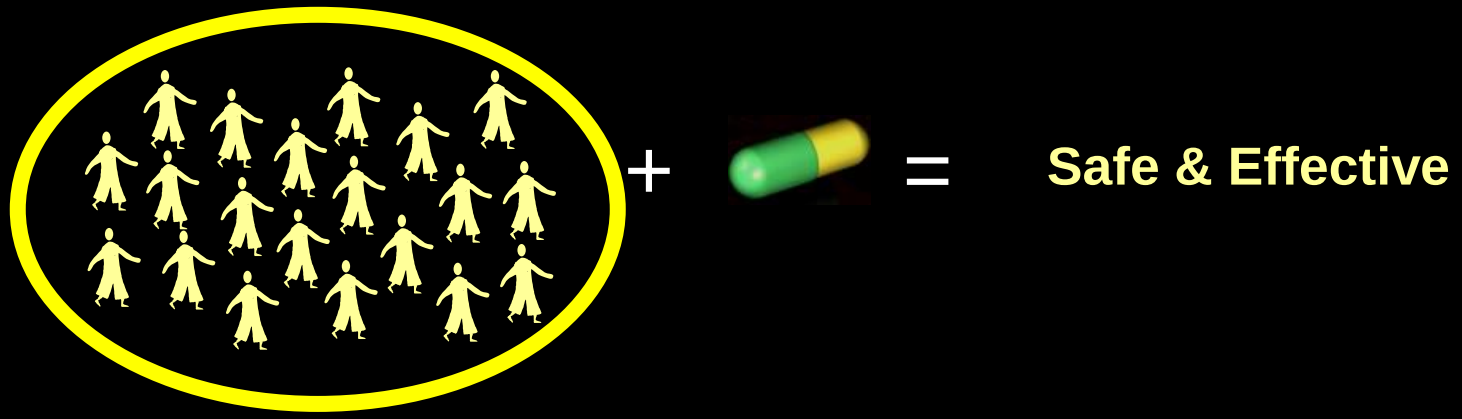
The Ideal Medication

Effectively treats or prevents disease

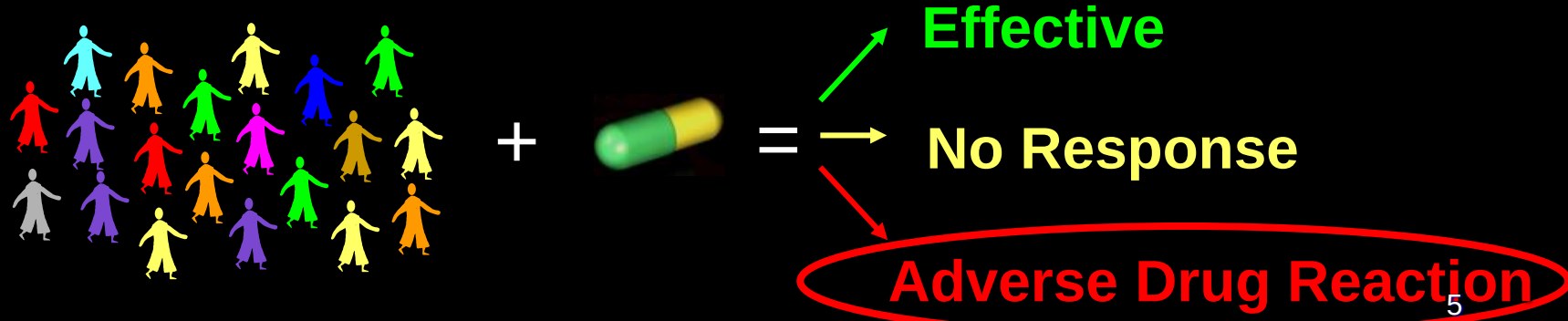
Has no adverse effects

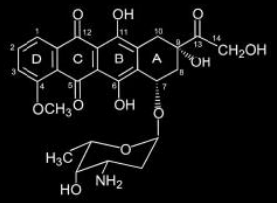
Paradox of Modern Drug Development

1. Clinical trials provide evidence of efficacy and safety at <u>usual doses</u> in ***populations***



2. Physicians treat ***individual*** patients who can <u>vary widely</u> in their response to drug therapy





Anthracyclines

- Doxorubicin, Daunorubicin, Idarubicin, Epirubicin, Valrubicin, Mitoxantrone
- Administered to 70% of all childhood cancer patients
- Adjuvant chemotherapy for 50-90% of breast cancer
  - 22,000 patients/year in Canada
- At least 970,000 patients receive it each year (N. America)

Highly effective

- Improved childhood cancer survival: from 30% in 1960s to >80% today
  - 1 in 750 young adults is childhood cancer survivor;

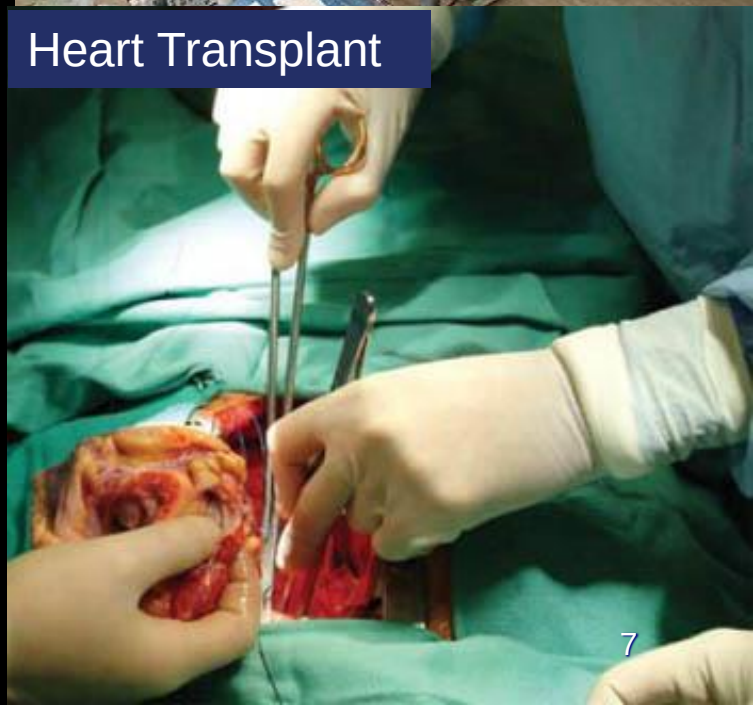
Kremer LC et al (2004), N Engl J Med 351: 120-121.
Lipshultz SE (2008) Heart 94:525-533.
Altekruse SF et al (eds): SEER Cancer Statistics Review, 1975-2007.

