

Autoimmune hemolytic anemia in Chronic Lymphocytic Leukemia

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CLL and Immune Status

BLOOD

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Special Article

**Chronic Lymphocytic Leukemia—an Accumulative Disease
of Immunologically Incompetent Lymphocytes**

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**Chronic Lymphocytic Leukemia—an Accumulative Disease
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By WILLIAM DAMESHEK

Clinical case

- 68-year-old male
- Past medical history: diabetes mellitus, hypertension, COPD, non-granulomatous anterior uveitis
- August 2012: Lymphocytosis (13.800/ μ l)
- November 2012: **CLL diagnosis**
 - Hb 14.9, WBC 22.690 (17.900 lymphocytes), platelet 241.000
 - Immunophenotype: CD5⁺, CD20⁺, CD79b⁺, CD23⁺, Kappa restriction

Clinical case

- No symptoms
- PS: 0/CIRS: 4
- No lymphadenopathy or organomegaly

Other laboratory parameters

- LDH 362 (N <243)
- Beta-2 microglobulin: 3.27mg/L (N < 1.80)
- ZAP-70: 50%
- Unmutated *IGHV* genes
- FISH: trisomy 12

CLL - Stage A(0)

February 2014-December 2014: Disease progression to CLL Stage B(I)

- **Symptoms:** Asymptomatic, PS: 0
- **Physical findings:** Generalized peripheral lymphadenopathy (~3 cm). No splenomegaly, no hepatomegaly
- **CT scan:** consistent with CLL (no bulky disease, SUV < 3)
- **Laboratory findings**
 - Hemoglobin: 12.8
 - Platelets: 196.000
 - WBC count: 40.690 (64% lymphocytes) (LDT < 1 yr)

January 2015 (Emergencies Department)

- **Symptoms:** Fever $> 38^{\circ}\text{C}$ with cough, fatigue, PS: 1
- **Physical findings:** Pallor, generalized cutaneous and oral mucosa petechiae, generalized peripheral lymphadenopathy (~3-4 cm). No splenomegaly, no hepatomegaly
- **Laboratory findings**
 - Hemoglobin: 6.2
 - Platelets: 4.000
 - WBC count: 43.810 (41.180 lymphocytes, 880 neutrophil count)

Clinical case

- Corrected reticulocyte count: 0.05%
- Positive Direct Antiglobulin Test (C3d++/IgG-)
- LDH 244 (N <243)
- Bilirrubin: 22 (N<17)
- Haptoglobins: 3.02 (N:0.3-2.0)

CLL - Stage C(IV)

(Evans syndrome: AHAI DAT-positive and ITP)

