Chronic Lymphocytic Leukemia

accumulation

of mature-appearing lymphocytes in the blood, marrow,

lymph nodes, and spleen.

CLL cells are:

monoclonal B lymphocytes
that express CD19. CD5, and CD23

weak or no expression
of surface immunoglobulin (Ig), CD20, CD79b, and FMC7

incidence of CLL varies throughout the world,

highest in North America and rare in the Far East

The disease is rare in young people

the incidence rises in the fourth decade and continues to rise exponentially

mean age at diagnosis being
69.6 years and >80% of patients being >60 years

PATHOPHYSIOLOGY

Predisposing Factors

no firm evidence linking an occupational exposure

infection in the etiology of this
disease?

family history of CLL or another
lymphoproliferative disorder
is a strong risk factor for CLL

1 in 10 patients *with* CLL has a family history of CLL or another lymphoproliferative disorder

30-fold increase in the risk of CLL in first-degree relatives of patients with CLL

13 to 18% of first-degree relatives have a peripheral blood CD5+ monoclonal B-cell lymphocytosis

Patients with familial CLL are ~10 years younger than those with sporadic CLL

Anticipation may occur in familial CLL

with affected children being 15 to 20 years younger than their parents at diagnosis

There is no consistency in the clinical features of affected members with familial CLL

the CLL cell is a memory B cell

two forms of CLL

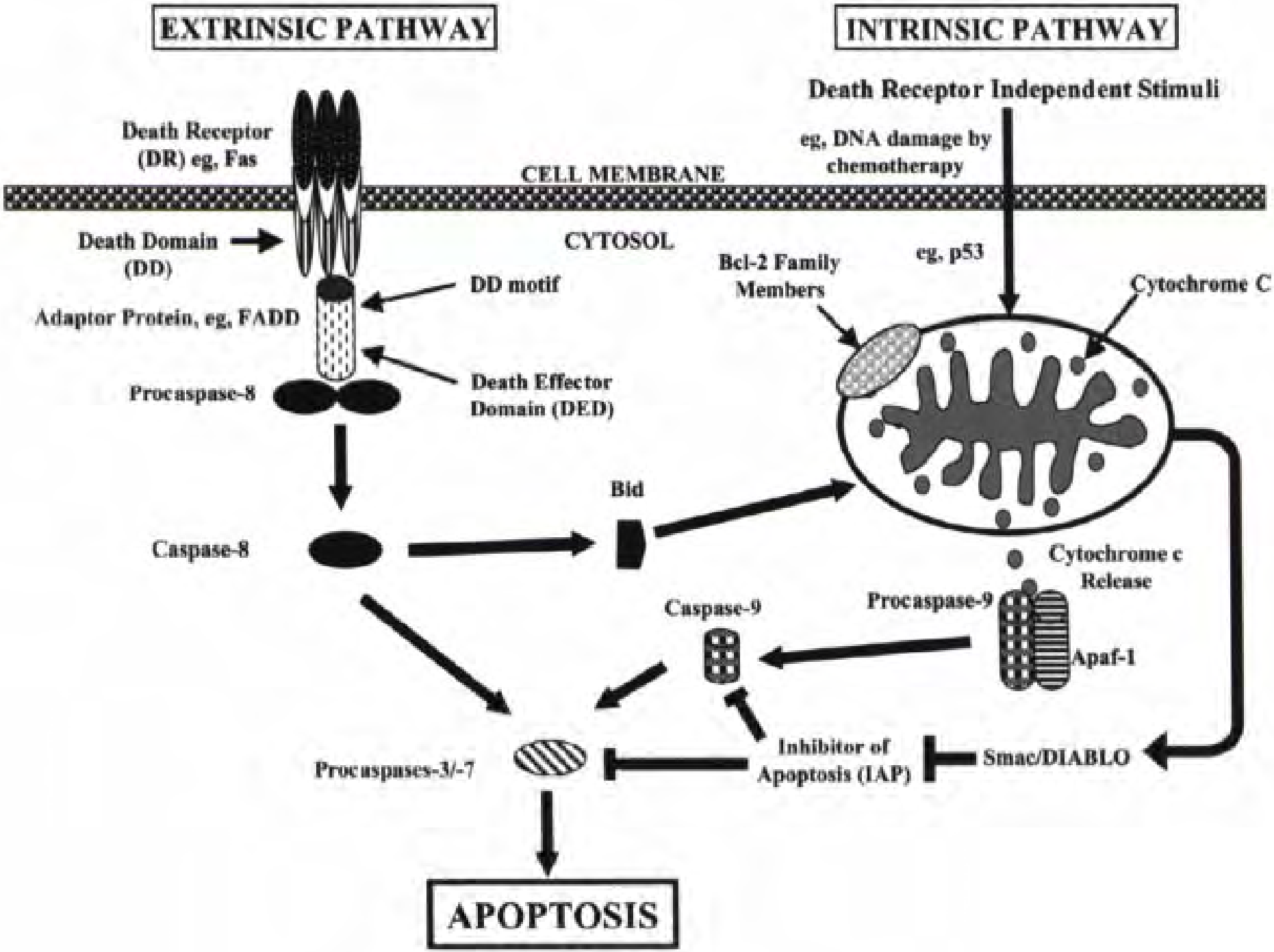
Pregerminal lymphocyte and lacking mutations of the *IgVHgene*

other having traversed the germinal center and containing mutations

Abnormalities in Apoptosis

defect in apoptosis typifies CLL with the majority of cells being long-lived Non cycling and in Go

there is a small fraction of replicating cells in the lymph nodes and marrow that is responsible for disease progression


