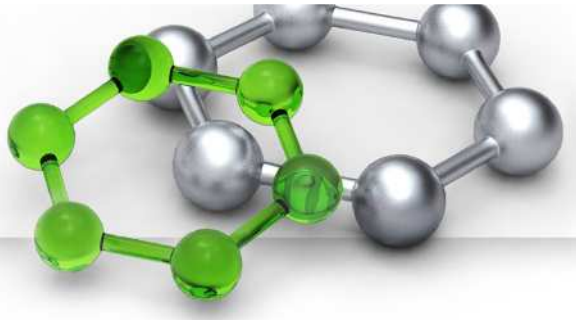

MOLECULAR GENETICS IN DIAGNOSTICS OF HEMATOONCOLOGICAL AND INHERITED DISEASES

Šárka Pavlová

Centre of Molecular Biology and Gene Therapy - Group of Tumor Genomics,
Dpt. of Internal Medicine - Hematooncology
University Hospital Brno and Medical Faculty, Masaryk University Brno

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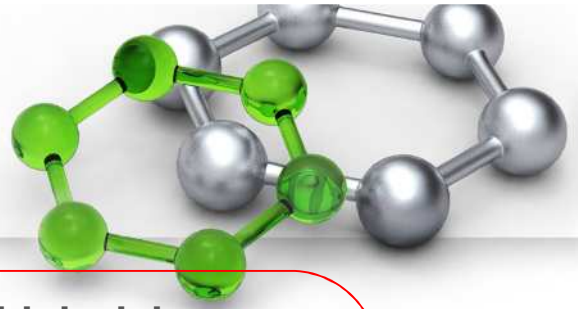
CEITEC: MOLECULAR MEDICINE



WP1: Development of novel therapeutic strategies for the high-risk cancer patients

WP2: Introduction of genomic approaches to cancer research and diagnostics

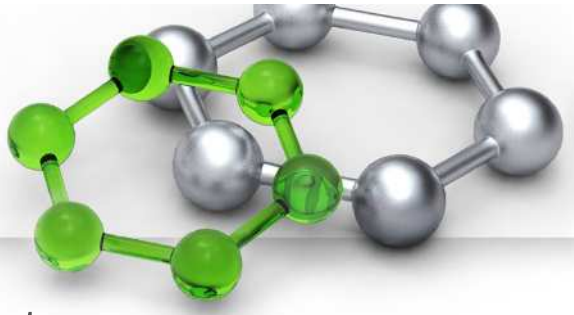
WP 3: Genetics and epigenetics of inherited disorders

WP4: Advanced microbiological studies

WP5: Molecular immunology and allergology

- Laboratory of Medical Genomics (Sarka Pospisilova)
  + GENOMIC CORE FACILITY
- Laboratory for Molecular Oncology (Martin Trbusek)
- Laboratory of Inherited Disorders (Lenka Fajkusova)
- Laboratory for Advanced Microbiological Studies (David Smajs)
- Laboratory for Molecular Immunology and Allergology (Tomas Freiberger)
- Laboratory of Genome Dynamics (Eduard Kejnovsky)

HEMATOLOGICAL MALIGNANCIES: leukemias and lymphomas



- acquired DNA changes in cells of hematopoietic system
- deregulation of hematopoiesis and accumulation of nonfunctional malignant cells in blood, bone marrow and/or lymphoid tissue

IMPAIRMENT OF BLOOD FUNCTIONS - immunity, hemocoagulation, transport of blood gases and other molecules

TREATMENT - chemotherapy, immunotherapy (monoclonal antibodies), HSCT (hematopoietic stem cell transplantation)

MOLECULAR GENETICS AT MANY LEVELS - diagnosis, prognostic markers, monitoring and predicting treatment response....

