



*Pharmacogenomics – new tools for  
improved patient management*

*The AmpliChip CYP 450 and Leukemia Tests*

**Walter Koch, Ph.D.  
Vice President,  
Head of Research  
Roche Molecular Diagnostics**

# Pharmacogenomics



Studies and describes the **interaction** of the **genome** (sum of all genes) and **drugs**

## Therapeutics Today

*“One size fits all” approach to drug dosing often fails*

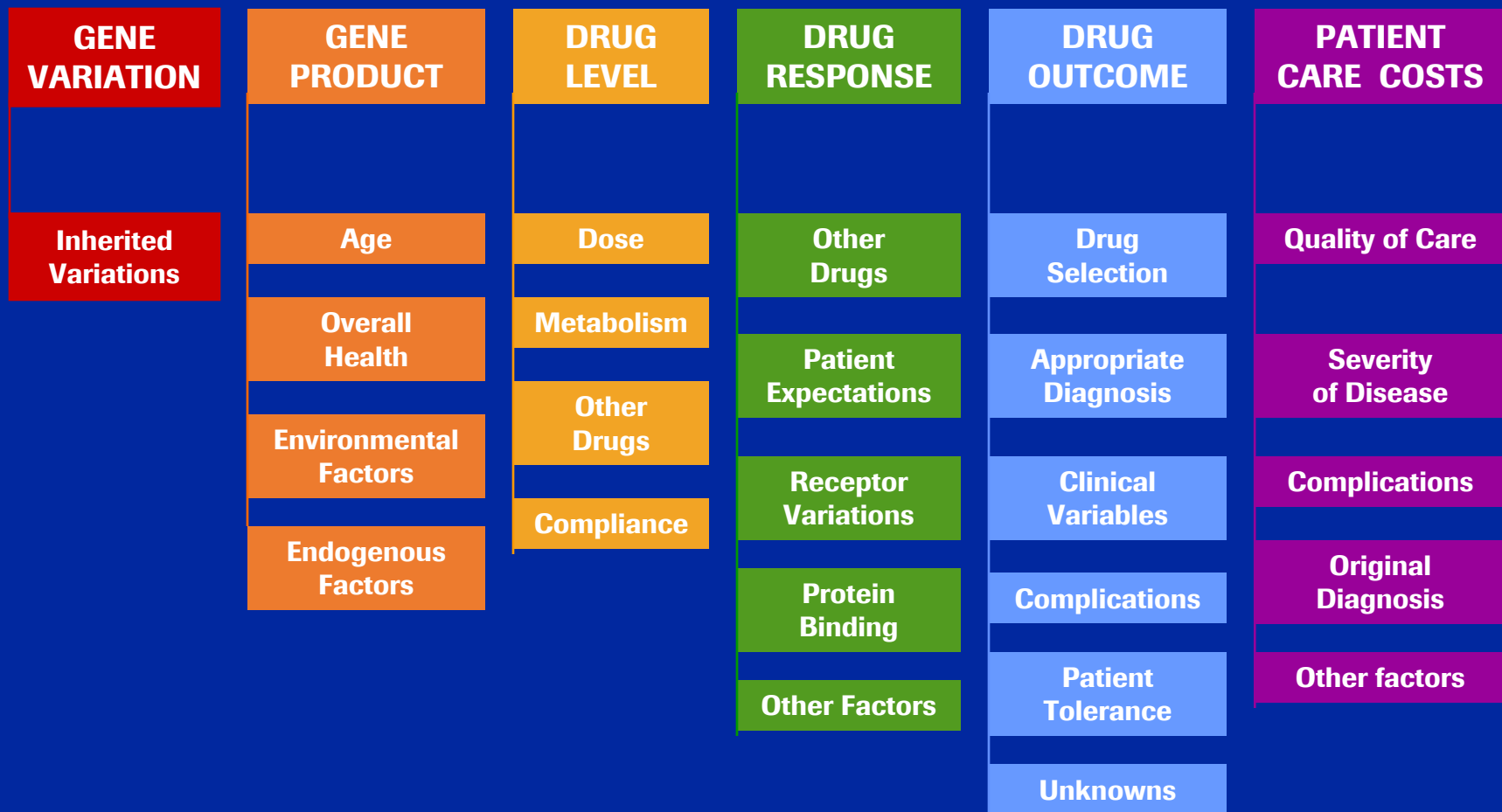
- Adverse drug reactions are the fifth leading cause of death
- Genetics can account for 20-95% of variability in drug disposition and effects



