VŠEOBECNÁ FAKULTNÍ NEMOCNICE V PRAZE

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Laboratory Hematology and Chronic Lymphocytic Leukemia

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Overview

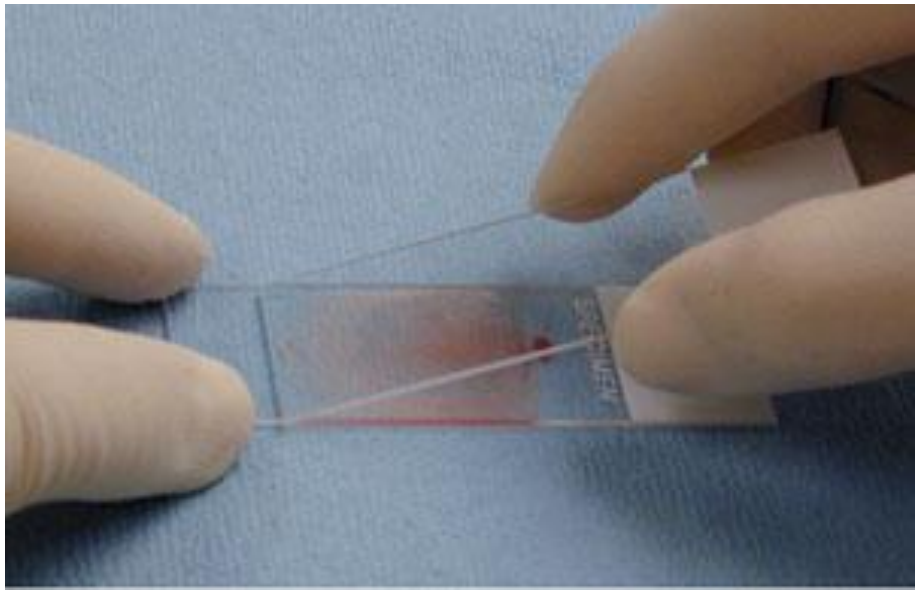
- Lab tests in hematology
  - Full blood count + differential, ICIS score
  - Coagulation tests
  - Flow cytometry (immunophenotyping)
- Chronic Lymphocytic Leukemia

FBC + Diff + Rtc

- **Full blood count**
  - Leukocytes: WBC
  - Erythrocytes: RBC, HGB, HCT, MCV, MCH, MCHC, RDW
  - Platelets: PLT, MPV, PCT, PDW; *IPF (Immature Platelet Fraction)*
  - *NRBC (Nucleated Red Blood Cell)*
- **FBC + Differential of leukocytes**
  - Hematology analyzer and/or microscopic differential (= blood smear)
- **Reticulocytes**
  - absolute number and percentage

Differential of Leukocytes

- Hematology analyzer
  - 5 or 6 main subpopulations (neutrophils, lymphocytes, monocytes, eosinophils, basophils, *IG = immature granulocytes*)
- Microscopic differential (= blood smear)
  - Manual



Differential of Leukocytes

- Hematology analyzer
  - 5 or 6 main subpopulations (neutrophils, lymphocytes, monocytes, eosinophils, basophils, *IG = immature granulocytes*)
- Microscopic differential (= blood smear)
  - Manual
  - Automatic (digital morphology)