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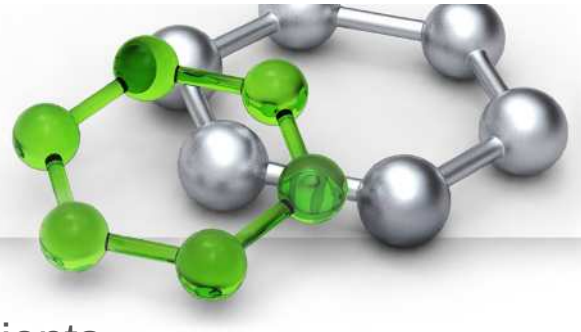
METHOD
FOR MOLECULAR DIAGNOSTICS
OF INVASIVE FUNGAL INFECTIONS

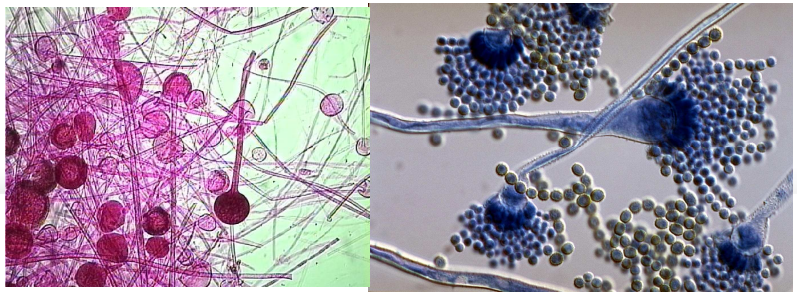


- **life-threatening complication** in hematooncological patients
- difficult **diagnostics, no reliable molecular diagnostic** method available

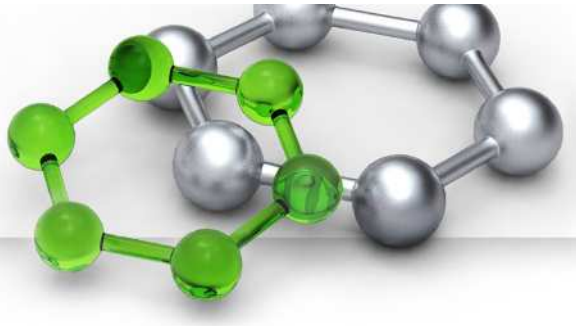
DEVELOPMENT AND OPTIMISATION:

- **species-specific real-time PCR** methods for detection of five most clinically important ***Aspergillus*** **spp.** and **zygomycetes**
- **real-time PCR with High Resolution Melting (HRM) analysis** for detection of clinically important **zygomycetes**

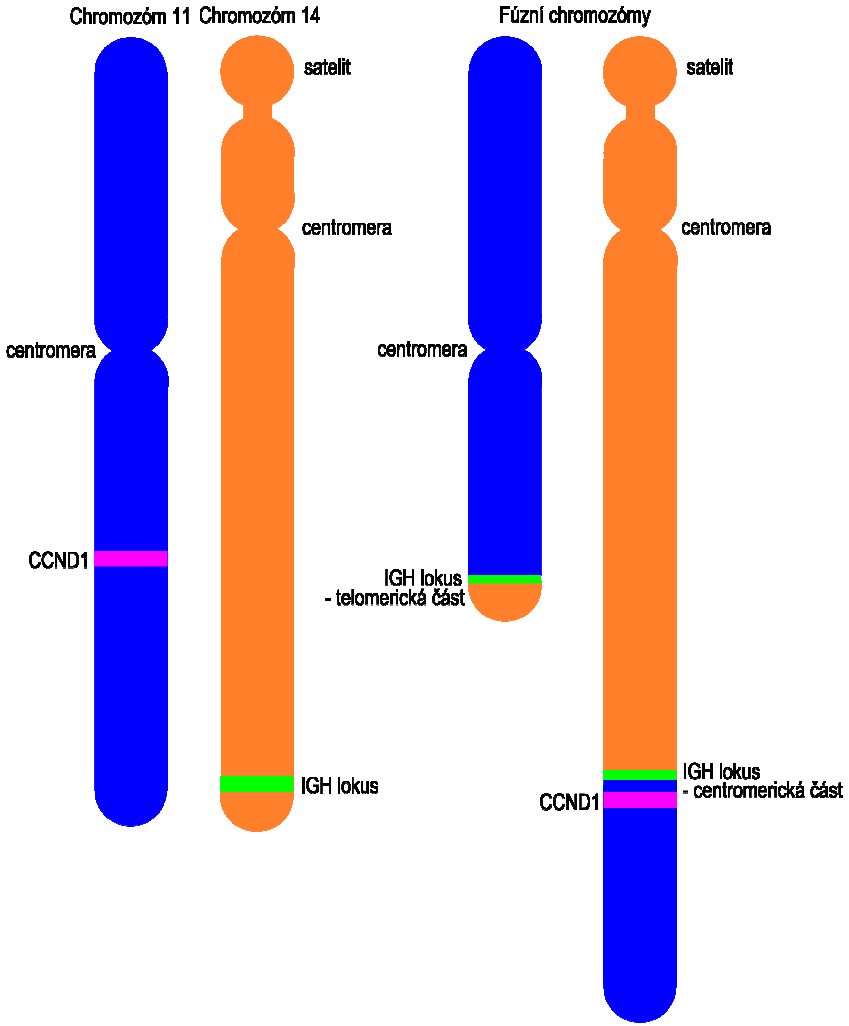
- **routine laboratory diagnostics - tissue, bronchoalveolar lavage**
- Czech and international patent in process