# Many factors affect efficacy & outcome

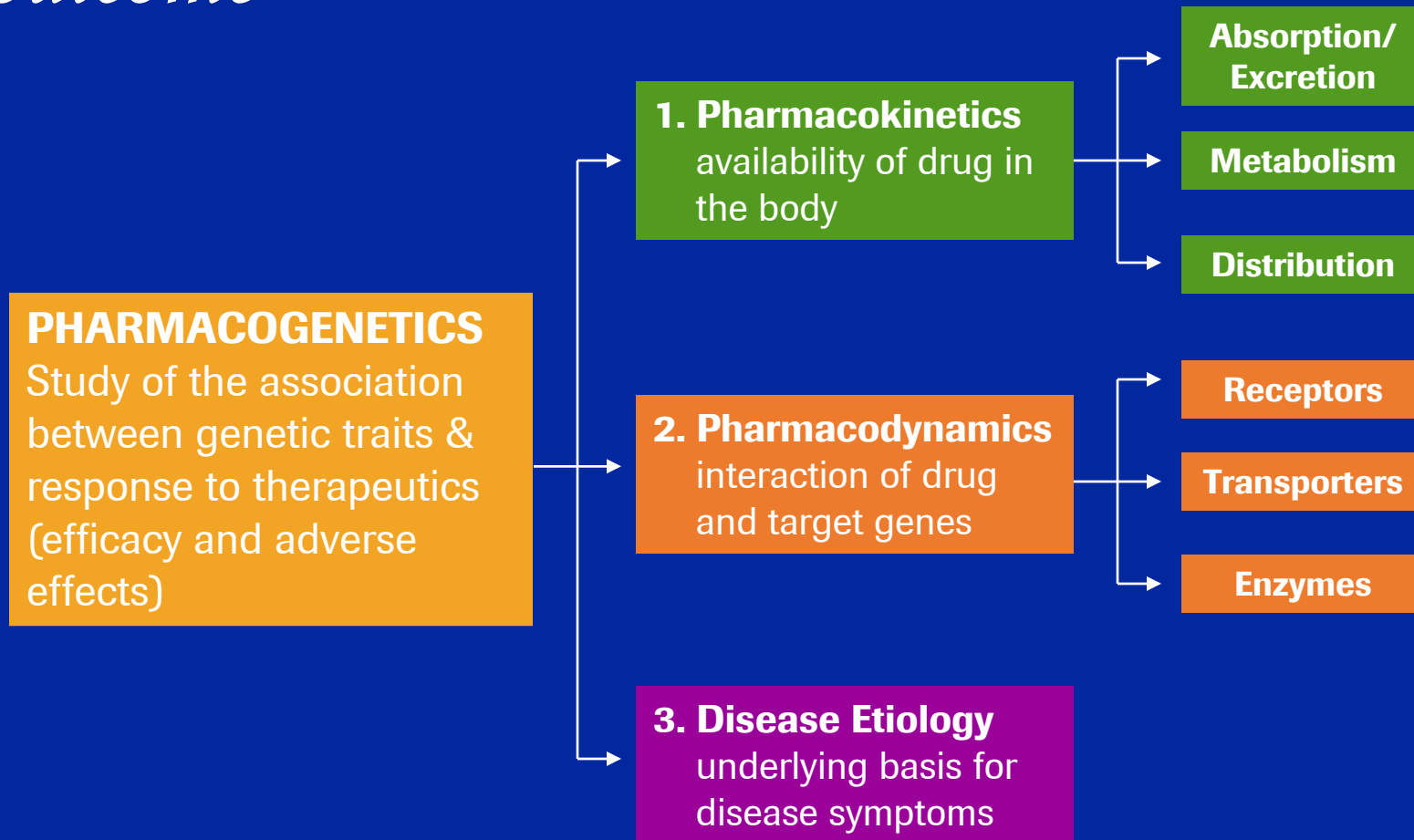
*Genetic and non-genetic factors are important*

## Influences of genetic & non-genetic factors



# Pharmacogenetics

*Using individual genetic information to improve outcome*



# Therapeutics Today

*“One size fits all” approach to drug dosing often fails*

## Drugs

- Patients who metabolize certain medications ***poorly***
  - are at increased risk of adverse events and possibly toxicity
- Patients who metabolize these medications ***too quickly***
  - may not achieve sufficient therapeutic levels with standard dosing, resulting in treatment failure

## Pro-drugs

- The opposite phenomenon occurs with pro-drugs (converted by CYP450 enzymes into active metabolites)
  - ***ultra-rapid*** metabolizers may suffer adverse events
  - ***poor*** metabolizers may not respond.