Differential of Leukocytes

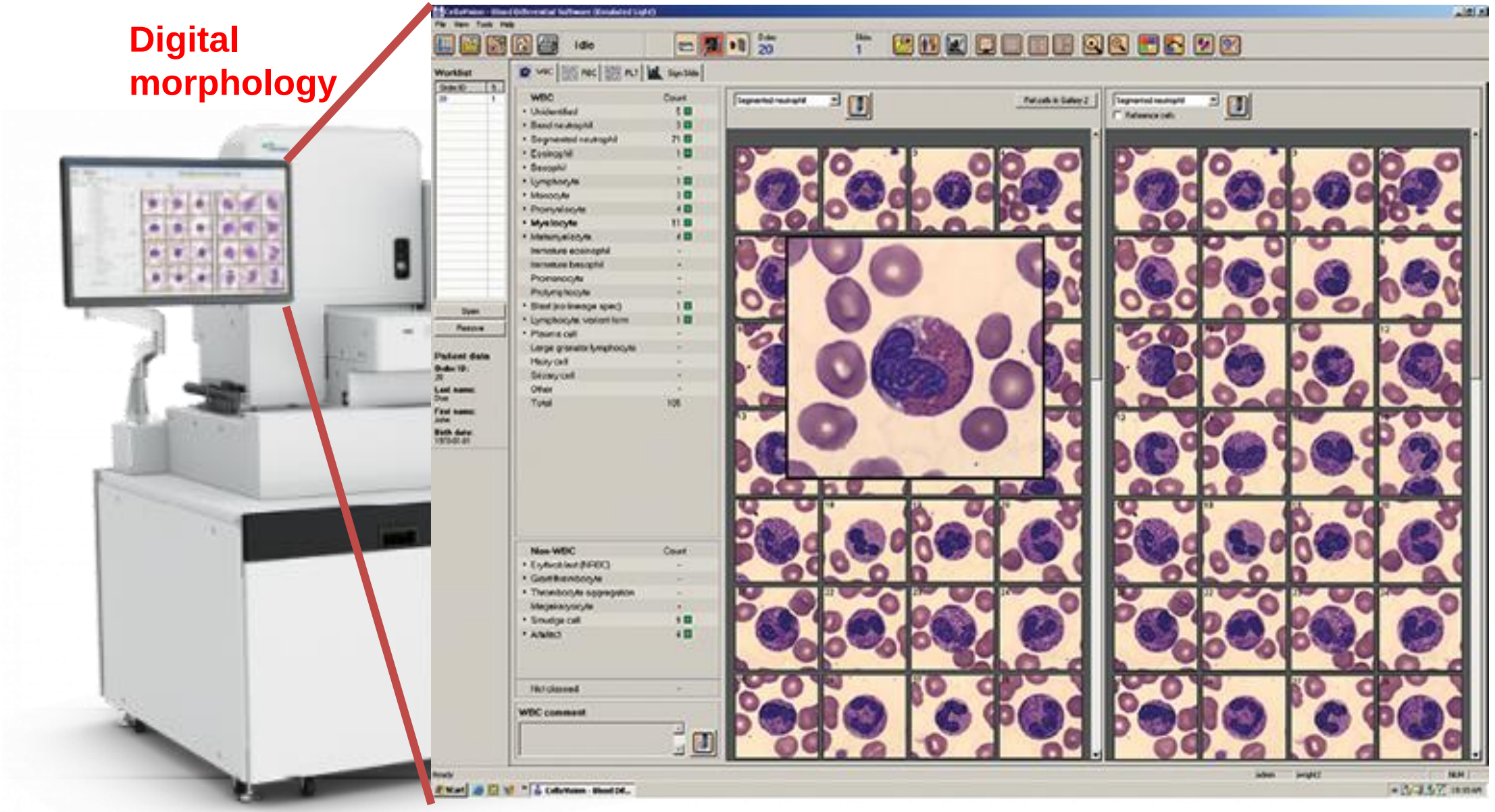
Digital morphology

Staining

Analyzer

Differential of Leukocytes

Digital morphology



FBC – New parameters

- **IPF** – Immature Platelet Fraction
- **NRBC** – Nucleated Red Blood Cell
- **IG** – Immature Granulocytes
- **ICIS** – Intensive Care Infection Score

IPF

- **IPF** – Immature Platelet Fraction
  – ↑ ITP, HIT, DIC…

- 34-year-old woman
- GP > dermatology > internal dpt
- Petechia

- Immune thrombocytopenia (ITP)

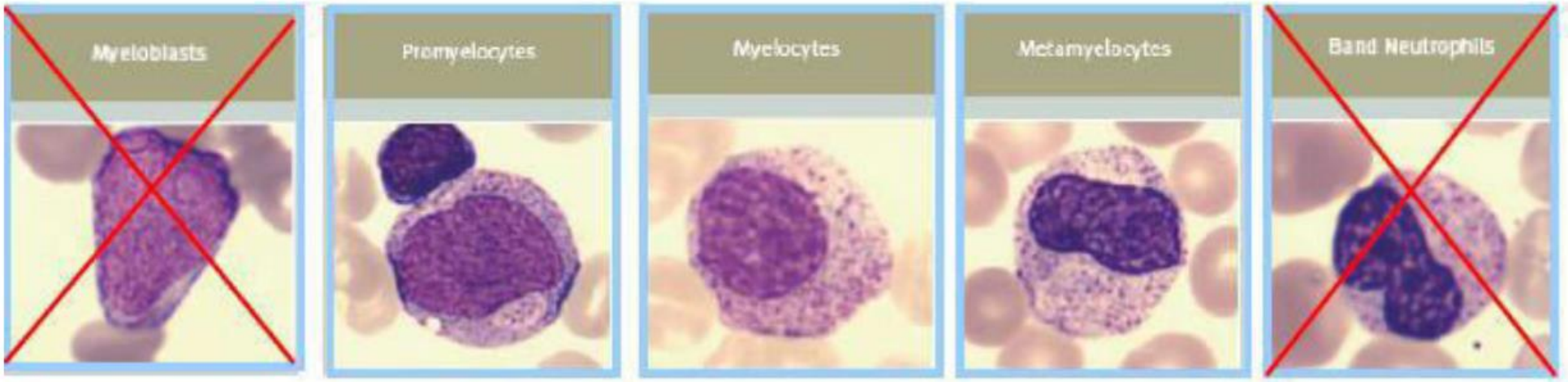
		Krevní obraz-perifer	
10^9/l	4,00..10,00	Leukocyty WBC	4,54
10^12/l	3,80..5,20	Erytrocyty RBC	4,59
g/l	120..160	Hemoglobin HGB	134
1	0,350..0,470	Hematokrit HCT	0,380
fl	82,0..98,0	Stř.obj.ery MCV	82,8
pg	28,0..34,0	Stř.mn.hem.v ery MCH	29,2
g/l	320..360	Stř.konc.hem. v ery MCH	353
%	10,0..15,2	Distr.křiv.ery RDW	12,3
10^9/l	150..400	Trombocyty PLT	2
%	0,8..6,3	Mladé frakce trombocytů **IPF**	34,4
fl	7,8..11,0	Stř.obj.trombo MPV	Txt+His
%	0,120..0,350	Tromb.hematokrit PCT	Txt+His
fl	9,0..17,0	Distr.křiv.tr. PDW	Txt+His
-		Shluky PLT	
		Dif.stroj. relativní	
%	45,0..70,0	Neutrofily NE	49,8
%	20,0..45,0	Lymfocyty LY	46,5
%	2,0..12,0	Monocyty MO	3,3
%	0,0..5,0	Eozinofily EO	0,0
%	0,0..2,0	Bazofily BA	0,4
		Dif.stroj. absolutní	
10^9/l	2,00..7,00	Neutrofily abs. NE	2,26
10^9/l	0,80..4,00	Lymfocyty abs. LY	2,11
10^9/l	0,08..1,20	Monocyty abs. MO	0,15
10^9/l	0,00..0,50	Eozinofily abs. EO	0,00
10^9/l	0,00..0,20	Bazofily abs. BA	0,02
		Diferenciál manuální	<+>
		Ostatní hematologie-	
%	0,0..0,6	Nezralé granulocyty	0,2
10^9/l	0,00..0,09	Nezralé granulocyty abs.	0,01
-	0,00..0,00	Normoblasty / 100 L	0,00
10^9/l	0,00..0,00	Normoblasty abs. strojov	0,00
%	5..25	RTC stroj.	10
10^12/l	0,025..0,100	RTC abs. stroj.	0,044
pg	28,0..38,0	Koncentrace hem. v RET	29,2