Anthracyclines-induced cardiotoxicity (ACT)

- 1 in 2 patients develop detectable cardiac abnormalities (57%)
- 1 in 5 patients suffer Congestive heart failure
- May require intra-ventricular assist device or heart transplant
- Increased risk in children, especially under 4 years old

ECMO Unit

Heart Transplant



Lefrak EA *et al*, (1973). Cancer 32: 302-314.
Lipshultz SE *et al*, (2008). Heart 94: 525-533.
Felker GM *et al*, (2000). N Engl J Med 342: 1077-1084.

Aminkeng F et al, Nature Genetics 2015 Sep;47(9):1079-84

Research Question

What are the genetic factors that modify the risk of ACT and can potentially inform treatment decisions?

Analysis Plan Implemented in SVS from Golden Helix

Step 1: Quality Control (Samples & SNPs) - Exclude poor quality data

Step 2: Discovery - Screening & Prioritisation of Candidates for follow-up

Step 3: Replications – Identification of associated gene region

Step 4: Fine mapping - To identify the causal variants within the region

Step 5: Biological Plausibility – Functional relevance of gene & variant

EXAMPLE FROM ONE OF OUR GWAS

Stage 1. Quality Control (Sample and SNP) using SVS

Before QC: Sample size = 434 patients and 740 000 variants

Detail QC Step takes place right after calling the genotypes

Samples

- Sample Call Rates
- Gender Misspecification Check
- Cryptic Relatedness Verification
- Population Stratification

SNP

- SNP call rates
- Minor allele frequency
- Hardy-weinberg Equilibrium

After QC: Sample size = 280 European ancestry patients and 657,694 variants

GWAS Work Flow Using SVS

Stage 1. Quality Control (Sample and SNP)

Stage 2. Screening and Prioritisation of Candidates for follow-up in an initial patient cohort

Genetic Association Analyses

- Genotype distribution
- Regression Analysis
- Multiple testing correction or select top candidates by *p*-value

SVS provides a variety of statistical tests to perform all these analysis which largely depends your data set and research question

Screened and Prioritized candidates variants in specific genomic regions for follow-up in an independent patient cohort

GWAS Work Flow Using SVS

Stage 1. Quality Control (Sample and SNP)

Stage 2. Screening and Prioritisation of Candidates for follow-up

3. Identifying associated gene regions using another patient cohort

Genetic Association Analyses

- Genotype distribution
- Regression Analysis
- May or may not implement multiple testing correction

SVS provides a variety of statistical tests to perform all these analysis which largely depends your data set and research question

We Identified a new genomic region

GWAS Work Flow Using SVS

Stage 1: Quality Control (Samples and SNPs) - Exclude poor quality data

Stage 2: Discovery - Screening and Prioritisation of Candidates for follow-up

Stage 3: Replication - Identifying associated gene regions

Stage 4: Fine mapping to identify the causal variants within the region

Imputation

- Available scripts for import and export of data for any of the imputation software programs
- Data analysis after imputation

Sequencing

- SVS can also analyzed sequence data

The causal or most important variant is studies for the mechanistic basis of the ADR phenotype

Patients (Children treated with Anthracyclines)

Stage 1 Discovery

Europeans from Canada
N = 280 patients: 32 cases & 248 controls

Stage 2 Replication

Europeans from The Netherlands
N = 96 patients: 22 cases & 74 controls

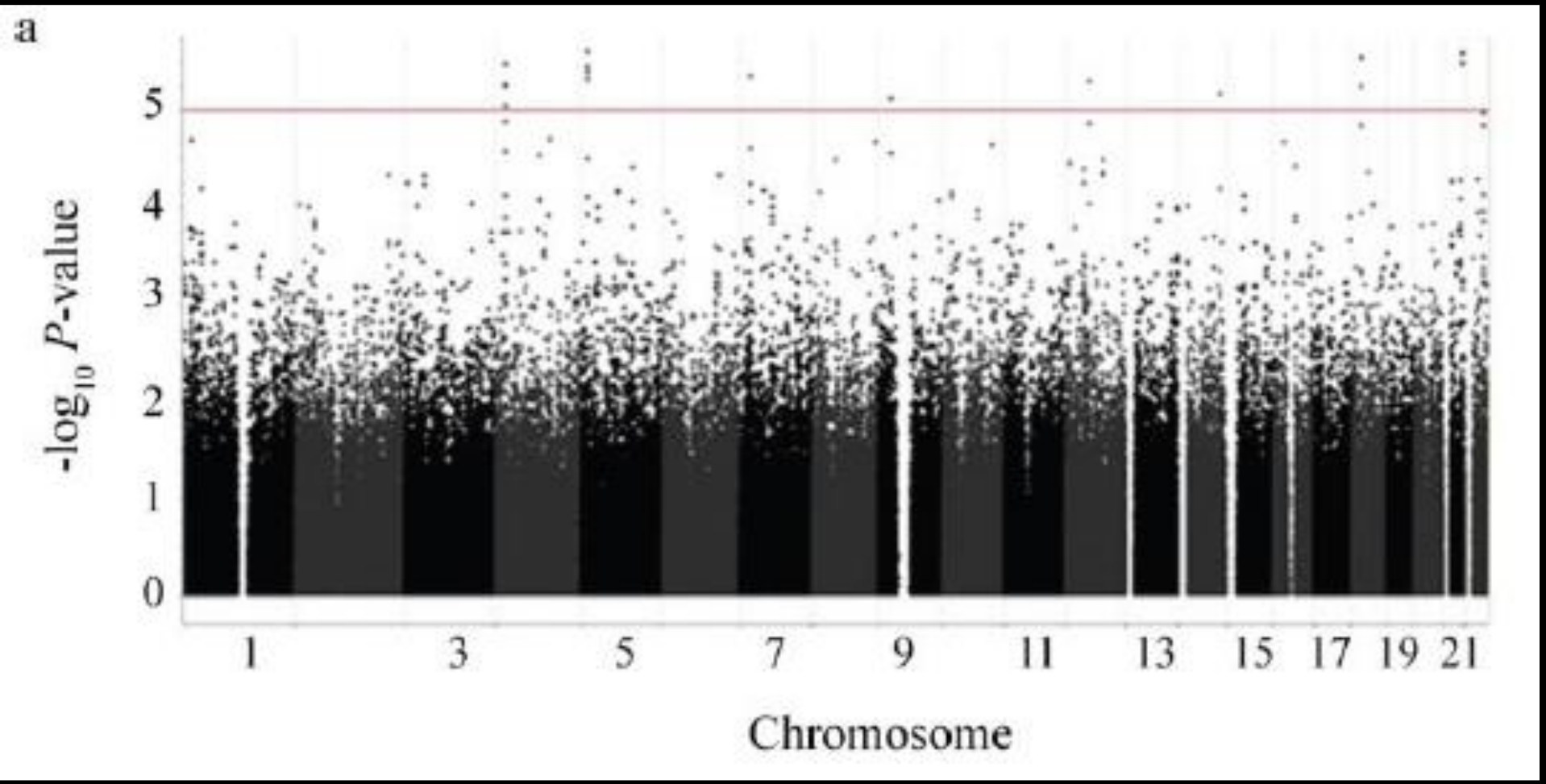
Stage 3 Replication

Africans	Latinos	A. Canadians	East Asians
11 patients	23 patients	15 patients	31 patients