METHOD FOR ACCURATE DETECTION OF CHROMOSOMAL TRANSLOCATION t(11;14)(q13;q32) IN MCL



- >95% **Mantle cell lymphoma (MCL) – translocation t(11;14)(q13;32)**
- cyclinD1/IgH
- exact position of the breakpoint – sequence for design of specific real-time PCR assay for **MRD (minimal residual disease) detection**
- till now – breakpoint in MTC (major translocation cluster) in 30-40% patients, clonal Ig in rest (low sensitivity 10^{-1-2})



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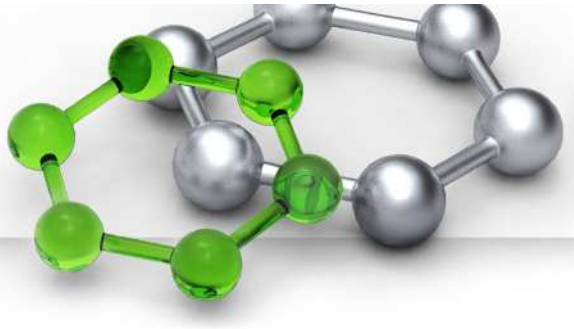

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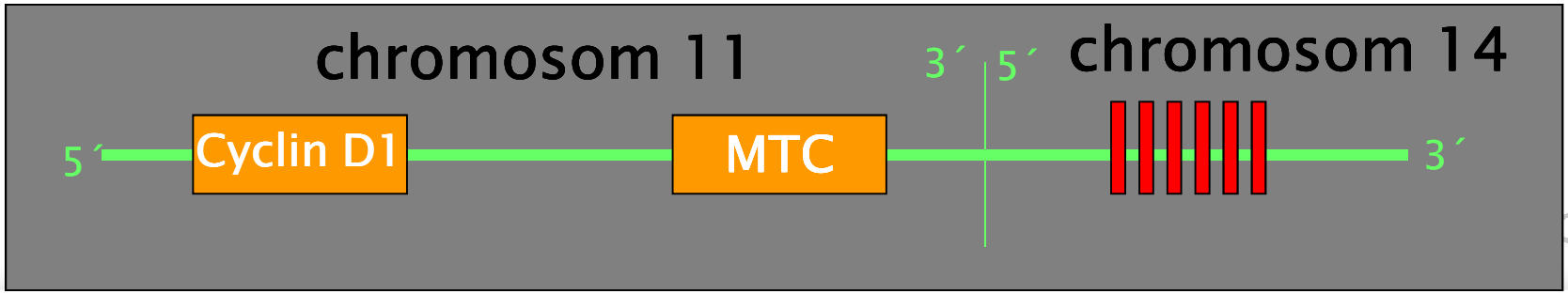

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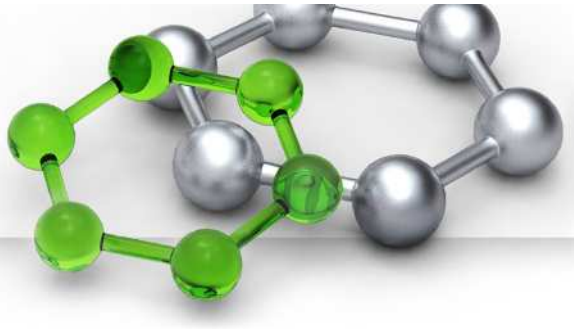