NRBC (**N**ucleated **R**ed **B**lood **C**ell)

- Erythroblasts = normoblasts = immature red blood cells
- NRBC → reticulocyte → erythrocyte
- Normal value = 0
  - ↑ reaction to any stress
  - ↑↑ hemolytic anemia, hematological malignancies

IG (**I**mmature **G**ranulocytes)

- Number of immature granulocytes
- Sixth subpopulation of the differential from the analyzer
- ↑ = ‚deeper' left shift (infection)



ICIS

- **Intensive Care Infection Score**
- 5 FBC+Rtc parameters
  - neutrophils (count, MFI), Ret-He, antibody secreting lymphocytes, IG
- New approach for discrimination between sepsis and non-infectious systemic inflammation
- ICIS ≤5 indicates low risk of sepsis/bacterial infection

Normal ranges

- **WBC:** 4-10 x 10^9/l
- **Hgb:** men 135-175 g/l, women 120-160 g/l
  - Significant anemia:
- **PLT:** 150-400 x 10^9/l
  - Increased risk of spontaneous bleeding:
- Neutrophils: 2,0-7,0 x 10^9/l
  - Neutropenia (grade 3):

Normal ranges

- **WBC:** 4-10 x 10^9/l
- **Hgb:** men 135-175 g/l, women 120-160 g/l
  - Significant anemia: < 80 g/l
- **PLT:** 150-400 x 10^9/l
  - Increased risk of spontaneous bleeding: < 20 x 10^9/l
- Neutrophils: 2,0-7,0 x 10^9/l
  - Neutropenia (grade 3): < 1,0 x 10^9/l

Coagulation tests

- APTT (activated partial thromboplastin time)
- Prothrombin time (PT, Quick, INR)
- Thrombin time (TT)
- Fibrinogen
- Antithrombin
- D-dimer