Genotyping: Genome-wide Association Study

- GWAS examines genetic variation across the entire genome
- Unbiased and hypothesis-free
- Specifically target common variations
- Potential to discover novel genes, variants, pathways & inform drug development
- Illumina Infinium HumanOmniExpress assay (738,432 SNPs)
- First genome-wide study of anthracycline-induced cardiotoxicity

Genome-wide Association Study



We screened with statistical tests implemented in SVS and prioritized with *P* < 1.0E-05

GWAS Uncovered *RARG* as Novel Gene for Cardiotoxicity

Stage 1& 2 – Discovery & Replication, European Patients

Canada → The Netherlands → Combined

Gene	Variant	Canada O.R.	Canada *P*-value	The Netherlands O.R.	The Netherlands *P*-value	Combined O.R.	Combined *P*-value
RARG	**rs2229774**	**7.0**	**4.1E-08**	**4.1**	**0.0042**	**5.4**	**1.2E-09**

Aminkeng F et al, 2015. Nature Genetics Sep;47(9):1079-84

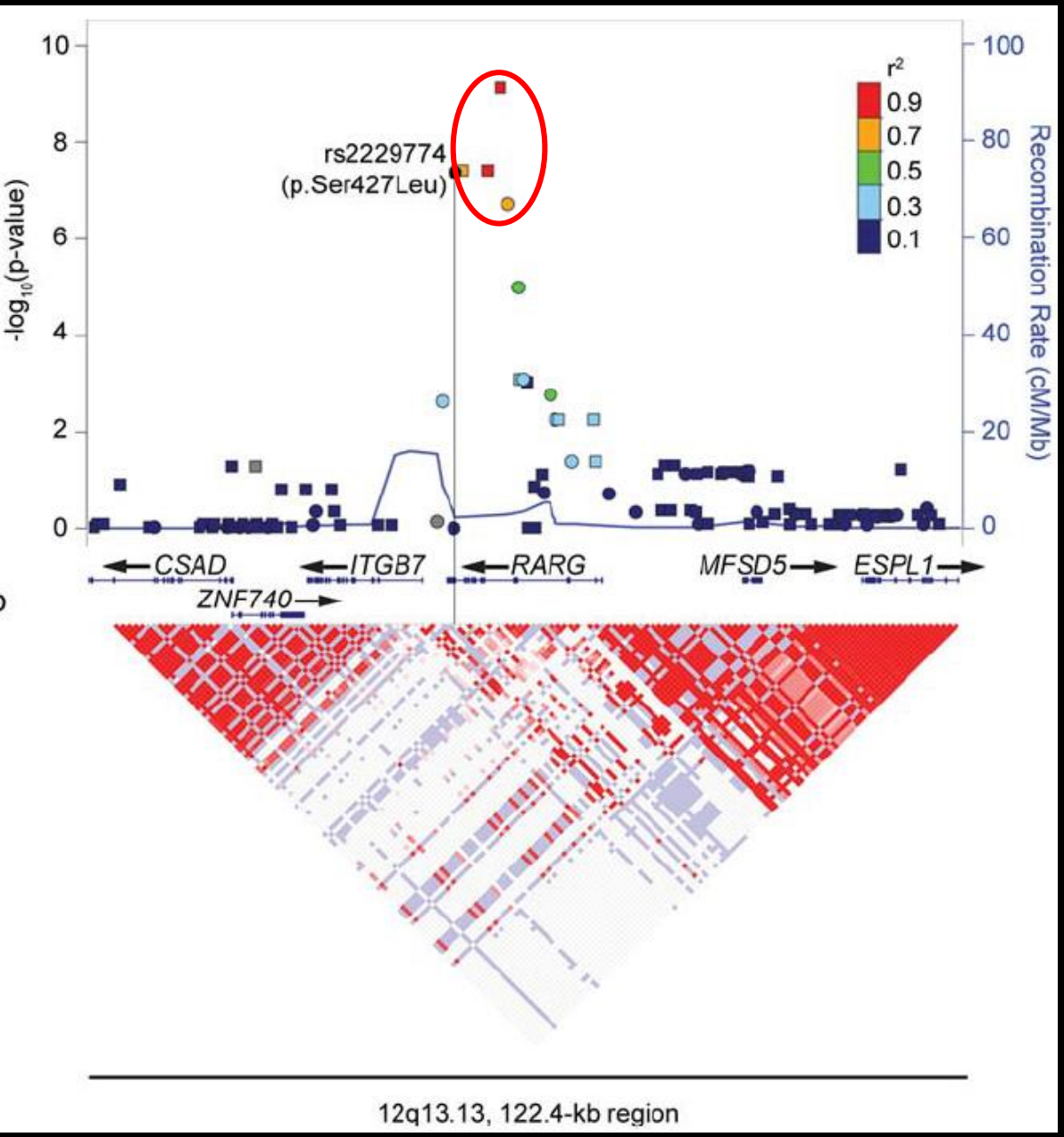
Stage 3 – Replication, Worldwide:

Variant	O.R.	*P*-value
rs2229774	**> 6**	**0.00012**

Africans | Hispanics | Aboriginal Canadians | East Asians

Variant	Africans O.R.	Africans *P*-value	Hispanics O.R.	Hispanics *P*-value	Aboriginal Canadians O.R.	Aboriginal Canadians *P*-value	East Asians O.R.	East Asians *P*-value
rs2229774	**9.5**	**0.026**	**12.3**	**0.052**	**9.9**	**0.012**	**5.9**	**0.085**

