CML Horizons 2013

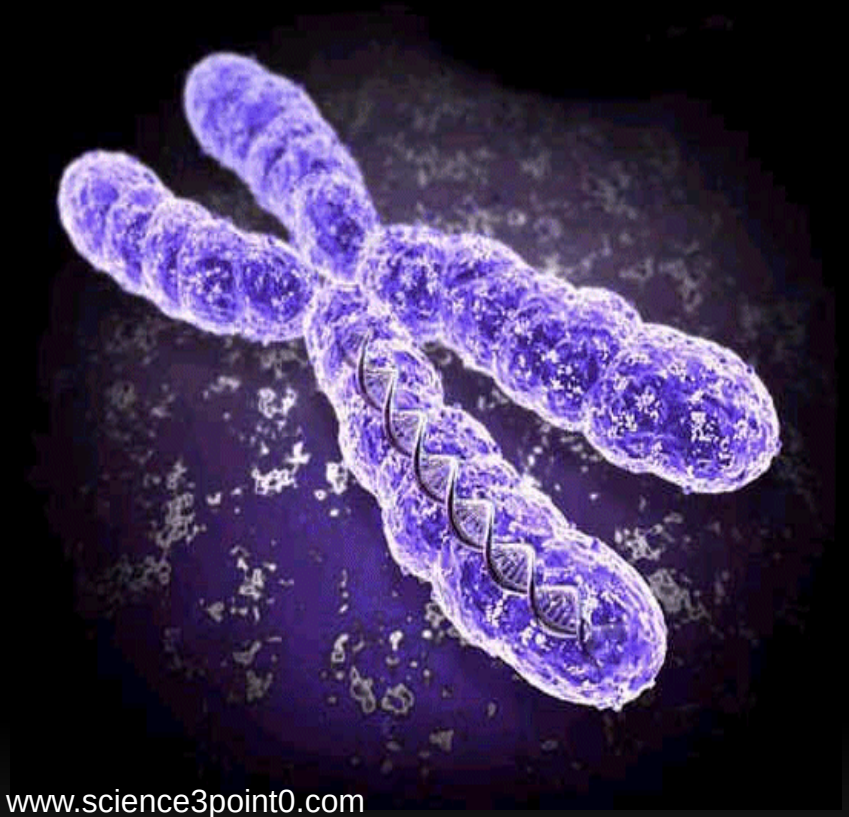
The basic monitoring before we can talk about "cure"

Katerina Machova Polakova

Laboratory for molecular diagnostics and monitoring of CML and Ph+ leukemias
ELN reference laboratory for the Czech Republic
EUTOS-CMR laboratory