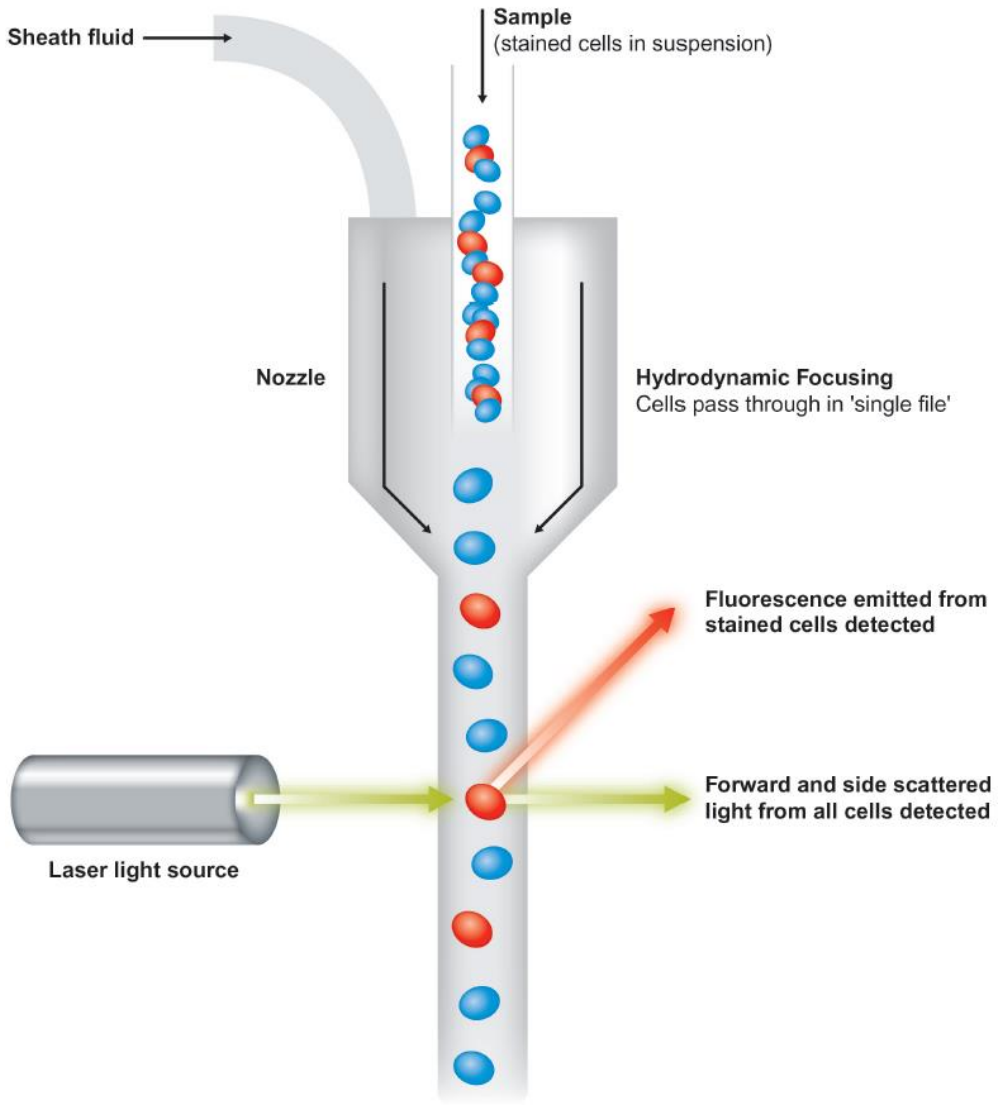
- Anti-Xa

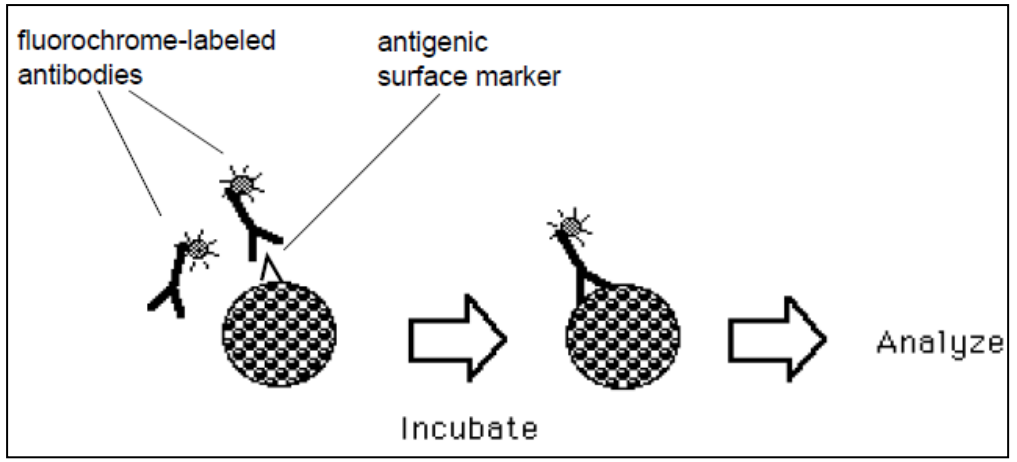
Coagulation tests

- APTT: ↑ *heparin treatment*
- Prothrombin time (PT, Quick, INR): ↑*warfarin*
- Thrombin time (TT): ↑ *heparin*
- Fibrinogen: ↑ *inflammation*
- Antithrombin: ↓ *thrombophilia*
- D-dimer: ↑ *DVT*

- Anti-Xa: *blood sampling 2 to 4 hours after LMWH*

Immunophenotyping





Immunophenotyping

- Test used to identify cells using fluorochrome conjugated antibodies
- CD – „cluster of differentiation“
- Samples
  - PB, BM, lymph nodes, CSF, etc.
- Diagnosis, prognosis, minimal residual disease monitoring

Immunophenotyping

- Hemato-Oncology
  - **Lymphoproliferative disease (lymphocytosis)**
  - Acute leukemia
  - Multiple myeloma
  - Myelodysplastic syndrome
  - PNH (paroxysmal nocturnal hemoglobinuria)
- Hematology
  - HIT (heparin induced thrombocytopenia)
  - Fetal erythrocytes

Case

- 72-year-old woman
- Admitted to hospital for fever

Krevní obraz-perifer:
- Leu: 23,07
- Ery: 2,59
- HB: 86
- HTC: 0,275
- MCV: 106,2
- MCH: 33,2
- MCHC: 313
- RDW: 21,3
- Plt: 74
- MPV: 11,7
- PCT: 0,090
- PDW: 14,9