Fine Mapping and Haplotype Analysis

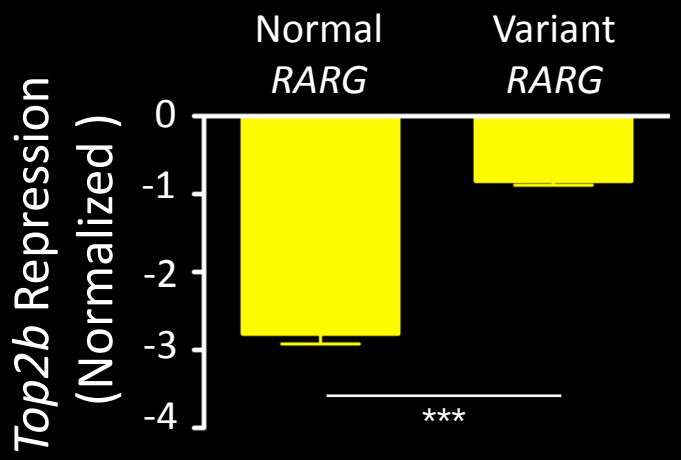


- GWAS and imputation identified putative **haplotype (5 SNP)** associated with ACT
- rs2229774 **(S427L)** only **coding** variant in haplotype

Aminkeng F et al, 2015. Nature Genetics Sep;47(9):1079-84

RARG regulates *TOP2B*, ACT causative gene

- We showed that *RARG* transcriptionally regulates *Top2b*
- *Top2b* critical to development of ACT *(Zhang et al. 2012 Nat Med 18:1639)*
- *RARG* variant impaired in *Top2b* regulation



n = 12
*** = $P < 0.0001$

Aminkeng F et al, 2015. Nature Genetics Sep;47(9):1079-84

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Summary of Main Findings

- Genetic Association: Novel gene (*RARG*), Novel variant (rs2229774) & Novel haplotype (5 SNPs) for ACT
- Functional Validation: *RARG* & rs2229774 regulates *Top2b* expression; *Top2b* - known ACT-susceptibility gene
- Conclusion: *RARG* rs2229774 is a novel pharmacogenetic biomarker & provides novel insight into the pathophysiology of ACT

Next Steps: Ongoing Projects

- Genetic Association (Drs. Carleton BC, Ross CJD and Aminkeng F): We are current studying the genetic association of *RARG* rs2229774 in adult breast cancer patients

- In vitro & in vivo functional studies (Drs. Ross CJD and Bhavsar AP): Mechanistic studies of *RARG* & rs2229774 are ongoing & will inform future drug development in the following ways:
  - Development of less heart failure prone cancer treatments
  - Development of more advance cardio protectants

- Patients Studies (Dr. Bernstein D): A collaboration with a Stanford based NIH project is studying the role of *RARG* & rs2229774 in cardiotoxicity using real world patient populations

Next Steps: Ongoing Projects

- Personalized Medicine Project (Drs. Carleton BC, Rassekh RS, Ross CJD): Pilot project on implementation of PGX Testing

What is currently done?

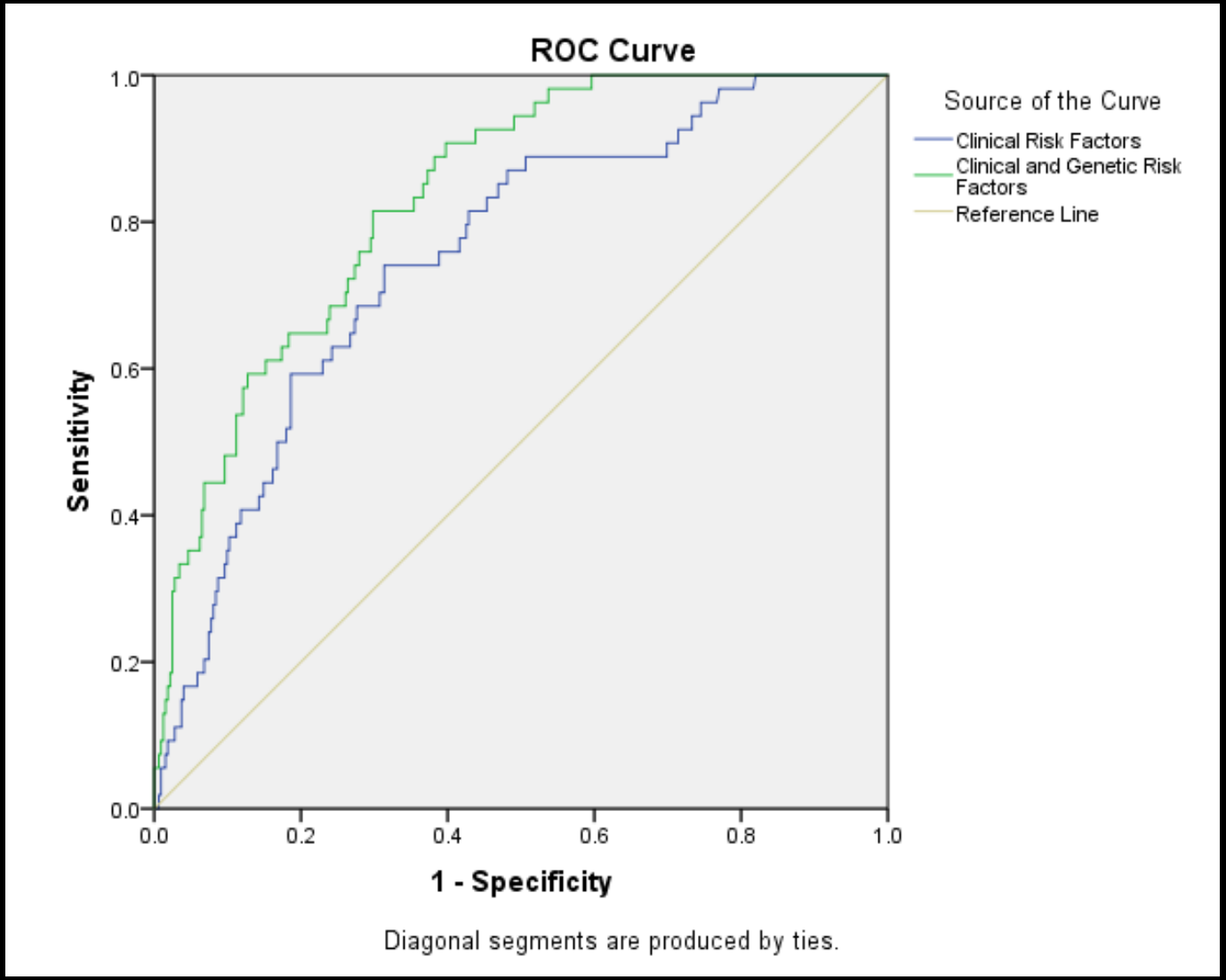
- Treatment decisions based on clinical risk factors