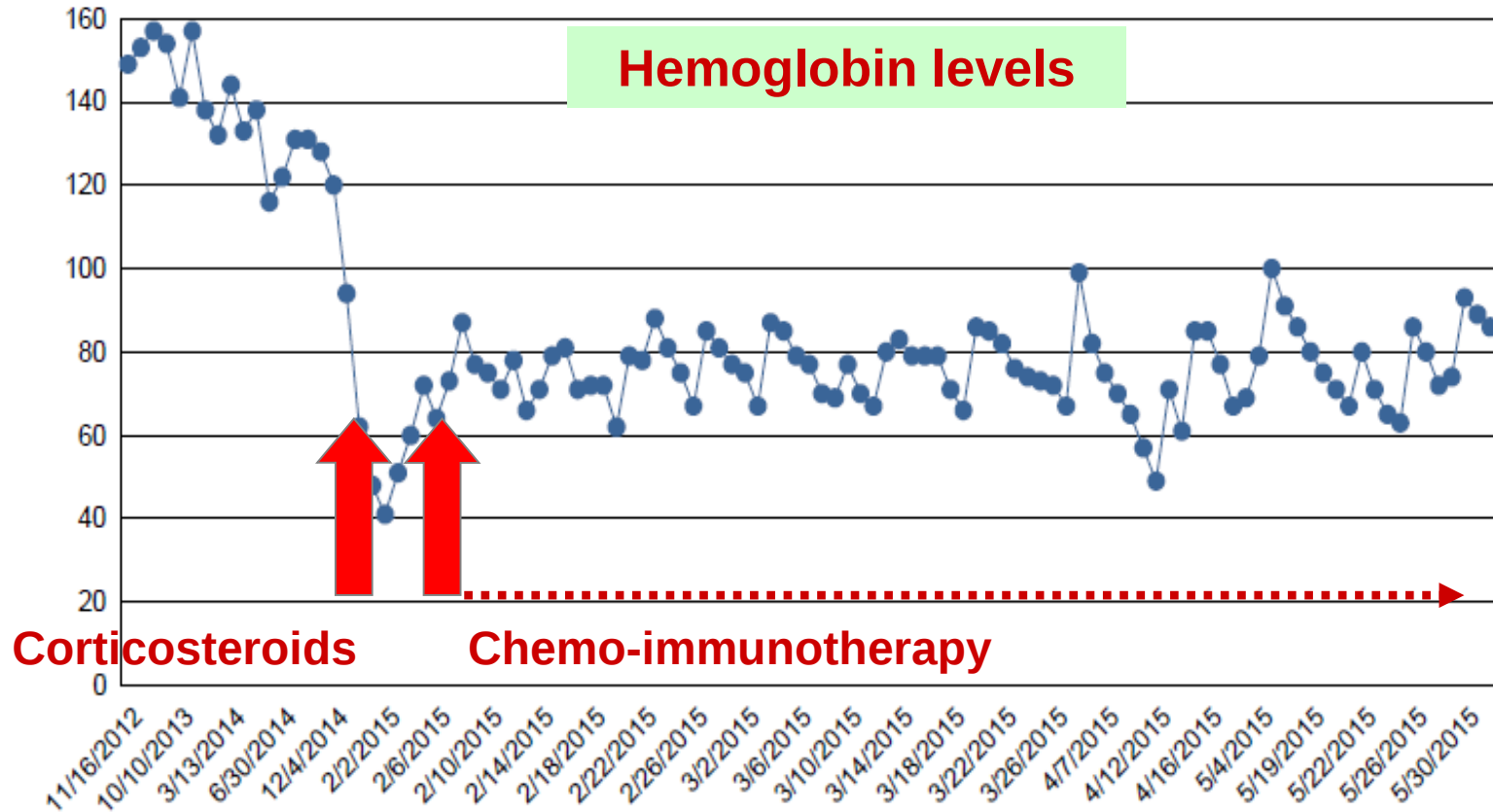
Therapy and treatment results

- 31 January 2015: Prednisone 1mg/kg/day and Immunoglobulin infusion 1gr/Kg on days 1 and 2

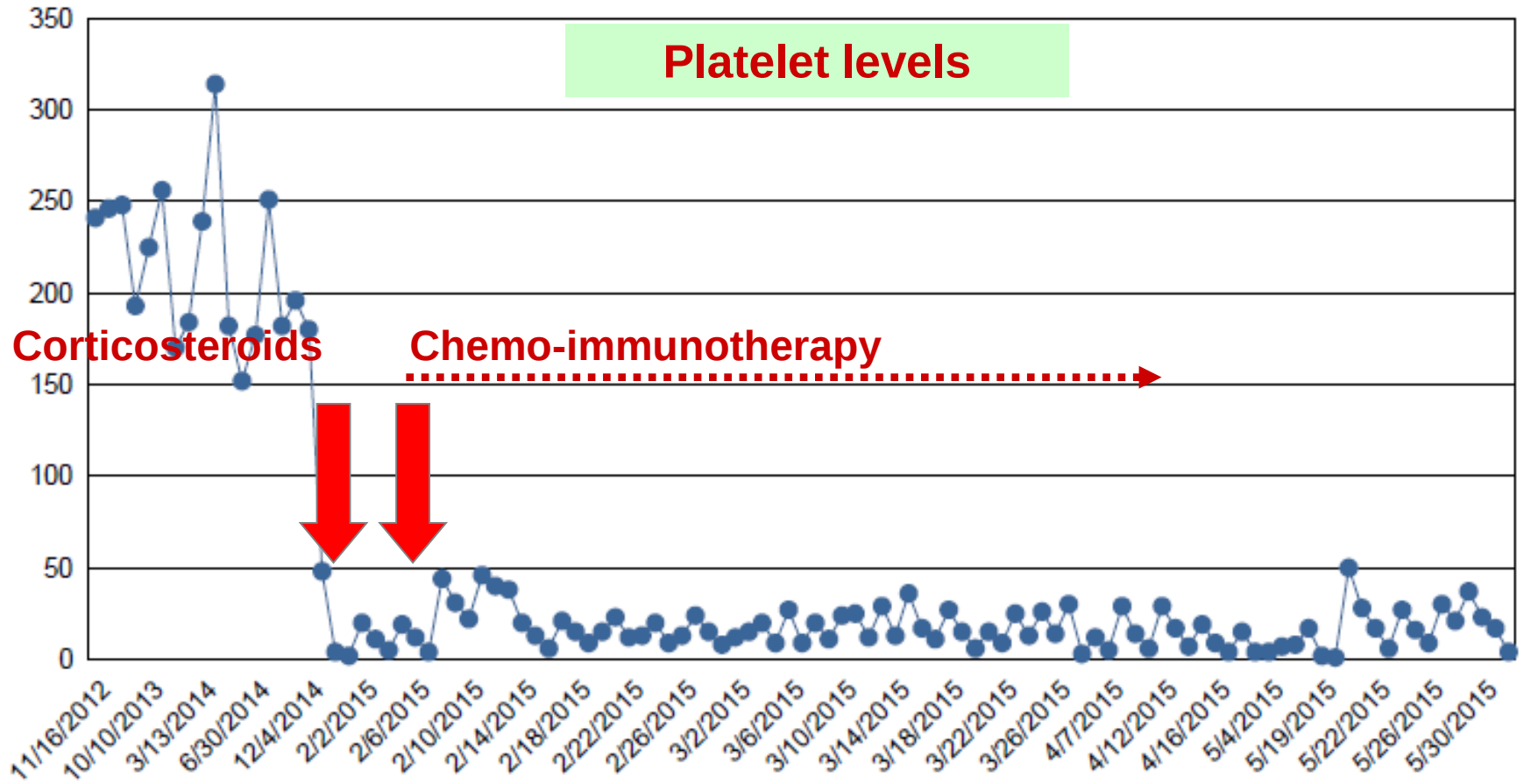
No response

- Bone marrow aspiration: massive infiltration by CLL cells.
- 6 February 2015: chemo-immunotherapy with Bendamustine (70mg/m² days 1 and 2) and Rituximab (375mg/m²).