Dif.stroj. relativní:
- Ne: 12,6
- Ly: 86,5
- Mo: 0,8
- Eo: 0,0
- Ba: 0,1

Dif.stroj. absolutní:
- Ne abs.: 2,91
- Ly abs.: 19,95
- Mo abs.: 0,19
- Eo abs.: 0,00
- Ba abs.: 0,02

Diferenciál manuální:
- SEG: 36,4
- T: 2,1
- Ly: 55,9
- Mo: 4,2
- Eo: 0,7
- My: 0,7

Ostatní hematologie-:
- Nezralé granulocyty %: 0,4
- Nezralé granulocyty abs.: 0,10
- Retikulocyty strojově: 9
- Retikulocyty abs. strojově: 0,024

Case

- 72-year-old woman
- Admitted to hospital for fever

- Leukocytosis (lymphocytosis)
- Macrocytic anemia
- Thrombocytopenia

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- Leu: 23,07
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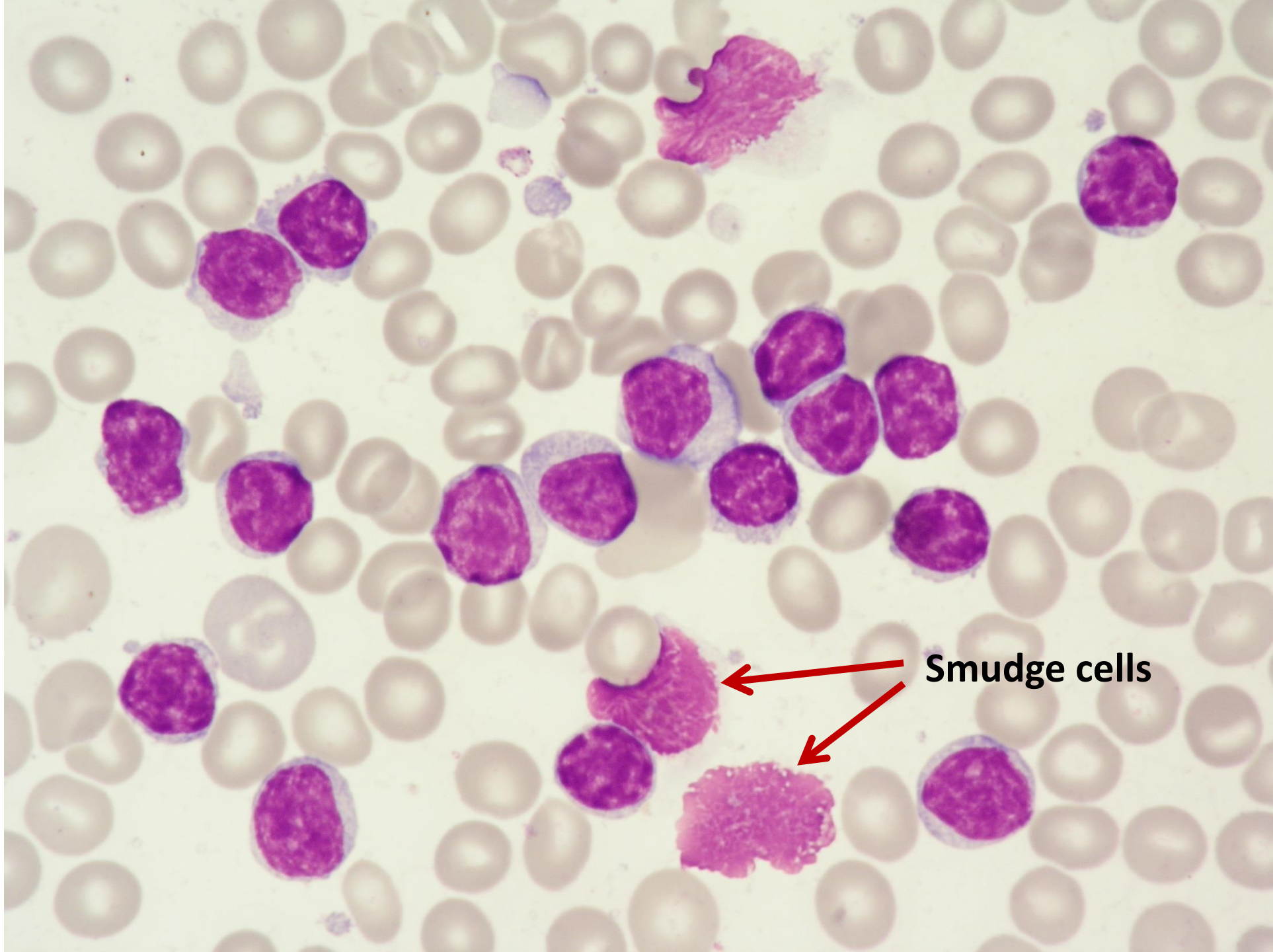
Case