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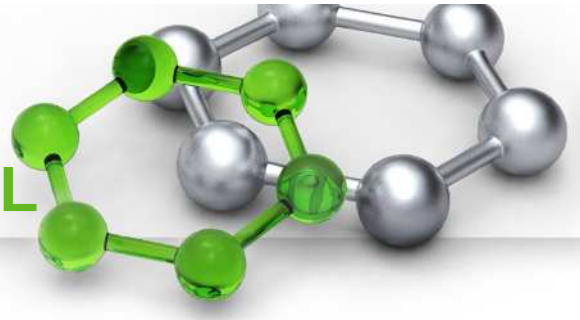
NEW TECHNIQUE DEVELOPMENT - multiplex long range PCR

approx. 80% of translocation detected – detection sensitivity 10^{-3}

- Czech patent in process

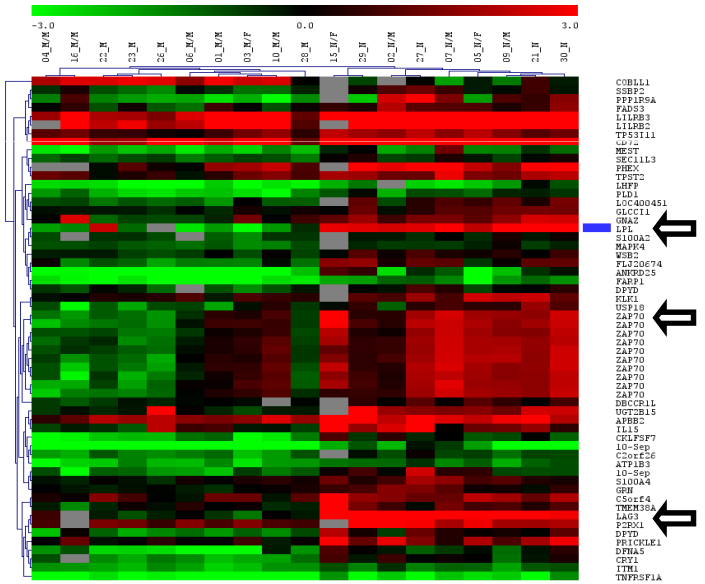
SET OF 3 GENES WITH PROGNOSTIC SIGNIFICANCE IN CLL



- mutational status of Ig heavy chain (IgVH) – important prognostic marker in Chronic lymphocytic leukemia (median survival 8 and 25 years)
- time-consuming analysis

DESCRIPTION OF 3-GENE SET (LAG3, LPL, ZAP70)

- expression by Real-time PCR - prognostic significance comparable to IgVH mutational status
- Czech patent



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Kotaskova J., Tichy B., Trbusek M., et al: ***High Expression of Lymphocyte-Activation Gene 3 in Chronic Lymphocytic Leukemia Cells is Associated with Unmutated IGHV and Reduced Treatment-Free Survival.*** *Journal of Molecular Diagnostics (2010), 12(3):328-34.*

SET OF 3 GENES
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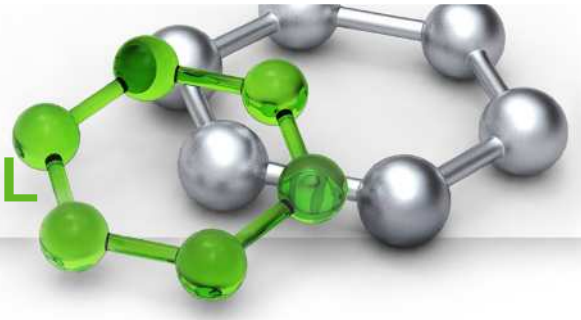

