Universität Rostock Traditio et Innovatio

Universitätsmedizin Rostock

Jahrestagung der DGHO, OeGHO, SGMO und SGH 2017

HCL, HCLv und SMZL: Zusammenspiel von Immunphänotypisierung, Zytomorphologie und Molekulargenetik

Sebastian Böttcher

sebastian.boettcher@med.uni-rostock.de

Stuttgart, 02.10.2017

Universitätsmedizin Rostock, Zentrum für Innere Medizin
Medizinische Klinik III - Hämatologie, Onkologie, Palliativmedizin

HCL, HCLv, SMZL

„indolente“ Non-Hodgkin-Lymphome der B-Zell-Reihe

- Milzbeteiligung
- Typischerweise fehlende Lymphknoteninfiltration
- Blut- und Knochenmarkbeteiligung
- Zytopenien
- Lymphomzellen mit „haarigen“ Ausläufern

WHO-Klassifikation der Lymphome 2016

THE UPDATED WHO CLASSIFICATION OF HEMATOLOGICAL MALIGNANCIES

The 2016 revision of the World Health Organization classification of lymphoid neoplasms

Steven H. Swerdlow,[1] Elias Campo,[2] Stefano A. Pileri,[3] Nancy Lee Harris,[4] Harald Stein,[5] Reiner Siebert,[6] Ranjana Advani,[7] Michele Ghielmini,[8] Gilles A. Salles,[9] Andrew D. Zelenetz,[10] and Elaine S. Jaffe[11]

[1]Division of Hematopathology, Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [2]Department of Pathology, Hospital Clinic, University of Barcelona, August Pi i Sunyer Biomedical Research Institute, Barcelona, Spain; [3]Haematopathology Unit, European Institute of Oncology, Milan, and Department of Experimental, Diagnostic and Specialty Medicine, Bologna University Medical School, Bologna, Italy; [4]Department of Pathology, Harvard Medical School and Massachusetts General Hospital, Boston, MA; [5]Pathodiagnostik, Berlin, Germany; [6]Institute of Human Genetics, Christian Albrechts University Kiel, Kiel, Germany; [7]Division of Oncology, Department of Medicine, Stanford University, Stanford, CA; [8]Department of Medical Oncology, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [9]Department of Hematology, Hospices Civils de Lyon, and Université Claude Bernard Lyon-1, Lyon, France; [10]Department of Medicine, Memorial Sloan Kettering Cancer Center and Weill Cornell Medical College, New York, NY; and [11]Hematopathology Section, Laboratory of Pathology, National Cancer Institute, Bethesda, MD

A revision of the nearly 8-year-old World Health Organization classification of the lymphoid neoplasms and the accompanying monograph is being published. It reflects a consensus among hematopathologists, geneticists, and clinicians regarding both updates to current entities as well as the addition of a limited number of new provisional entities. The revision clarifies the diagnosis and management of lesions at the very early stages of lymphomagenesis, refines the diagnostic criteria for some entities, details the expanding genetic/molecular landscape of numerous lymphoid neoplasms and their clinical correlates, and refers to investigations leading to more targeted therapeutic strategies. The major changes are reviewed with an emphasis on the most important advances in our understanding that impact our diagnostic approach, clinical expectations, and therapeutic strategies for the lymphoid neoplasms. (*Blood*. 2016;127(20):2375-2390)

WHO-Klassifikation der Lymphome 2016

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

Mature B-cell neoplasms

- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Monoclonal B-cell lymphocytosis*
- B-cell prolymphocytic leukemia
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- *Splenic B-cell lymphoma/leukemia, unclassifiable*
  - *Splenic diffuse red pulp small B-cell lymphoma*
  - *Hairy cell leukemia-variant*
- Lymphoplasmacytic lymphoma
  - Waldenström macroglobulinemia
- Monoclonal gammopathy of undetermined significance (MGUS), IgM*
- μ heavy-chain disease
- γ heavy-chain disease
- α heavy-chain disease
- Monoclonal gammopathy of undetermined significance (MGUS), IgG/A*
- Plasma cell myeloma
- Solitary plasmacytoma of bone
- Extraosseous plasmacytoma
- Monoclonal immunoglobulin deposition diseases*
- Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Nodal marginal zone lymphoma
  - *Pediatric nodal marginal zone lymphoma*
- Follicular lymphoma
  - In situ follicular neoplasia*
  - Duodenal-type follicular lymphoma*
- Pediatric-type follicular lymphoma*
- *Large B-cell lymphoma with IRF4 rearrangement**
- Primary cutaneous follicle center lymphoma
- Mantle cell lymphoma
  - In situ mantle cell neoplasia*
- Diffuse large B-cell lymphoma (DLBCL), NOS
  - Germinal center B-cell type*
  - Activated B-cell type*
- T-cell/histiocyte-rich large B-cell lymphoma
- Primary DLBCL of the central nervous system (CNS)
- Primary cutaneous DLBCL, leg type
- EBV^+ DLBCL, NOS*
- *EBV^+ mucocutaneous ulcer**
- DLBCL associated with chronic inflammation
- Lymphomatoid granulomatosis
- Primary mediastinal (thymic) large B-cell lymphoma
- Intravascular large B-cell lymphoma
- ALK^+ large B-cell lymphoma
- Plasmablastic lymphoma
- Primary effusion lymphoma
- *$HHV8^+$ DLBCL, NOS**
- Burkitt lymphoma
- *Burkitt-like lymphoma with 11q aberration**
- High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
- High-grade B-cell lymphoma, NOS*
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Swerdlow et al., Blood, 2016

WHO-Klassifikation der Lymphome 2016

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

Mature B
Chroni
Monoc
B-cell
Splenic
Hairy c
Splenic
Sple
Hair
Lymph
Walc
Monoc
μ heav
γ heav
α heav
Monoc
Plasma cell myeloma
Solitary plasmacytoma of bone
Extraosseous plasmacytoma
Monoclonal immunoglobulin deposition diseases*
Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
Nodal marginal zone lymphoma
Pediatric nodal marginal zone lymphoma

Splenic marginal zone lymphoma

Hairy cell leukemia

Splenic B-cell lymphoma/leukemia, unclassifiable

Splenic diffuse red pulp small B-cell lymphoma

Hairy cell leukemia-variant

Follicular lymphoma
In situ follicular neoplasia*

Intravascular large B-cell lymphoma
ALK^+ large B-cell lymphoma
Plasmablastic lymphoma
Primary effusion lymphoma
*$HHV8^+$ DLBCL, NOS**
Burkitt lymphoma
*Burkitt-like lymphoma with 11q aberration**
High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
High-grade B-cell lymphoma, NOS*
B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Swerdlow et al., Blood, 2016

WHO-Klassifikation der Lymphome 2016

Table 1. 2016 WHO classification of mature lymphoid, histiocytic, and dendritic neoplasms

Mature B-cell neoplasms

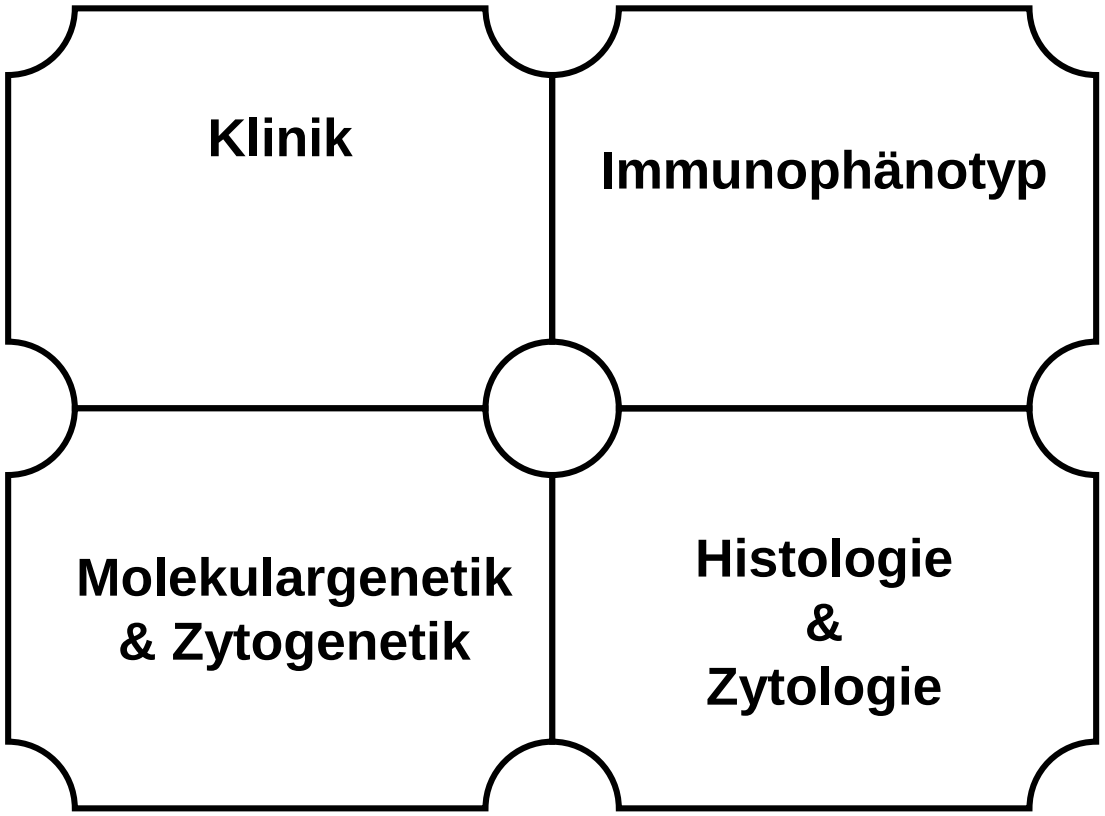
- Chronic lymphocytic leukemia/small lymphocytic lymphoma
- Monoclonal B-cell lymphocytosis*
- B-cell prolymphocytic leukemia
- Splenic marginal zone lymphoma
- Hairy cell leukemia
- *Sp*
- Ly
- M
- μ
- γ
- α
- M
- Pl
- So
- Ex
- Monoclonal immunoglobulin deposition diseases*
- Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma)
- Nodal marginal zone lymphoma
  - *Pediatric nodal marginal zone lymphoma*
- Follicular lymphoma
  - In situ follicular neoplasia*
  - Duodenal-type follicular lymphoma*
- Pediatric-type follicular lymphoma*
- *Large B-cell lymphoma with IRF4 rearrangement**
- Primary cutaneous follicle center lymphoma
- Mantle cell lymphoma
  - In situ mantle cell neoplasia*
- Diffuse large B-cell lymphoma (DLBCL), NOS
- Primary effusion lymphoma
- *HHV8$^+$ DLBCL, NOS**
- Burkitt lymphoma
- *Burkitt-like lymphoma with 11q aberration**
- High-grade B-cell lymphoma, with *MYC* and *BCL2* and/or *BCL6* rearrangements*
- High-grade B-cell lymphoma, NOS*
- B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

Table 2. Highlights of changes in 2016 WHO classification of lymphoid, histiocytic, and dendritic neoplasms

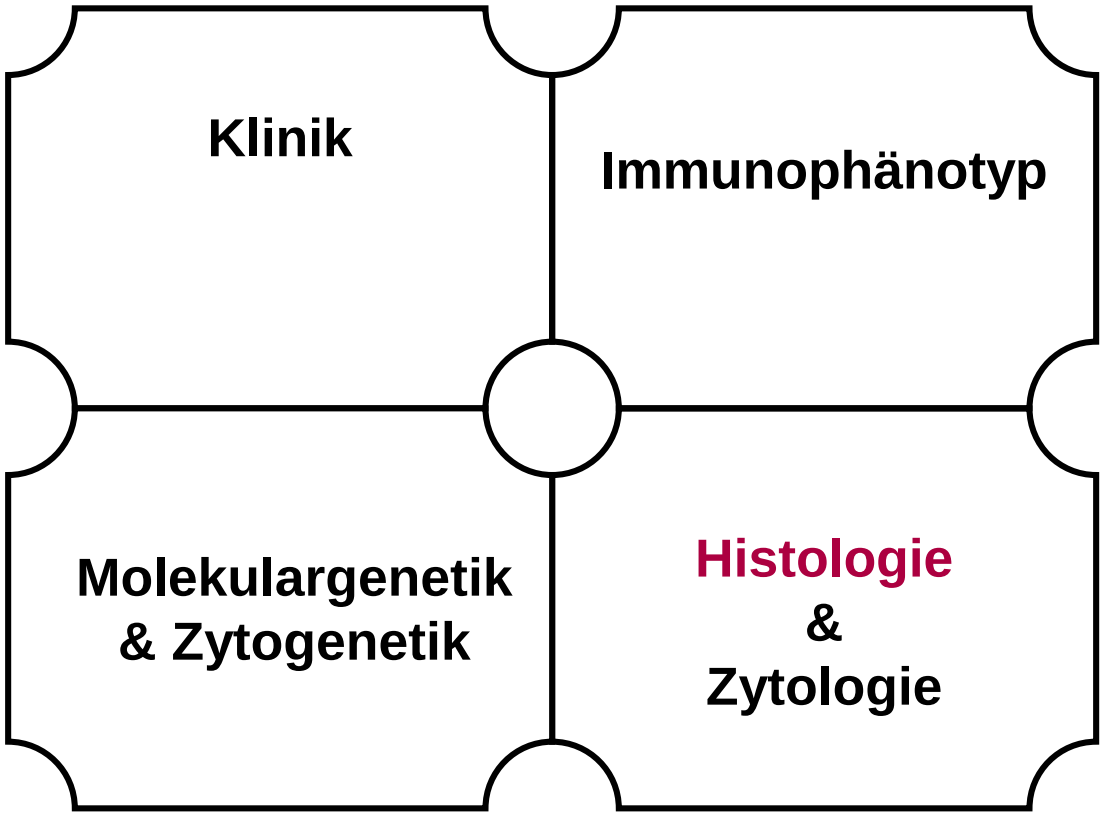
Entity/category	Change
CLL/SLL	• Cytopenias or disease-related symptoms are now insufficient to make a diagnosis of CLL with $<5 \times 10^9$/L PB CLL cells. • Large/confluent and/or highly proliferative proliferation centers are adverse prognostic indicators. • Mutations of potential clinical relevance, such as *TP53*, *NOTCH1*, *SF3B1*, *ATM*, and *BIRC3*, have been recognized.
Monoclonal B-cell lymphocytosis	• Must distinguish low-count from high-count MBL. • A lymph node equivalent of MBL exists.
Hairy cell leukemia	• *BRAF* V600E mutations in vast majority of cases with *MAP2K1* mutations in most cases that use IGHV4-34 and lack *BRAF* mutation.