EXTRINSIC PATHWAY
INTRINSIC PATHWAY
Death Receptor Independent Stimuli
Death Receptor (DR) eg, Fas
eg, DNA damage by chemotherapy
CELL MEMBRANE
Death Domain (DD)
CYTOSOL
Bcl-2 Family Members
eg, p53
Cytochrome C
DD motif
Adaptor Protein, eg, FADD
Procaspase-8
Death Effector Domain (DED)
Bid
Caspase-8
Cytochrome c Release
Procaspase-9
Caspase-9
Apaf-1
Procaspases-3/-7
Inhibitor of Apoptosis (IAP)
Smac/DIABLO
APOPTOSIS

Genomic Abnormalities

Conventional cytogenetic proved difficult because CLL cells have a very

low proliferative index

clonal chromosomal

abnormalities are detected in -50% of cases

Analysis by Conventional Cytogenetic

The most common clonal abnormality was trisomy 12 (36%)

structural abnormalities of chromosome 13(20%)

structural abnormalities of chromosome 14 (16%)

The 13q abnormalities usually involved a

13q14 deletion (site of the *Rb gene)*

patients with abnormal karyotyping had a worse prognosis than those with normal cytogenetic,

trisomy 12 was usually seen in those 15% of cases with CLL variants, (CLL /PLL)
or "atypical" CLL

those with chromosome
14 abnormalities had the worst prognosis

11q22-q23 deletion have been detected in 13% of patients

these patients had disease progression and
poor survival

Analysis by Fluorescence in Situ Hybridization

most present-day studies use FISH to identify and quantify the genetic defects in CLL

Evaluation of 325 CLL patients for deletions of 6q21, 11q22-q23 ,13q14, and

17p13;

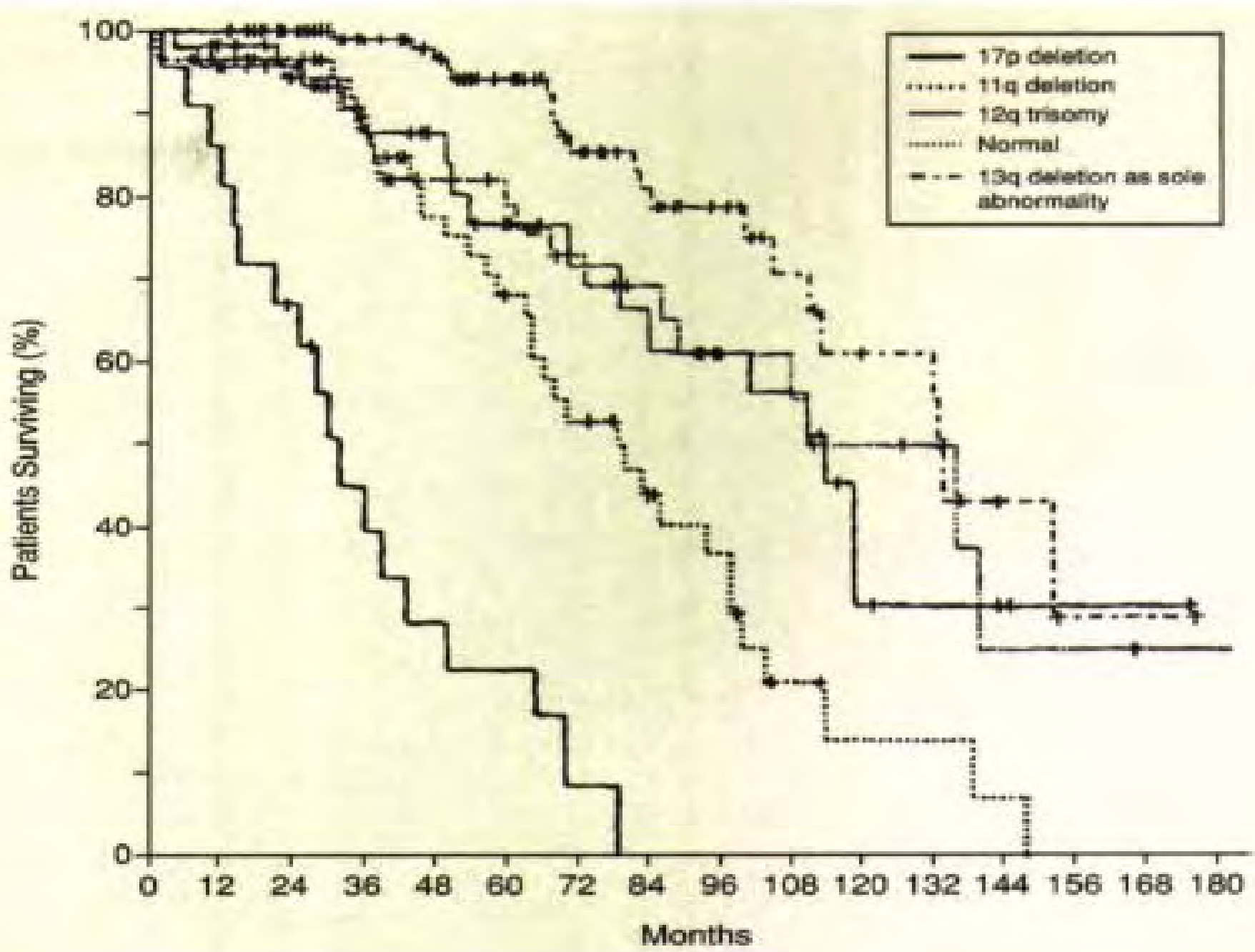
268 (82%) had abnormalities

deletion 13q14 being most frequent (55%)

deletion 11q22-q23 (18%)

trisomy 12q13 (16%), deletion 17p13 (7%)

deletion 6q21 (7%)