RECOMMENDED FREQUENCY OF ECHOCARDIOGRAM OR MUGA SCAN

Age at Treatment*	Radiation with Potential Impact to the Heart§	Anthracycline Dose†	Recommended Frequency
<1 year old	Yes	Any	Every year
	No	<200 mg/m²	Every 2 years
		≥200 mg/m²	Every year
1-4 years old	Yes	Any	Every year
	No	<100 mg/m²	Every 5 years
		≥100 to <300 mg/m²	Every 2 years
		≥300 mg/m²	Every year
≥5 years old	Yes	<300 mg/m²	Every 2 years
		≥300 mg/m²	Every year
	No	<200 mg/m²	Every 5 years
		≥200 to <300 mg/m²	Every 2 years
		≥300 mg/m²	Every year
Any age with decrease in serial function			Every year

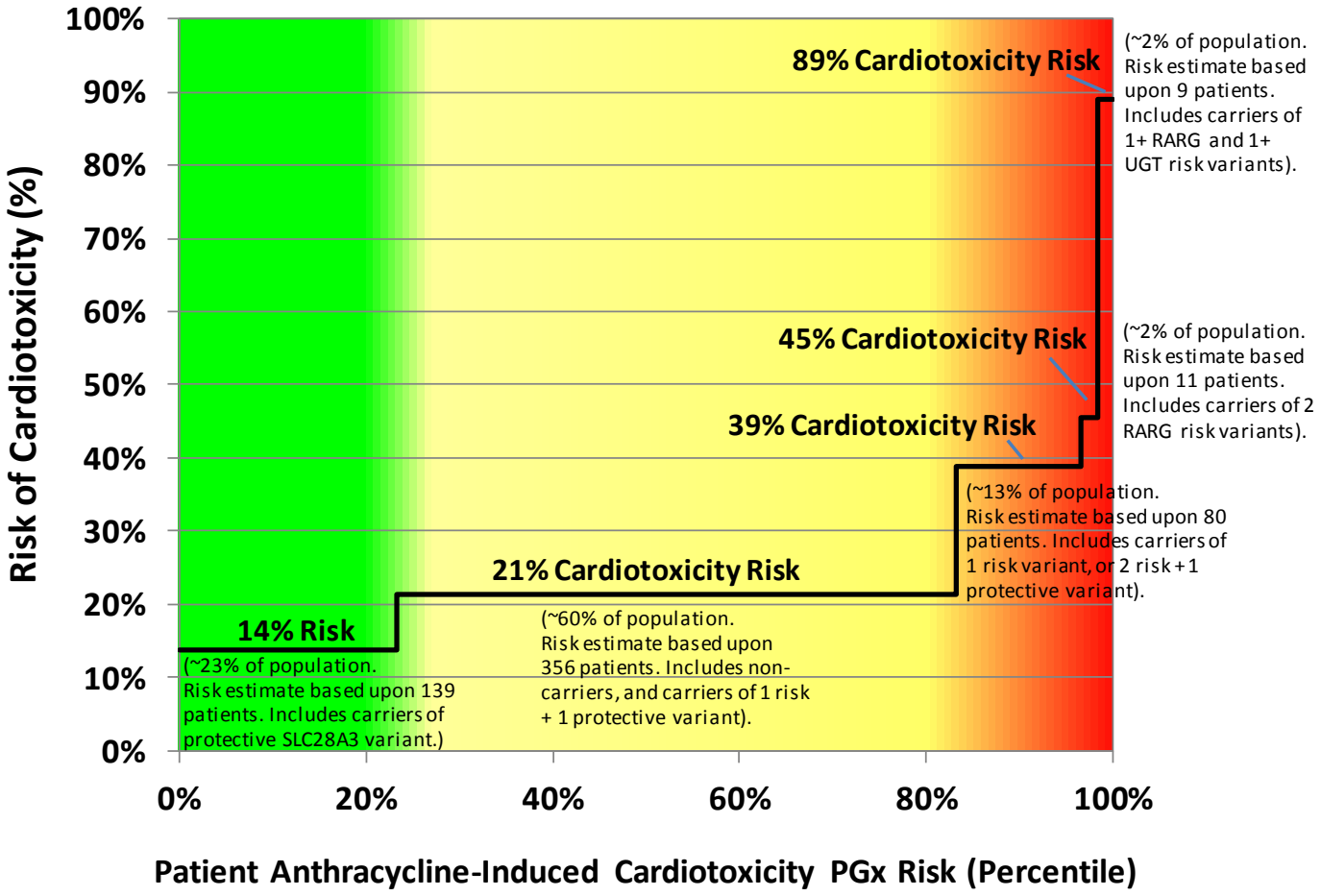
Children's Oncology Group Long-Term Follow-up Guidelines v3.0

Prediction Based on Clinical & Genetic Risk Factors



Genetic factors improve the prediction of ACT beyond clinical factors and can potentially inform treatment decisions

Genetic Risk Stratification for Anthracycline-induced Cardiotoxicity in Children

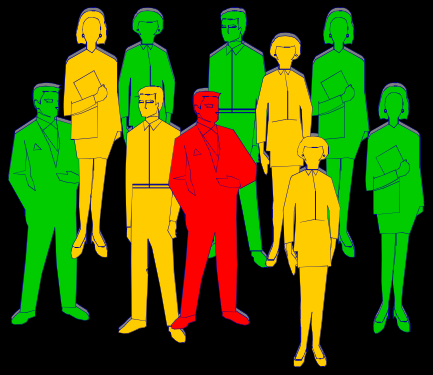


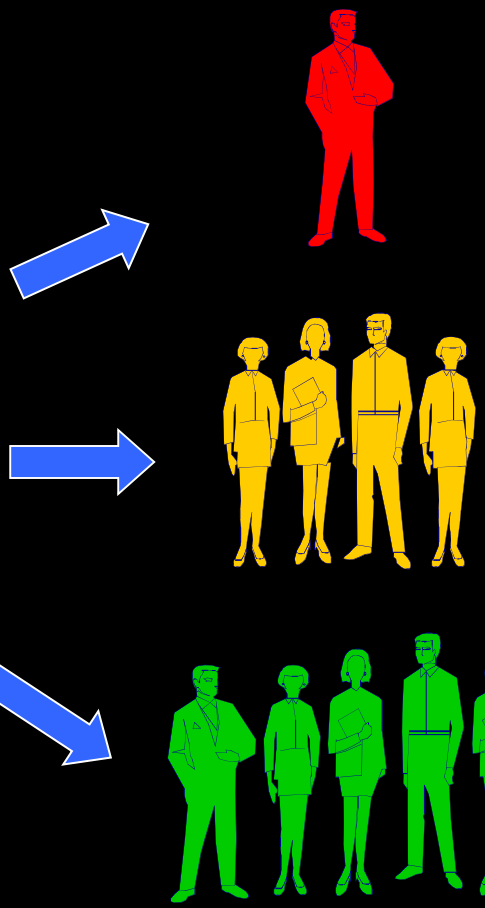
Clinical Pharmacogenomic Testing

- Avoid adverse drug reactions
- Maximize drug efficacy for individual patients

Pharmacogenetic Risk Profile:

All Patients Treated with Same Drug



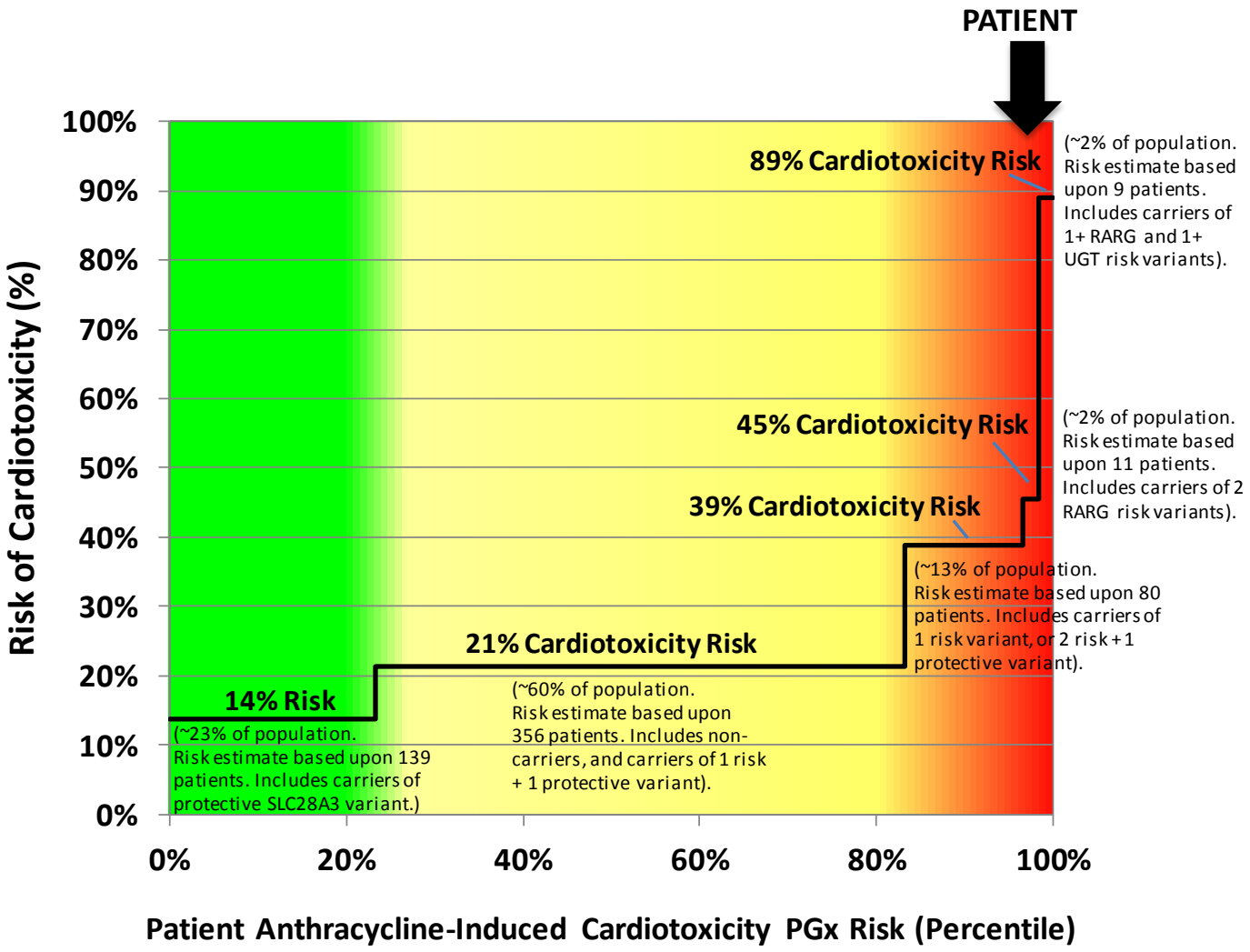


High risk of ACT

Moderate risk of ACT

Low risk of ACT

Genetic Risk Stratification for Anthracycline-induced Cardiotoxicity in Children



Importance of SNP & Variation Suite

SNP & Variation Suite

- Well suited for big & complex data analysis, visualization & interpretation
- Important application for pharmacogenomic studies to uncover the genetic & mechanistic basis of drug response

10 reasons SVS remains our software of choice

1. Very user friendly and great for beginners
2. Detailed user manuals available which is also easy to use
3. Wide range of statistical tests available
4. Great for data visualization
5. Very fast and have lots of computation power
6. Great variety of data manipulating tools

10 reasons SVS remains our Software of Choice

7. Technical support is great & rapid

8. Lots of tutorials and training available on the website

9. Constantly investing heavily in educations & training of its customers on a wide variety of topics in medical and biomedical sciences

10. With the amount of genetic data growing faster than computation capacity of most standard statistical software packages, SVS is the software of choice, especially for scientist & clinicians who are neither statisticians nor bio-informaticians