Swerdlow et al., Blood, 2016

Artdiagnose



Artdiagnose



Splenic marginal zone lymphoma: from genetics to management

Luca Arcaini,[1,2] Davide Rossi,[3] and Marco Paulli[1,4]

Diagnosis

Definitive diagnosis of SMZL relies on spleen histology; if that is not available, diagnosis requires integration of BM histology with cell morphology and immunophenotype in the blood and BM.[11,15]

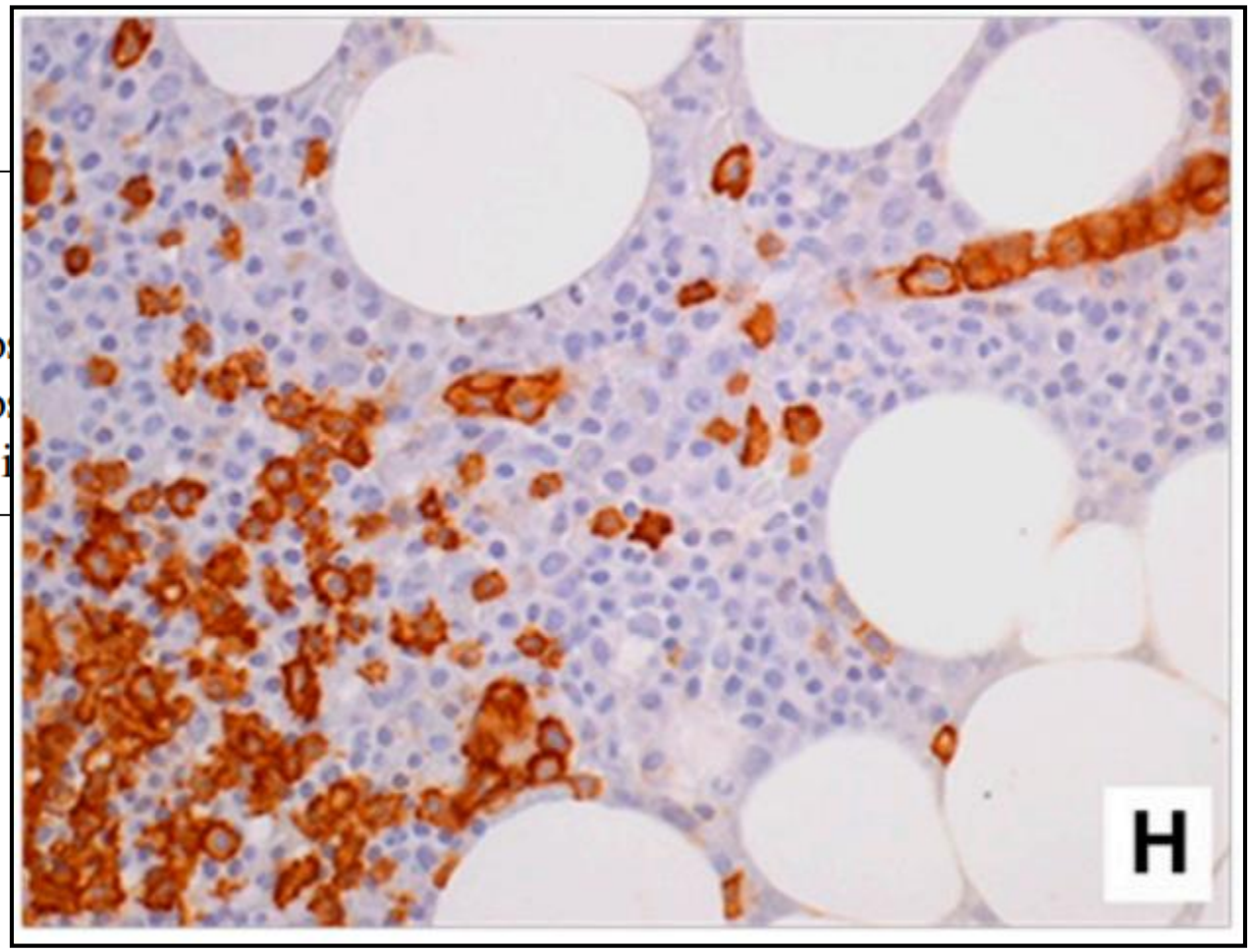
Arcaini et al., Blood, 2016

Splenic marginal zone lymphoma: from genetics to management

Luca Arcaini,[1,2] Davide Rossi,[3] and Marco Paulli[1,4]

