

*Introducing Therapeutic Diagnostics™*



# Discovery of Biomarkers for a Rare Adverse Drug Reaction

Benjamin A. Salisbury, Ph.D.

Senior Director, Genetics and Biostatistics

# Developing Biomarkers for Safety or Efficacy

- Biomarkers need to be ‘validated’ or ‘qualified’ to a level ‘fit for purpose’
- Will describe a development plan for a genetic test for a rare, but serious, adverse event
- Genetic test will fit criteria for clinical use
  - Sensitivity/specificity
  - Reproducibility
  - Robust
  - Accurate/precise
  - Inexpensive
  - Biologically plausible

# Clozapine

- Considered the “gold standard” for treatment of schizophrenia
- Indicated for treatment-resistant schizophrenia and for the reduction in the risk of recurrent suicidal behavior in schizophrenia or schizoaffective disorders
  - Use is limited, in part, due to the side effect of agranulocytosis

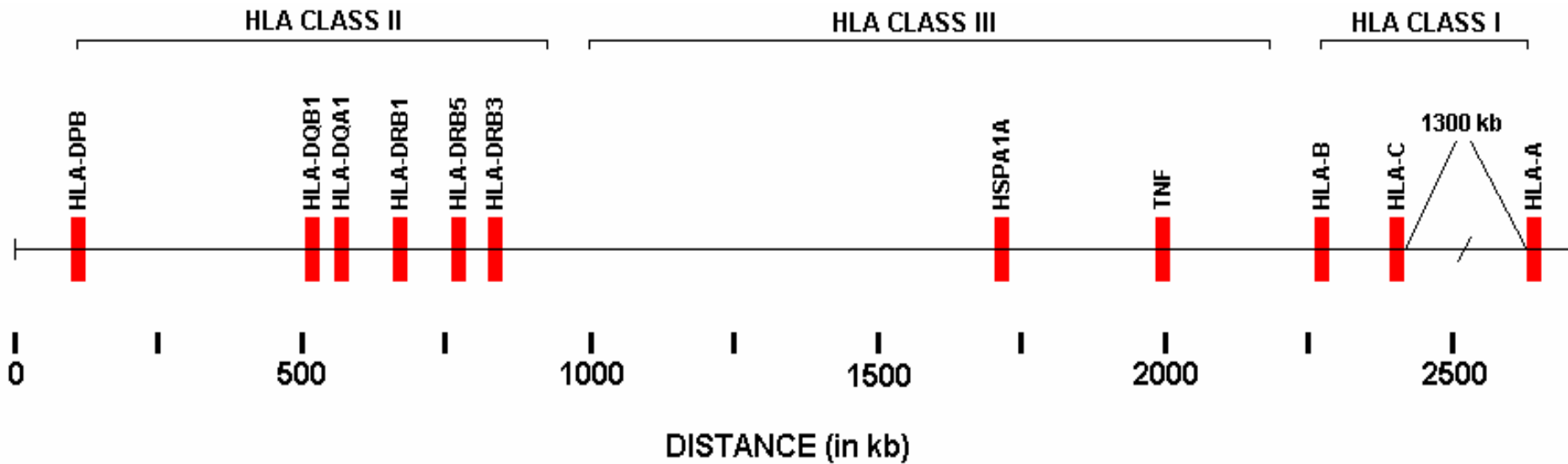
# Clozapine-Induced Agranulocytosis (CIA)

- Defined as an Absolute Neutrophil Count  $<500/\text{mm}^3$ 
  - Incidence of  $\sim 1.3\%$  without monitoring (pre-market testing)
  - Incidence of  $0.3\% - 0.4\%$  with weekly monitoring
  - Incidence rises steeply for first 2 months and peaks during the third month of treatment
- Progression from a loss of neutrophils to onset of agranulocytosis is  $\sim 2-5$  days
- Recovery from agranulocytosis generally takes 14-24 days

# Evidence for a Genetic Component to CIA

- Per the package insert: a disproportionate number of the U.S. cases occurred in patients of Jewish background
- Inter-individual variability in the onset of CIA
- Published evidence for genetic associations

# Published Associations in the HLA Complex



# Select Published Associations with CIA

Gene	N Cases	N Controls	Raw p-value	OR
HLA-DRB1/B5 <sup>1</sup>	25	19	0.01	5.95
HLA-C <sup>2</sup>	31	77	0.02	2.85
NQO2 <sup>3</sup>	18	80	<0.001	*
MPO <sup>4</sup>	49	78	0.04	5.30

<sup>1</sup> Yunis, et al. Blood 1995

<sup>2</sup> Dettling, et al. Pharmacogenetics 2001

<sup>3</sup> Ostrousky, et al. Tissue Antigens 2003

<sup>4</sup> Mosyagin, et al. J Clin Psychopharm 2004

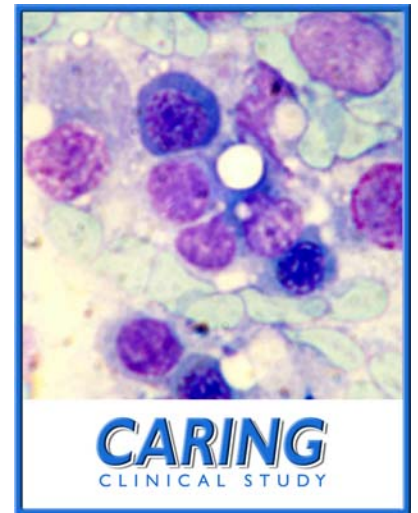
\*Not defined (zero-cell)