Institue of Hematology and Blood Transfusion, Prague

Chromosome

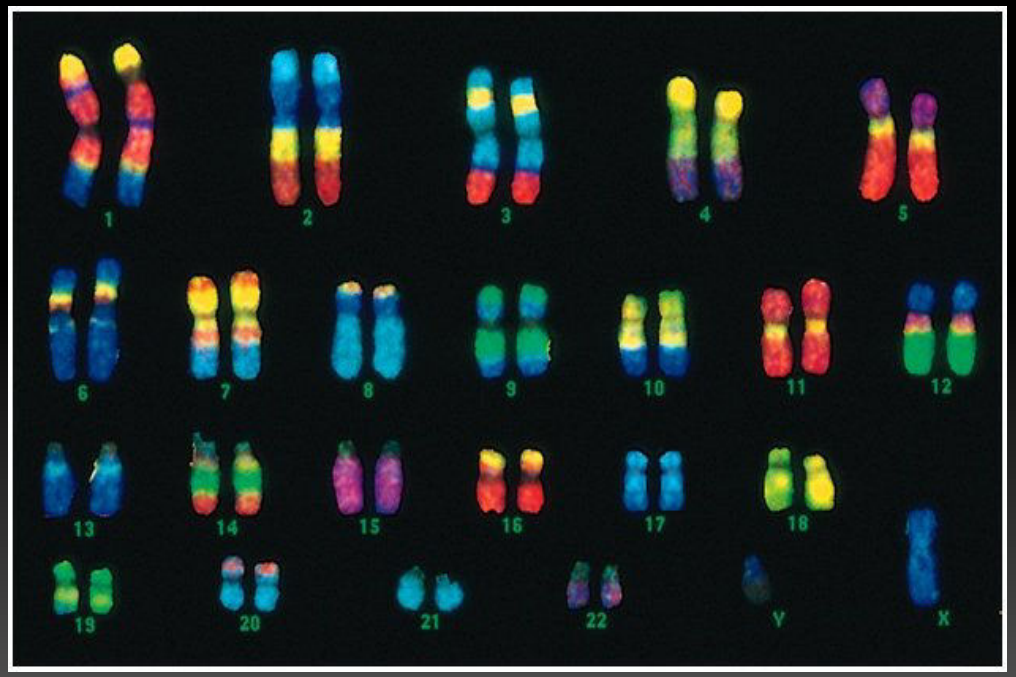


www.science3point0.com

Cytogenetics

- studies of structure and function of the cell

Spectral karyogram of a human male

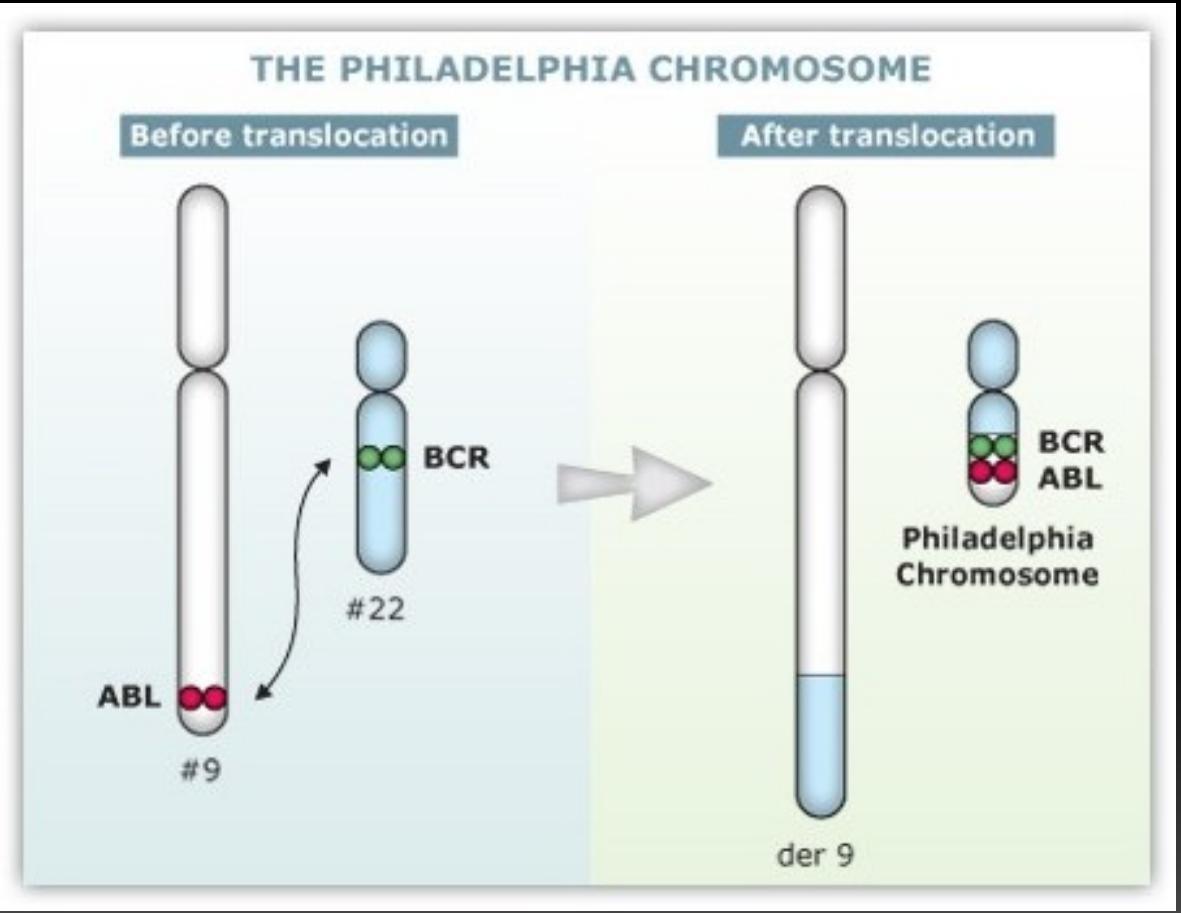


Chromosome

- an organized structure of DNA and proteins

Chronic myeloid leukemia

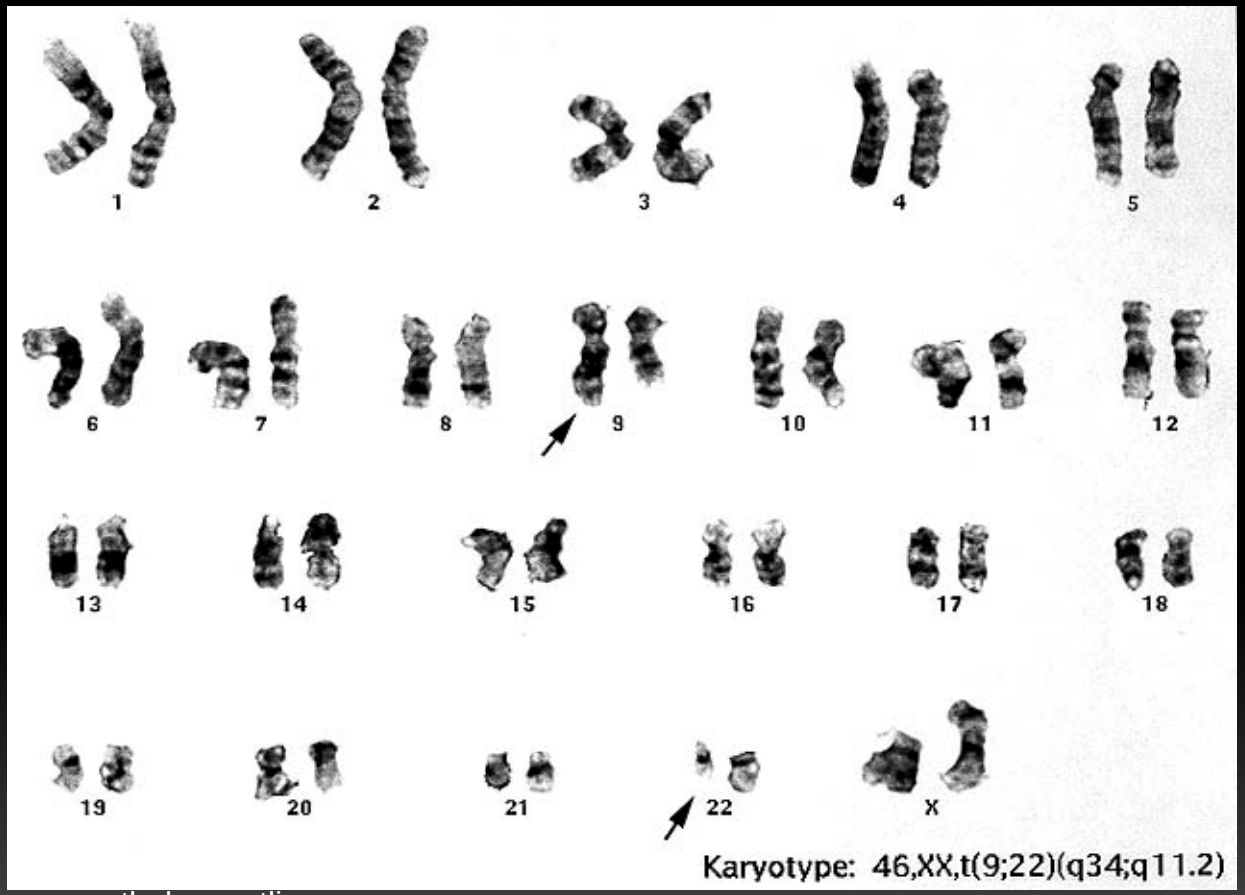
Cytogenetics



www.cmlalliance.com

Chronic myeloid leukemia

Cytogenetic analysis



www.pathologyoutlines.com

How do we monitor CML patients today?

Cytogenetic analysis

- Cytogenetic analysis of minimal 22 metaphases from bone marrow

46,XY,t(9;22)(q34;q11) [3]

46,XY [19]

3/22=14% leukemic cells in BM

46,XX,t(9;22)(q34;q11) [15]

46,XX [7]

15/22= 68% leukemic cells in BM

How do we monitor CML patients today?