# CYP2D6 genetic variation

*Frequencies among people of different geographical origin*

## Poor Metabolizers

- Caucasians 7-8%
- Japanese ~1%
- Chinese ~1%
- U.S. Blacks ~6%

## Gene Duplications

- Ethiopians 16.0%
- Saudi Arabians 10.4%
- Spaniards 10%
- Italians 8.3%
- Zimbabweans 2%
- Germans 1.8%
- Chinese 1.3%

# Why Diagnostics?

*Pick out the poor metabolizer in this picture*



## A Guessing Game



For concept illustration purposes only. Not a real or intended diagnosis.



# Only an informed physician knows for sure

*Eliminating the guesswork for patients & doctors*



New screening tools  
like the  
Amplichip CYP450 test  
can inform



For concept illustration purposes only. Not a real or intended diagnosis.

# How molecular diagnostics can help

## *A broader scope of actionable health information*

- New pharmacogenetic tools for predicting individual response to drugs
  - AmpliChip CYP450 test
- New tools for differential diagnosis and personalized treatment
  - Disease manifests itself the same, but has important differences at molecular level
  - Identifying disease sub-types
  - AmpliChip Leukemia test



# AmpliChips (Microarrays)

## Microarrays can be designed to

- genotype =  
determining which of specific genetic variations are present in the individual (AmpliChip CYP 450)
- analyze gene expression patterns =  
which genes are turned on or off in a given tissue or disease state (AmpliChip Leukemia test)
- confirm gene sequences =  
which molecular building blocks (base pairs) are present in a sample, important for identifying specific variants of an virus or bacteria



**First pharmacogenetic diagnostic tool  
for predicting individual response to drugs**

*AmpliChip CYP450 test*



# Roche AmpliChip™ CYP450 Microarray

*Determines genotype and predicted phenotype for two important drug metabolizing enzymes*

- **CYP 450**  
stands for the Cytochrome 450 gene family including the two genes **CYP2D6** and **CYP2C19**
- CYP2D6 and CYP2C19 code for two enzymes which play an important role in drug metabolism
- They are extensively studied and exist in many different variants in humans



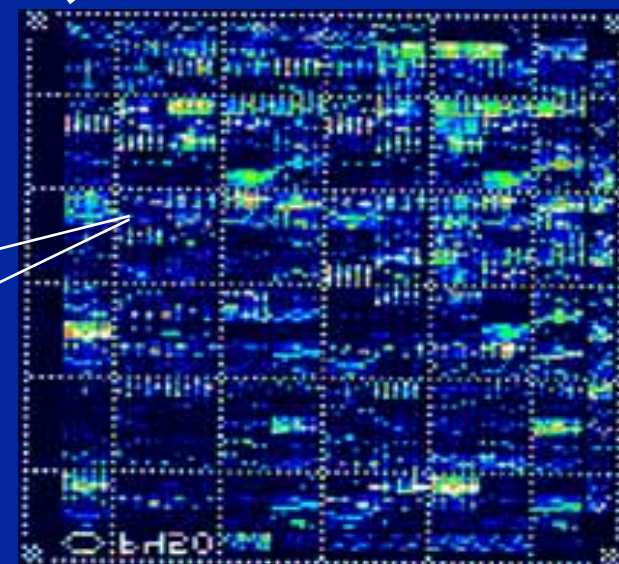
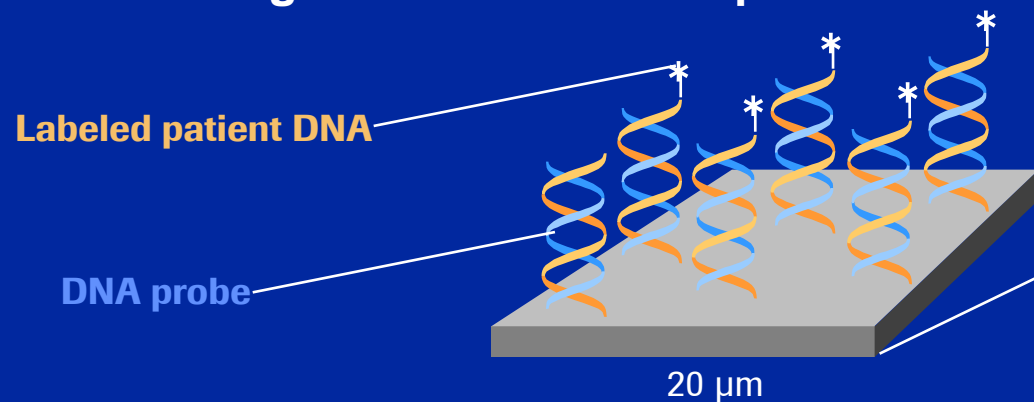