17p deletion
11q deletion
12q trisomy
Normal
13q deletion as sole abnormality
Patients Surviving (%)
100
80
60
40
20
0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180
Months

The median survival times

17 p deletion → 32 months

11q22-q23 deletion → 79 months

trisomy 12q → 114 months

normal karyotype → 111 months

13q deletion → 133 months

deletion 13q as the only abnormality had similar survival to those with normal chromosomes

only one third required therapy

Patient with 17p13 or 11q22-q23 deletions

Have the poorest survival , more

marked lymphadenopathy splenomegaly, and are more

likely to be symptomatic with night sweats and weight loss

correlation between genomic abnormalities and presence of *IgV/I* gene mutations

and alterations in cell morphology

Genomic Abnormality	Incidence (%)		Affected Genes	Clinical Features
	Classical Cytogenetics[a]	Fluorescence *in Situ* Hybridization[b]		
Normal	50[c]	18	—	—
13q deletion	10	55	*Rb, MiR-15a, MiR-16-1*	Good prognosis
11q deletion	8	18	*ATM*	Younger; bulky lymphadenopathy; poor prognosis
12q trisomy	13	16	*mdm2*	"Atypical" morphology; end-stage disease
17p deletion	4	7	*P53*	CLL/PLL morphology; drug resistance; very poor prognosis
6q deletion	4	6	—	—

Clonal Evolution

additional chromosomal
abnormalities develop with disease progression

16 to 39% incidence of new
or additional chromosomal abnormalities, which
develop over several years from initial diagnosis

By FISH analysis, one quarter of

patients will have the development of new abnormalities after 5 years

particularly in patients who are ZAP-70-positive, CD 38 positive, and those with un mutated *IgVH*

patients with un mutated *IgVH have an increased risk of developing*

deletions of 11q22-q23 or 17p13,

those with

mutated *IgVH develop deletions of 13q*

treatment with fludarabine increases the

expression of p53-dependent genes, increasing the risk of

selecting out 17p13-deleted cells

42% of fludarabine-resistant CLL patients

have either p53 mutations or deletions

CLINICAL FINDINGS

most CLL patients are elderly

-10% of patients are <50 years old

the presenting features are similar regardless of age

70 to 80% of patients are diagnosed incidentally and they have early-stage

The most frequent complaint is fatigue or a vague sense of being unwell

Less frequently:

enlarged nodes or the development of an infection is the initial complaint

.

Fever and weight loss are uncommon at presentation but may occur with advanced and drug-resistant disease

Enlargement of the cervical and supraclavicular nodes occurs more frequently than axillary or inguinal lymphadenopathy

mild to moderate enlargement of the spleen

splenic infarction is uncommon

Less common manifestations:

enlargement of the tonsils

abdominal masses due to mesenteric or retroperitoneal lymphadenopathy

skin infiltration.

Anemia: related to marrow replacement or autoimmune hemolysis and aplasia

bruising or bleeding secondary to :

thrombocytopenia, acquired von Willebrand disease, or an acquired inhibitor to factor VIII

paraneoplastic syndrome

nephrotic syndrome, paraneoplastic pemphigus, or angioedema

- **LABORATORY FINDINGS**

Peripheral Blood

The median lymphocyte count at diagnosis is 30000 /mL,

in most patients there is a continuous increase in the lymphocyte count over time

In half >12 months for the lymphocyte count
to double

cyclic fluctuations of up to 50 x 109/L can occur in the lymphocyte counts of untreated patient

in others, the count
may remain stable for years

~~CLL cells:~~

normal small to medium-sized lymphocytes

clumped chromatin

inconspicuous nucleoli

small ring of
cytoplasm

Smudge cells are commonly seen

Appear to be caused by a decrease in vimentin

Relationship between ~30% smudge cells
And mutated *IgVH and better prognosis than*