Therapy and treatment results



Therapy and treatment results

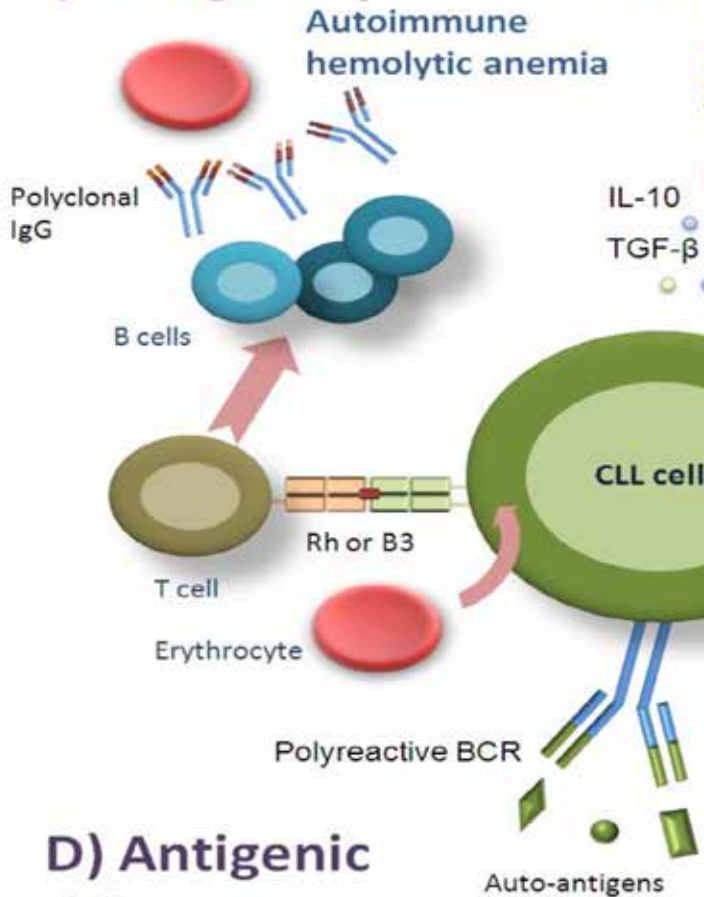


Therapy and treatment results

- After two cycles of chemo-immunotherapy with bendamustine and rituximab
 - Quick disappearance of lymphadenopathy (+2 months after therapy)
 - Improvement of anemia and persistent thrombocytopenia.
 - Corrected reticulocyte count: 1.3%
- Eltrombopag treatment planned