Cytogenetics

Cytogenetic response evaluation

❑ None	>95% Ph+ metaphases
❑ Minimal	66-95% Ph+ metaphases
❑ Minor	36-65% Ph+ metaphases
❑ Partial	1-35% Ph+ metaphases
❑ Complete	no Ph+ metaphases

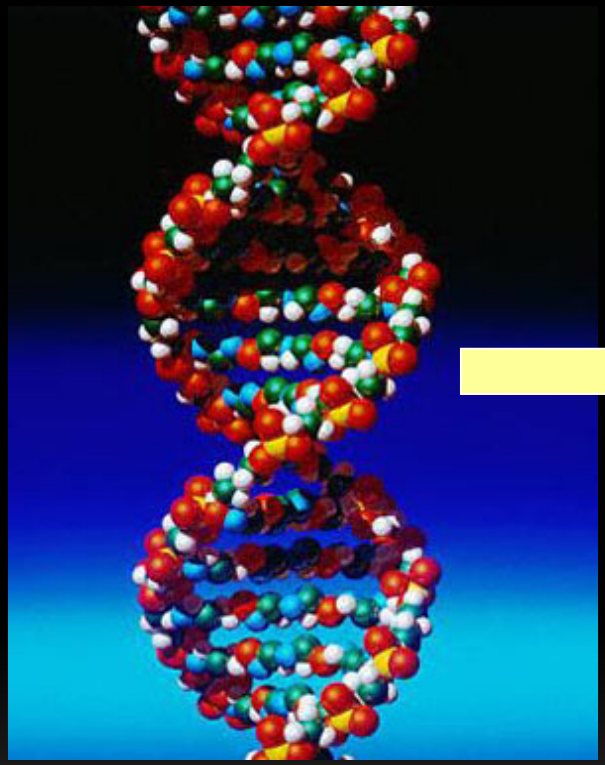
Monitoring

- ❑ Monitoring after 3, 6 and 12 months of therapy
- ❑ After CCgR achievement only once per year

ELN reccomendations, Baccarani et al. J Clin Oncol 2009

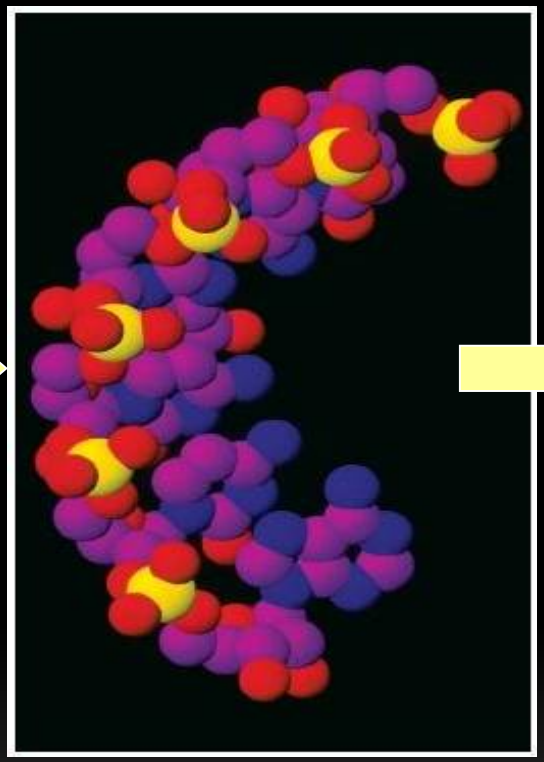
Molecular biology

DNA



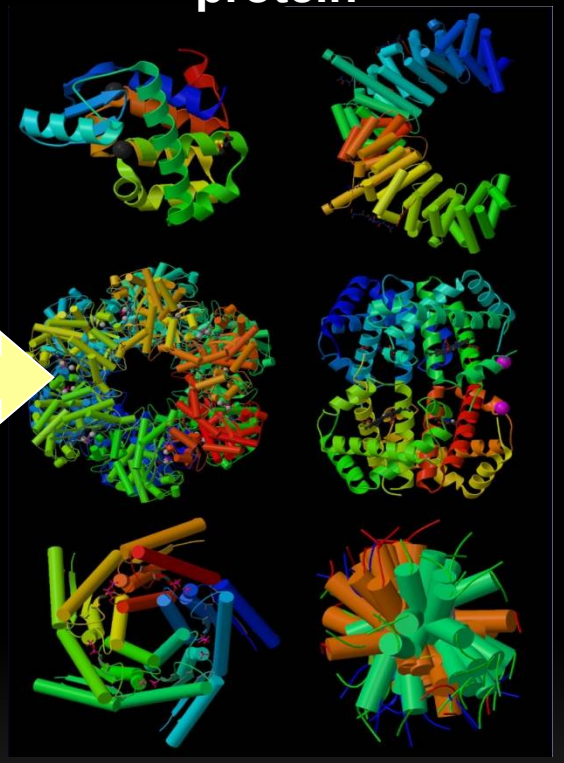
DeoxyriboNucleic Acid encodes genetic information

mRNA



messenger RNA is the key intermediary in gene expression, translating the DNA's genetic code into the amino acids that make up proteins

protein



Proteins are required for the structure, function, and regulation of the body's cells, tissues, and organs

How do we monitor CML patients today?

Molecular biology

BCR-ABL detection from peripheral blood:

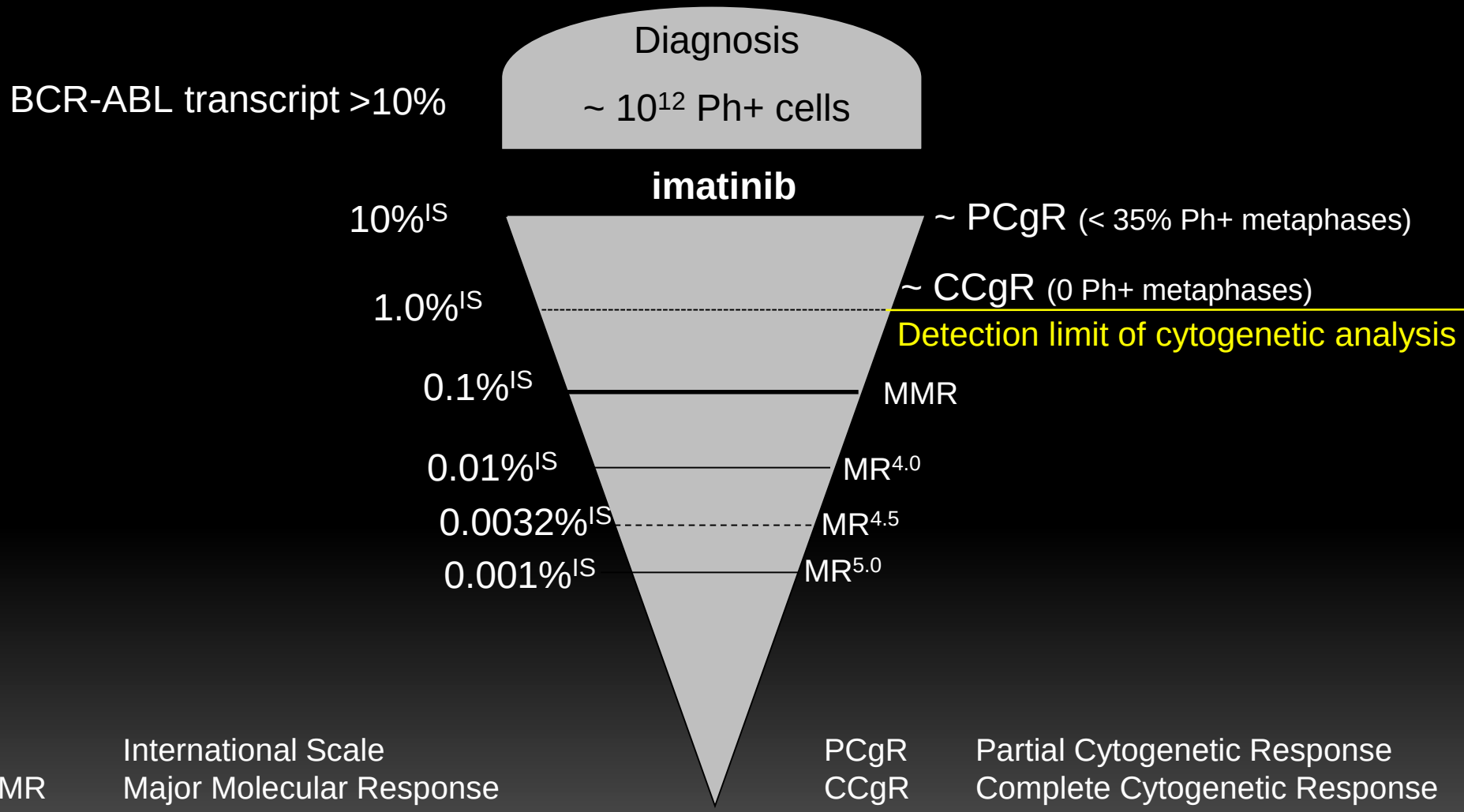
1) CML confirmation and characterisation of BCR-ABL transcript at diagnosis

2) BCR-ABL transcript level monitoring during CML treatment

3) Mutation detection in BCR-ABL kinase domain

How do we monitor CML patients today?

BCR-ABL transcript level monitoring



IS	International Scale	PCgR	Partial Cytogenetic Response
MMR	Major Molecular Response	CCgR	Complete Cytogenetic Response
MR	Molecular Response		

Modified: S. Branford and T. Hughes 2010 Seminar Hematol

How do we monitor CML patients today?

BCR-ABL transcript level monitoring

WHY?

- ❑ monitoring response to treatment and minimal residual disease (patients in complete molecular remission or after stem cell transplantation)

WHAT MATERIAL?

- ❑ total leukocytes from peripheral blood

WHEN?

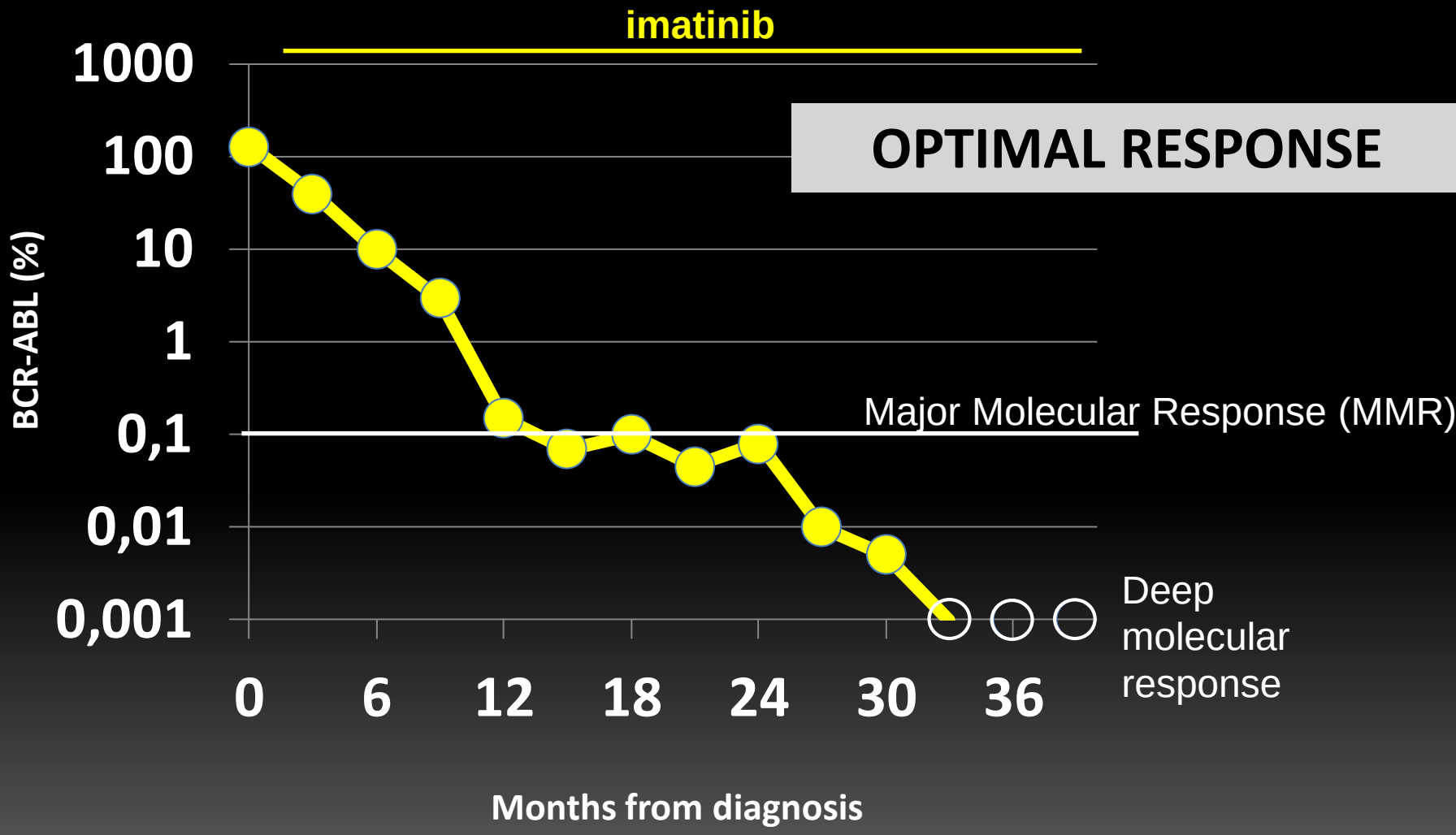
<u>Imatinib (nilotinib, dasatinib) treated patients</u>