# Roche AmpliChip™ CYP450 Microarray

*Determines genotype and predicted phenotype for two important drug metabolizing enzymes*

Distinguishes 29 known variants in the CYP2D6 gene and 2 major variants in the CYP2C19 gene

Each 20  $\mu\text{m}^2$  cell on the array can contain millions of DNA fragments, or “probes” to detect the gene variants of the patient





## Metabolizer phenotypes

*The most comprehensive analysis available with the AmpliChip CYP450 test*

- **Extensive metabolizers (EM)**

Possess at least one normal functional allele (one variation of a gene located at a particular site on a chromosome)

- **Intermediate metabolizers (IM)**

Possess one reduced activity allele and one null allele

- **Poor metabolizers (PM)**

Carry two mutant alleles which result in complete loss of enzyme activity

- **Ultrarapid metabolizers (UM)**

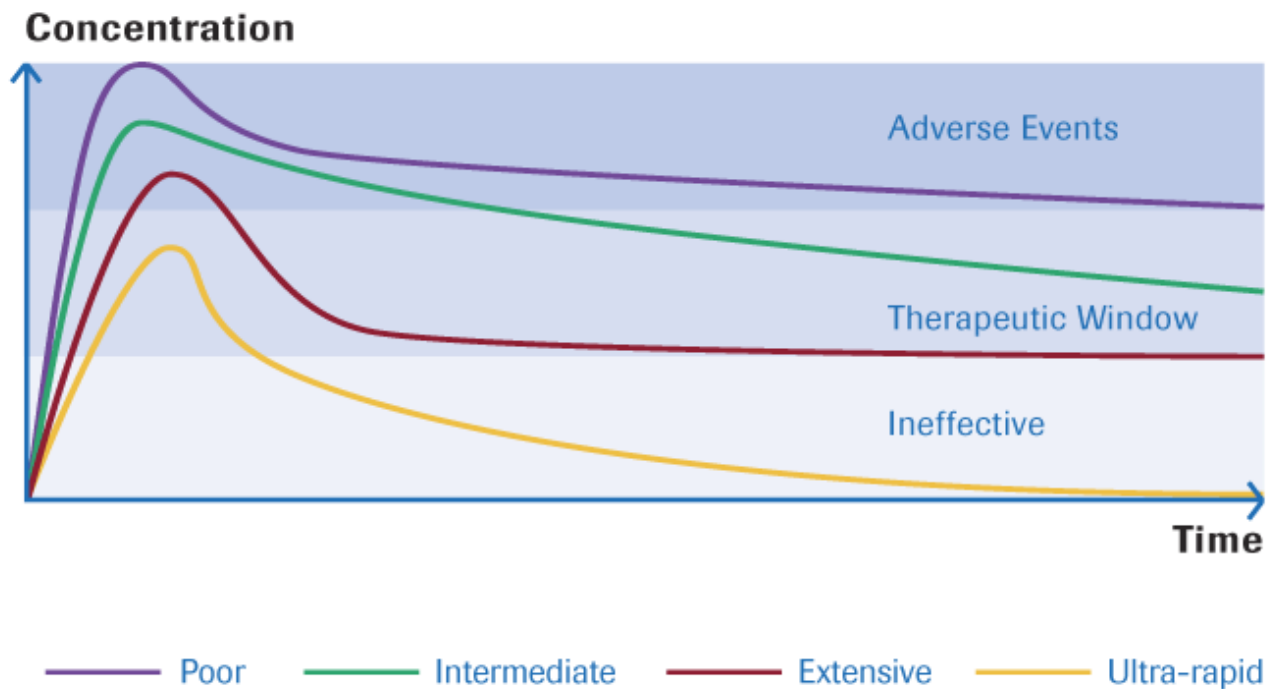
Carry multiple copies (3-13) of functional alleles and produce excess enzymatic activity