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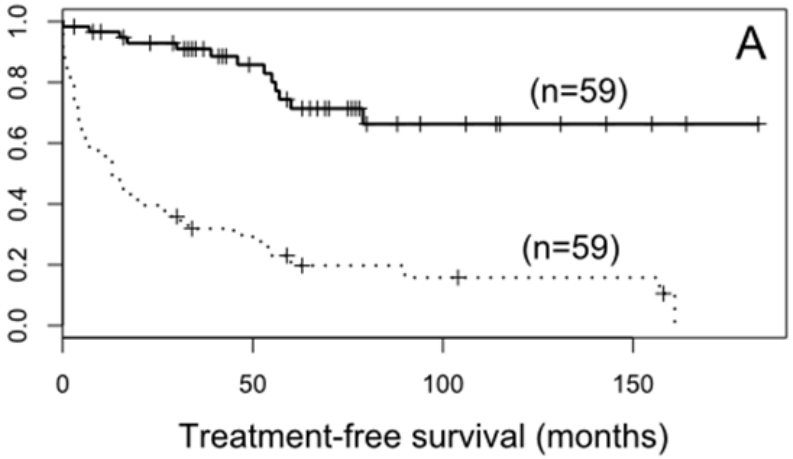
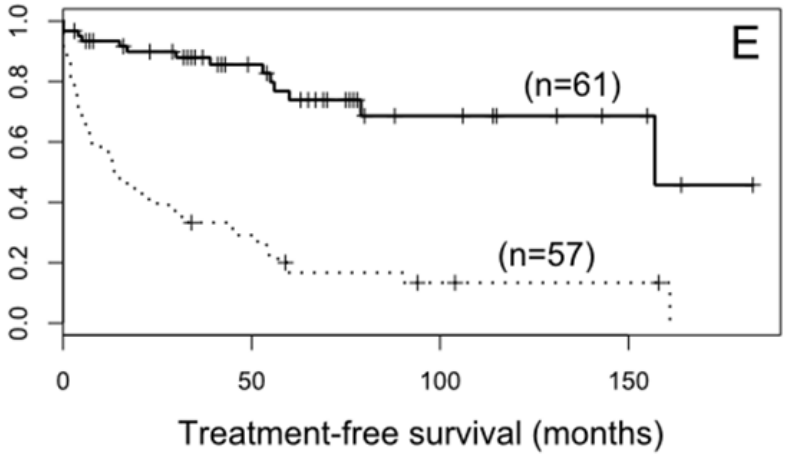
Survival according to IgVH mutational status



Survival according to ZAP70, LAG-3, LPL expression



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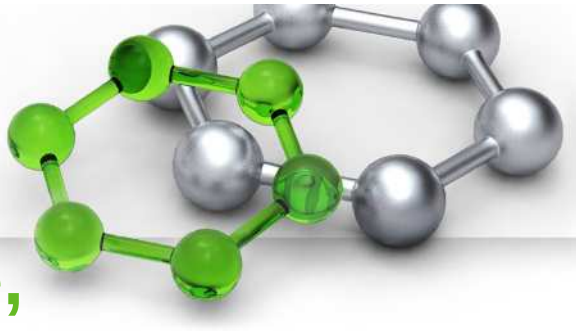

INHERITED DISEASES: neuromuscular, neurodegenerative, and metabolic diseases

- Change in DNA in all cells of the body = inherited

MOLECULAR GENETICS:

- Diagnosis of this change at DNA level
  → **treatment if available**
  → prenatal diagnosis

- Biological impact of detected changes, confirmation of their relation to the disease

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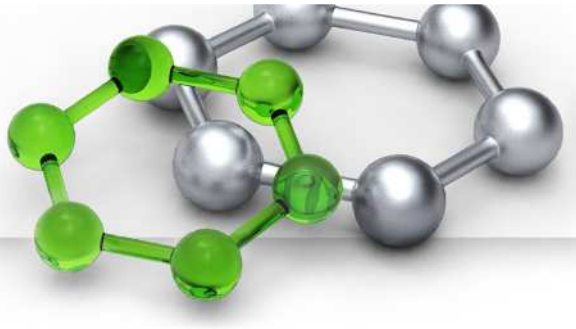
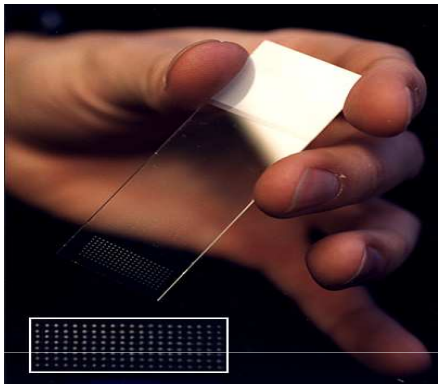
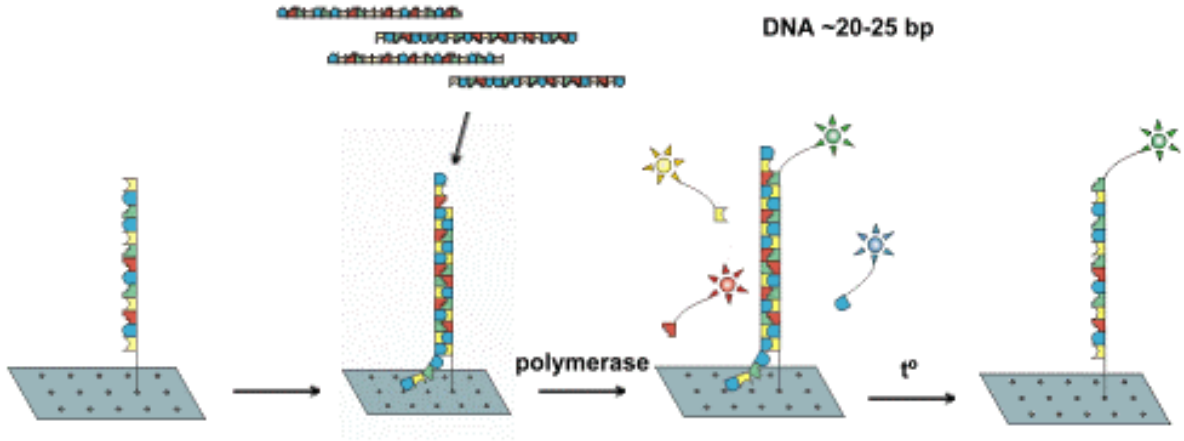
GENOTYPING CHIP FOR WILSON DISEASE AND FAMILIAR HYPERCHOLESTEROLEMIA