# Development Plan

- Pharmacogenetic ‘proof of principle’ for assessing patients’ susceptibility to rare adverse drug reactions
- Develop a sensitive and specific test for CIA
  - Discover genetic marker(s) for CIA
  - Replicate the genetic marker(s) in an independent cohort
  - Release a genetic test as a risk assessment tool

# CARING

Clozapine and Agranulocytosis  
Relationships Induced by Genetic  
Variants



# Steering Committee

## **John Kane, M.D.**

Chair, Department of Psychiatry, Hillside Hospital  
Professor of Psychiatry, Albert Einstein College  
of Medicine

## **Stanton Gerson, M.D.**

Director, Case Comprehensive Cancer Center &  
Director, Ireland Cancer Center  
Case Western Reserve University & University Hospitals of  
Cleveland

## **Anil Malhotra, M.D.**

Director, Psychiatry Research, Hillside Hospital  
Associate Professor of Psychiatry, Albert Einstein College  
of Medicine

## **Ron Diamond, M.D.**

Medical Director  
Dane County Mental Health Center

# Study Design: Case-Control

- Case: ANC<500 and clozapine use was discontinued
  - Not immune compromised
  - No known bone marrow disease
- Control: Treatment at least one year with WBC>5000 and ANC>1500
  - Minimum dose of 250mg/day
  - Age-, sex- and ethnicity-matched to cases

# Study Design: Sample Size

- Analysis of 30 agranulocytosis cases versus 60 matched controls
- Designed to discover
  - Markers with odds ratio = 16
    - Sensitivity and specificity 80%
  - 99% power at  $\alpha = 0.001$

# CARING Study: Actual Sample

- 33 cases and 54 controls
  - Still >99% power
- Near complete matching achieved
  - Balanced ethnicity between cases and controls by self report and genomic control
- Used logistic regression with matching variables as covariates

# CARING Cohort Characteristics

		Cases n=33	Controls n=54
<b>Sex</b>	Male	17	33
	Female	16	21
<b>Ethnicity</b>	American Indian or Alaskan Native	4	4
	Black or African American	1	2
	White	28	48
<b>Age</b>	Median (range) in years	36 (20-58)	35 (18-54)
<b>Nadir ANC</b>	Median (range)	237 (0-506)	N/A
<b>Diagnosis</b>	Schizophrenia/Schizoaffective Disorder	32	53
	Bipolar Disorder	1	1
<b>Time to CIA</b>	Median (range) in months	2.2 (0.5-62.6)	N/A

# CARING Candidate Genes

- 74 candidate genes
  - Genes involved in clozapine metabolism
  - Genes involved in promyelocytic differentiation
  - Genes for which associations with CIA have been previously reported
- Sequenced functional regions to discover and genotype all SNPs and small insertion/deletions

# Haplotype Analysis

- Full haplotypes built with all polymorphisms
- Haplotype markers with up to 4 polymorphisms analyzed
- Logistic regression
  - dominant mode: (0 vs. 1 or 2 copies), and
  - recessive mode (0 or 1 vs. 2 copies)
  - Covariates: Ethnicity, age, sex
- Multiple comparisons adjusted for by permutation tests
- Two-gene models assessed for significant markers