Pathogenesis of autoimmune phenomena

A) Antigenic presentation

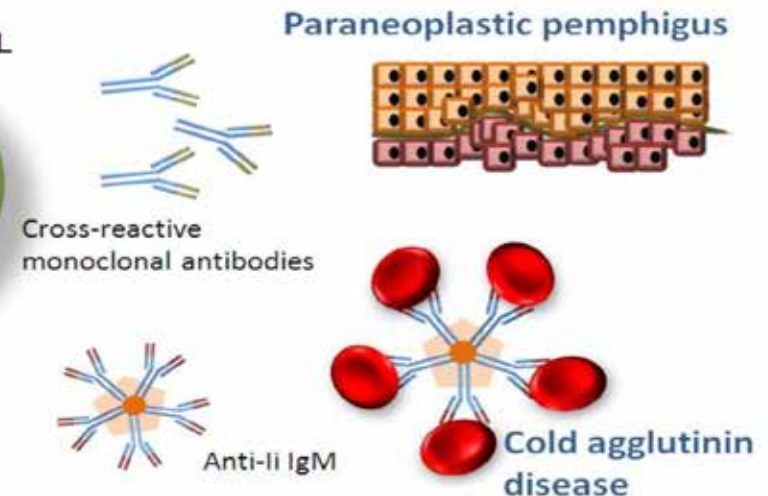


D) Antigenic drive

Loss of tolerance

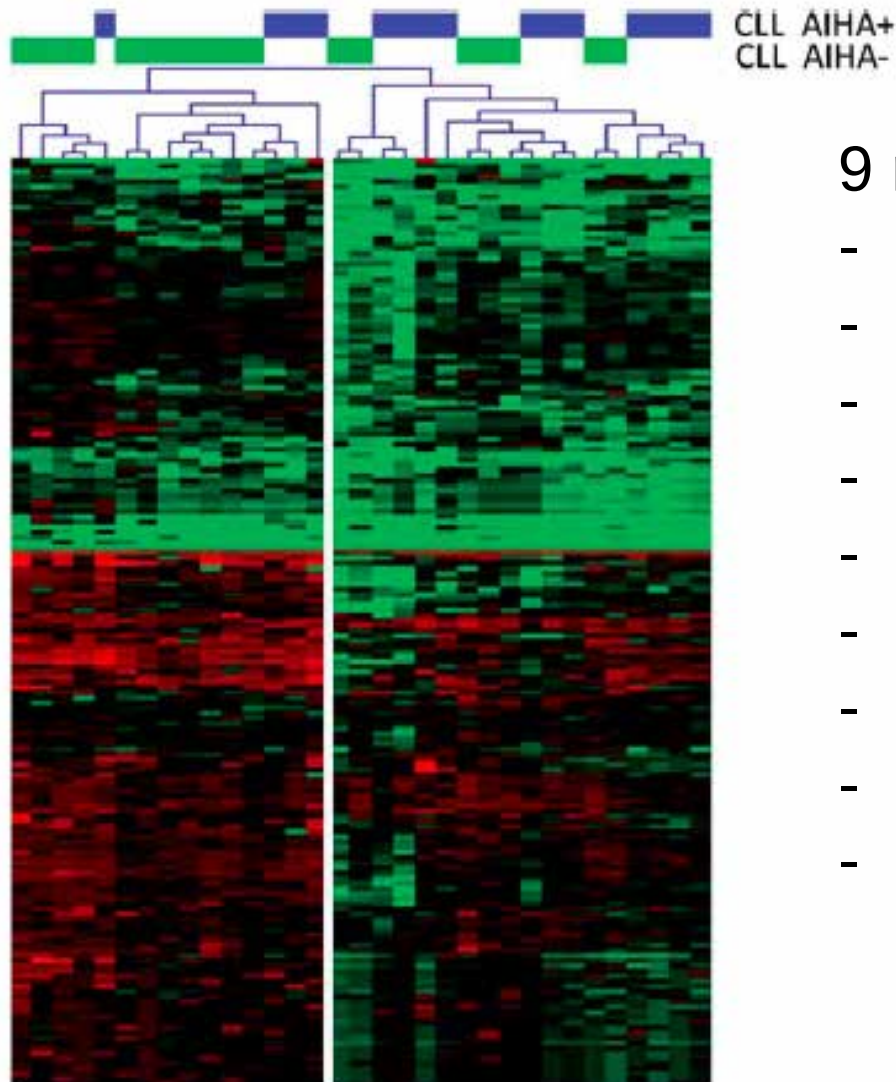


B) Cytokine secretion and cell-cell contact



C) Autoantibody secretion

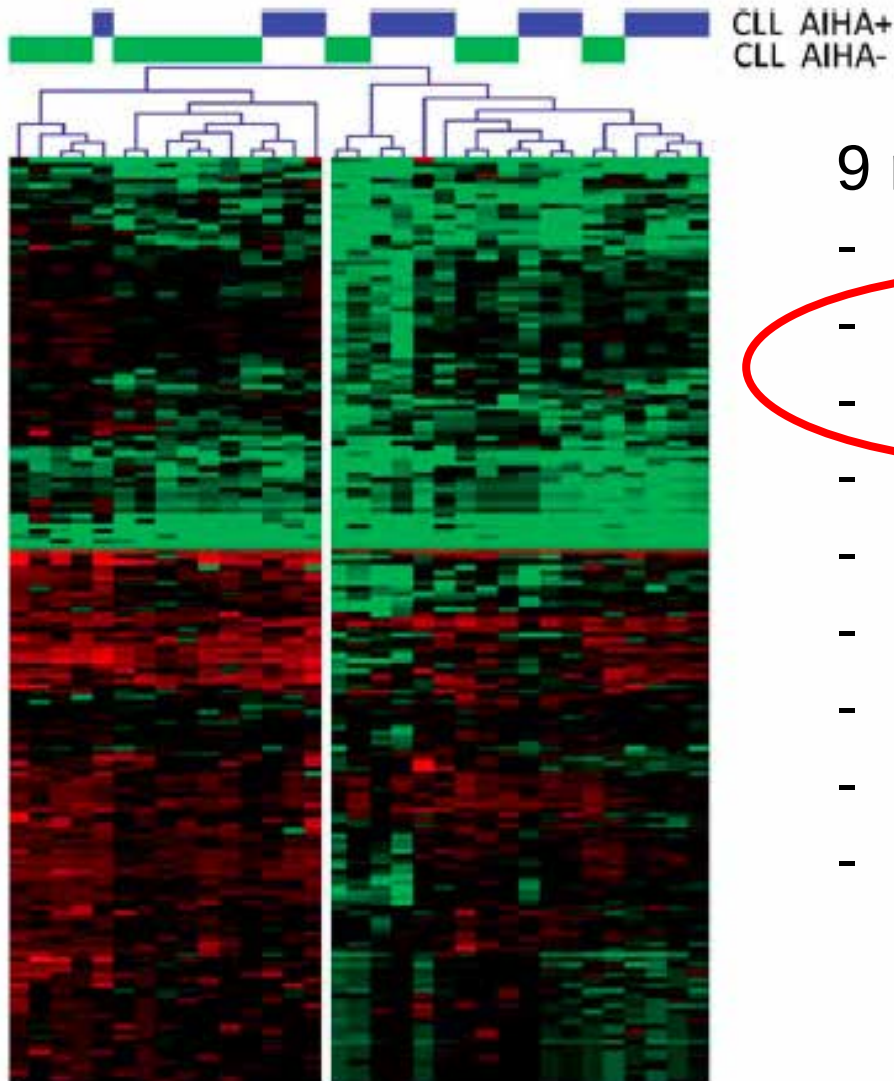
MicroRNA signature in autoimmune hemolytic anemia (AIHA) and CLL



9 miRNAs downregulated

- mir-19a
- mir-20a
- mir-146b-5p
- mir-29c
- mir-186
- mir-223
- mir-324-3p
- mir-484
- mir-660