<http://www.imm.ki.se/cypalleles/cyp2d6>

# Pharmacokinetic impact

## Drug Levels Based On Phenotype

*Phenotype influences the concentration of drug in a patient's blood.*





# Improving efficacy, reducing adverse reactions

## *The importance of understanding genetic variations*

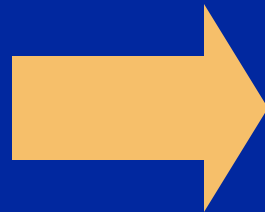
- Genetic variations can play a major role in outcome by contributing to differing:
  - rates of efficacy
  - types of side effects
  - severity of side effects
  - types of serious adverse reactions
  - severity of adverse reactions (fatal and non-fatal)
- Some less serious side effects are expected and unavoidable, even with appropriate dosing.

# Avoiding Adverse Reactions

*A primary goal of pharmacogenetics*

## Side Effects

- Dry mouth
- Rash
- Fatigue
- Constipation
- Nausea
- Vomiting
- Dizziness
- Weight Gain
- Tachycardia
- Arrhythmias



## Possible Results

- Discontinuation of chosen therapy (patient can't tolerate or won't accept side effects)
- More doctor visits
- More hospital re-admissions
- Longer hospital stays
- Higher costs
- Transient or permanent impairment from adverse reactions
- Death



## Some of the widely prescribed drugs metabolized by CYP2D6

Beta Blockers	Antidepressants	Antipsychotics	Others
Carvedilol	Amitriptyline	Haloperidol	Atomoxetine
Metoprolol	Clomipramine	Risperidone	Codeine
Propafenone	Desipramine	Thioridazine	Dextromethorphan
Propranolol	Imipramine		Flecainide
Timolo	Nortriptyline		Mexiletine
	Paroxetine		Ondansetron
	Venlafaxine		Tamoxifen
			Tramadol

## Some of the widely prescribed drugs metabolized by CYP2C19

Proton Pump Inhibitors	Anti-epileptics	Others	
Omeprazole	Diazepam	Amitriptyline	
Lansoprazole	Phenytoin	Clomipramine	
Pantoprazole	Phenobarbitone	Cyclophosphamide	
		Progesterone	



# The good news

## *Dose can be adjusted based on CYP2D6 genotype*

<b>CYP2D6 Genotype-Based Dose Adjustments for Anti-Depressant Drugs</b>					
<b>Drug</b>	<b>Usual Dose (mg)</b>	<b>Poor</b>	<b>Intermediate</b>	<b>Extensive</b>	<b>Ultra-Rapid</b>
<b><u>Tricyclics</u></b>					
Amitriptyline	150 (50-150)	50 %	(90 %)	120 %	
Clomipramine	150 (100-200)	60 %	(90 %)	120 %	
Desipramine	150 (10-100)	30 %	30 %	130 %	260 %*
Fluvoxamine	100 (100)	90 %	(100 %)	110 %	
Imipramine	150 (25-100)	30 %	(80 %)	130 %	
Nortriptyline*	50 (25-150)	50 %	70 %	140 %	230 %
<b><u>SSRI</u></b>					
Fluoxetine*	20 (20-60)	70 %	(90 %)	110 %	
Paroxetine	20 (30)	70 %	(90 %)	110 %	
<b><u>Mixed-Function</u></b>					
Venlafaxine	150 (20-225)	20 %	(80 %)	130 %	

**NOTES:** \* denotes single-dose recommendations. Recommendations in brackets are estimations and require clinical confirmation