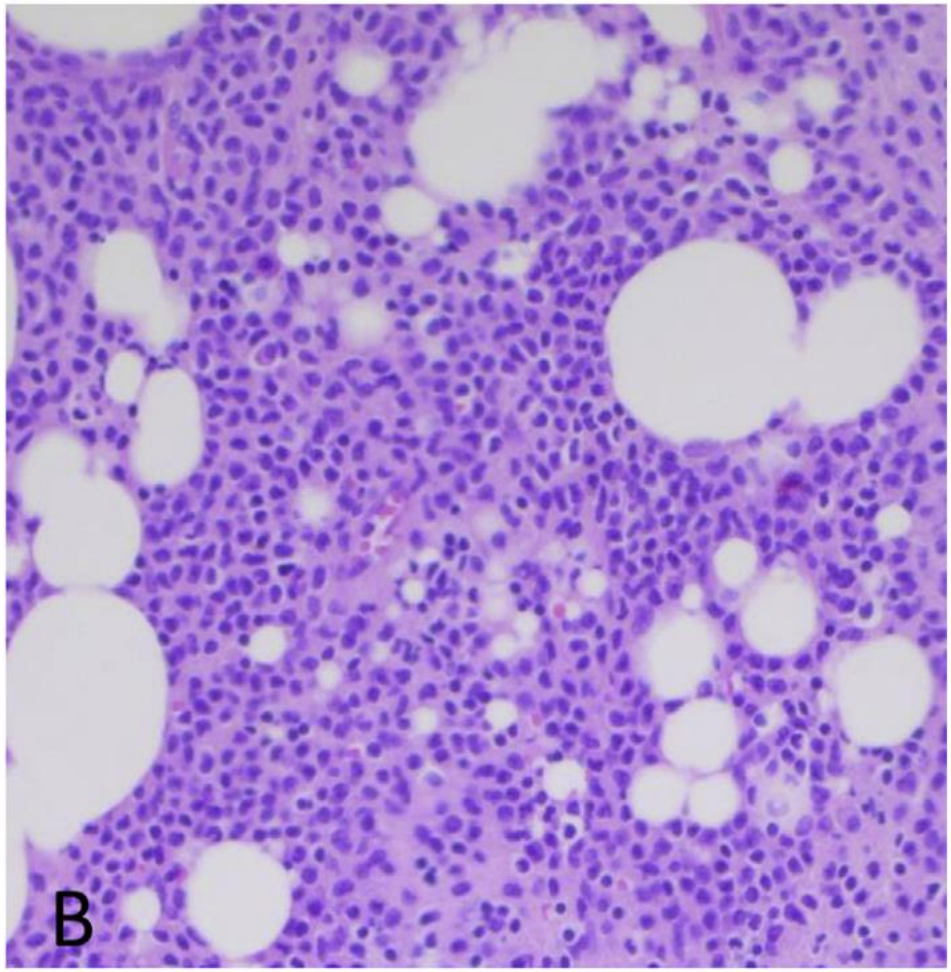
Diagnosis

Definitive diagno
available, diagno
morphology and i



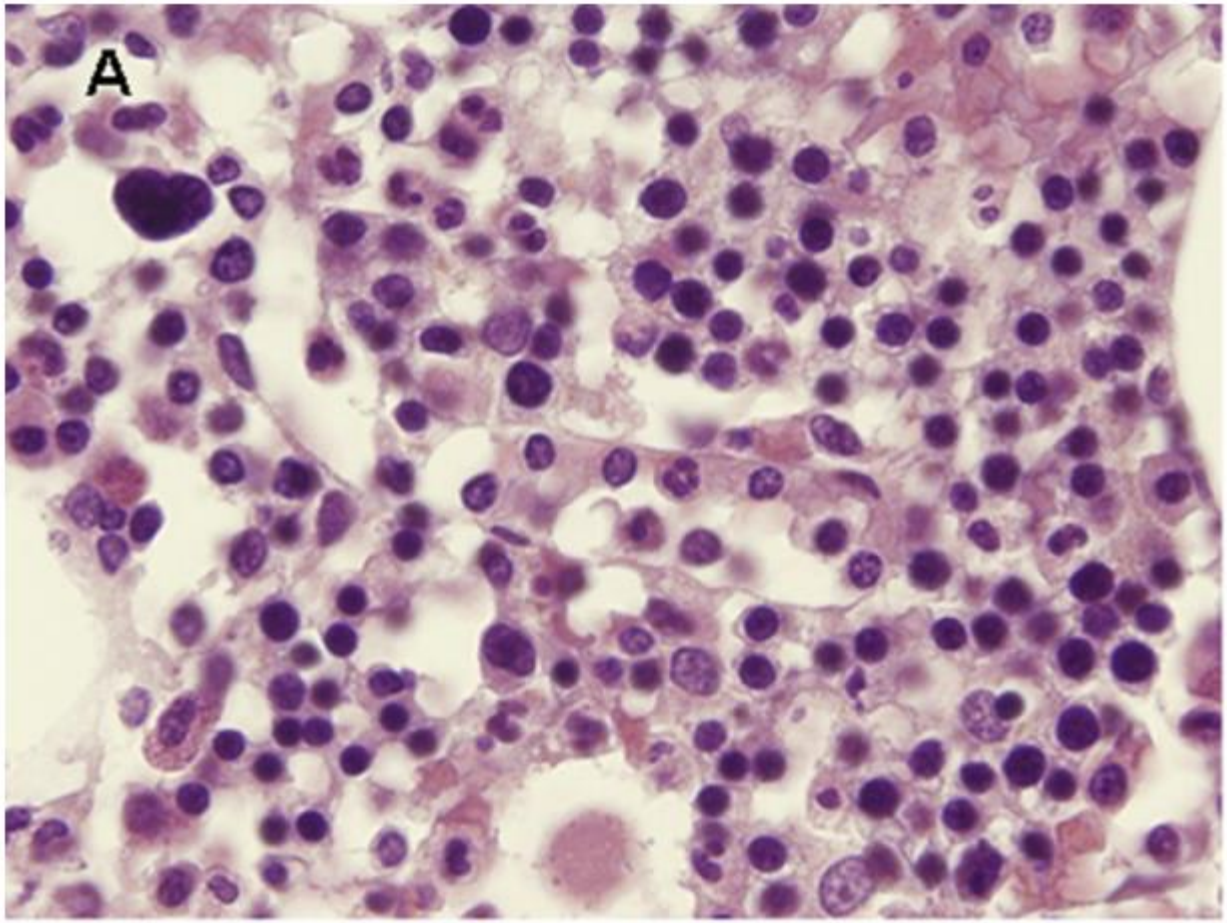
Arcaini et al., Blood, 2016

Histologie: HCL



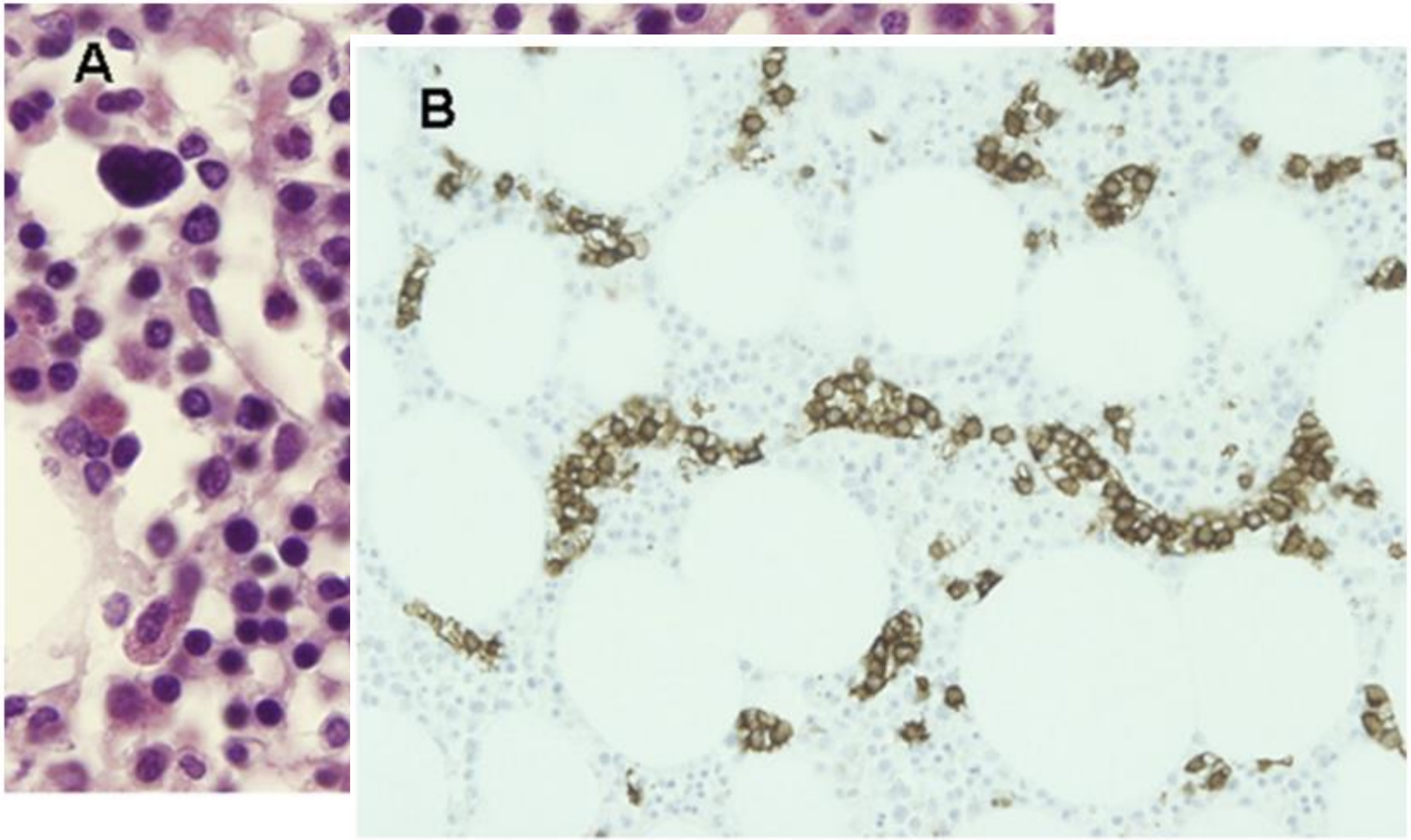
Quest &Johnston, Best Practice & Research Clinical Haematology 28 (2015)

Histologie: HCLv



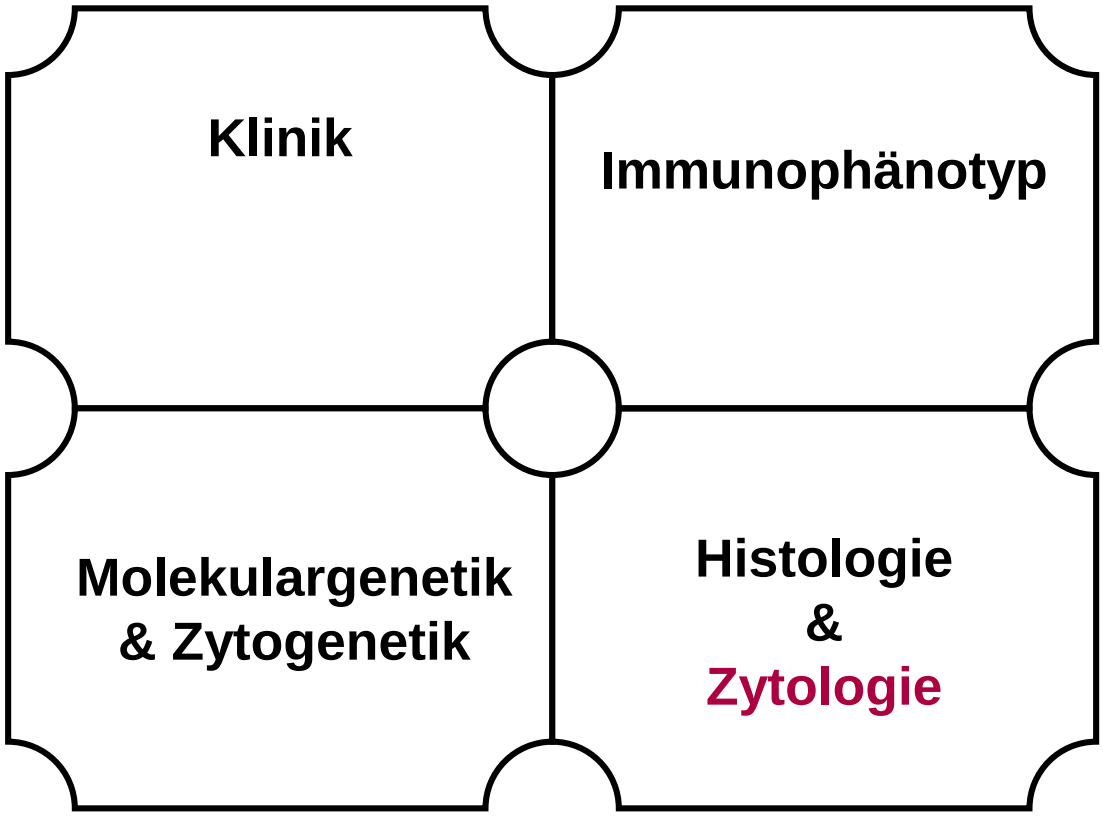
Matutes et al., Best Practice & Research Clinical Haematology 28 (2015)

Histologie: HCLv



Matutes et al., Best Practice & Research Clinical Haematology 28 (2015)