MicroRNA signature in AIHA and CLL



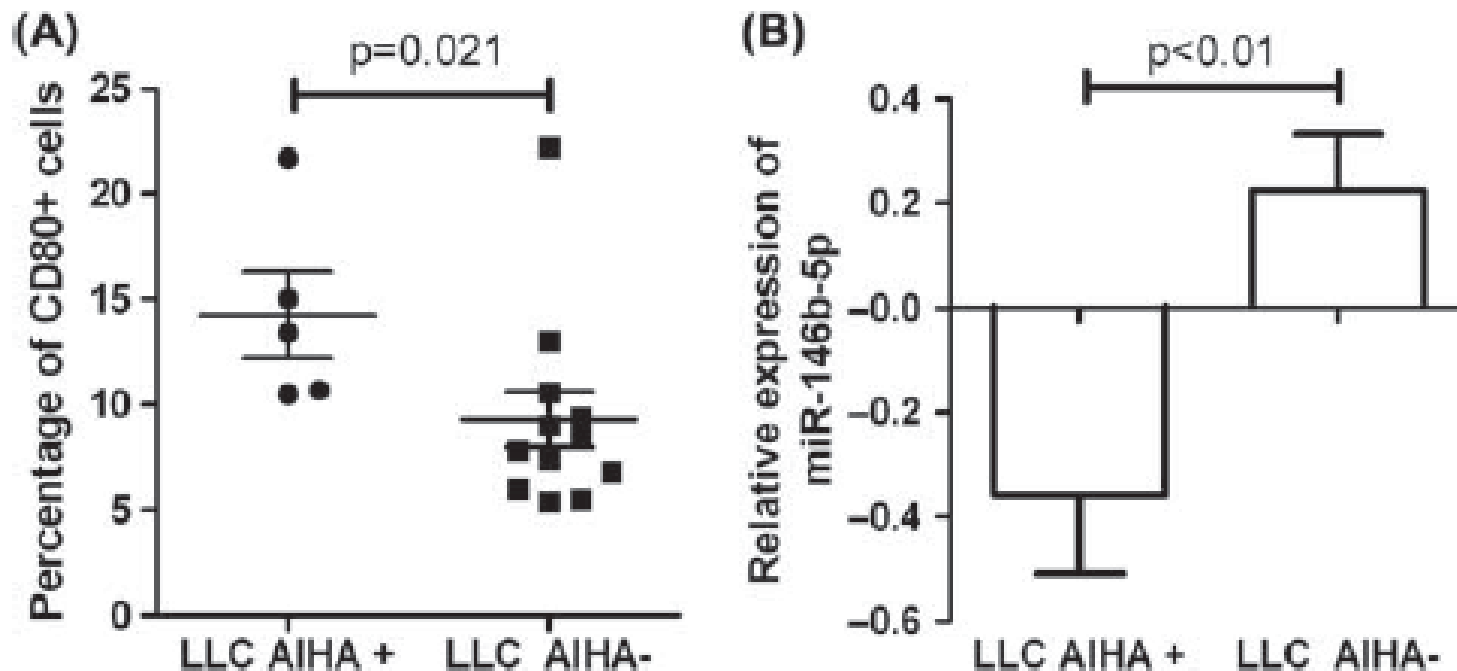
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- mir-484
- mir-660

Involved in autoimmune phenomena

MicroRNA signature in AIHA and CLL

mir-146b-5p modulates the expression of CD80 a molecule that regulates B-T cell synapse and is associated with APC capacity of CLL cells



Autoimmune phenomena in CLL

- **Common**

- AIHA (4.5% to 11%)
- Immune thrombocytopenia (2%-5%)
- PRCA (< 1%)
- Immune neutropenia (?) (LGL)

- **Infrequent**

- Autoimmune disorders preceeding CLL
(e.g. pernicious anemia)
- Concomitant autoimmune disorders/CLL
(e.g. Cold agglutinin disease, paraneoplastic pemphigus, neuropathies)

Anemia/Thrombocytopenia: Immune or marrow failure?: Some clues

	Immune	Bone marrow f.
Prior history of IC	Yes	No
Ongoing or recent Rx	No	Yes
Onset	Abrupt	Gradual
Plt count/Hb level	Very low	Moderately low
Bone marrow	Not massively infiltrated Glicoforine ++ / Factor VIII	Packed
Indirect signs hemolysis	Yes; <u>but not always!</u>	No
Espherocytes/Large Plts.	Yes; not striking	No
Laboratory tests	AIHA : DAT(+) ITP : No reliable tests	DAT(-)
Dissociated Hb /Plt count	Possible	No
Response to corticosteroids	Yes	No