# Genotyping will become an integral part of diagnosis and treatment

## *Treatment Flow*



**Diagnosis**



**AmpliChip  
CYP450  
Genotyping**



**Treatment &  
Drug  
Considerations**



**Drug Dosing  
Decision**



**Order may be reversed  
depending on drug profile**

# **AmpliChip Leukemia Test**

*Differential diagnosis of  
adult and pediatric leukemia*



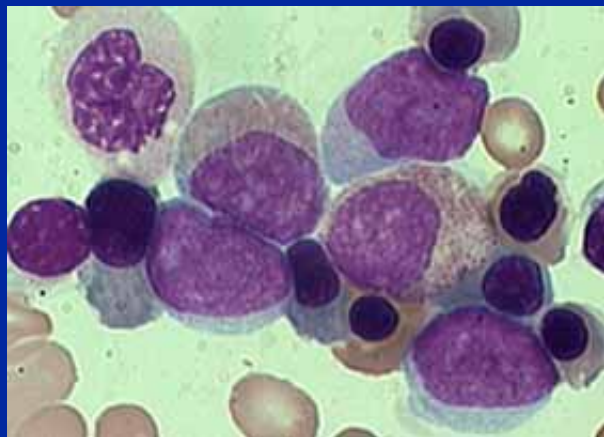
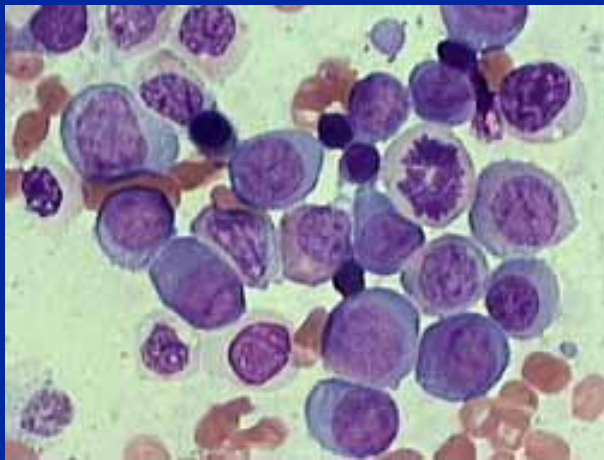
# AmpliChip Leukemia Test

A microarray designed to analyze gene expression patterns

=

which genes are turned on or off in a given tissue or  
disease state

# The value of molecular classification



**Acute Myelotic  
Leukemia (AML)**

**Good Prognosis**

**Acute Myelomonocytic  
Leukemia (AMML)**

**Poor Prognosis**

# AmpliChip Leukemia test

*Reduction of complexity in diagnostic labs*

## Today

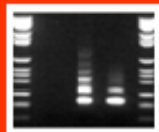
**Morphology**



**Cytochemistry**



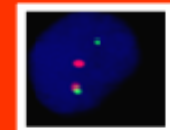
**PCR**



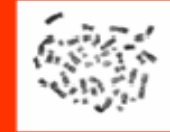
**Q-PCR**



- Many sophisticated highly specialized tests for one differential diagnosis
- Highly skilled workforce
- Up to 5 days for accurate sub-classification
- Therapy decisions based on descriptive results
- Cost for one diagnosis: CHF > 2,000



**FISH**



**Cytogenetics**



**24-color FISH**


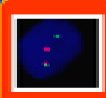


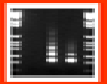





**Immuno phenotyping**

# AmpliChip Leukemia test

*Reduction of complexity in diagnostic labs*

**Today**

<p><b>Morphology</b></p> 	<ul style="list-style-type: none"> <li>• Many sophisticated highly specialized tests for one differential diagnosis</li> <li>• Highly skilled workforce</li> <li>• Up to 5 days for accurate sub-classification</li> <li>• Therapy decisions based on descriptive results</li> <li>• Cost for one diagnosis: CHF &gt; 2,000</li> </ul>	 <p><b>FISH</b></p>
<p><b>Cytochemistry</b></p> 		 <p><b>Cytogenetics</b></p>
<p><b>PCR</b></p> 		 <p><b>24-color FISH</b></p>
<p><b>Q-PCR</b></p> 		 <p><b>Immuno phenotyping</b></p>

## Future

### Gene Expression Analysis On Chips

Roche will launch an in vitro diagnostic product in 2007 distinguishing > 20 sub-classes