- Biochemistry:
  - LDH ↑, bilirubin (unconjugated) ↑
- Peripheral blood film:
  - Lymphocytosis with mature appearance
  - Smudge cells (damaged cells in film preparation)

Peripheral blood film:

- Mature lymphocytes
- Smudge cells



Case

- Hemolytic anemia
- Lymphoproliferative disorder

Case

- Direct antiglobulin test (Coombs): positive
- Flow cytometry (immunophenotyping)
  - 80% of monoclonal B cells, positive markers: CD19, CD20 (dim), CD5, CD23, CD200, CD43, CD22 (dim), CD25 (dim) a LC Lambda (dim)

- Conclusion: chronic lymphocytic leukemia and autoimmune hemolytic anemia

Chronic Lymphocytic Leukemia

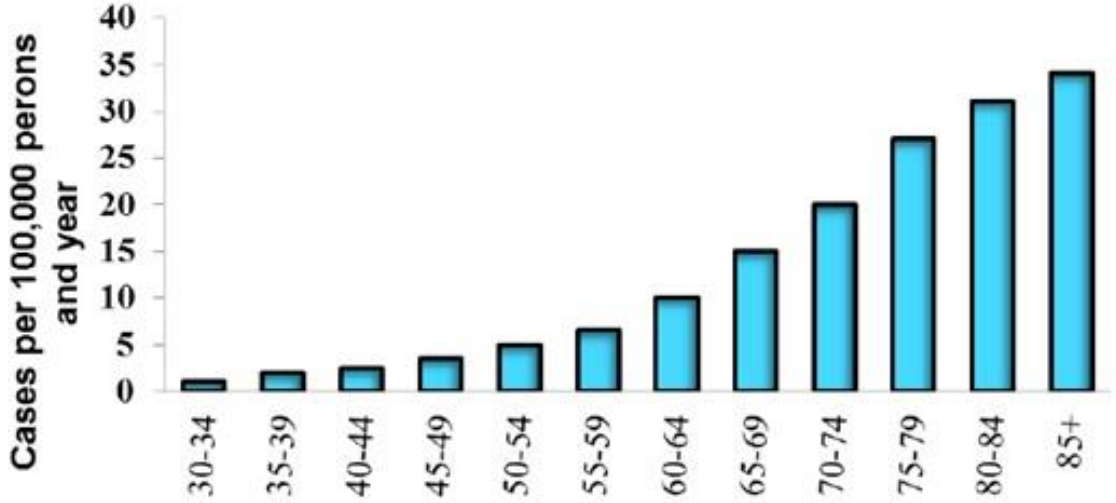
- Statistics
- Clinical features
- Diagnosis
- Staging, prognosis
- Treatment
- Complications

Statistics

- Most frequent leukemia in Western world
- 25 to 30% of all leukemias
- Incidence rate: 5-6/100 000/ year
- More common in men than women (2:1 ratio)

Statistics

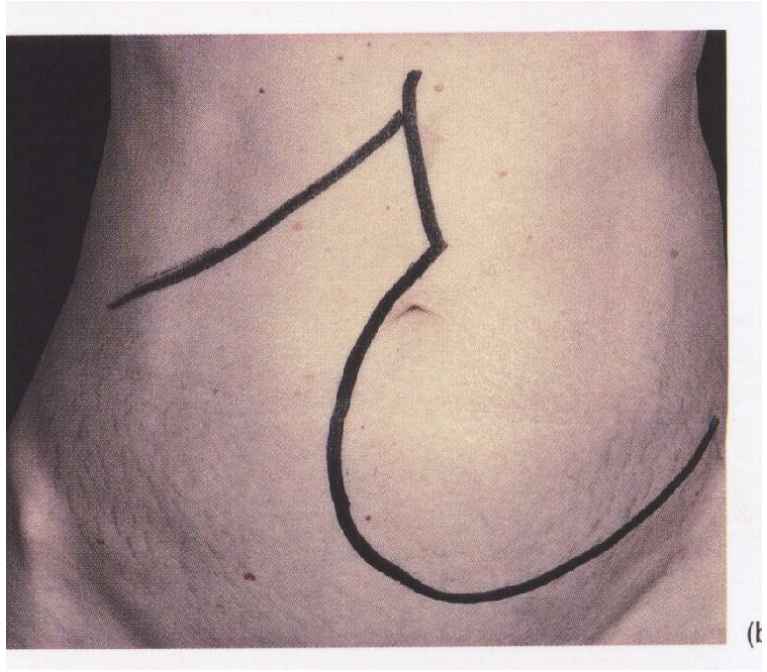
- CLL incidence increases with age
- Median age at diagnosis 72 years



Increasing age-adjusted incidence

Clinical features

- Asymptomatic: 50 % or more (at diagnosis)
- In more advance disease:
  - Fatigue, fever, weight loss, night sweats
  - Lymphadenopathy (painless)
  - Splenomegaly / hepatomegaly
  - Anemia
  - Thrombocytopenia, bleeding



Clinical features

- Recurrent infections
- Autoimmune diseases
- Infiltration of the tonsils (rare)

Diagnosis of CLL

- Presence of at least $5x10^9$ B lymphocytes/L (5000/µL) in the peripheral blood
- Typical immunophenotype

- Tests to establish diagnosis:
- Complete blood count and differential (blood smear)
- Immunophenotyping of lymphocytes

What is the prognosis of patients with CLL?