French/American/British classification

Classical CLL: are small >90% of cells

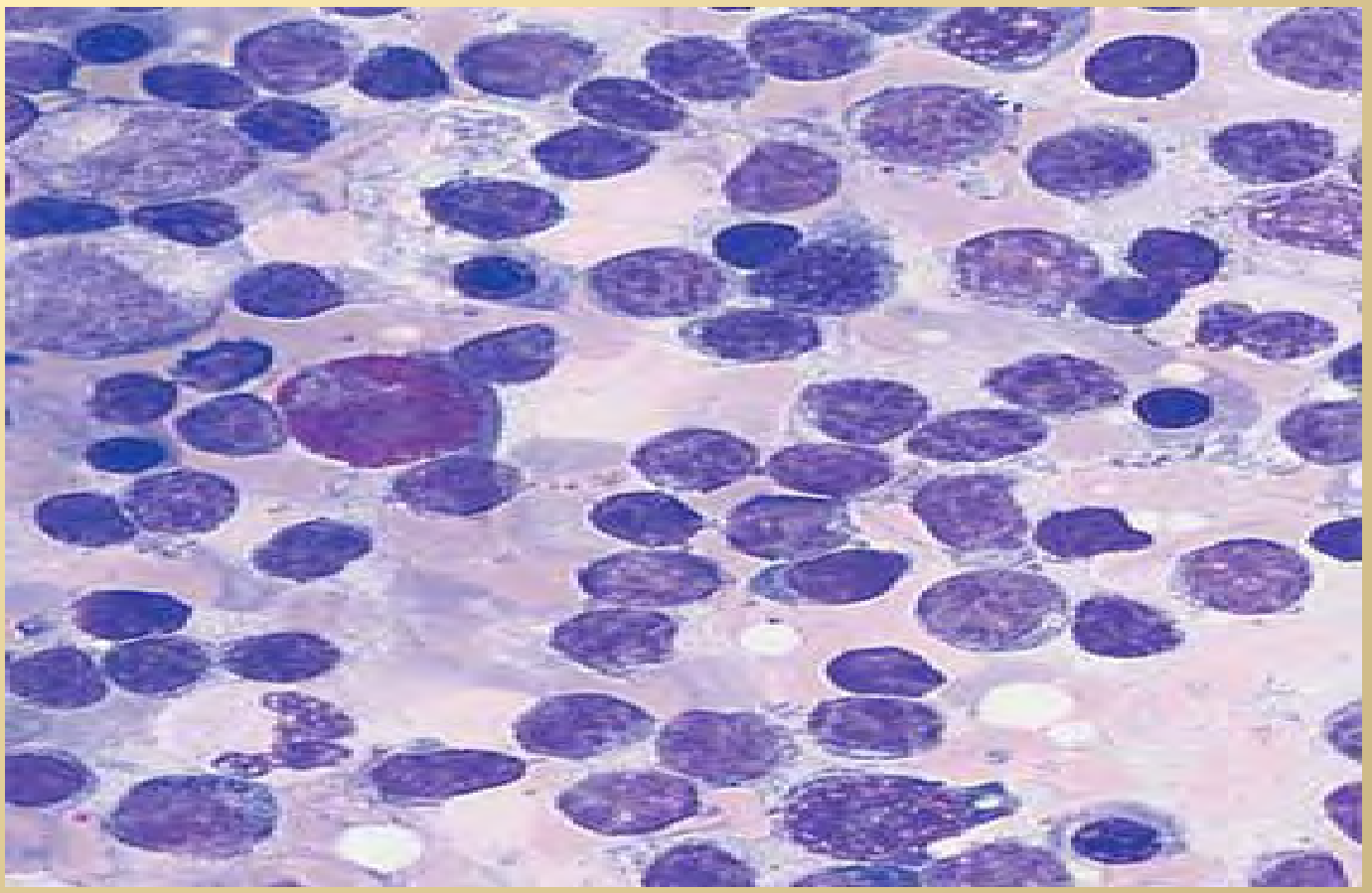
the cells are *CLL/PLL:* when 11 to 54% of e pro lymphocytes

atypical CLL: >15% of the lymphocytes are plasmoid or cleaved and <10% are prolymphocyts