- ❑ regulary every 3 months
- ❑ shorter intervals when indicated – e.g. marked rise of BCR-ABL

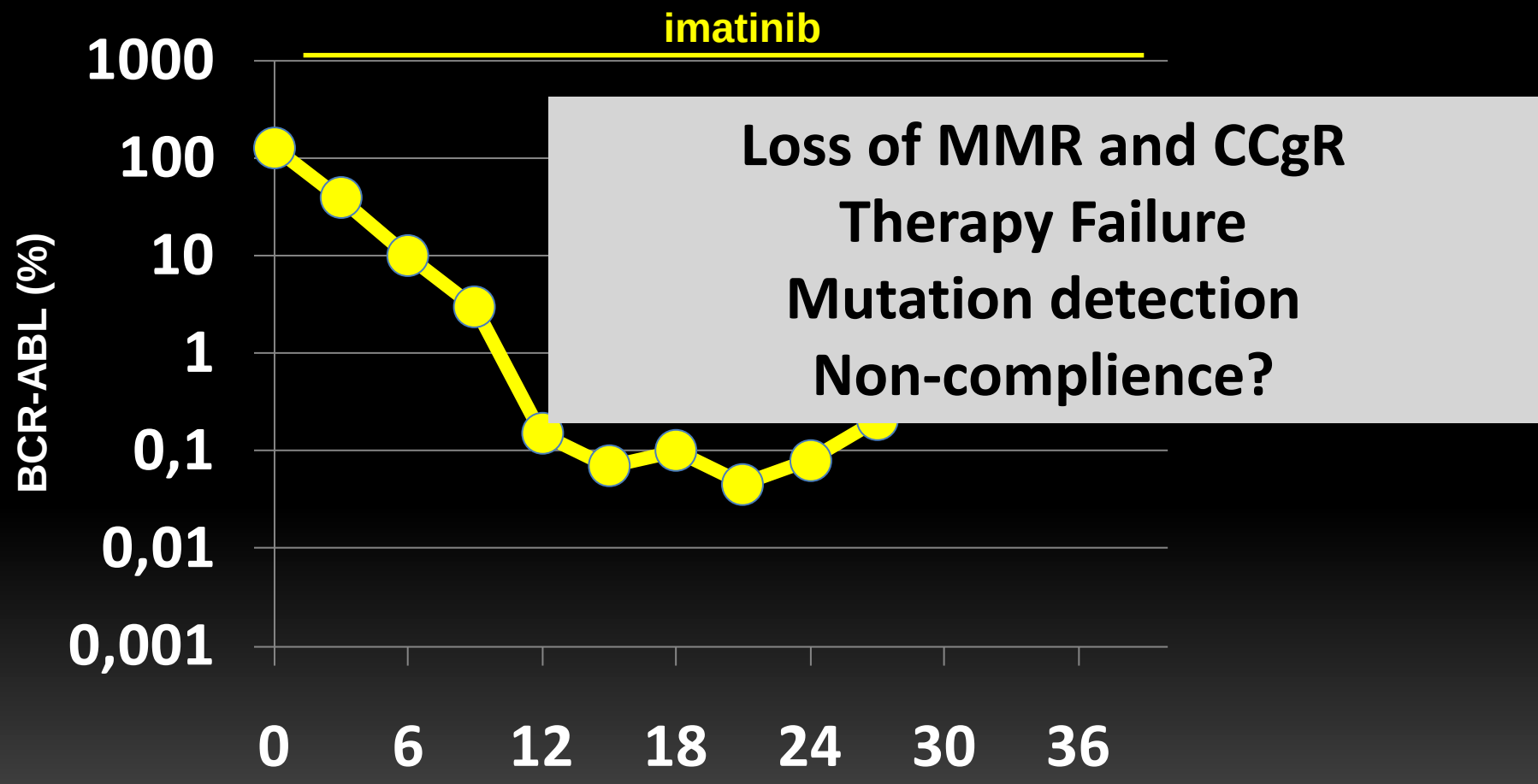
<u>After bone marrow transplantation</u>