- Survival 5 years after diagnosis approx. 90%
- Up to a third of CLL patients never require treatment
- We only treat active disease
- Most patients die from causes other than CLL (infection)

Prognostic factors

- We are able to predict patients with good and poor prognosis at diagnosis

- Poor prognosis:
- ↑ Clinical stage (Rai or Binet)
- Unmutated IGHV
- TP53 gene aberration (mutation or deletion)

CLL staging system: Rai

Stage (Rai)	Description
0	Lymphocytosis
I	Lymphocytosis + lymphadenopathy
II	Lymphocytosis + spleno/hepatomegaly
III	Lymphocytosis + anemia (Hb < 110 g/L)
IV	Lymphocytosis + thrombocytopenia (PLT < $100x10^9$/L)

Rai KR et al. Clinical staging of chronic lymphocytic leukemia, Blood 1975

CLL staging system: Rai

Stage (Rai)	Description	Median survival (yrs.)
0	Lymphocytosis	> 10
I	Lymphocytosis + lymphadenopathy	7-11
II	Lymphocytosis + spleno/hepatomegaly	7-8
III	Lymphocytosis + anemia (Hb < 110 g/L)	4-5
IV	Lymphocytosis + thrombocytopenia (PLT < $100x10^9$/L)	4-5

Rai KR et al. Blood 1975.
Nabhan, Rose, JAMA 2014
Wierda et al, Blood 2007
Mayo Clinic CLL database 1995-2015

Treatment options

- **Targeted treatment** (small molecules)
  - BCR and BCL2 inhibitors

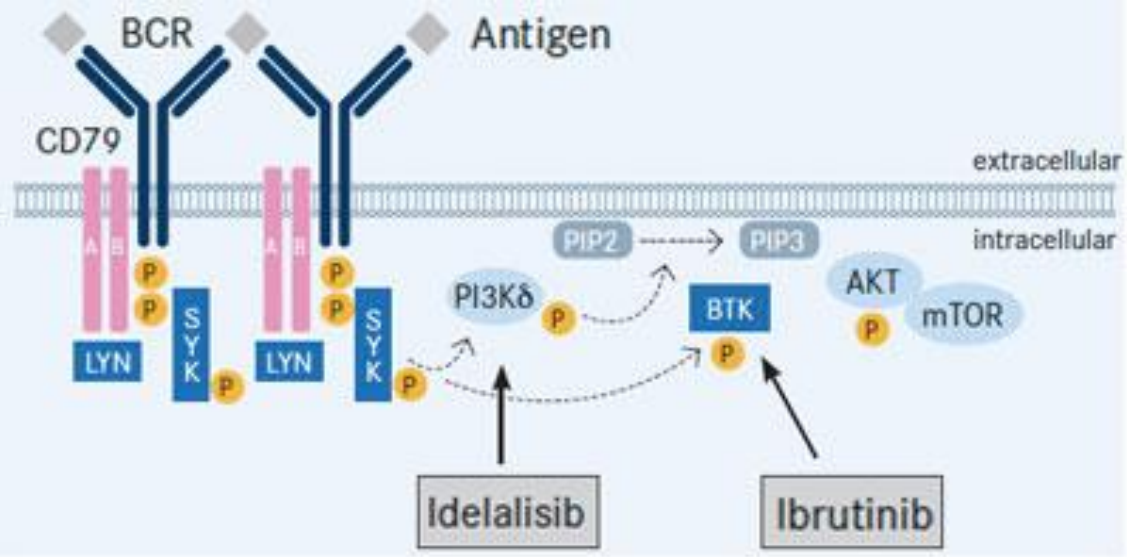
- Chemoimmunotherapy
  - anti-CD20 antibody (rituximab/obinutuzumab) + chemotherapy
- Allogenic stem cell transplantation
  - Fit young CLL patients with poor prognosis

Targeted treatment (small molecules)

- Acalabrutinib (Calquence)
- Ibrutinib (Imbruvica)
- Idelalisib (Zydelig)
- Venetoclax (Venclyxto)
- Zanubrutinib (Brukinsa)