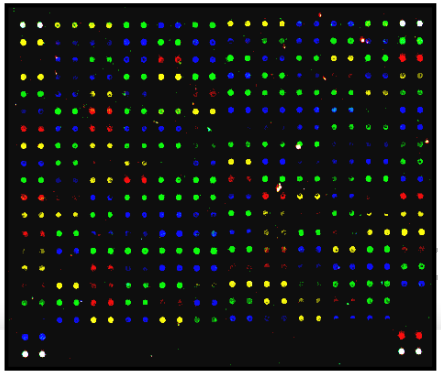


- hybridization reaction between DNA sample and sequence specific probes, immobilized on the chip → simultaneous detection of broad spectrum of mutations



Arrayed Primer Extension (APEX) reaction



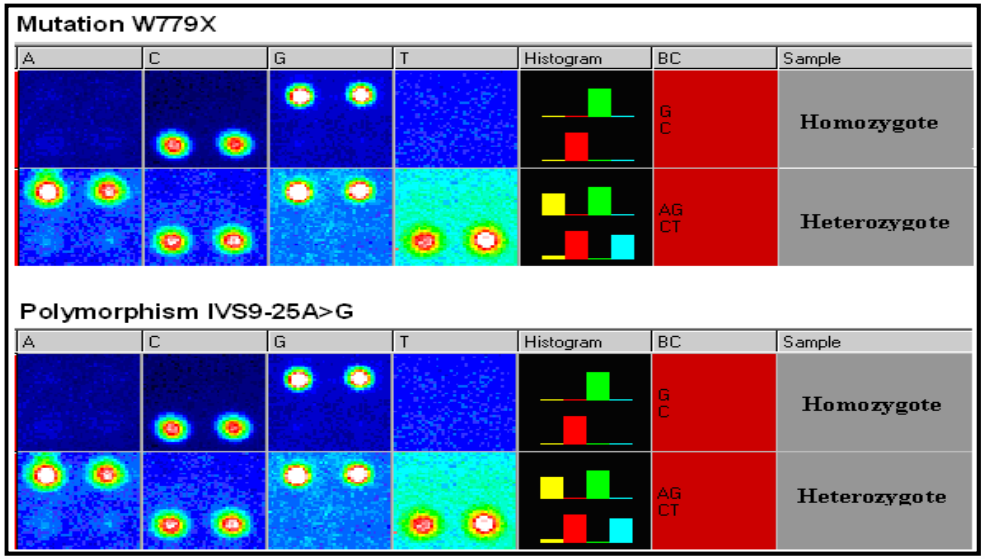


WD chip
Wilson disease

- autosomal recessive inherited disorder of copper metabolism
- 87 mutations and 17 polymorphisms in the *ATP17B* gene

FH chip
Familial hypercholesterolemia

- autosomal dominant inherited disorder caused by mutations in the apolipoprotein B gene and in the *LDLR* gene
- 1 mutation in *ApoB gene*, 169 mutations and 3 polymorphisms in *LDLR* gene