- ❑ monthly till complete molecular remission

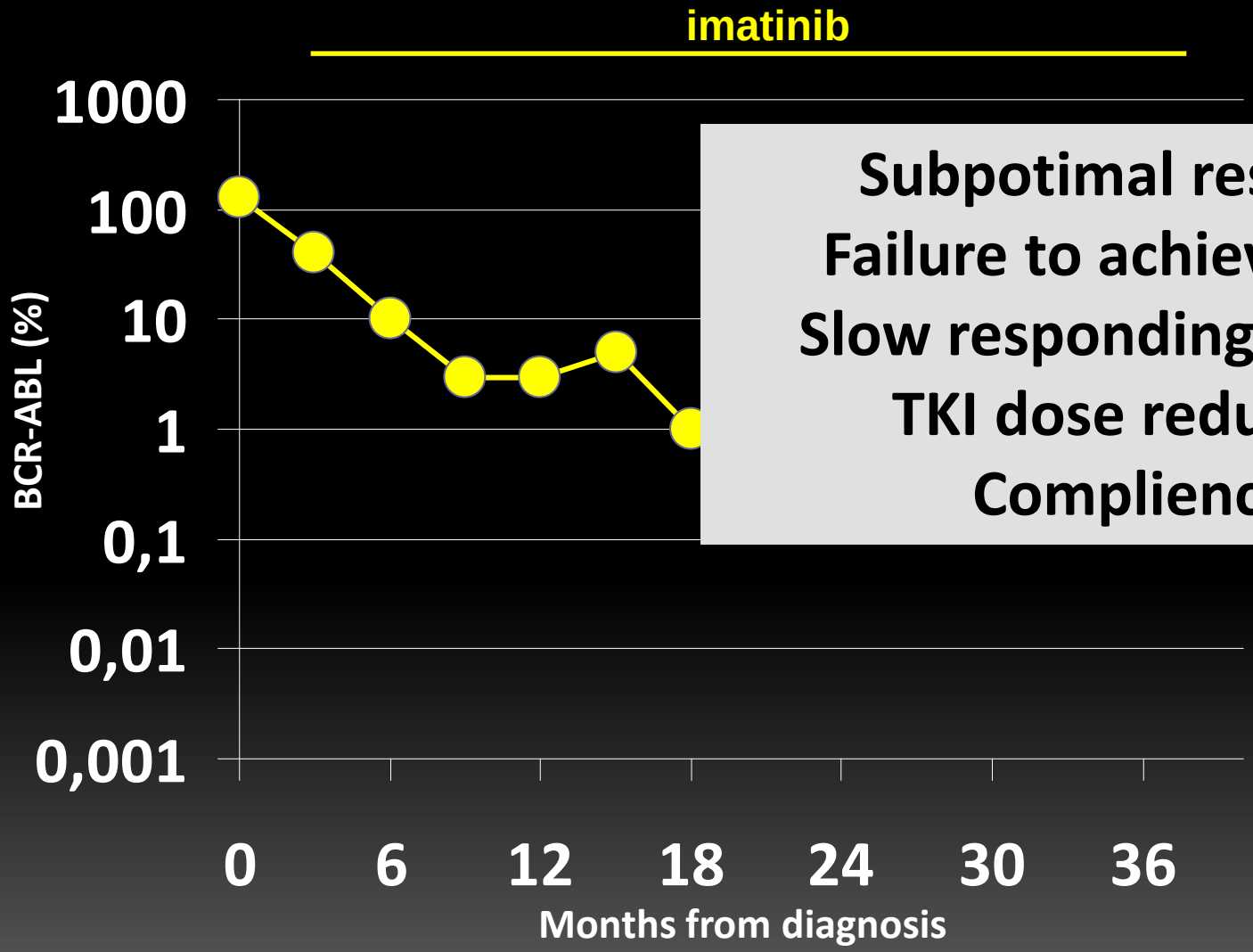
BCR-ABL transcript level monitoring



BCR-ABL transcript level monitoring



BCR-ABL transcript level monitoring



Subpotimal response
Failure to achieve MMR
Slow responding patient?
TKI dose reduction
Complience?

What are the differences between all available tests?

Pro's and con's

Cytogenetic analysis

- Necessary for CML diagnosis
- Complete cytogenetic response achievement
  → good prognostic factor
- Detects additional chromosomal abnormalities
  → bad prognostic factor
- Specimen - bone marrow aspirates
- Not enough sensitive

Molecular monitoring

- High senstivity (1 CML cell in 1 million cells)
- Major molecular response achievement
  → good prognostic factor
- Early detection of resistance development and disease progression
  → early therapy management
- Specimen – peripheral blood
- Measurement variability
- Necessity of interlaboratory standardization

Do we still need cytogenetics?

It depends…

Cytogenetic analysis after CCgR achievement

- In case of additional chromosomal abnormalities in Ph negative cells
  → monitoring of changes

- When resistance development is suspected (significant molecular and hematological progression)

BUT

In case of stable molecular response and without presence of additional chromosomal abnormalities:

- Repeated cytogenetic analysis have quite low prognostic value
- It is recommended a regular molecular monitoring without cytogenetic analysis

What are the different types of PCR tests available?

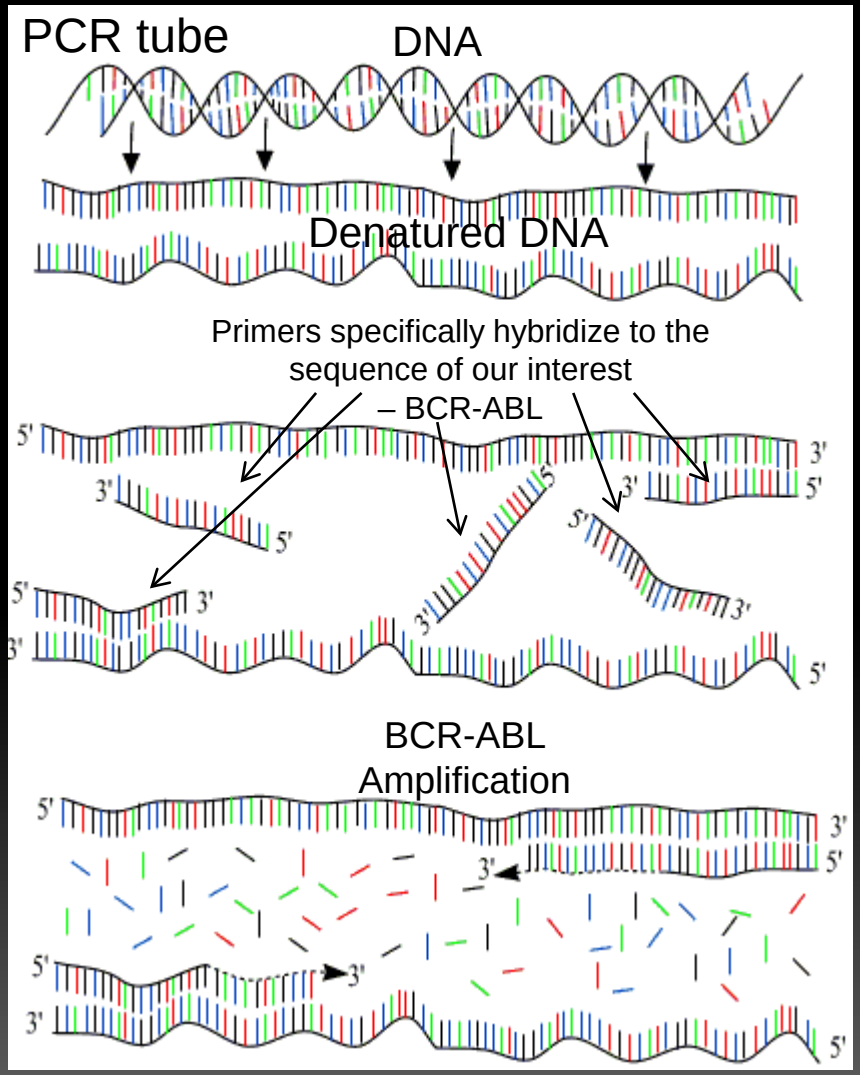
quantitative, qualitative, nested

Qualitative PCR says, if BCR-ABL gene is present in the sample:

- ❑ Regular PCR
-1 type of BCR-ABL sequence amplified

- ❑ Multiplex PCR
- More than 1 type of BCR-ABL sequence amplified

- ❑ Nested PCR
- After regular PCR, PCR product is the template for second PCR
- The most sensitive technique