Artdiagnose



Zytologie: SMZL

Bild: Prof. H.A. Horst, UKSH, Kiel

Zytologie: HCL

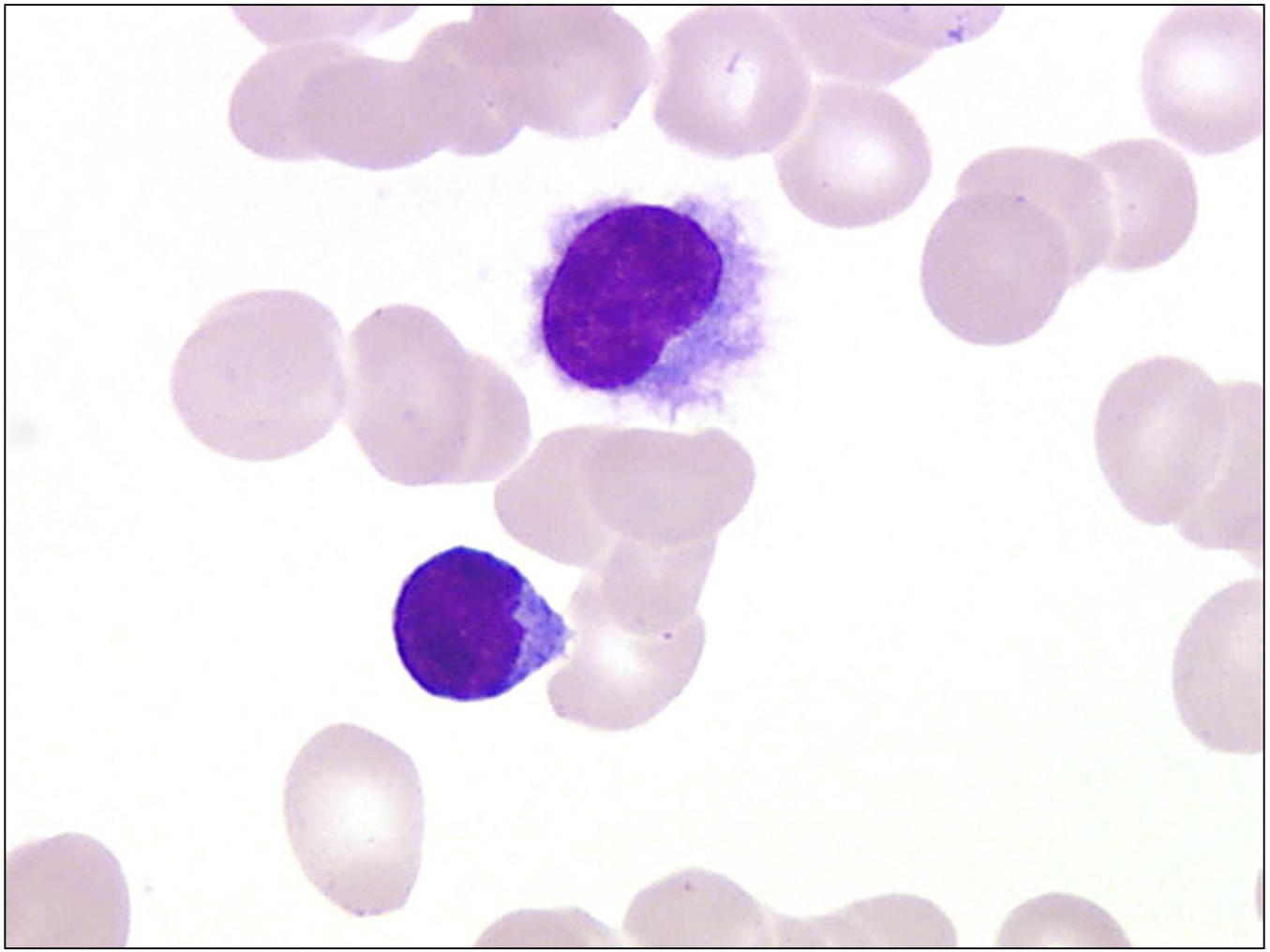


Bild: Prof. H.A. Horst, UKSH, Kiel

Zytologie: HCLv

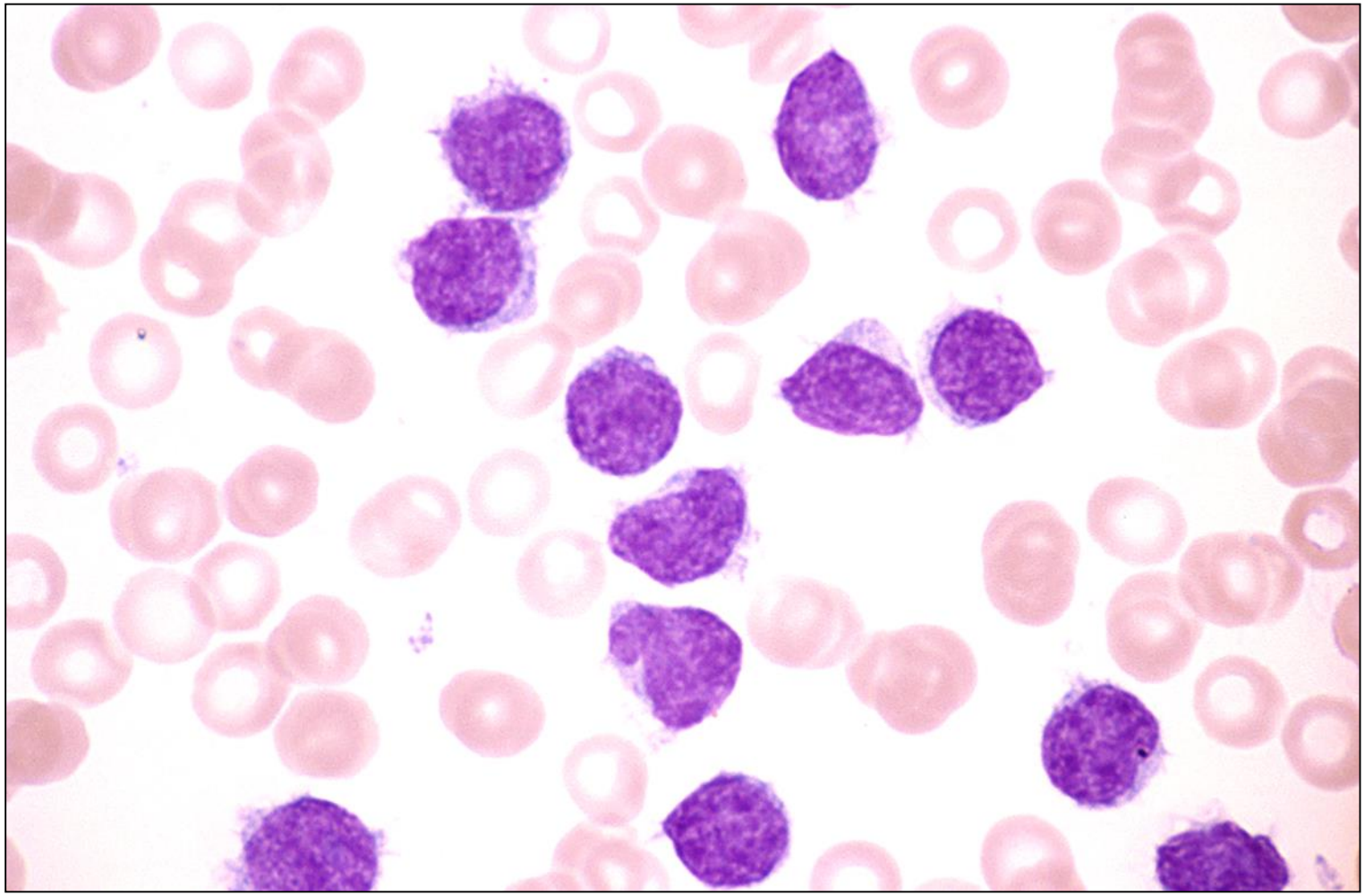
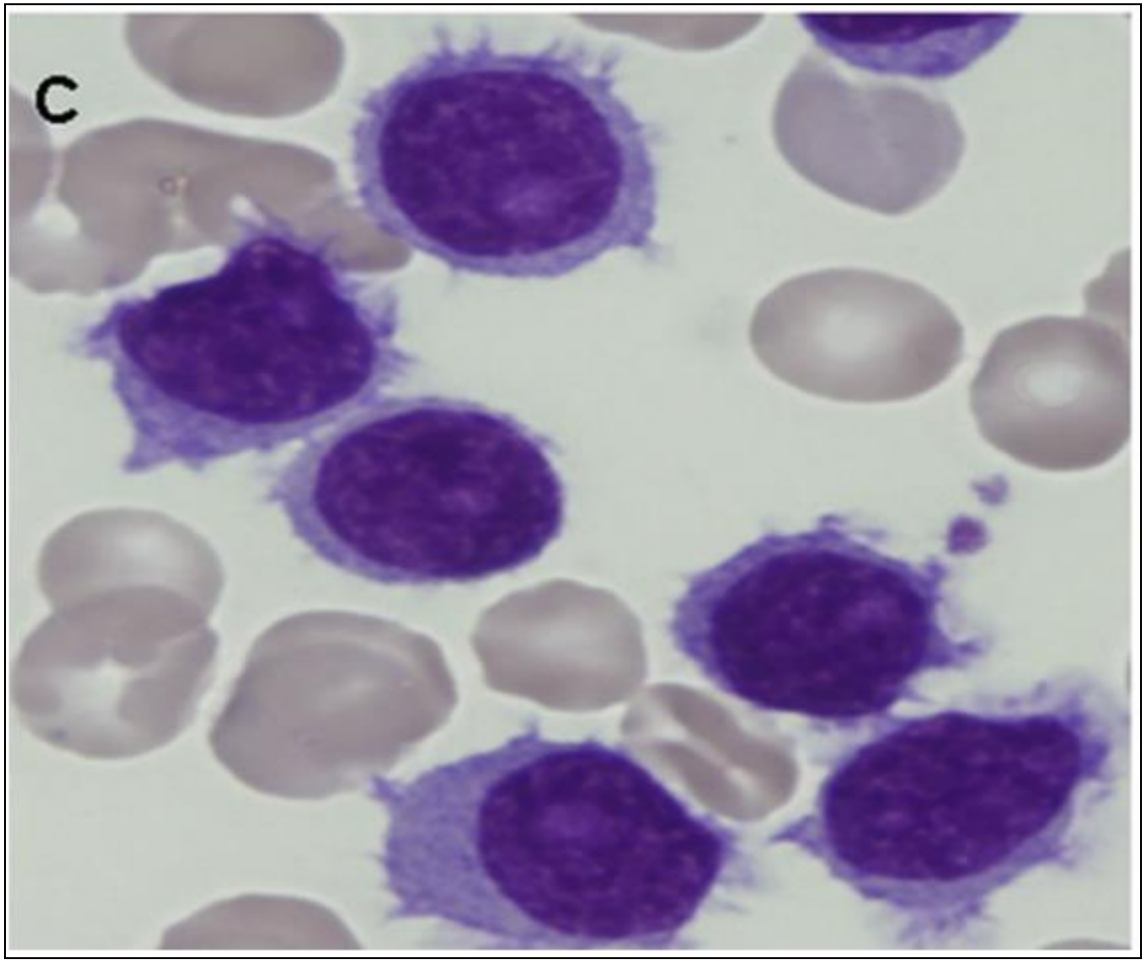


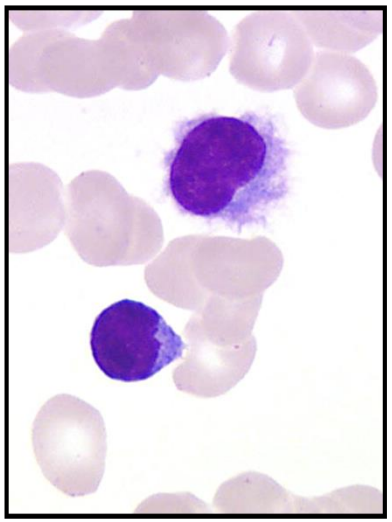
Bild: Prof. H.A. Horst, UKSH, Kiel

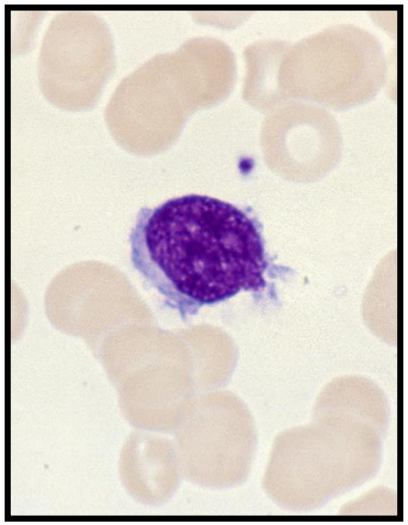
Zytologie: HCLv

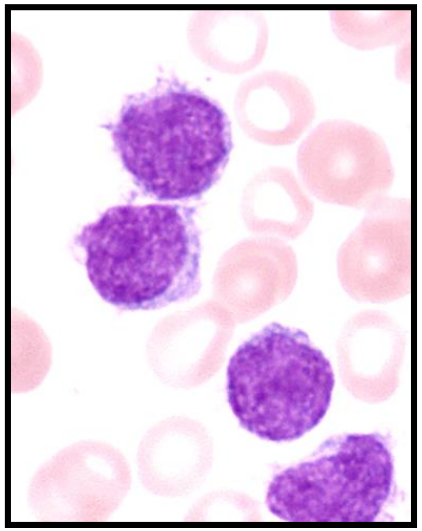


Matutes et al., Best Practice & Research Clinical Haematology 28 (2015)

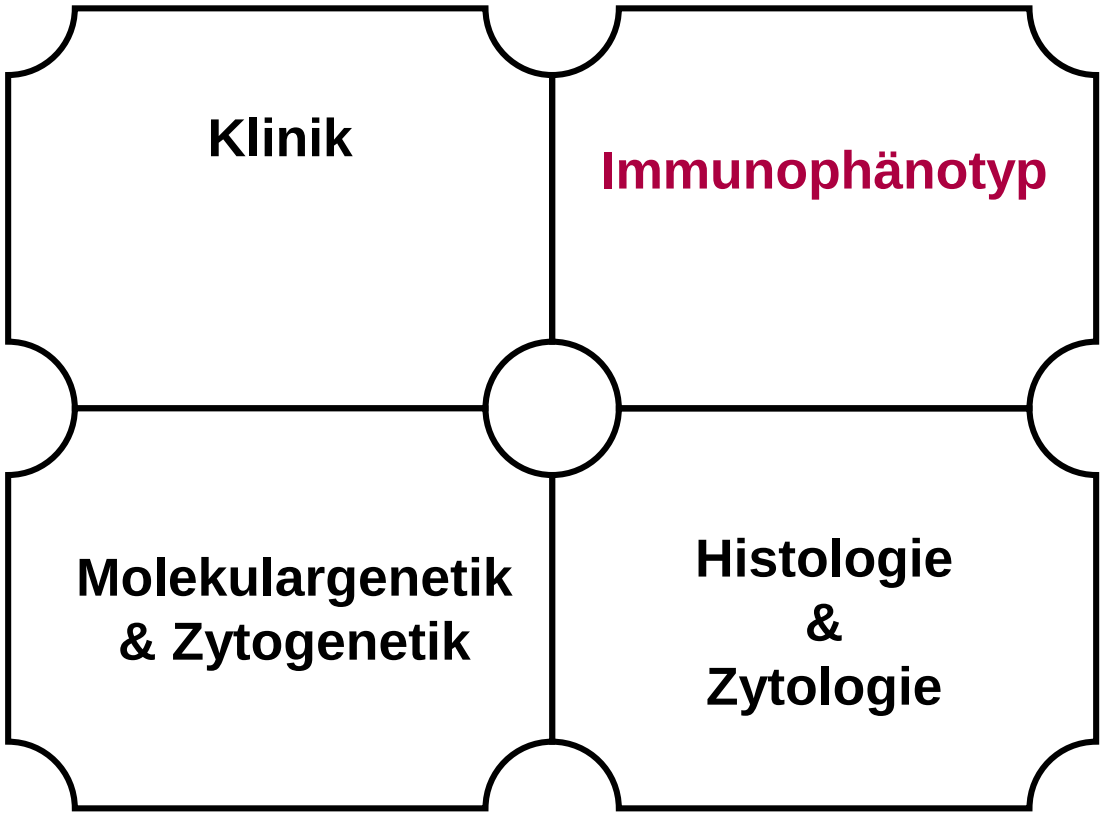
Zytologie: HCL, SMZL, HCLv





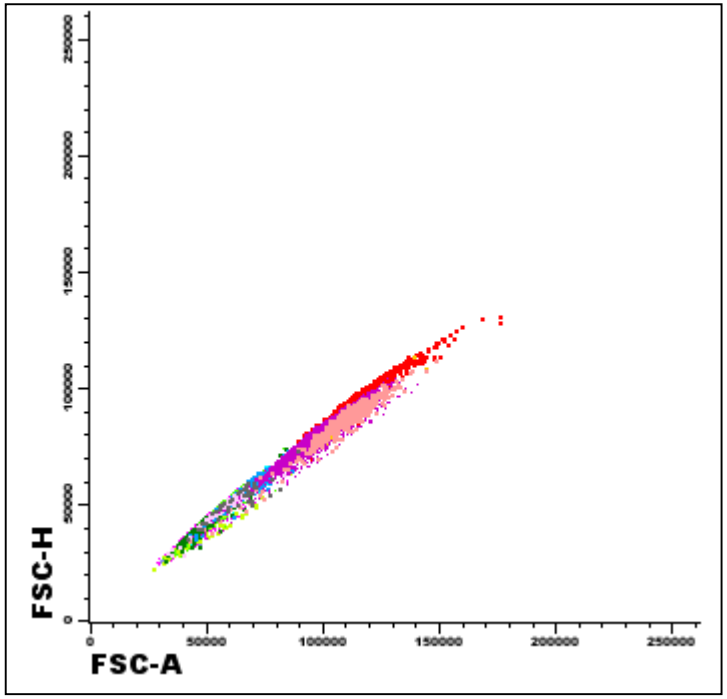


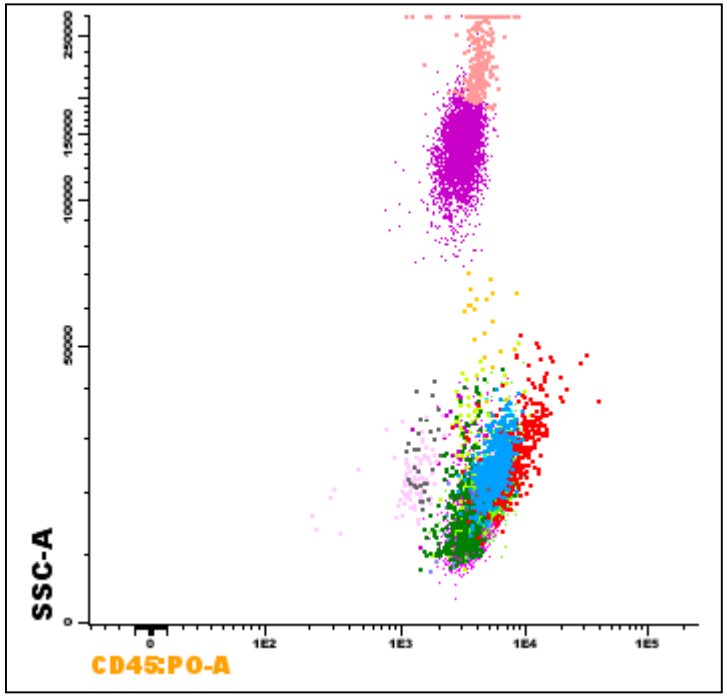
Dignitätsdiagnose



Durchflusszytometrie: Dignitätsdiagose

EuroFlow



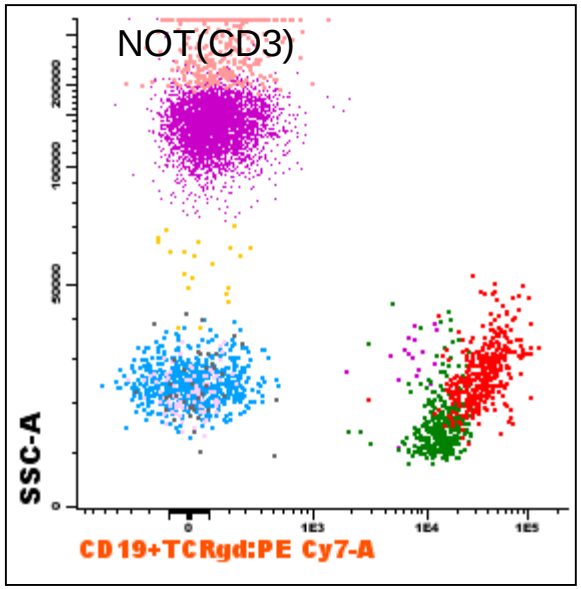


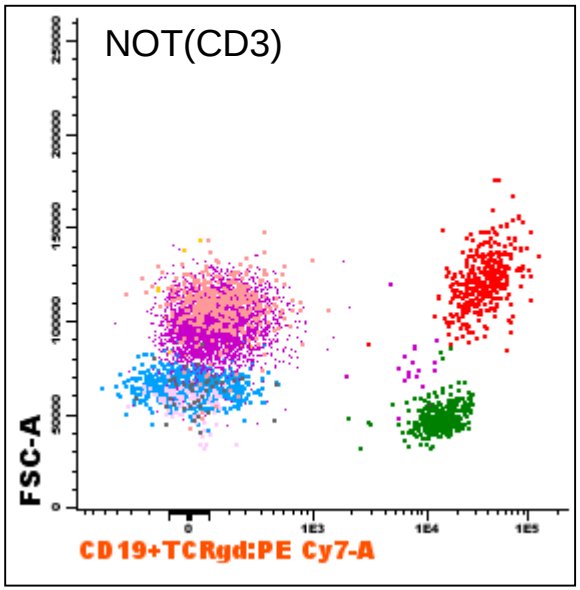
	PacB	PacO	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7
LST	CD20/CD4	CD45	λ/CD8	κ/CD56	CD5	CD19 /TCRγ/δ	CD3	CD38