prolymphocytic leukemia: ~55% of the cells are pro lymphocytes,

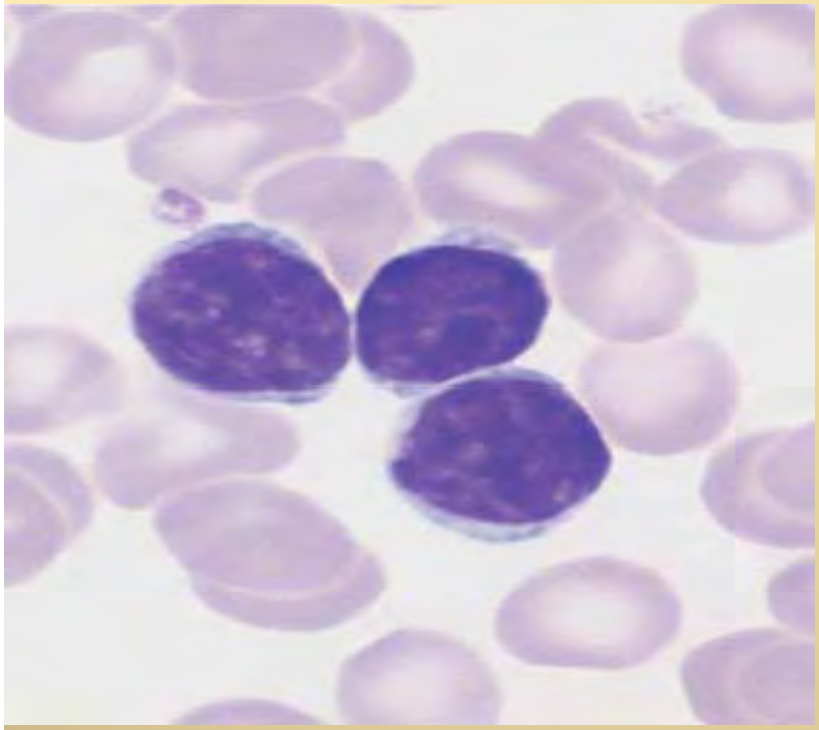
There is always strong

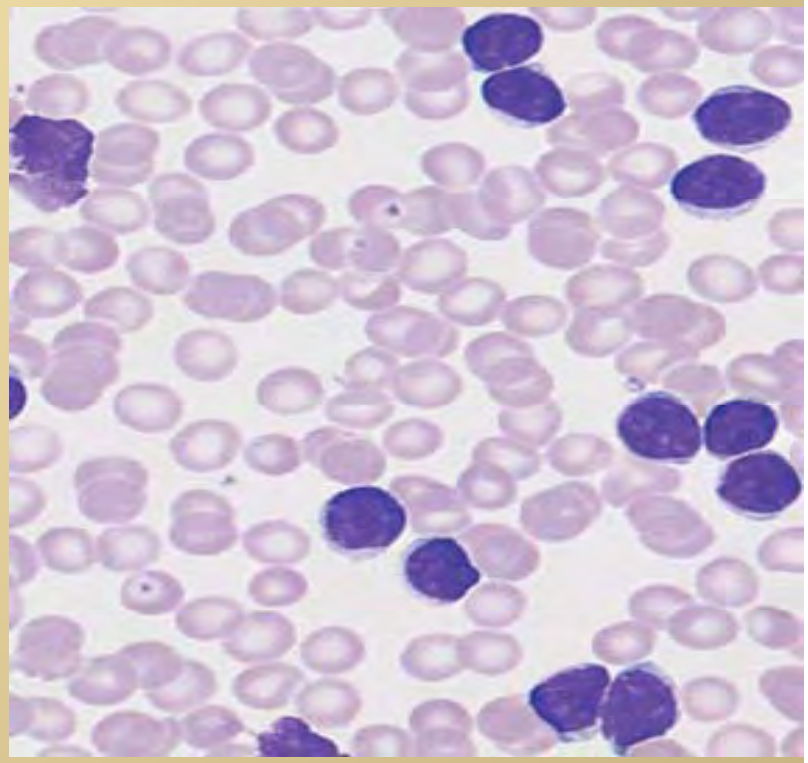
proliferation of the typical small lymphocytes, which are usually spread out diffusely.



densely structured nuclei and little variation in CLL cells

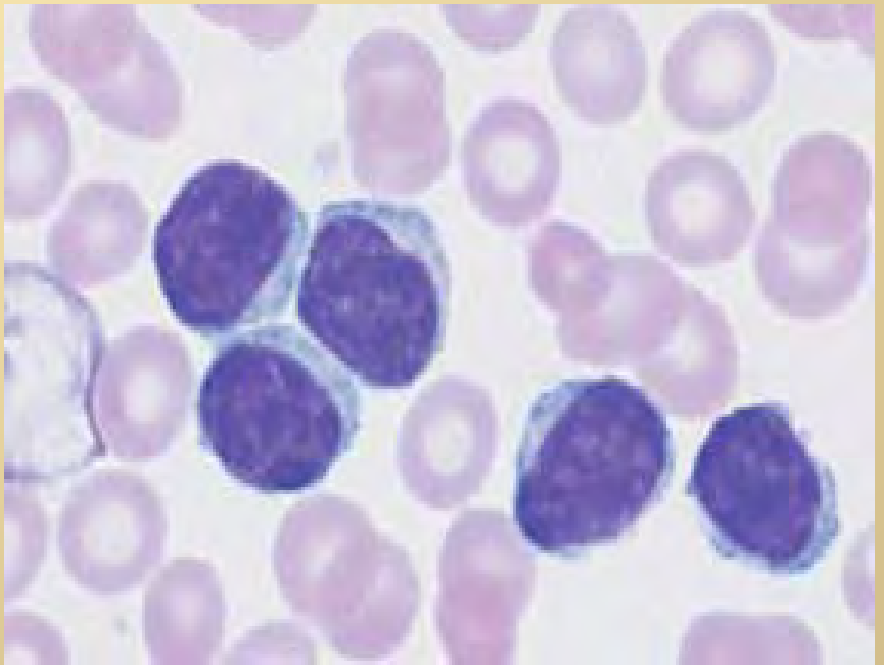
small cytoplasmic layer





Slightly

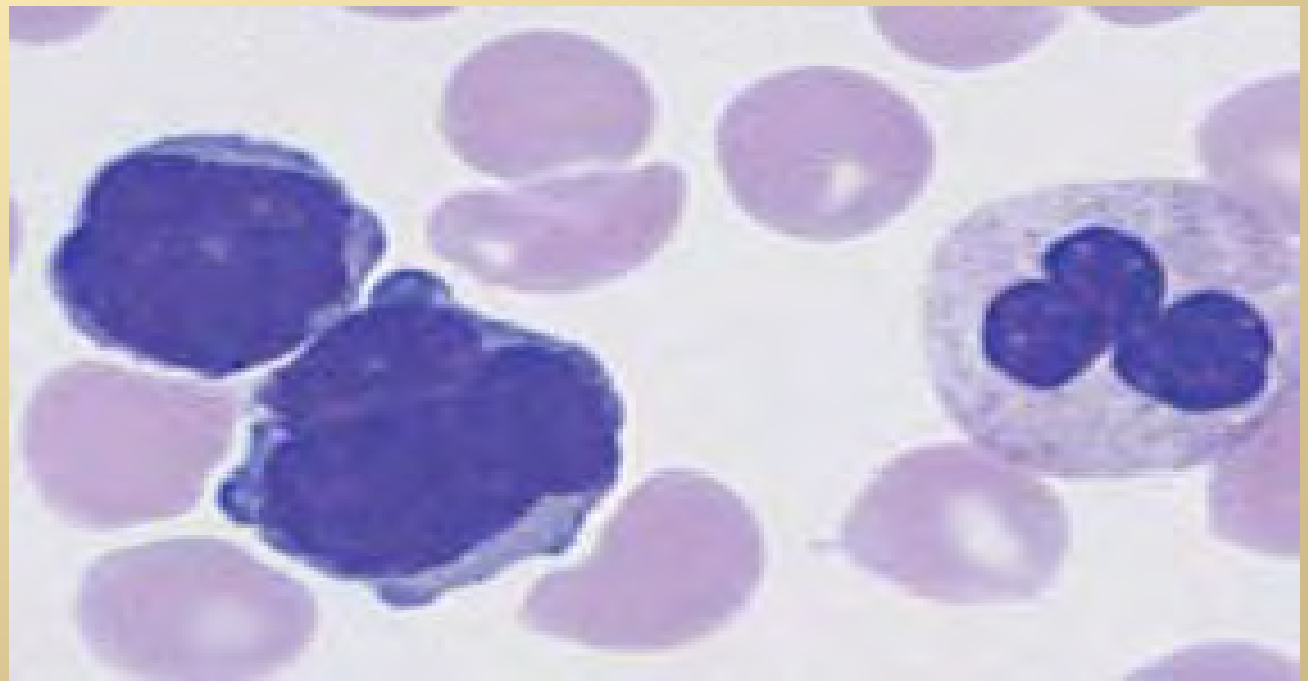
eccentric enlargement of the cytoplasm in the lymphoplasmacytoid variant of CLL



large lymphocytes with irregularly structured nucleus, well-defined nucleolus, and wide cytoplasm (transitional form CLL/PLL)