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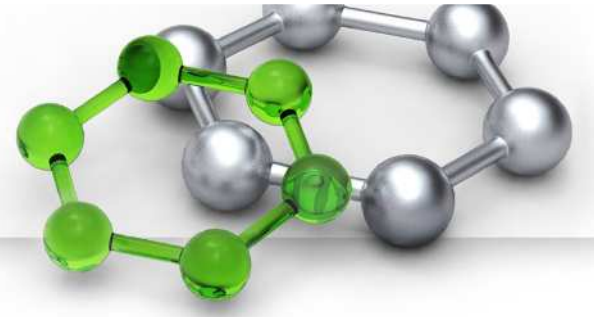
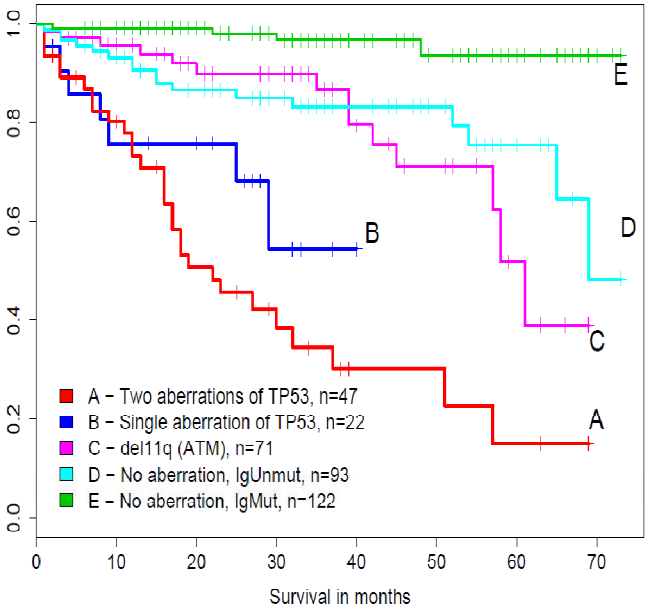
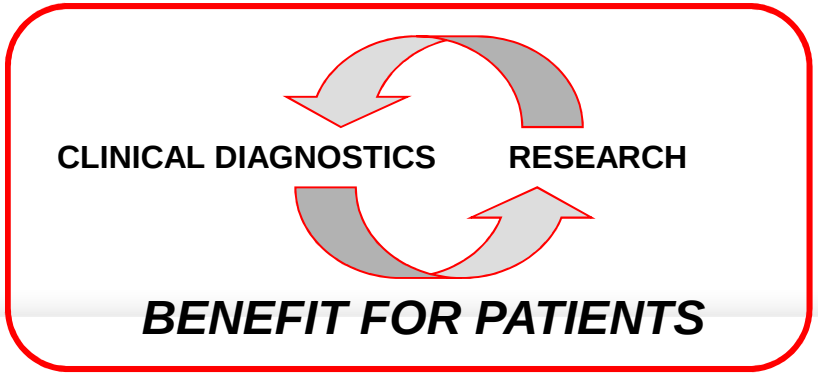
⇨ developed in cooperation with Asper Biotech Ltd., Estonia

p53 DEFECTS IN CHRONIC LYMPHOCYTIC LEUKEMIA



- CLL patients with p53 mutation - **the poorest prognosis (P<0.0001) and response to therapy**
- routine analysis of gene deletions by FISH not sufficient
- weak response to cytotoxic therapy, may profit from monoclonal antibodies or alloHSCT
- *de novo* mutations – short survival
- *Malcíková J., Smardova J., Rocnova L. et al:* ***Monoallelic and biallelic inactivation of TP53 gene in chronic lymphocytic leukemia: selection, impact on survival, and response to DNA damage.*** *Blood. 2009 Dec 17;114(26):5307-14.*





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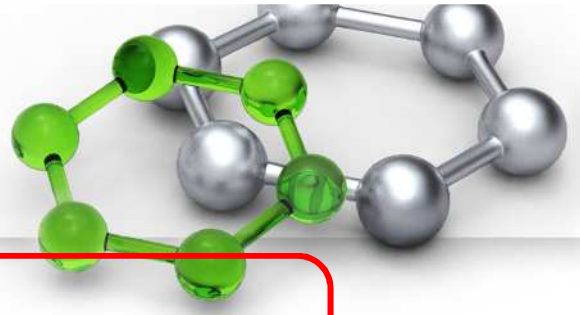

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Sarka Pospisilova

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