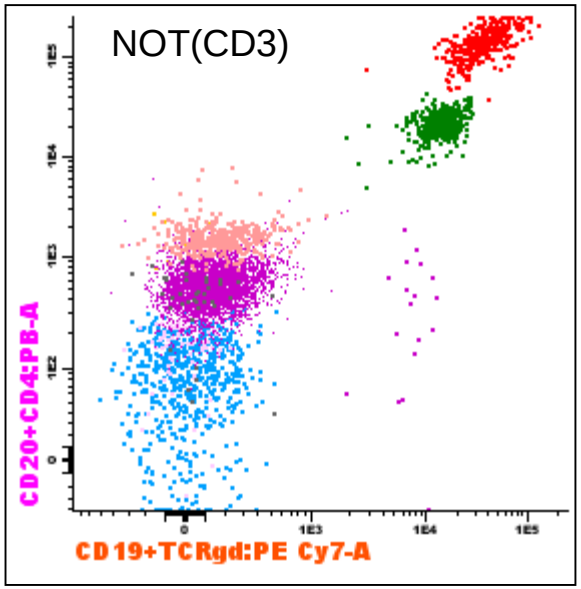
Flores J, et al. in van Dongen et al. ***Leukemia*** 2012

Durchflusszytometrie: Dignitätsdiagose

EuroFlow



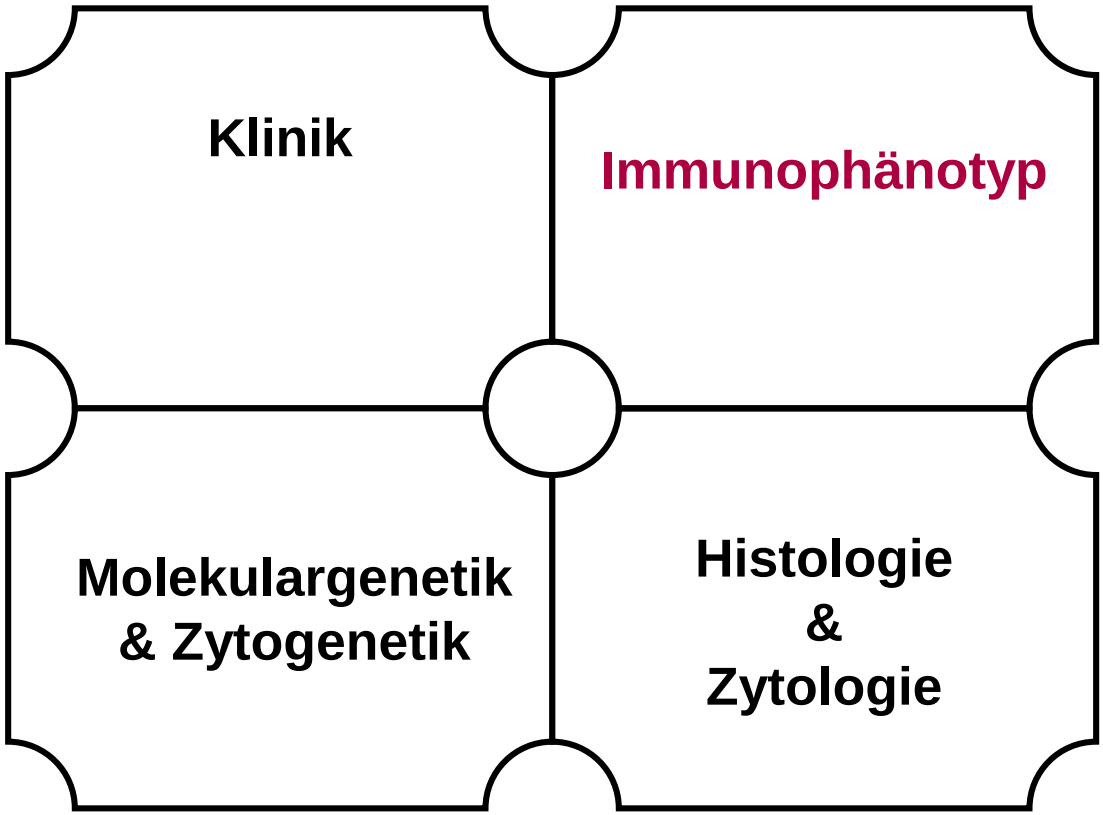




	PacB	PacO	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7
LST	CD20/CD4	CD45	λ/CD8	κ/CD56	CD5	CD19 /TCRγ/δ	CD3	CD38

Flores J, et al. in van Dongen et al. ***Leukemia*** 2012

Artdiagnose



Artdiagnose: Durchflusszytometrie

	SMZL	CLL	MCL	HCL	HCL-v
sIg	Strong	Weak	Strong	Strong	Strong
CD5	+	+++	+++	−	−
CD23	+	+++	−	−	−
FMC7	+++	+	+++	+++	+++
CD11c	++	−	−	+++	+++
CD103	−	−	−	+++	++
CD123	−	−	−	+++	−
CD25	+	−	−	+++	−
CD27	++	+++	+++	−	++
CD200	−	+++	−	+++	−

Arcaini et al., Blood, 2016

Durchflusszytometrie: Artdiagose

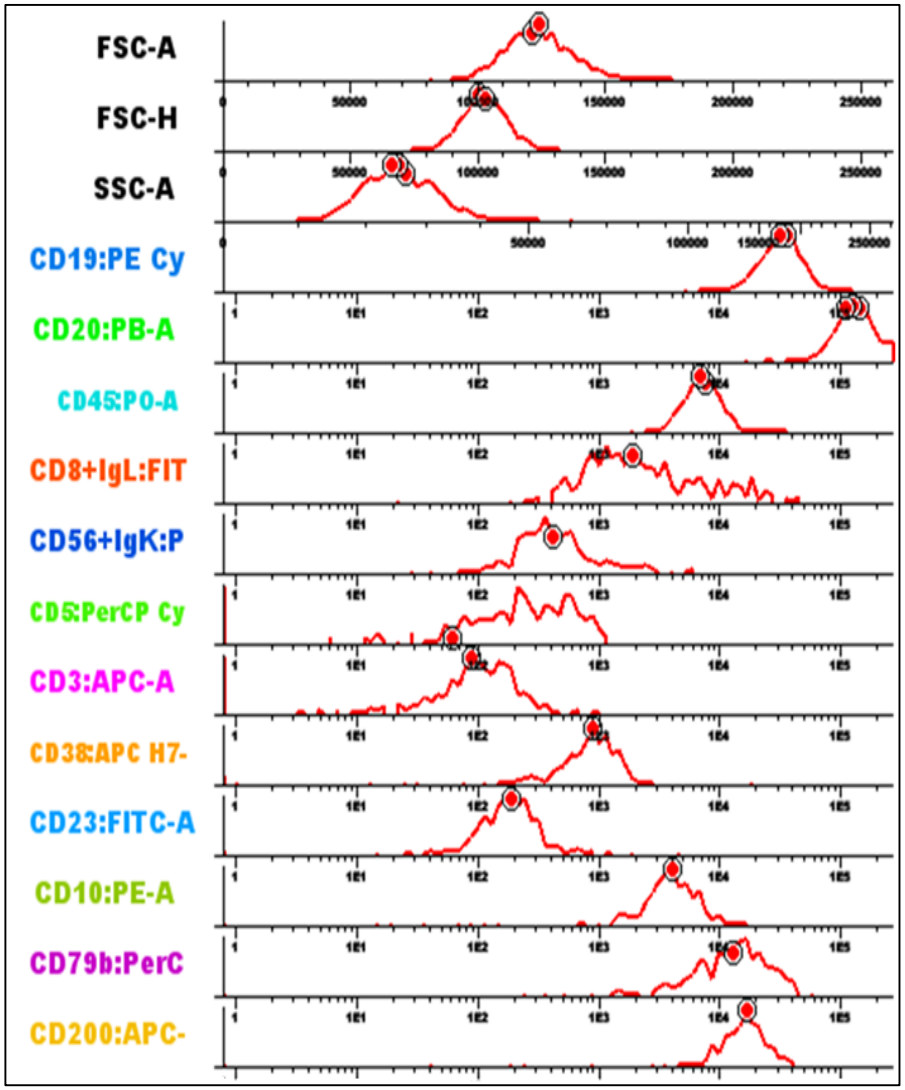
EuroFlow

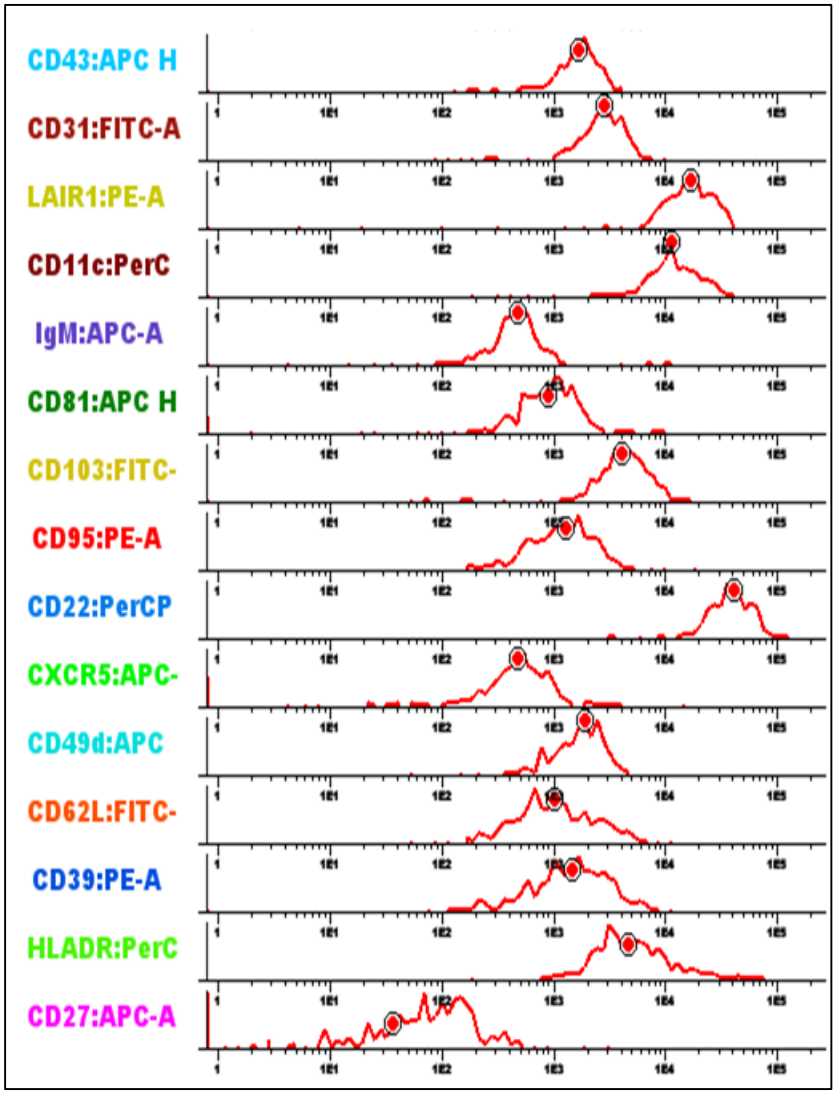
	PacB	PacO	FITC	PE	PerCP-Cy5.5	PECy7	APC	APC-H7
LST	CD20/CD4	CD45	λ/CD8	κ/CD56	CD5	CD19 /TCRγ/δ	CD3	CD38
2	CD20	CD45	CD23	CD10	**CD79b**	CD19	**CD200**	CD43
3	CD20	CD45	CD31	**LAIR**	**CD11c**	CD19	IgM	CD81
4	CD20	CD45	**CD103**	CD95	CD22	CD19	CXCR5	CD49d
5	CD20	CD45	CD62L	CD39	HLA-DR	CD19	**CD27**	