Prolymphocytic

leukemia of the T-cell series (T-PLL) with indented nuclei and nucleoli



Bone Marrow

There are four patterns of marrow involvement in CLL

Interstitial

Nodular: the least common

Mixed: most common

Diffuse: effacement of the fat spaces by tumor, carries the worst prognosis

Immunophenotyping

leukemic cells have the B-cell markers

Cd19, Cd20 (low), Cd43, Cd79b (low), and, by definition, must be Cd5+

weak expression of sIgM and sIgD

Cd23+ and Cd IO –

These cells are also Cd27+:
Suggesting that these cells are memory B cells

.

Recommendation for the use of five markers to differentiate CLL from
other B-cell malignancies

Typical CLL should be:

surface Ig (weak)

Cd5+, Cd23+

Cd79b or Cd22 (weak)

FMC7_

membrane Cd23 over expression

is an activation marker

may play a role in the decreased apoptosis observed in this disease

is useful to differentiate CLL from mantle cell lymphoma

FMC7 is usually strongly expressed in hairy cell leukemia and

prolymphocytic leukemia,

16% of CLL cases also stain positively

those patients who are FMC7+have high levels of

Surface IgM, low expression of Cd23, and poor prognosis

The Cd 5 antigen is most commonly associated with mature T

cells and is expressed weakly on thymocytes

Normal B cells carrying the CD 5 marker are located in the mantle zone

of the lymph node, and

in the peripheral blood

The Cd5 molecule has been

appears to be involved in the activation of T lymphocytes

function of Cd5 on the B cell remains unknown

studies suggested that 5% of cases of CLL could be Cd5-

These cases were more likely to be FMC7+, Cd23-, Cd11b+, and Cd13+ and had a poor prognosis

Marker	Marker Intensity	Score	Marker Intensity	Score
Surface immunoglobulin	Weak	1	Strong	0
CD5	+	1	−	0
CD23	+	1	−	0
CD22/CD79b	Weak	1	Strong	0
FMC7	−	1	+	0

+, present; −, absent.

NOTE: Diagnosis of chronic lymphocytic leukemia requires a score of 4 or 5.

A score of 4 or 5 had an accuracy of 97 percent for the diagnosis of CLL, while most of the other non-CLL B-cell lymph proliferative diseases had scores of zero to two

Condition	sm/g	CD5	CD10	CD11c	CD19	CD20	CD22	CD23	CD25	CD43	CD79b	CD103'	FMC7
CLL	Dim	++	-	-/+	++	Dim	-/+	++	+/-	+	-	-	-/+
Waldenström macroglobulinemia	++	-	-	-/+	++	++	+	-	-/+	+/-	+	-	+
Prolymphocytic leukemia	+++	-/+	-/+	-/+	++	+++	++	++	-/+	+	++	-	+
HCL	+++	-	-	++	+++	+++	+++	-	+++	+	+	+++	+++
HCL variant	+++	-	-	++	+++	+++	+++	-	-	+	+	+++	+++
Splenic lymphoma with villous lymphocytes	++	-/+	-/+	+/-	++	++	++	+/-	-/+	+	++	-/+	++
Marginal zone lymphomas	++	-	-	+/-	++	++	+/-	+/-	-	-/+	++	-	+
Mantle cell lymphoma	++	++	-/+	-	++	++	++	-	-	+	++	-	++
Follicular lymphoma	++	-/+	++	-	++	++	++	-/+	-	-	++	-	++

Approximately 50% of

patients had >30% Cd38+ cells, and these patients had un mutated

IgVh and had a worse prognosis than those with <30% Cd38+ cells

Cd 38 acts as a receptor and enzyme and can produce

cell replication and survival with a variety of signals

Activation

of Cd38+ cells, through sIgM induces

apoptosis and through IgD prolongs cell survival and induces differentiation

Cd38 can be measured easily by flow cytometry

disagreement about the number of cells that are required to define positivity,

with values

ranging from 5 to 30%

Moreover, the number of

Cd38-positive cells can vary over time

ZAP-70 is a member of the Syk-ZAP-70 protein kinase family

is expressed in T and natural killer cells

Is important in T-cell signaling

~~recent evidence suggests that normal B cells may also express ZAP-70, particularly when activated~~

Gene expression studies in CLL have demonstrated that cells with unmutated *IgVu have increased* expression of ZAP-70

70 to 90 % correlation between ZAP-70 expression and *IgVh mutational status*,

whether ZAP-70 was measured by flow cytometry (>20% cells positive), Western blot analysis, or immunohistochemistry