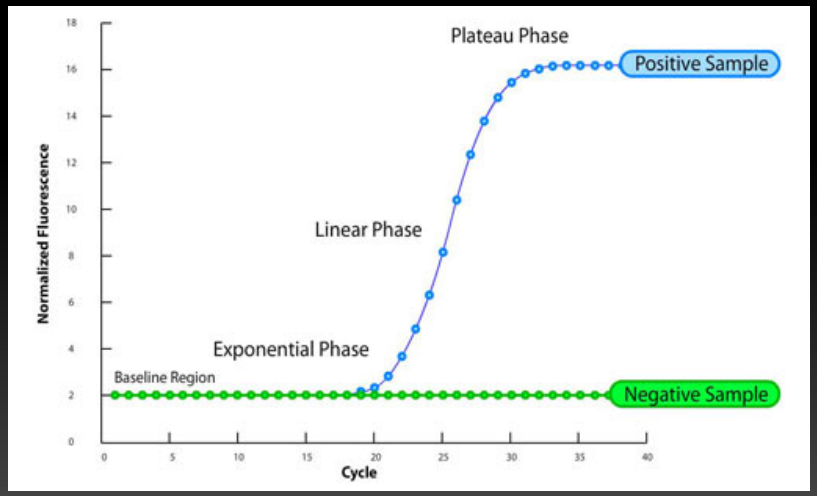
Andy Vierstraede, 1999

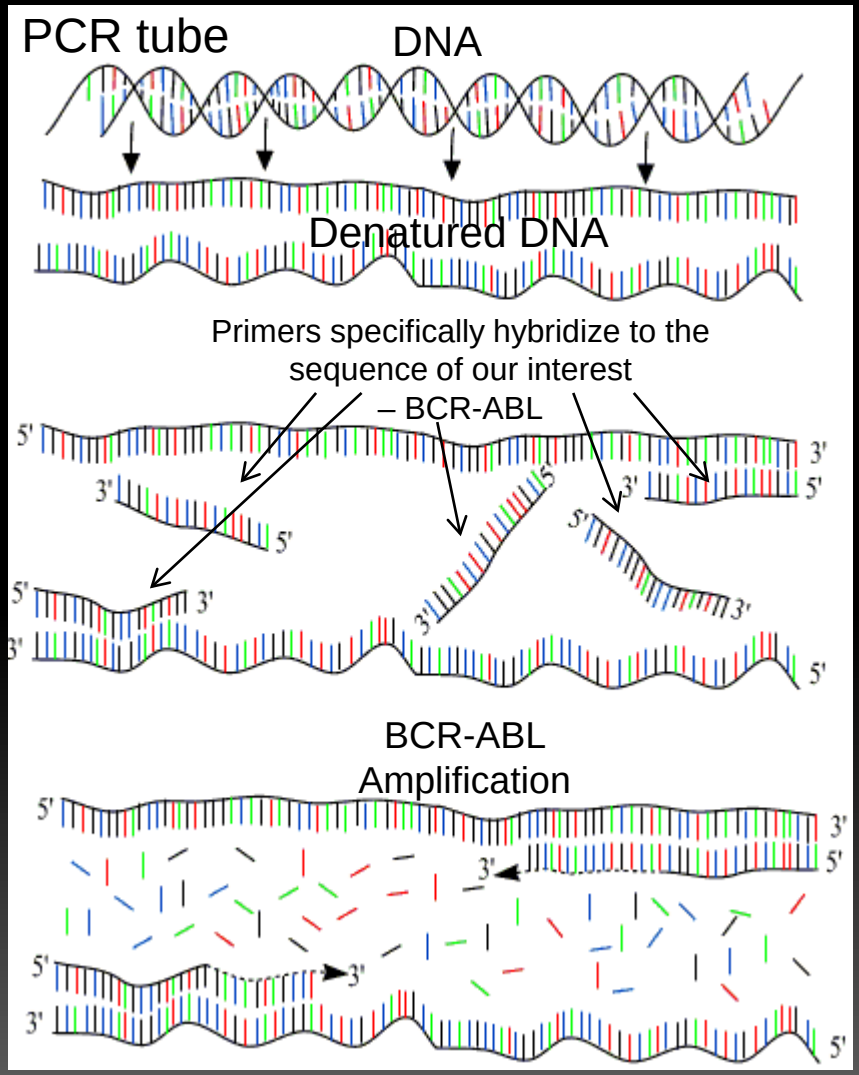
What are the different types of PCR tests available?

quantitative, qualitative, nested

Quantitative PCR says, how much of BCR-ABL gene is present in the sample:

- ❑ Real-time PCR
- Much more sensitive than regular PCR
- Precise quantification of BCR-ABL copies
- Nested real-time PCR