IC: immune cytopenia

Rx: treatment

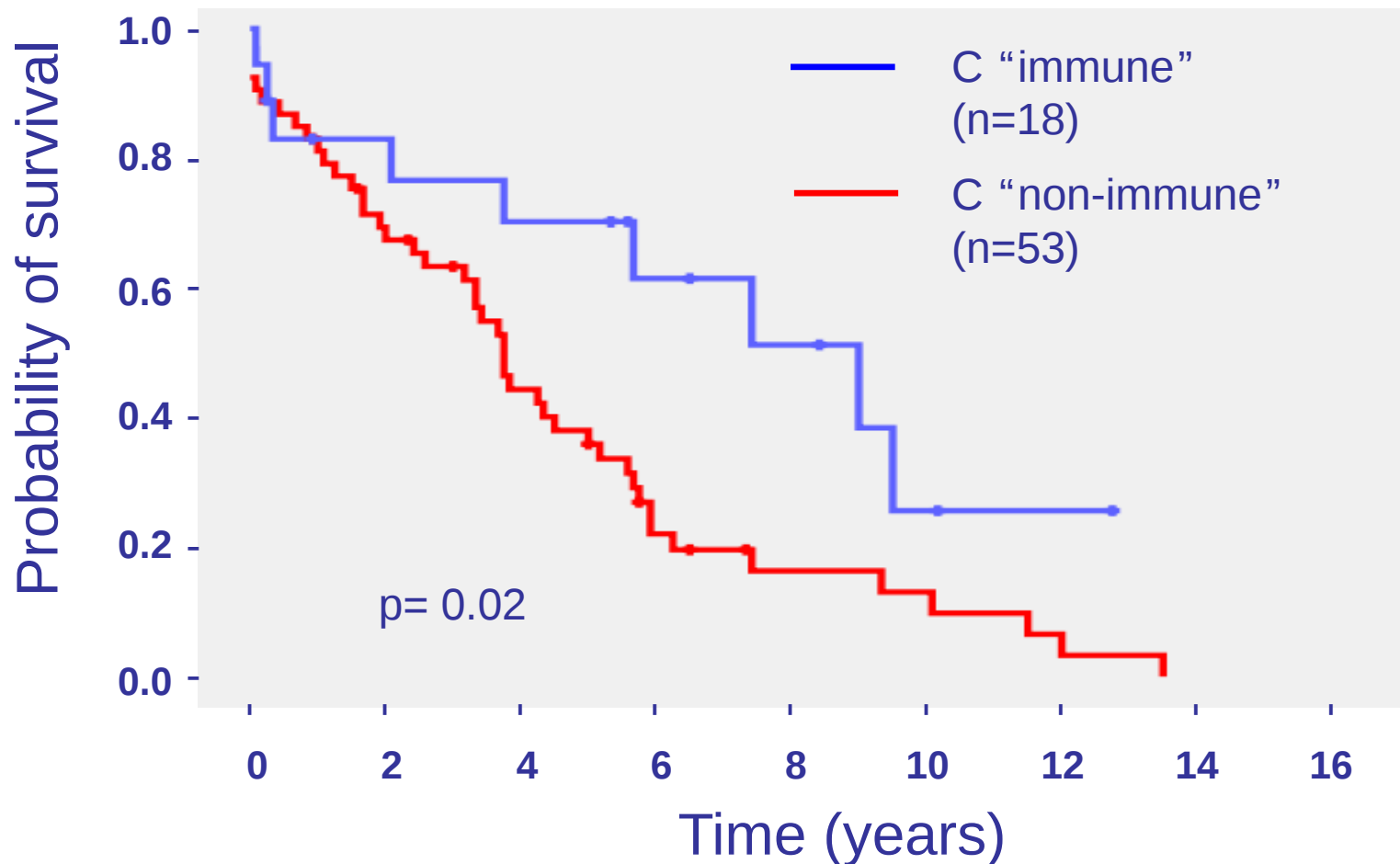
Prognostic significance of autoimmune cytopenia

	Autoimmune cytopenia	Clinical and biological associations	Impact on survival	C “immune” vs C “non-immune”
Mauro et al. Blood 2000	AHAI	Older age Male High white cell count	No	NA
Zent et al. BJH 2008	AHAI ITP	Advanced stage Male High ZAP-70 Unmutated <i>IGHV</i> genes Poor risk cytogenetics	No	Yes (better outcome)
Visco et al. Blood 2008	ITP	High white cell count High ZAP-70 Unmutated <i>IGHV</i> genes	Negative	NA
Visco et al. Haematologica 2010	AHAI	Unmutated <i>IGHV</i> genes	Negative	NA
Dearden et al. Blood 2010	AHAI	Older age Beta 2 microglobulin increased	Negative	NA
Moreno et al. Blood 2010	AHAI ITP	High white cell count Short doubling lymphocyte count High CD38 Beta 2 microglobulin increased	No	Yes (better outcome)
Visco et al. Leukemia and Lymphoma 2014	AHAI ITP	No associations	No	Yes (better outcome)