ACKNOWLEDGEMENTS

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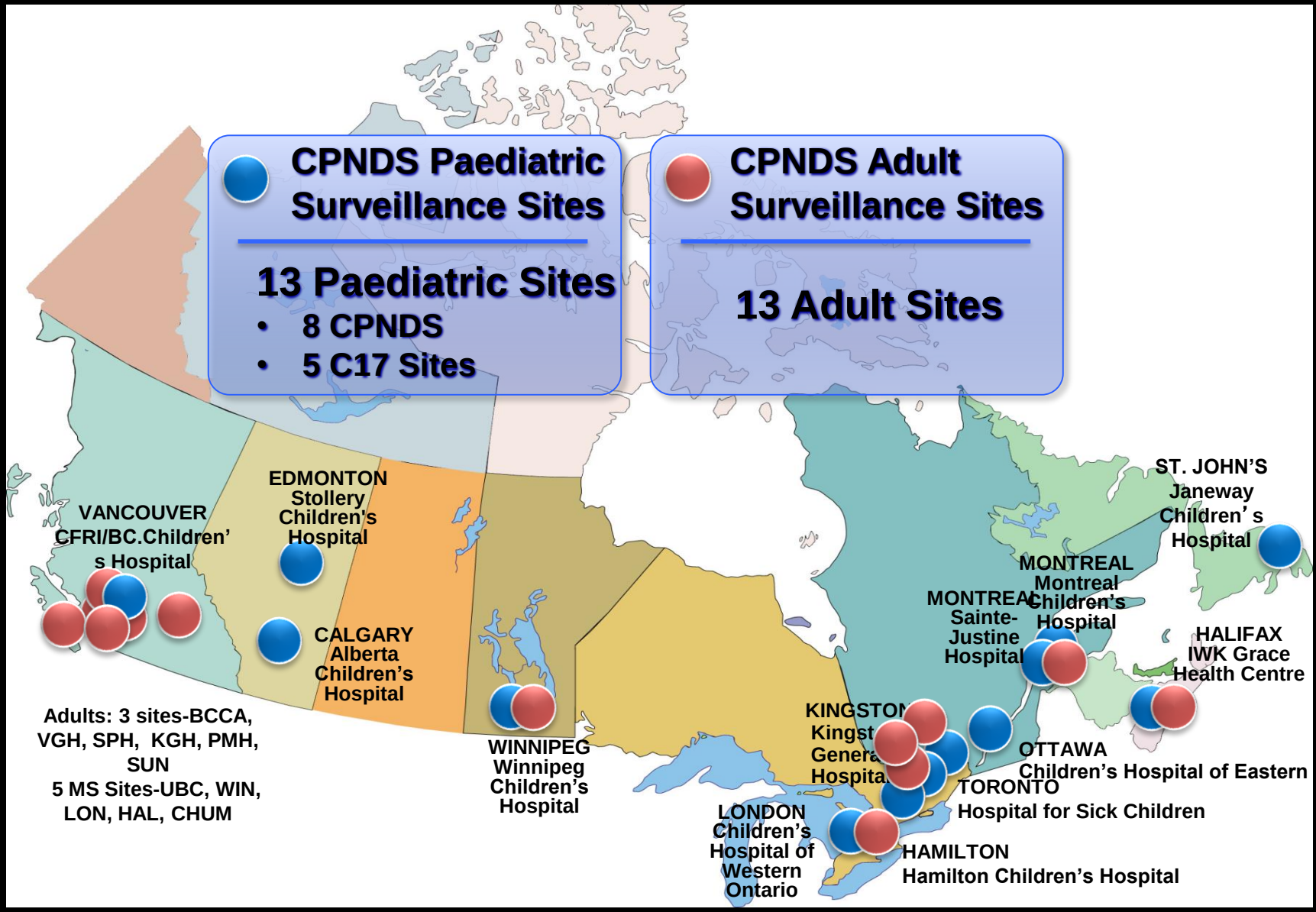
CPNDS Network in Canada



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International Collaborations

Question Time

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Backup Slides

Anthracycline-induced Cardiotoxicity

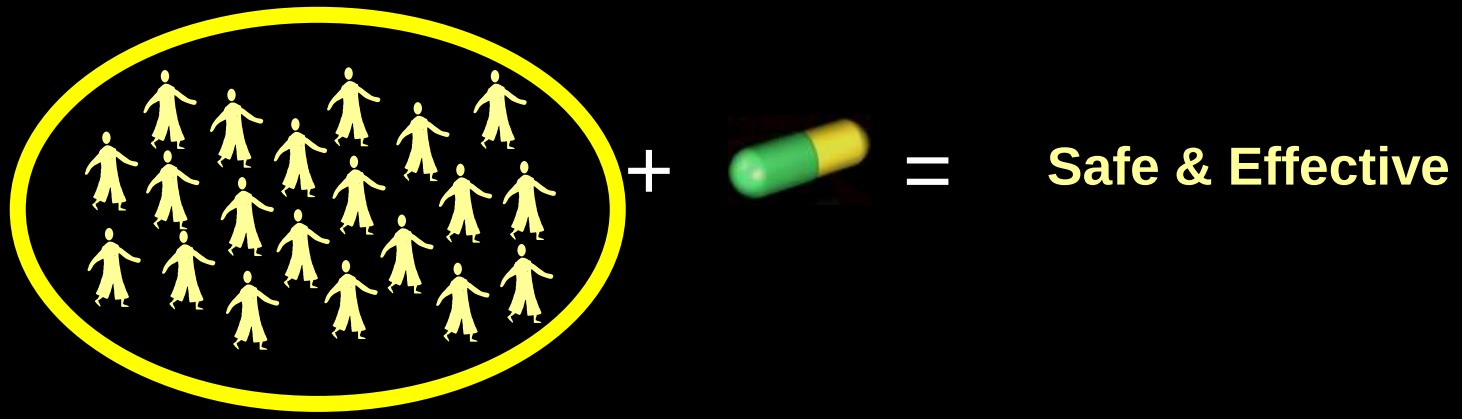
many risk factors

Risk factor	Risk
Cumulative dose	Cumulative doses >500 mg/m2 associated with significantly elevated long term risk
Length of follow-up	Incidence of clinically significant cardiotoxicity increases progressively post-therapy
Radiation therapy	Cumulative radiation dose (>30 Gy); prior or concomitant anthracycline treatment
Age	Both young and advanced age at treatment are associated with elevated risk
Sex	Females are at greater risk than males
Rate of anthracycline administration	Prolonged administration to minimise circulating dose volume may decrease toxicity; results are mixed
Individual anthracycline dose	Higher individual anthracycline doses are associated with increased late cardiotoxicity, even when cumulative doses are limited
Type of anthracycline	Liposomal encapsulated preparations may reduce cardiotoxicity. Conflicting data exist about anthracycline analogues and cardiotoxicity differences
Concomitant therapy	Trastuzumab, cyclophosphamide, bleomycin, vincristine, amsacrine, and mitoxantrone may increase susceptibility/toxicity.
Pre-existing cardiac risk factors	Hypertension; ischaemic, myocardial, and valvular heart disease; prior cardiotoxic treatment
Comorbidities	Diabetes, obesity, renal dysfunction, pulmonary disease, endocrinopathies, electrolyte and metabolic abnormalities, sepsis, infection, pregnancy
Additional factors	Trisomy 21; African American ancestry