Andy Vierstraede, 1999

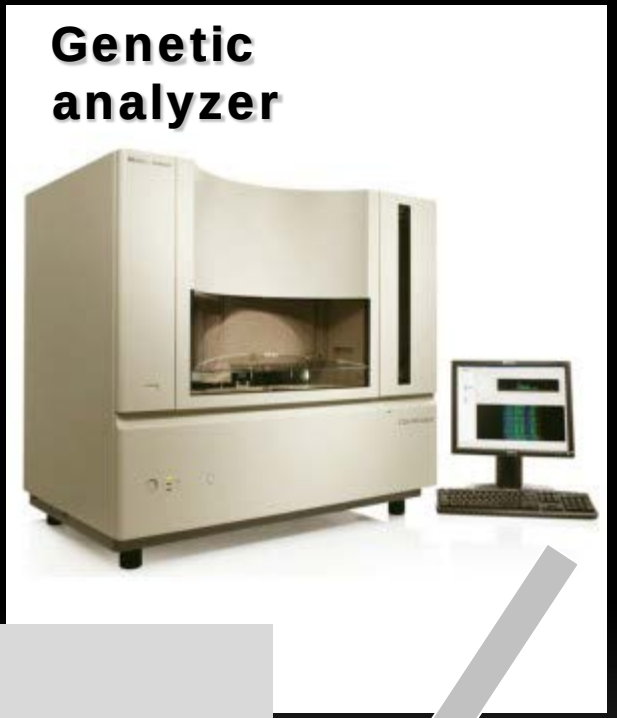
The basics of mutations testing

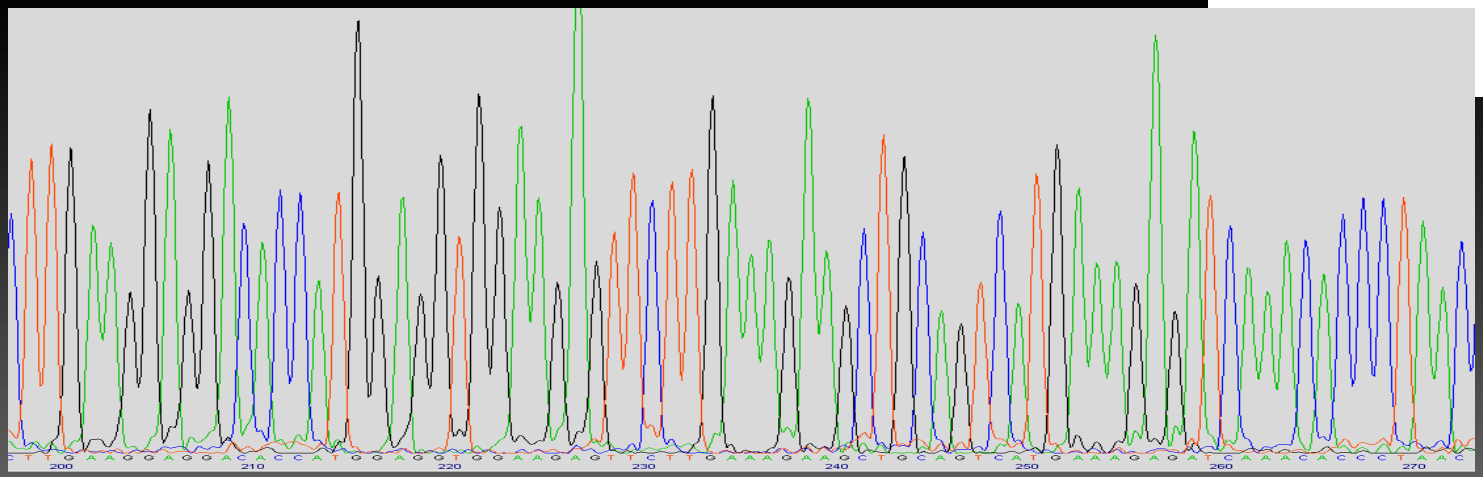
Sanger Sequencing

Sequencing

- ❑ “reading“ of DNA
- ❑ nucleotides **C**, **G**, **A**, **T**

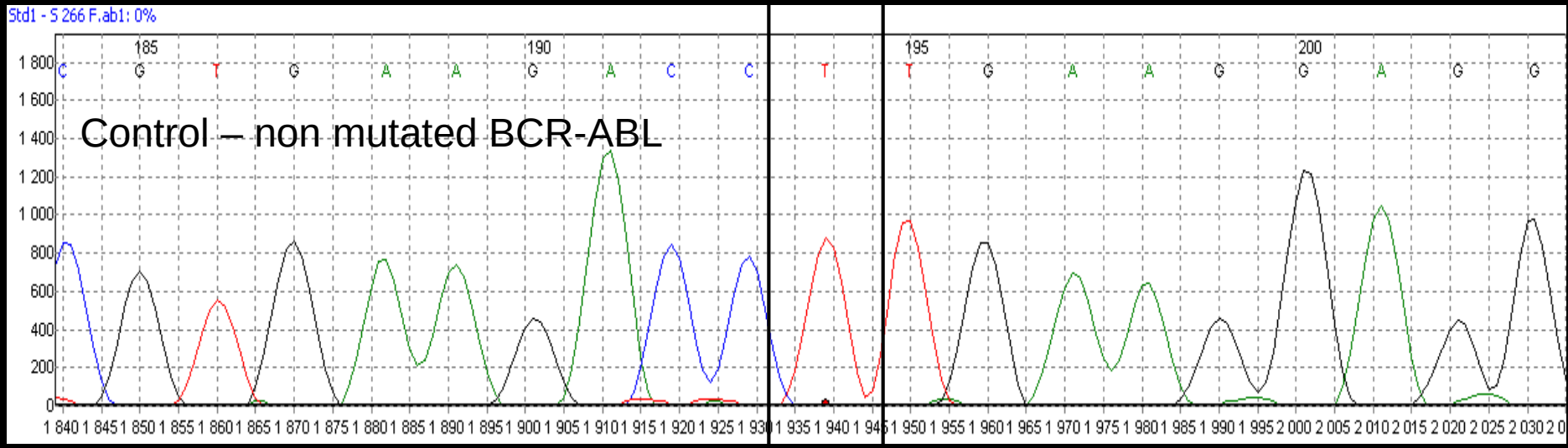
→ building blocks of DNA

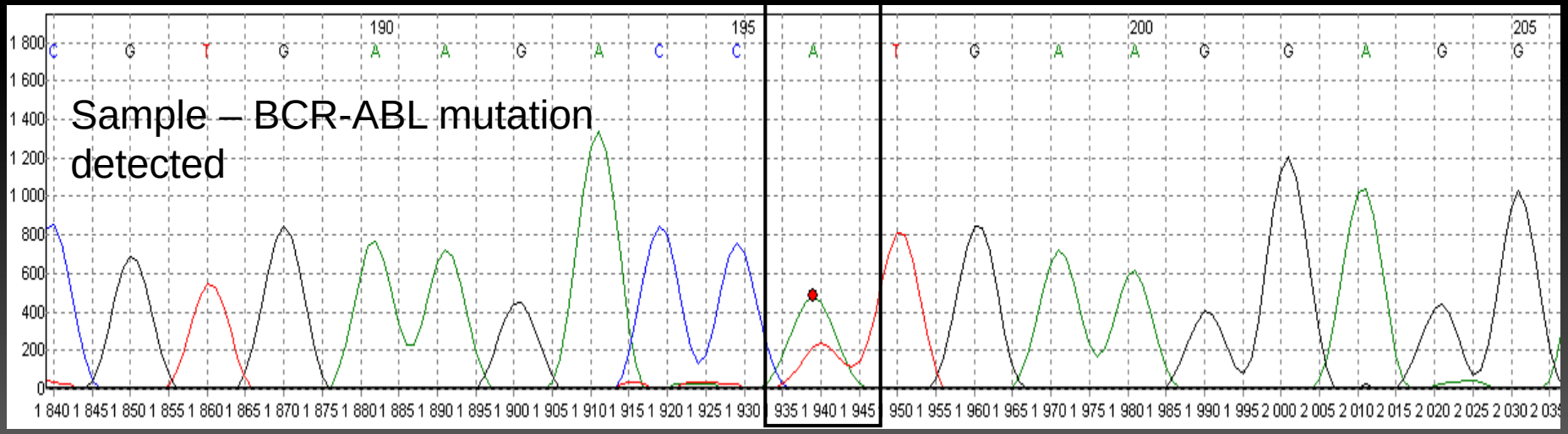




The basics of mutations testing

Sanger Sequencing





Mutations monitoring

Sanger Sequencing