# CARING Discovery Associations

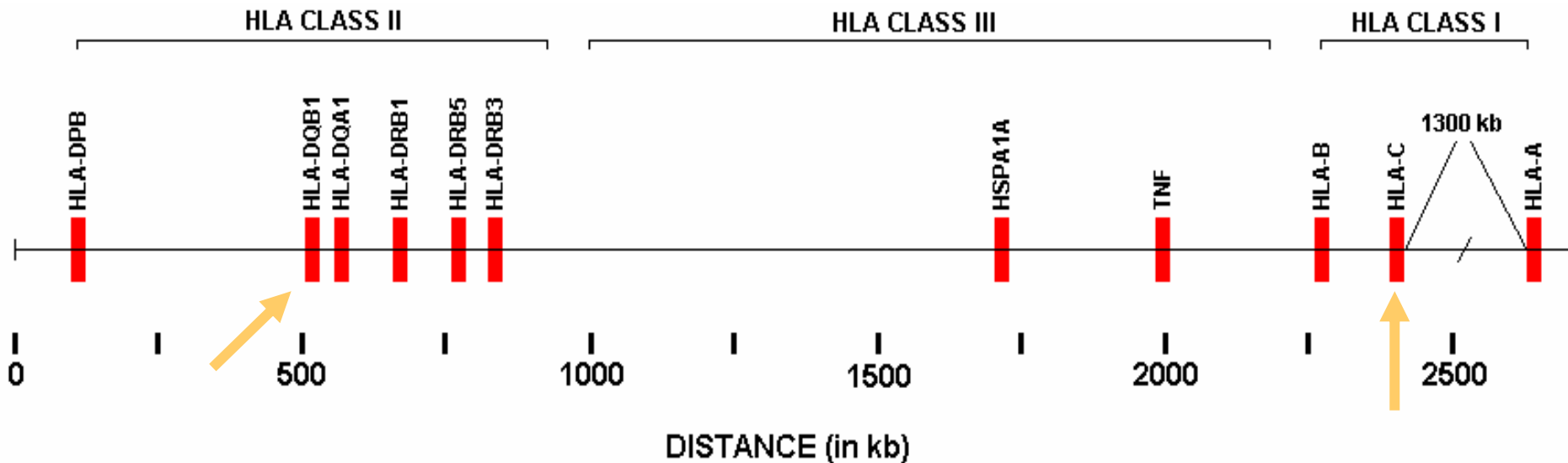
Gene(s)	Raw p-value	Perm. p-value	Adj. OR	Sens.	Spec.	100 – NPV*	% Test Positive
HLA-DQB1	0.000013	0.010	12.9	67%	83%	0.16%	16.9%
CSF2RB	0.00012	0.0040	26.8	39%	96%	0.25%	3.9%
DRD1	0.0022**	0.010	***	18%	100%	0.33%	0.1%
NTSR1	0.00065	0.0079	11.8	36%	94%	0.27%	5.7%
HLA-C	0.000051	0.032	12.8	82%	63%	0.12%	37.2%
HLA-DQB1 and CSF2RB	0.0000010	N/A	20.9	79%	80%	0.11%	20.7%

\* Assuming 0.4% incidence

\*\* From Fisher's exact test

\*\*\* Not defined (zero-cell)

# Published Associations in the HLA Complex



# Replication Study Design

- 49 cases and 78 controls
  - Case: ANC<500 and clozapine use was discontinued
  - Control: Treatment at least two years with WBC>5000 and ANC>1500 at a minimum dose of 250mg/day
  - >99% power at  $\alpha=0.002$  (assume ~25 markers to be assessed) for best marker (80% sensitivity and 80% specificity)
- Significance Definition for Replication
  - Permutation correction of p-values within genes
  - FDR (Benjamini-Hochberg) < 0.05 across genes

# Discovery and Replication Overview

Study	Cases <sup>3</sup>	Controls <sup>4</sup>	Genes and Markers Considered <sup>5</sup>	Multiple Comparison Adjustment	Results
Discovery <sup>1</sup>	33	54	SNPS and haplotypes in 74 candidate genes	Permutation within genes	4 genes selected for replication
Replication <sup>2</sup>	49	78	28 specific markers in 4 genes	Permutation within gene; FDR between genes	1 significantly associated gene <sup>6</sup> (perm p = 0.010 < 0.0125) 1 marker selected

<sup>1</sup>Discovery cohort collected in the US

<sup>2</sup>Replication cohort collected in Germany

<sup>3</sup>Cases: ANC<500

<sup>4</sup>Controls: treated with clozapine at least one year without reduction in WBC

<sup>5</sup>Logistic regression

<sup>6</sup>Results from an additional gene, CSF2RB (GM-CSF receptor) were not statistically significant, but warrant further study

# Discovery and Replication Results

## Best Marker in Combined Cohort

HLA-DQB1 Two-SNP Marker	Raw p-value	Crude OR	Sens (%)	Spec (%)	PPV* (%)	NPV* (%)	100 – NPV* (%)	% at High Risk
Discovery	0.000021	9.0	70	80	1.36	99.85	0.15	20.5
Replication	0.0078	2.9	51	74	0.77	99.73	0.27	26.5
Combined	0.0000015	4.6	59	76	0.98	99.78	0.22	23.9

Crude OR: odds of being a case in marker + group/odds of being a case in the marker - group  
 Sens: chance of being marker positive if case  
 Spec: chance of being marker negative if control  
 PPV: chance of being case if M+

NPV: chance of being control if M-  
 100-NPV: chance of being case if M- (Interpret as predicted incidence if genetic testing implemented)  
 \*Assumes an incidence of 0.4%

# Summary

- Demonstrated an effective method to discover biomarkers for rare side effects
- Initial release as a risk assessment tool will allow real-world understanding of test performance
- These markers may be relevant to other drug-induced neutropenia

# Contributors

Clinical Sites and  
Patients

## Collaborators

**Michael Dettling, M.D.**

Klinik und Hochschulambulanz für  
Psychiatrie und Psychotherapie  
Charite-Universitätsmedizin Berlin  
Berlin, Germany

**Ingolf Cascorbi, M.D., Ph.D.**

**Igor Mosyagin, M.Sc.**

Institute of Pharmacology  
University Clinic Schleswig-Holstein  
Kiel, Germany

## Clinical Data, Inc.

**Carol Reed, M.D.**

Chief Medical Officer

**Maria Athanasiou, Ph.D.**

Vice President, Biomarker Development

**Bradley Dain, Ph.D.**

Director, Biostatistics

**Janet Carr, Ph.D.**

Senior Manager Data Analysis and  
Reporting