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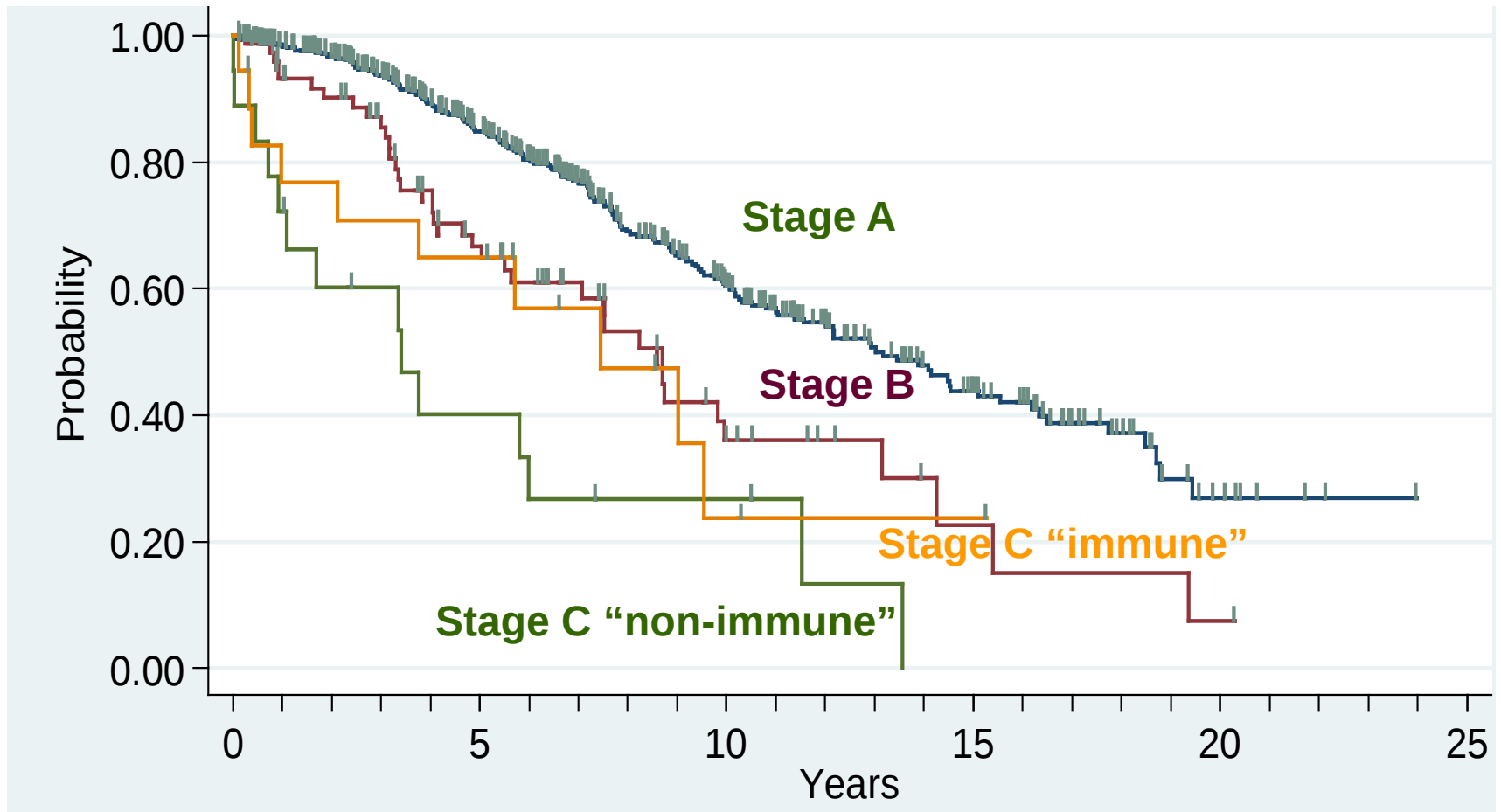
C “immune” vs. C “non-immune” (at diagnosis, no prior therapy)



Moreno et al. Blood 2010;

See also Zent et al. BJH 2008; Visco et al. Leukemia and Lymphoma 2014

Modified Binet staging system including stage C “immune” category



Moreno et al. Blood 2010

Dearden C Blood 2010 (Inside Blood)