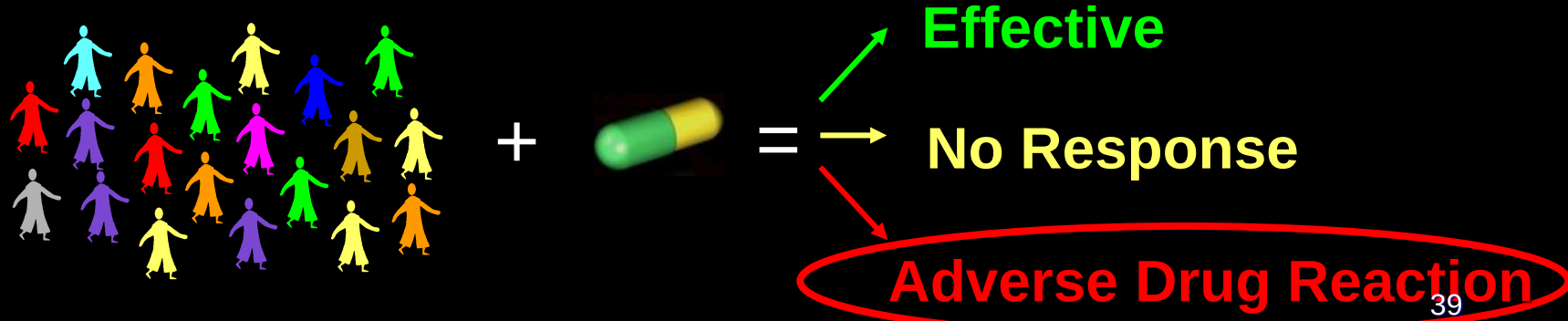
Adapted from: Lipshultz et al. *Heart*. 2008;94(4):525-33.

Paradox of Modern Drug Development

1. Clinical trials provide evidence of efficacy and safety at <u>usual doses</u> in ***populations***

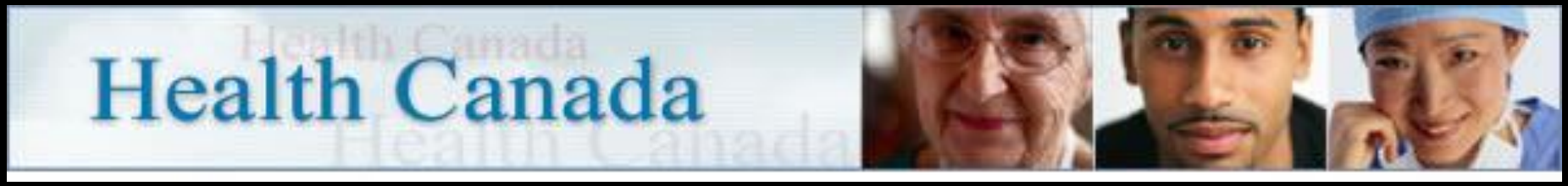


2. Physicians treat ***individual*** patients who can <u>vary widely</u> in their response to drug therapy



Health Canada

Canada



50% of newly approved therapeutic health products have ***serious ADRs,*** discovered only after the product is on the market

- Health Canada, 2007

Reports of Severe ADRs are Increasing

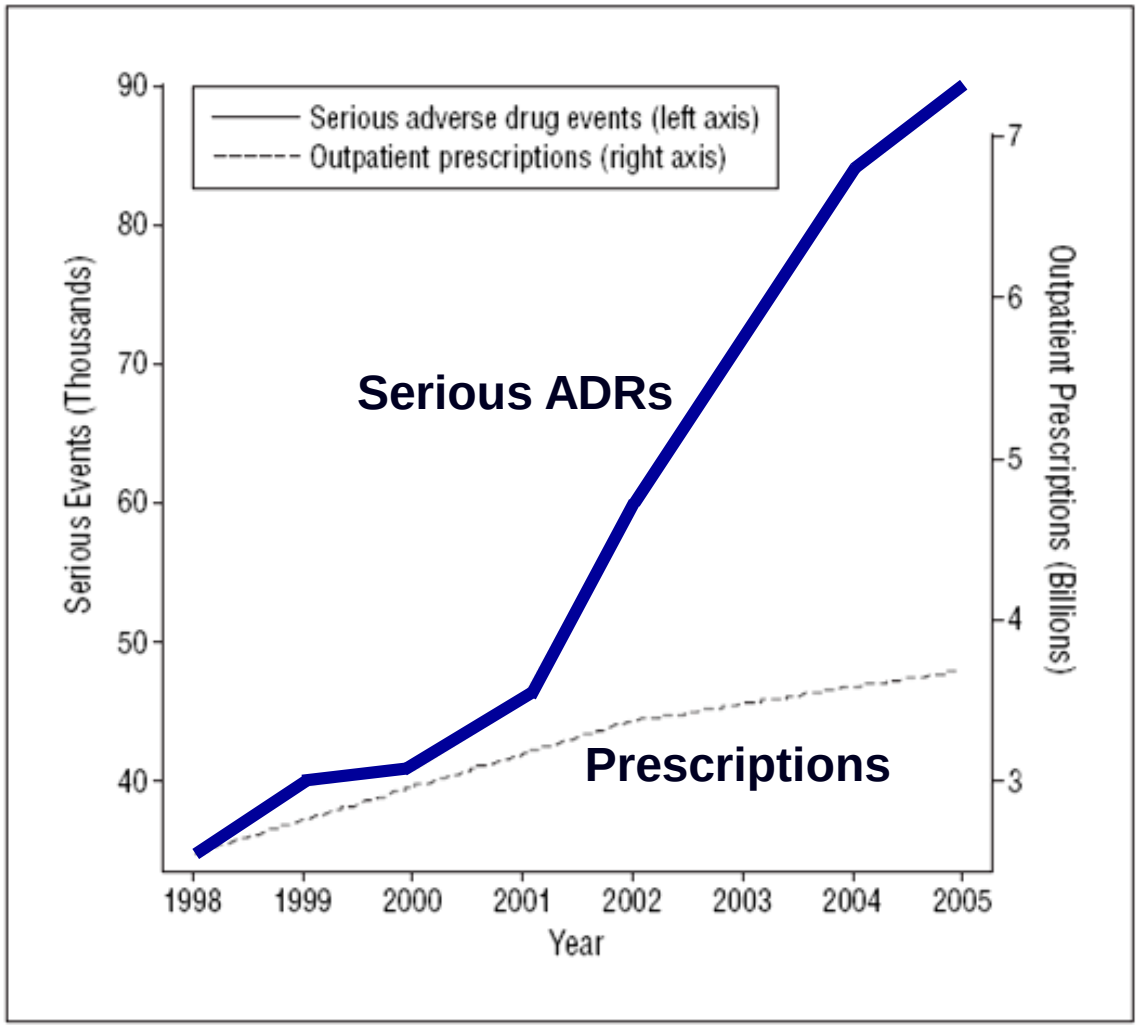


Figure 1. Reported serious events vs outpatient prescriptions, 1998-2005.

Moore et al, FDA, 2007