Böttcher S, et al. in van Dongen et al. ***Leukemia*** 2012

Durchflusszytometrie: Artdiagose

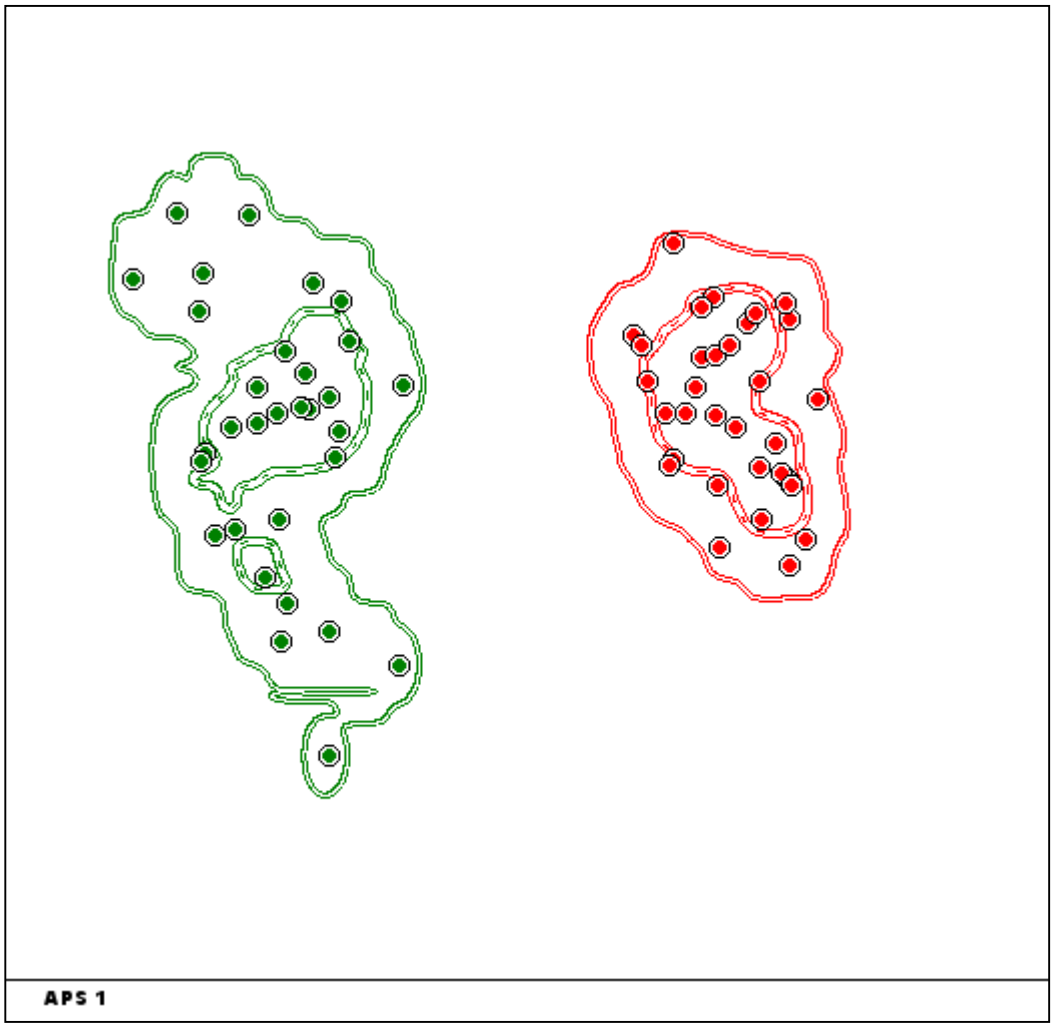
EuroFlow





Durchflusszytometrie: SMZL vs HCL (n = 70)

EuroFlow

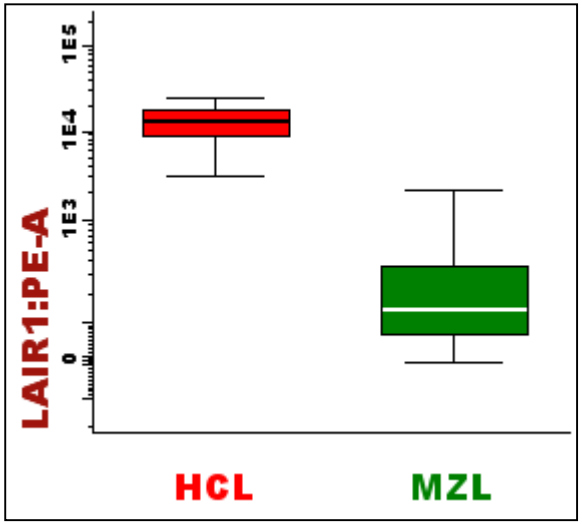


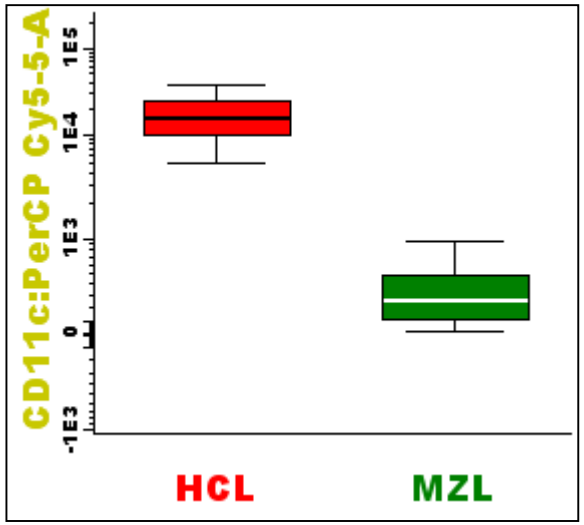
General | PC1 | PC2

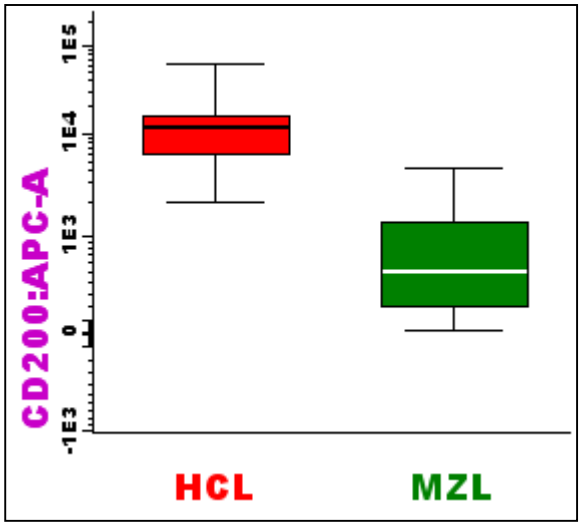
Parameter	Significance
LAIR1:PE-A LOGICAL	13.39
CD11c:PerCP Cy5-5-A LOGICAL	12.76
CD200:APC-A LOGICAL	9.85
CD103:FITC-A LOGICAL	7.58
CD22:PerCP Cy5-5-A LOGICAL	7.23
CD31:FITC-A LOGICAL	6.06
CD43:APC H7-A LOGICAL	5.19
CD20:PB-A LOGICAL	4.22
CD49d:APC H7-A LOGICAL	3.86
CXCR5:APC-A LOGICAL	3.72
IgM:APC-A LOGICAL	3.55
CD19:PE-Cy7-A LOGICAL	3.32
CD38:APC H7-A LOGICAL	3.10
CD27:APC-A LOGICAL	3.05
CD10:PE-A LOGICAL	2.97

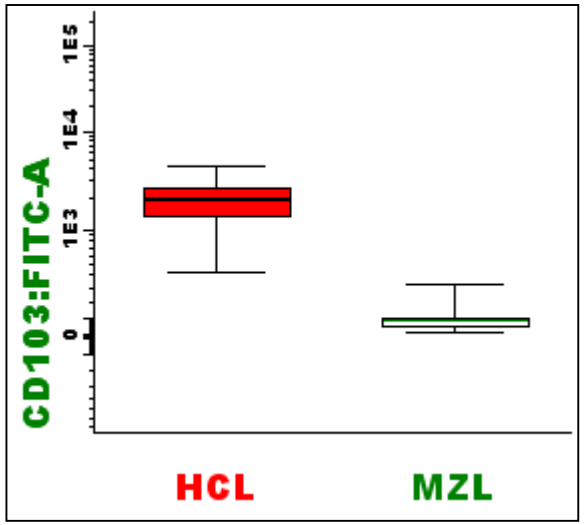
Durchflusszytometrie: SMZL vs HCL (n = 70)

EuroFlow



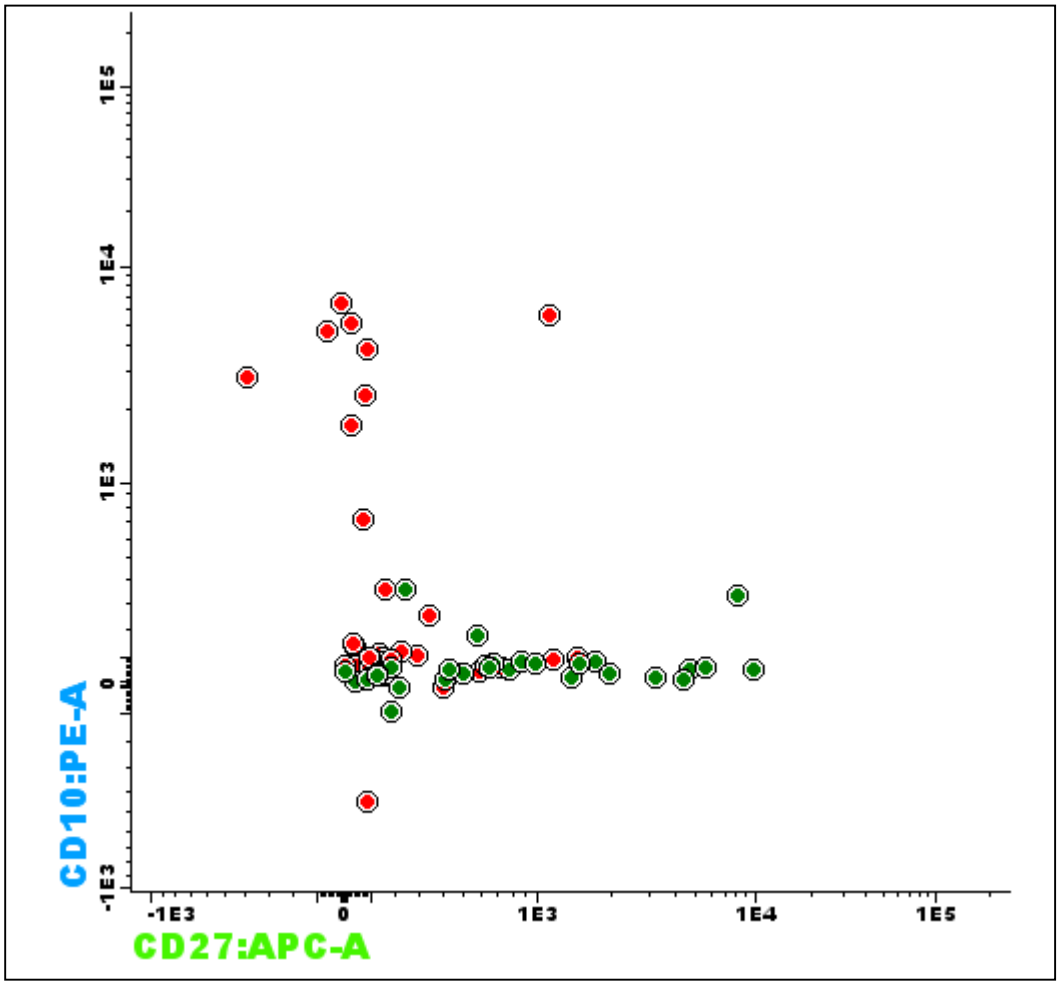






Durchflusszytometrie: SMZL vs HCL (n = 70)

EuroFlow



Separation power by 1 x 1 comparison (n=399)

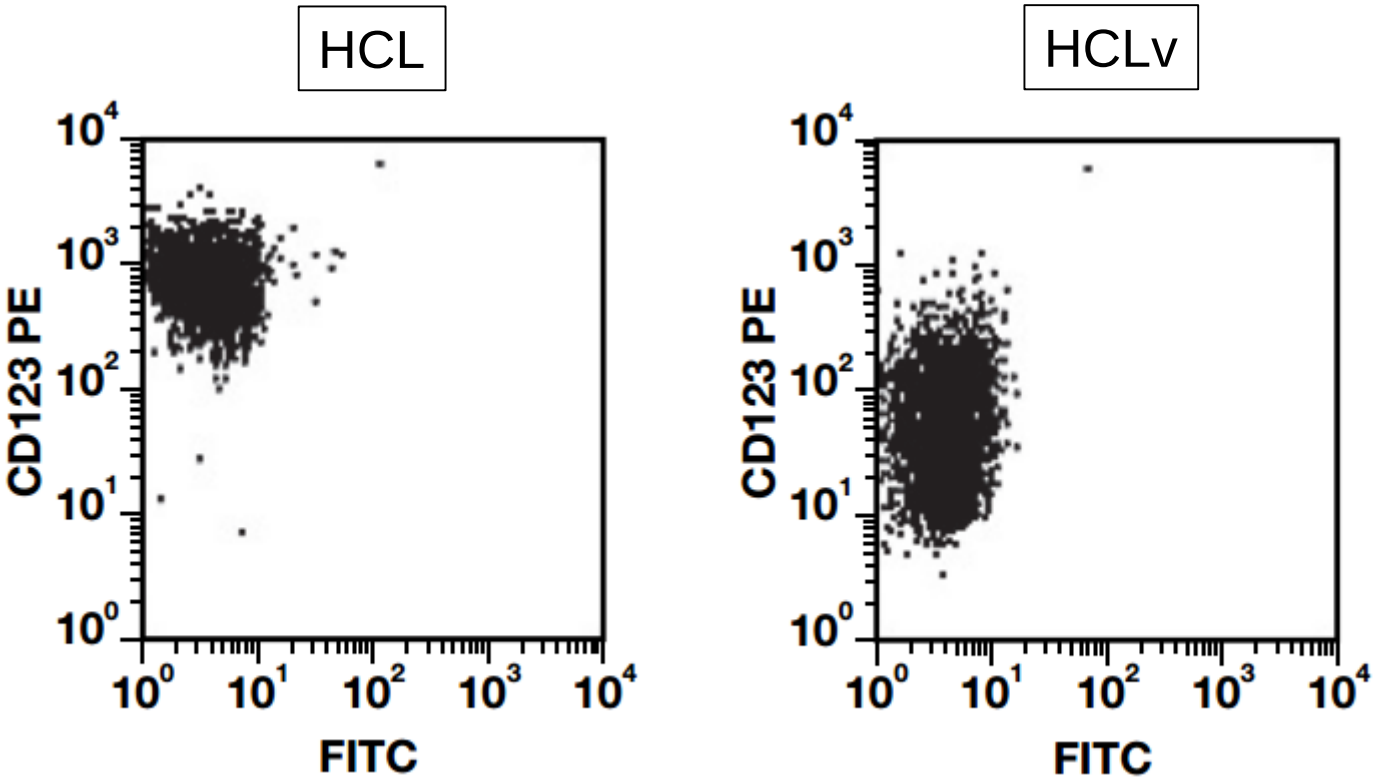
EuroFlow

	CLL	DLBCL CD10+	DLBCL CD10-	FL	HCL	LPL	MCL	MZL
BL								
CLL								
DLBCL CD10 +								
DLBCL CD10 -								
FL								
HCL								
LPL								
MCL								