AIHA/ITP in CLL: Treatment

Corticosteroids

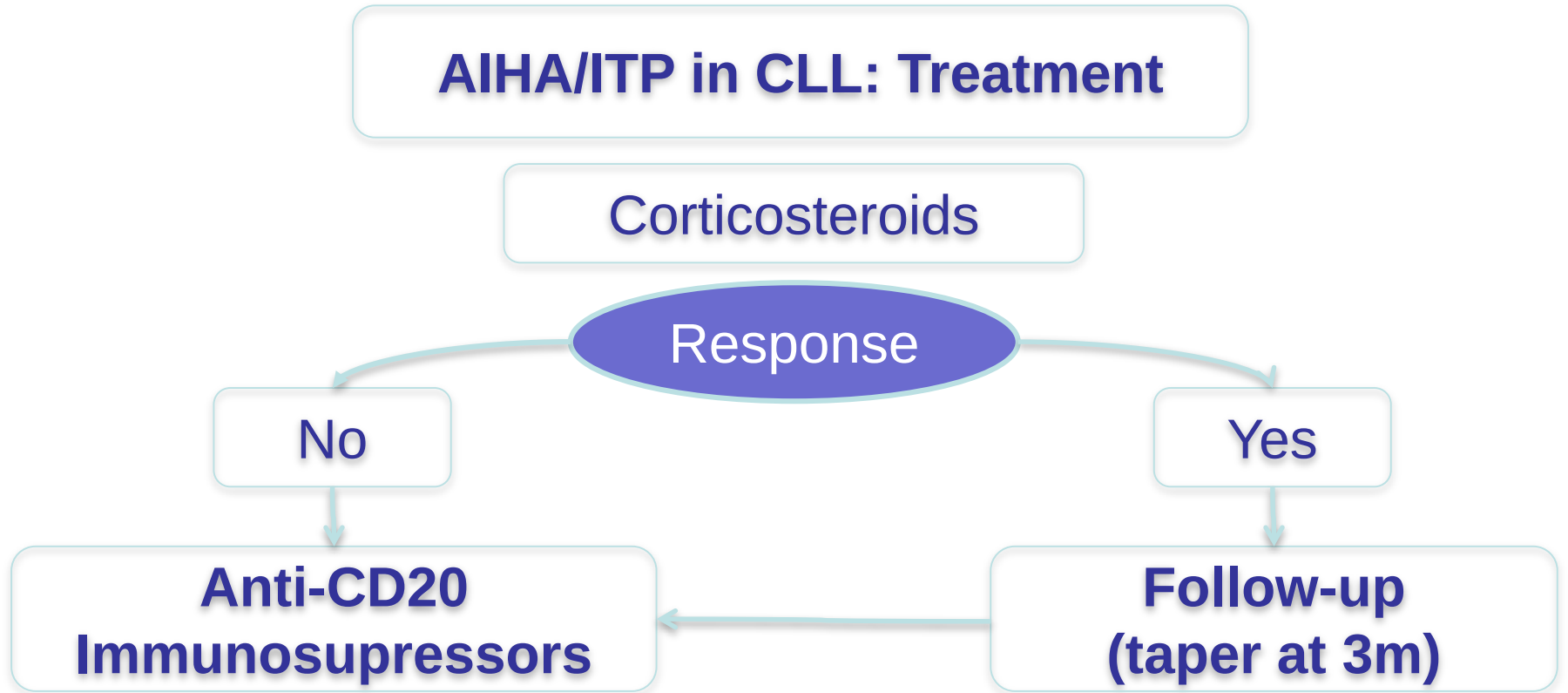
Response

No

Yes

**Anti-CD20
Immunosuppressors**

**Follow-up
(taper at 3m)**



AIHA/ITP in CLL: Treatment

Corticosteroids

Response

No

Yes

Anti-CD20
Immunosuppressors

Follow-up
(taper at 3m)

No response

R-based *light* therapy

Splenectomy*

Thrombopoietin
analogues

- Eltrombopag
- Romiplostin

* Role of splenectomy increasingly controversial

AIHA/ITP in CLL: Treatment

Corticosteroids

Response

No

Yes

Anti-CD20
Immunosuppressors

Follow-up
(taper at 3m)

No response

R-based *light* therapy

Splenectomy

Thrombopoietin
analogues

No response

bopag

- Romiplostin

CLL Treatment

Treatment of autoimmune cytopenia

- Combination therapies:
 - Corticosteroids + rituximab may increase reponse rate and its duration.
 - Rituximab, cyclophosphamide, dexamethasone (RCD): high *overall* response rate.
 - Bendamustine +Rituximab is effective in AIHA
 - Rituximab, dexamethasone and cyclosporine: high response in ITP
 - BCRi (i.e Ibrutinib)- case report showing controversial results, including effectiveness in AIHA
- Other:
 - Intravenous immunoglobulin (bleeding!).
 - Platelets/Red-blood cells tranfusion.

Modern therapy minimizes the risk of developing AIHA

Treatment regimen	AIHA prevalence	Remarks
Chlorambucil	5-12%	Unselected patients
Chlorambucil	2-12%	Selected patients (trials) Previously untreated
Fludarabine	11-23%	Unselected Advanced and heavily pretreated
Fludarabine	8-11%	Selected patients (trials) Previously untreated
Fludarabine plus Cyclophosphamide	1-5%	Selected patients (trials) Previously untreated
FCR	<1-5.8%	Selected patients (trials) Previously untreated

Di Raimondo et al. Leukemia and Lymphoma 1993; Myint et al. BJH 1995; Catovsky et al. Blood 2004; Leporrier et al. Blood 2001; Moreno et al. Blood 2010; Mauro et al. Blood 2000; Eichhorst et al. Blood 2006; Dearden et al. Blood 2008; Eichhorst et al. Blood 2009; Borthakur et al. BJH 2007; Hallek et al. Lancet 2010

Modern therapy minimizes the risk of developing AIHA

Treatment regimen	AIHA prevalence	Remarks
FCR+ibrutinib	NA	Selected patients (trial)
Ibrutinib vs Ofatumumab	0 vs 2%	Selected patients (trial)
Ibrutinib	1.5%	Selected patients (trial)
Ofatumumab	2-10%	Selected patients (observational study) Heavily pretreated
GA-101+Chlorambucil	NA	Selected patients (trial)

Brown J et al. Blood 2015; Montillo et al. Blood 2014 (ASH abstract); Rogers et al. Blood 2014 (ASH abstract); Moreno C et al. Haematologica 2015; Goede et al. N Engl J Med 2014

Take-home messages

- Autoimmune cytopenias (AC) frequently complicate CLL.
- The risk of AC is much lower with current than with older therapies.
- The possibility of cytopenia of immune origin needs to be taken into account in cases *advanced* disease (Rai III, IV; Binet C) without high tumor burden.
- The prognostic significance of autoimmune cytopenia may vary depending on the cohort investigated (i.e., pre-treated, whole series, advanced disease).
- Autoimmune cytopenia not responding to conventional therapy is an indication for CLL therapy.

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1st **ERIC** WORKSHOP ON ***TP53*** ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

Technical approaches and data interpretation, troubleshooting, predictive and therapeutic implications

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