One objective diagnostic result in 2 days; new information



# **AmpliChip Leukemia test**

## *Product Performance Goals*

### **Utilize gene expression profiling to diagnostically classify leukemia patients**

- Should correlate with cytogenetics, cytochemistry, immunophenotyping
- Provide actionable information to patient and physician (prognostic and/or therapeutic value)
- Encompass all major types of leukemia - AML, CML, ALL, CLL
- Differentiate myelodysplastic syndrome from AML or normal marrow
- Yield accurate and objective results

### **Intended Use:**

**First diagnosis of patients under suspicion of Leukemia  
sub-classification according to expression profile**

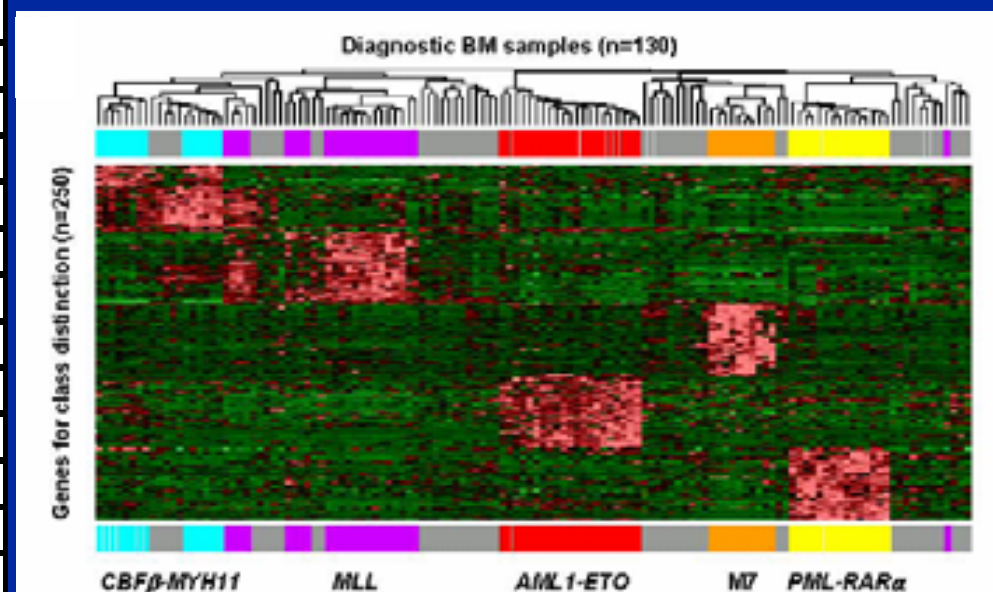
# Leukemia Cytogenetics

- Cytogenetic defects in leukemia often involve chromosome translocations (genetic changes)  
and dictate specific treatments
  - 9:22 (Bcr:abl); Treatment with Gleevec
  - 1:19 (E2a:PBX); Treatment with Chemotherapy
  - 15:17 (PML/RARa); Treatment with all-*trans*-retinoic acid
- Effected genes often encode transcription factors or signaling molecules
- Such defects are reflected in altered gene expression profiles

# Leukemia Cytogenetic Subclasses to be detected by the Amplichip test

	Class	Prognosis
1	ALL t(8;14)	Intermediate
2	ALL 11q23/MLL	Poor
3	ALL B t(9;22)	Poor (Gleevec)
4	T-ALL	Intermediate
5	T-ALL cortical	Favorable
6	ALL t(12;21)	Favorable
7	ALL t(1;19)	Intermediate
8	ALL hyperdiploid	Favorable
9	ALL other/normal	Mixed
10	AML t(8;21)	Intermediate
11	AML t(15;17)	Favorable (RA analogues)
12	AML inv(16)	Intermediate
13	AML MLL (11q23)	Poor
14	AML other/normal	Poor
15	AML complex	Poor
16	CLL 13q-	Favorable
17	CLL 17p-	Poor
18	CLL other/normal	Intermediate
19	CML	Gleevec
20	MDS	
21	Non-leukemia	

## Gene expression "Heat map"



inv16 11q23 t(8;21) | t(15;17)  
complex

Red: increased expression

Green: decreased expression

# Conclusions

## *Future AmpliChip Diagnosis of Leukemia*

- Gene expression profiling can reproduce today's leukemia classification based on
  - morphology
  - immunophenotype
  - genetic markers
- Specificity and sensitivity is high for all subcategories with specific therapeutic consequences
- AmpliChip-based gene expression profiling is a very promising platform for the diagnosis of leukemia and other cancers