No overlap
< 10% of cases overlap
≥ 10% of cases overlap

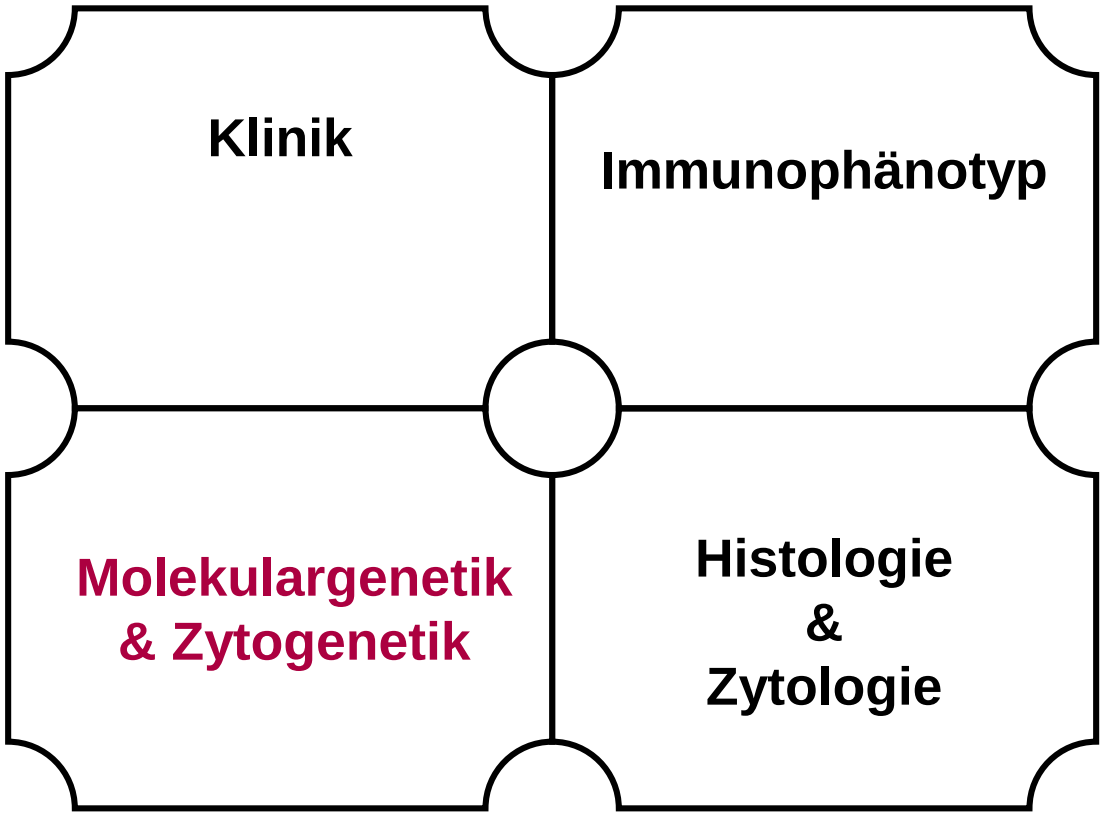
Böttcher et al., EHA, 2015

Durchflusszytometrie: HCLv vs HCL
- CD25 und CD123 -



Venkataraman et al., Am J Clin Pathol2011;136:625-630

Artdiagnose



The NEW ENGLAND JOURNAL of MEDICINE

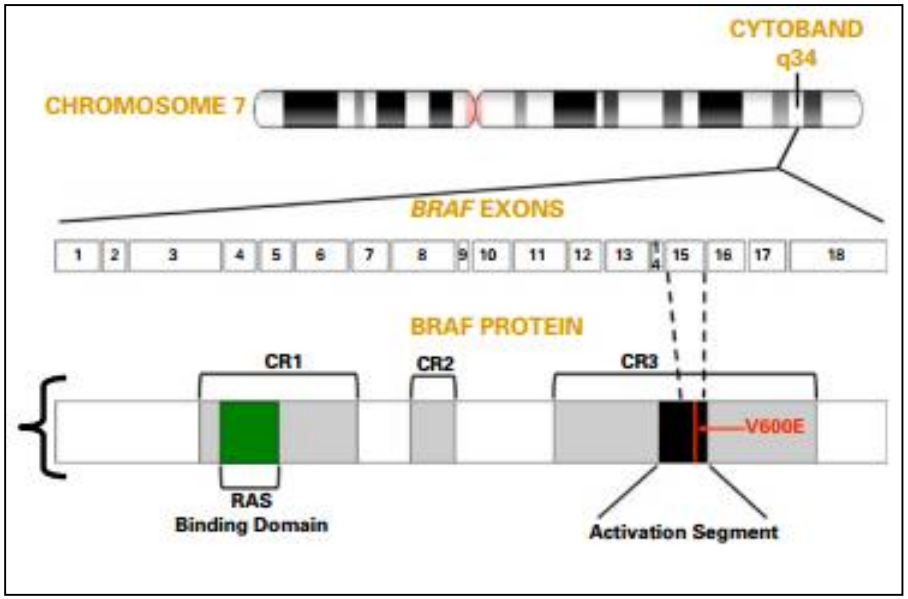
ORIGINAL ARTICLE

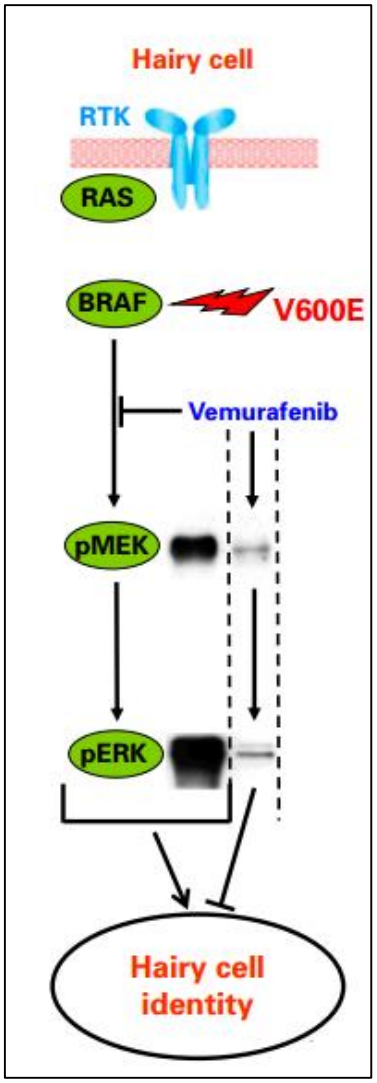
BRAF Mutations in Hairy-Cell Leukemia

Enrico Tiacci, M.D., Vladimir Trifonov, Ph.D., Gianluca Schiavoni, Ph.D., Antony Holmes, Ph.D., Wolfgang Kern, M.D., Maria Paola Martelli, M.D., Alessandra Pucciarini, Ph.D., Barbara Bigerna, B.Sc., Roberta Pacini, B.Sc., Victoria A. Wells, B.Sc., Paolo Sportoletti, M.D., Valentina Pettirossi, Ph.D., Roberta Mannucci, Ph.D., Oliver Elliott, M.Sc., Arcangelo Liso, M.D., Achille Ambrosetti, M.D., Alessandro Pulsoni, M.D., Francesco Forconi, M.D., Livio Trentin, M.D., Gianpietro Semenzato, M.D., Giorgio Inghirami, M.D., Monia Capponi, M.D., Francesco Di Raimondo, M.D., Caterina Patti, M.D., Luca Arcaini, M.D., Pellegrino Musto, M.D., Stefano Pileri, M.D., Claudia Haferlach, M.D., Susanne Schnittger, Ph.D., Giovanni Pizzolo, M.D., Robin Foà, M.D., Laurent Farinelli, Ph.D., Torsten Haferlach, M.D., Laura Pasqualucci, M.D., Raul Rabadan, Ph.D., and Brunangelo Falini, M.D.

Tiacci et al., N Engl J Med 2011;364:2305-15

BRAF V600E bei der Haarzellleukämie

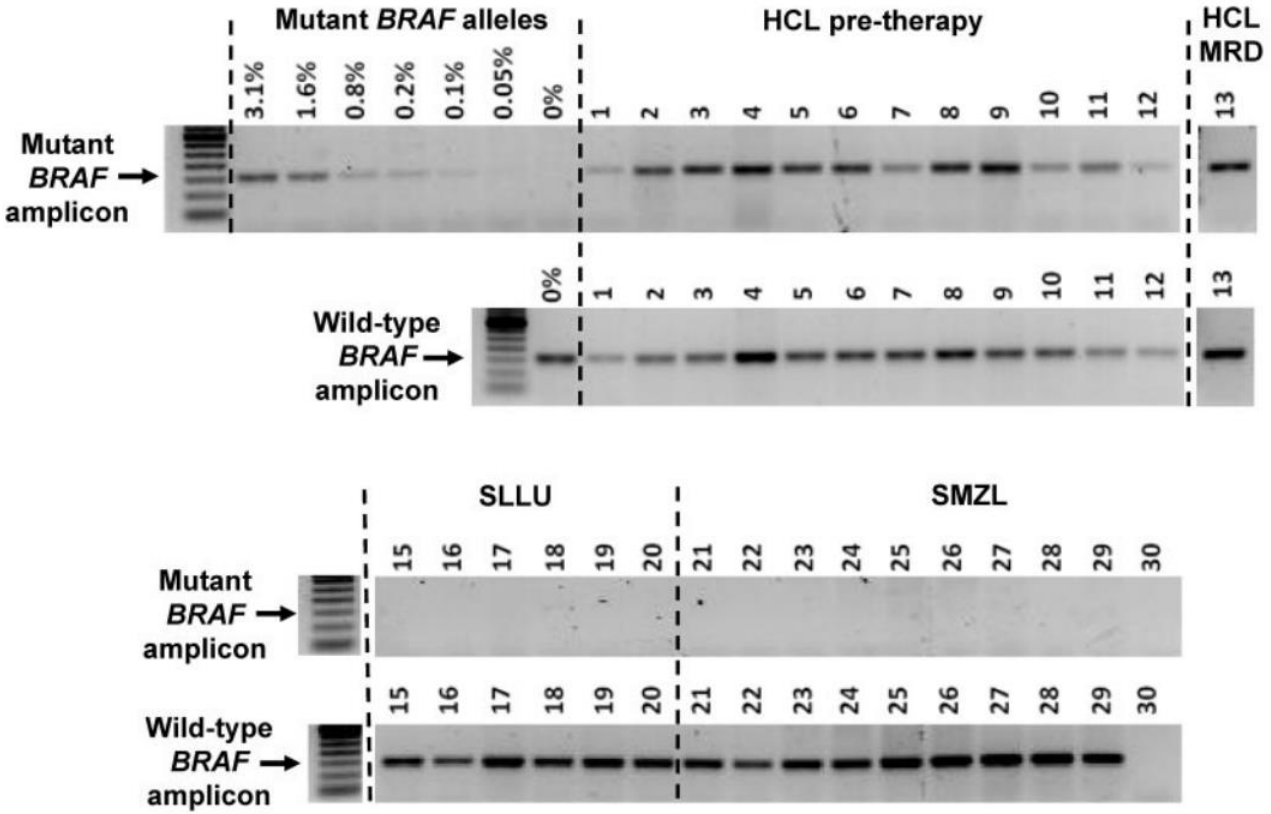




Tiacci et al., JCO 2017;35:1002-1010

Simple genetic diagnosis of hairy cell leukemia by sensitive detection of the *BRAF*-V600E mutation