ZAP-70 positivity correlates the presence

of poor-risk cytogenetic, del llq22-q23, del and trisomy 12 and del 17 P13

unlike Cd38, the ZAP-70

status appears stable over time

Functional Immune **Abnormalities**

80% of patients have recurrent infections

sepsis being the major cause of death

CLL patients are susceptible to:

typical
bacterial infections

opportunistic infections, particularly if they have received nucleoside analogs or monoclonal antibodies

hypogammaglobulinemia is The major cause of infection

,

CLL patients

also have abnormalities in T cells, complement, and neutrophil function

Hypogammaglobulinemia and agammaglobulinemia

the severity increases with the duration
and stage of disease

The Ig levels are all decreased

within the IgG class, reduced levels of IgG2 and IgG4
Correlate best with the risk of infection

the decline in IgA levels is
the most important predictor of infection

pathogenesis of
the hypogammaglobulinemi:

impaired B-cell function

regulatory abnormalities of T cells

suppress Ig secretion by normal B cells
in vitro by CLL-derived natural killer (NK)
cells

inversion of the T-helper to -suppressor cell ratio

increase in the number of T-suppressor cells may correlate with the degree of hypogammaglobulinemia

decreased T-helper function

abnormality in
the large granular lymphocyte population

levels of different complement components are decreased

multiple defects in neutrophil function have been described

Autoimmune Manifestations

CLL is the most common cause of autoimmune hemolytic anemia , causing 14% of cases

4to 25% of CLL patients develop AIHA

with a warm-type antibody against the Rhesus system

the incidence is higher in :

Men , lymphocyte > 60000 and >60 years old

Immune thrombocytopenia (ITP) occurs in 2% of patients

the diagnosis is based on increase in platelet size

In the PBS and an increase in megakaryocytes in the marrow

approximately one third of these patients with ITP have a positive Coombs test

Whether autoimmune neutropenia occurs in CLL is unclear

Pure red cell aplasia occurs in -1 % of cases and may be caused by a T-cell mechanism

Nephrotic syndrome related to membranous or membranoproliferative

acquired angioedema

autoimmune blistering skin diseases

DIAGNOSIS

peripheral blood lymphocyte count >5 x 109

<55% of the cells being atypical

Bcell specific differentiation antigens (CD19, CD20, and CD23) and be CD5+

bone marrow aspirate showing >30% lymphocytes

INVESTIGATIONS AND STAGING

investigations

complete blood count, review of the peripheral smear, and immunophenotyping are required for diagnosis

A marrow aspirate/
biopsy is not required for diagnosis but may be useful

under the following circumstances:

to establish the cause of anemia and
thrombocytopenia for patients with Rai stage III or IV
disease

To confirm that there is no paratrabecular
localization or cyclin DI staining in atypical cases

To assess the pattern of marrow infiltration, which is
of prognostic value

To assess response following chemotherapy

reticulocyte count

Coombs test, renal and liver function tests LDH , serum protein electrophoresis and/or immunoelectrophoresis, and Ig levels.

The plasma *B*-microglobulin level

as this is a simple and important prognostic marker and an indicator of response to therapy

A baseline chest radiograph

computed tomograph (CT) scans of the
chest and abdomen will depend on clinical indications

Positron emission tomography (PET) scans are not useful for staging in CLL

Before starting chemotherapy
screen for active viral infections (hepatitis Band C, cytomegalovirus)

Staging

The Rai staging system is generally used in North America

Rai System[a]

Stage	Description	Risk Status
0	Lymphocytosis, lymphocytes in blood > 15,000/mcL and > 40% lymphocytes in the bone marrow	Good
I	Stage 0 with enlarged node(s)	Intermediate
II	Stage 0-I with splenomegaly, hepatomegaly, or both	Intermediate
III[c]	Stage 0-II with hemoglobin < 11.0 g/dL or hematocrit < 33%	High
IV[c]	Stage 0-III with platelets < 100,000/mcL	High

Binet System[b]

Stage	Description
A	Hemoglobin ≥ 10 g/dL and Platelets ≥ 100,000/mm^3 and < 3 enlarged areas
B	Hemoglobin ≥ 10 g/dL and Platelets ≥ 100,000/mm^3 and ≥ 3 enlarged areas
C[c]	Hemoglobin < 10 g/dL and/or Platelets < 100,000/mm^3 and any number of enlarged areas

Rai Stage at Diagnosis	Percent of Patients Never Requiring Chronic Lymphocytic Leukemia Therapy	Expected Survival in Months From Initial Diagnosis
0. Lymphocytosis >5 x 10^9/L only	59	150
1. Lymph node (LN) enlargement	21	101
2. Spleen/liver (S/L) enlargement ± LN	23	71
3. (Anemia with hemoglobin <11 g/dL) ± LN or S/L	5	19
4. (Thrombocytopenia <100 x 10^{12}/L ± LN or S/L)	0	19

several points should be made

The staging is based on clinical examination and not on CT scans

patients with autoimmune

cytopenia have a better prognosis than patients with cytopenia related to marrow packing

The diagnosis of CLL was originally based on typical cell morphology peripherallymphocytosis

PROGNOSIS

Rai and Binet Staging

the median survival

of patients with stage A disease or in the low-risk group is > 10 years

40% of these patients will progress to a more advanced disease

50% of patients will require therapy at some point

25% will die from their diseas

Age and Sex

10% of CLL patients are <50 years old

clinical features and staging at presentation are similar
To those > 50 years

the proportion of deaths that can be
attributed directly to CLL is greater for the younger age group

The male-to-female ratio for CLL is 2:1

women are more likely to
have early-stage disease
women have a better prognosis than men
an increased incidence of *IgVH* mutated disease

good prognostic genetic markers in women

hormonal differences

lymphocyte Characteristics

Morphology :20% of patients have atypical CLL or CLL/PLL

more advanced stage.

a higher proliferative index

trisomy 12 or deletion of 17p13 (p53 mutation).