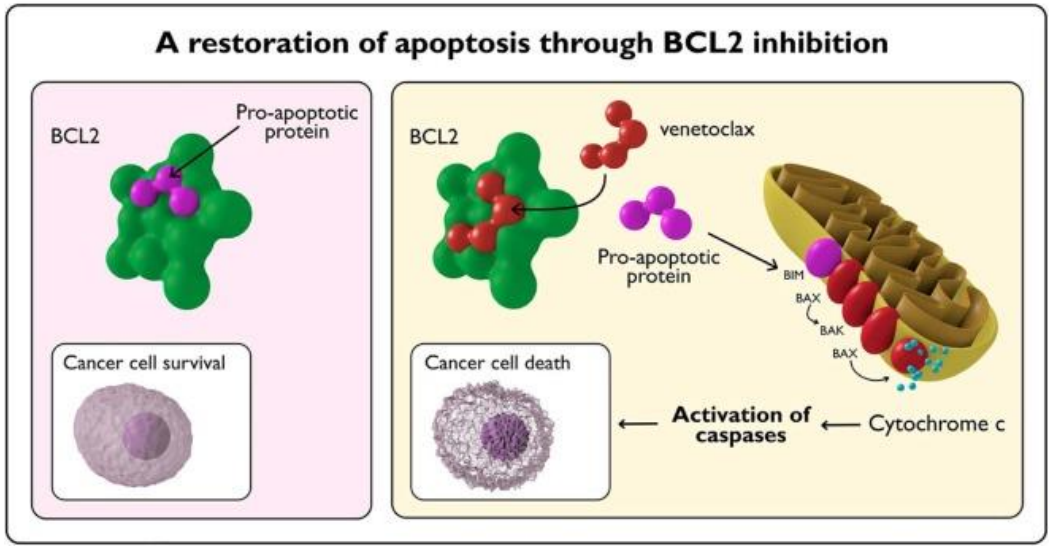
Small molecule inhibitors

- Excellent response in most patients including refractory disease or with poor prognostic features (TP53 del/mut)
- Option for all CLL patients (older, comorbidities)
- Specific adverse effects and drug interactions



Acalabrutinib
Zanubrutinib

Venetoclax - a BCL2 specific inhibitor

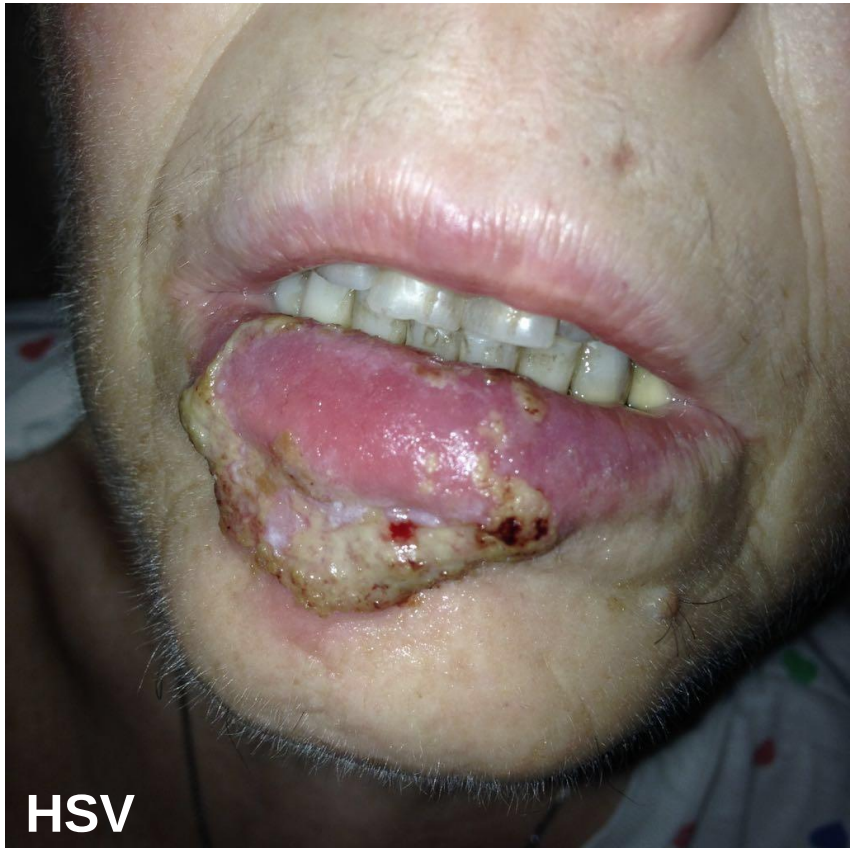


Complications in CLL

- **Histologic transformation (Richter‘s syndrome)**
  - Transformation to a highly aggressive lymphoma (DLBCL), up to 10% during CLL course
- **Infections**
  - Most frequent cause of morbidity and mortality in CLL
- **Autoimmune complications**
  - Occur in up to 25 % of CLL patients, AIHA, ITP
- **Other malignancies**
  - Up to 4 times more common than in the general population

Supportive care

- Even common infections can be severe in CLL patients
- Treat early and intensively
- Give antibiotics early



Supportive care

- Vaccination
  - *S. pneumoniae*, seasonal flu, COVID-19
  - Avoid all live vaccines
- Hypogammaglobulinemia
  - Immunoglobulin replacement therapy in patients with low IgG (< 5g/l) and recurrent bacterial infection
- Cancer screening
  - Higher risk of developing other malignancies

Conclusions

- Most common type of leukemia in western countries
- Disease typically occurs in elderly patients
- Highly variable clinical course
- Only patients with active or symptomatic disease require therapy
- New therapeutic agents – potential to improve the outcome of patients with CLL
- Supportive care is important

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Thank you