*Enrico Tiacci,[1] *Gianluca Schiavoni,[1] Francesco Forconi,[2] Alessia Santi,[1] Livio Trentin,[3] Achille Ambrosetti,[4] Debora Cecchini,[1] Elisa Sozzi,[2] Paola Francia di Celle,[5] Cristiana Di Bello,[5] Alessandro Pulsoni,[6] Robin Foà,[6] Giorgio Inghirami,[5] and Brunangelo Falini[1]



Tiacci et al., Blood. 2012;119(1):192-195

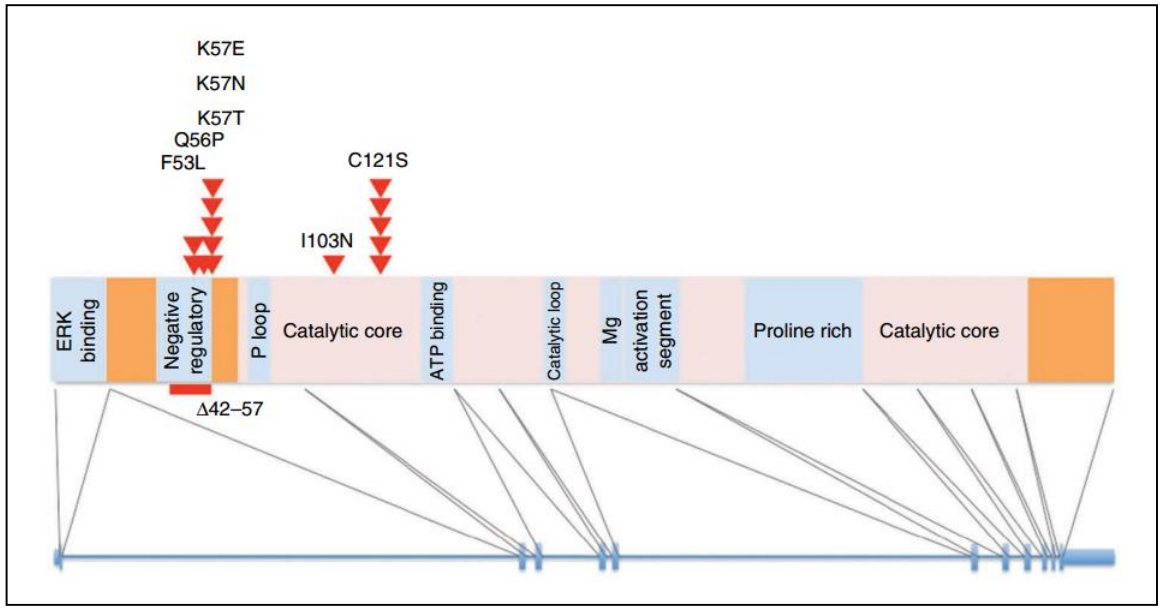
Sensitivität und Spezifität von BRAF V600E für HCL

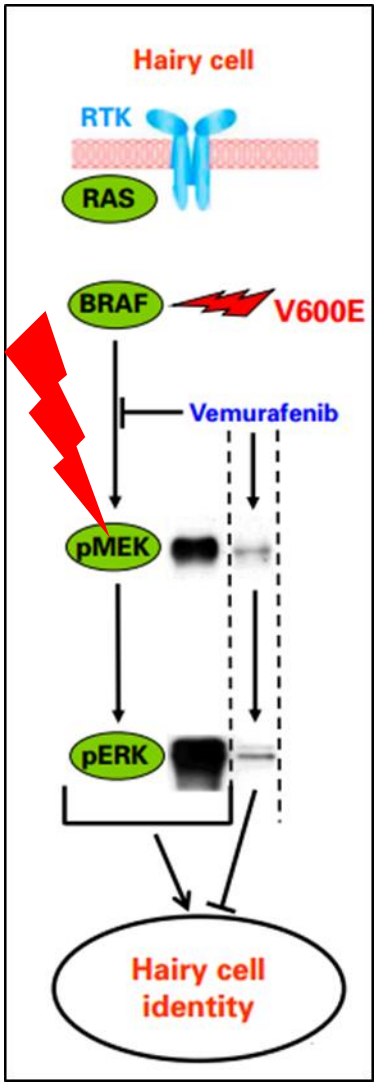
Sample type	Leukemic cells	No. of cases	Mutated cases, no. (%)
Hairy cell leukemia			
Blood, pretreatment	1%-90%	81	81 (100)
Blood, MRD	0.1%-13%		10 (100)
Blood, complete remission	Not detectable	16	0 (0)
BM biopsies	30%-80%	14	14 (100)
Purified leukemic cells	≥ 90%	17	17 (100)
Splenic marginal zone lymphoma	2%-97%	61	0 (0)
Splenic lymphoma/leukemia unclassifiable	15%-97%	18*	0 (0)
Chronic lymphocytic leukemia	18%-98%	31	0 (0)
CD5-negative mature B-cell neoplasm unclassifiable	15%-97%	5	0 (0)
Healthy blood donors	Not present	9	0 (0)

Tiacci et al., Blood. 2012;119(1):192-195

Akarca AU, et al., Br J Haematol 162:848-851, 2013; Blombery PA, et al., Haematologica 97:780-783, 2012; Boyd EM, et al., Br J Haematol 155:609-612,2011; Brown NA, Weigelin HC, Bailey N, et al, Appl Immunohistochem Mol Morphol 23: 590-600, 2015; Ewalt M, et al, Hematol Oncol 30:190-193, 2012; Langabeer SE, et al, Int J Lab Hematol 34:417-421, 2012; Okada K, et al, Intern Med 54:1397-1402, 2015; Rider T, et al., Hematol Oncol 32: 158-161, 2014; Schnittger S, et al., Blood 119: 3151-3154, 2012; Benjamin H, et al., Blood, epub, 2017

High prevalence of *MAP2K1* mutations in variant and IGHV4-34–expressing hairy-cell leukemias





Waterfall et al., Nat Genetics 2014;46:8-10

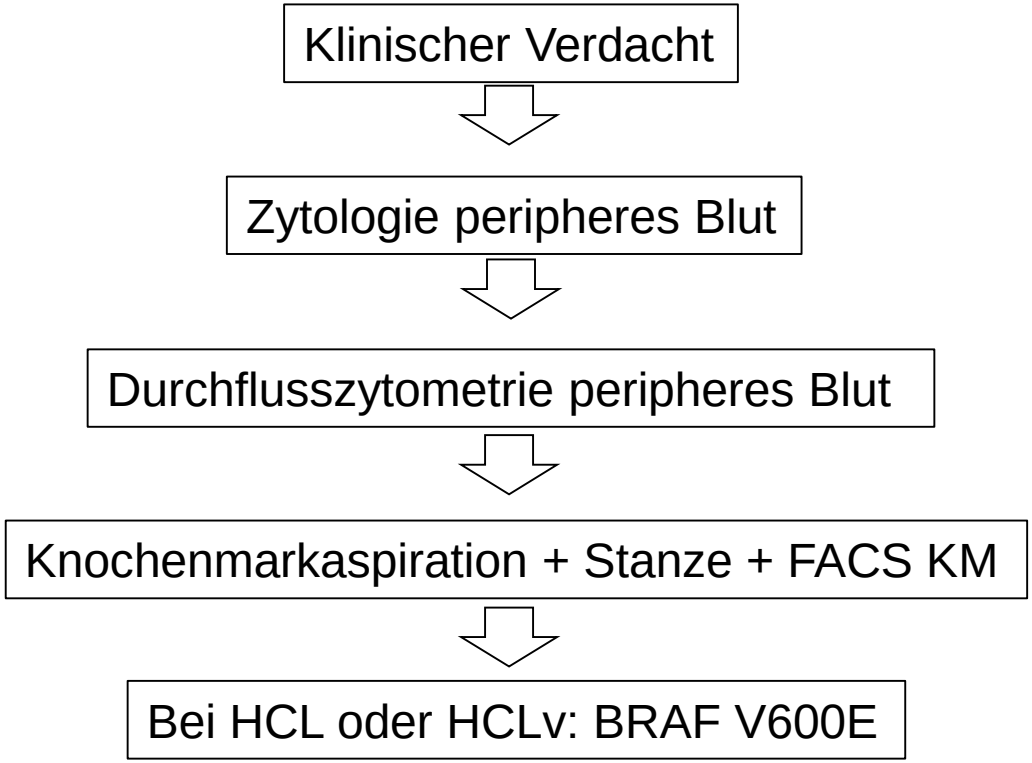
nach Tiacci et al., JCO 2017;35:1002-1010

(Molekular-)Zytogenetik: HCL, HCLv, SMZL

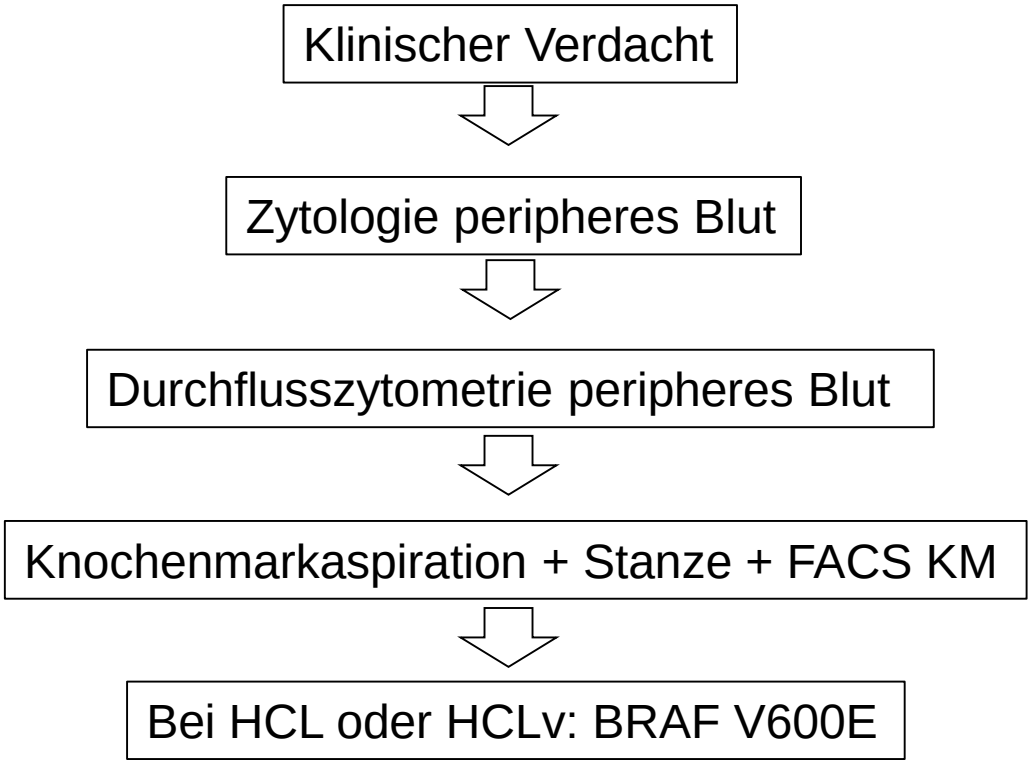
	HCL	**HCLv**	**SMZL**
BRAF V600E	> 97%	0%	0%
MAPK1 mut	bei *IGHV4-34*	48%	0%
del (7q)	< 10%	15%	30%

nach Tiacci et al., JCO 2017;35:1002-1010

Diagnose von HCL, HCLv, SMZL



Consensus guidelines for the diagnosis and management of patients with classic hairy cell leukemia



Grever et al., Blood. 2017;129(5):553-560

Vielen Dank

Rostock
- Christian Junghanß
- Hugo Murua Escobar
- Christina Große-Thie
- Larissa Henze
- Sandra Lange
- Catrin Roolfs

Prag
- Tomas Kalina
- Michaela Novakova

Lissabon
- Paolo Lucio

Rotterdam
- Ton Langerak
- Vincent van der Velden

Leiden
- Jacques van Dongen
- Alita van der Sluis

Salamanca
- Alberto Orfao
- Juan Flores-Montero

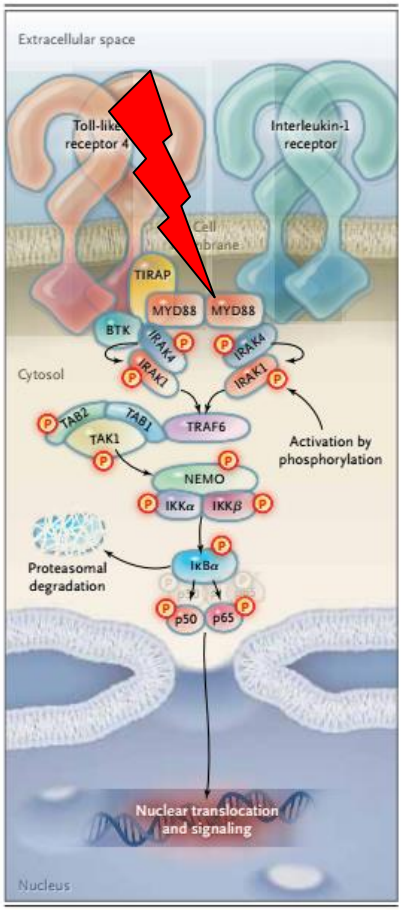
Leeds
- Andy Rawstron

Barcelona
- Neus Villamor

Kiel
- Michael Kneba
- Matthias Ritgen
- Torsten Raff
- Christiane Pott
- Monika Brüggemann

EuroFlow

MYD88 L265P Somatic Mutation in Waldenström's Macroglobulinemia



Treon et al., N Engl J Med 2012;367:826-33