CD38+

aberrant cell morphology :an independent prognostic marker

patients with >30% smudge cells have a better prognosis than with <30% smudge cells

Number

Survival decreases with increasing lymphocyte count

median survival is 8.6 years for a lymphocyte count <20000

3.7 years if the count is >40000

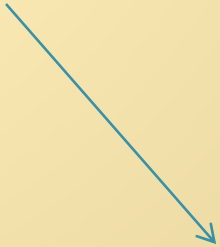
Doubling Time

patients with a lymphocyte doubling time

of <12 months have a significantly worse survival rate

Immune Markers

increase in surface Igs ,FMC7+, or increased intensity of CD20



associated with atypical morphology trisomy 12 and poor prognosis

CD38+ has been associated with:

shorter survival

correlates with increasing Rai stage.

intrathoracic and abdominal lymphadenopathy

short doubling time

increased ,B2-microglobulin levels

atypical morphology

the CD38 status is useful to predict

which patients within a particular clinical stage will progress

ZAP-70

70 to 90% correlation between ZAp-70 expression and absence of *IgVH mutations*

ZAP-70 positivity correlates moderately with CD38 positivity and the presence of poor-risk cytogenetics

The median survivals for ZAP-70-positive and ZAP-70-negative patients are 9.3 and 24.4 years

Molecular Genetics and Cytogenetics

p53, Ataxia Telangiectasia Mutation, Retinoblastoma

Mutations of p53 :in 10 to 15% of patient

predictive of resistance to CTx

The abnormality is associated with aberrant cell morphology and very poor survival

ATM and Rb protein levels are reduced in 34 and 42% of patients

these patients have advanced-stage disease and reduced survival

Marrow Histology

Nodular growth patterns: 90 months

interstitial growth patterns: 46months

diffuse infiltration : median survival time of only 28 months

Fibrosis of the marrow indicates an aggressive clinical course

The pattern of marrow involvement after chemotherapy is also prognostically important:

nodular partial remission :

persistent leukemia and have a shorter time to relapse compared

to those in complete remission

Fludarabine Resistance

Patients who do not respond to fludarabine or who relapse within 6 months of treatment have a median survival of 10 months

with standard chemotherapy

Serum Markers

Elevated *B*2microglobulin:

predict resistance to chemotherapy

Increased levels of LDH *B*2-microglobulin CD23 , and *TNF-a:*

patients who are at risk of disease progression.

TREATMENT

one third of CLL patients never require therapy,

one third need treatment as soon as they are seen,

one third have disease progression over the years and require therapy at some point

With the possible exception of allogeneic hematopoietic cell transplantation (HCT), CLL cannot be cured by current treatment options

immediate versus delayed treatment strategies:

no improvement in long-term survival with early treatment

Grade 2 or greater fatigue limiting life activities

B-symptoms persisting for 2 weeks or greater

Lymph nodes greater than 10 cm or progressively enlarging lymph nodes causing symptoms

Spleen or liver with progressive enlargement or causing symptoms

Anemia (Hemoglobin <11 g/dL) referable to CLL

Thrombocytopenia (Platelets <100 x 10_{12}/L) referable to CLL

Autoimmune hemolytic anemia or idiopathic thrombocytopenic purpura poorly responsive to traditional therapy

WBC > 300 x 10_{9}/L on two occasions two weeks apart if no alternative comorbid diseases increase morbidity of Treatment

Severe paraneoplastic (insect hypersensitivity, vasculitis, myositis, etc) process related to CLL not responsive to traditional therapies

Response Criteria and Minimal Residual Disease (MRD

RESPONSE DEFINITION AFTER TREATMENT FOR CLL[a]

Parameter	Complete response	Partial response	Progressive Disease	Stable Disease
Lymphadenopathy[b]	None above 1.0 cm	Decrease ≥ 50%	Increase ≥ 50%	Change from -49% to +49%
Liver and/or spleen size	Normal size	Decrease ≥ 50%	Increase ≥ 50%	Change from -49% to +49%
Constitutional symptoms	None	Any	Any	Any
Leukocytes	> 1500/mm^3	> 1500/mm^3 or > 50% improvement	Any	Any
Circulating B lymphocytes	Normal	Decrease ≥ 50% over baseline	Increase ≥ 50%	Change from -49% to +49%
Platelet count	> 100,000/mm^3	> 100,000/mm^3 or increase ≥ 50% over baseline	Decrease ≥ 50% over baseline	Change from -49% to +49%
Hemoglobin	> 11.0 g/dL (untransfused)	> 2 g/dL from baseline	Decrease of > 2 g/dL from baseline	Increase < 11.0 g/dL or < 50% over baseline, or decrease < 2 g/dL
Marrow	Normocellular, < 30% lymphocytes, no B-lymphoid nodules	Hypocellular, or ≥ 30% lymphocytes, or B-lymphoid nodules, or not done	Increase of lymphcytes to more than 30% from normal	No change of marrow infiltrate

MRD

estimated that 25% of patients with
a CR criteria will have MRD-positive disease

same prognosis as those in PR?

patients who achieve a marrow MRD-negative CR
will have sustained remissions and perhaps improved survival

two-color (CD19/CD5) flowcytometry to detect MRD, with a sensitivity of one CLL cell in 1 x 10 -2 leukocytes

four-color (CD19/CD5/CD20/CD79b) flowcytometry can detect one CLL cell in 5 x 10 -5 leukocytes

new four-color (CD81/CD22/ CD19/CD5) flow - cytometry assay

ongoing studies are required to develop a simple and reproducible assay for MRD that can be used universally

more intense treatments to achieve an MRD-negative Marrow ?

Treatment options include

purine analogs

alkylating agents

monoclonal antibodies

combinations of these agents