Citopenie: le forme acquisite

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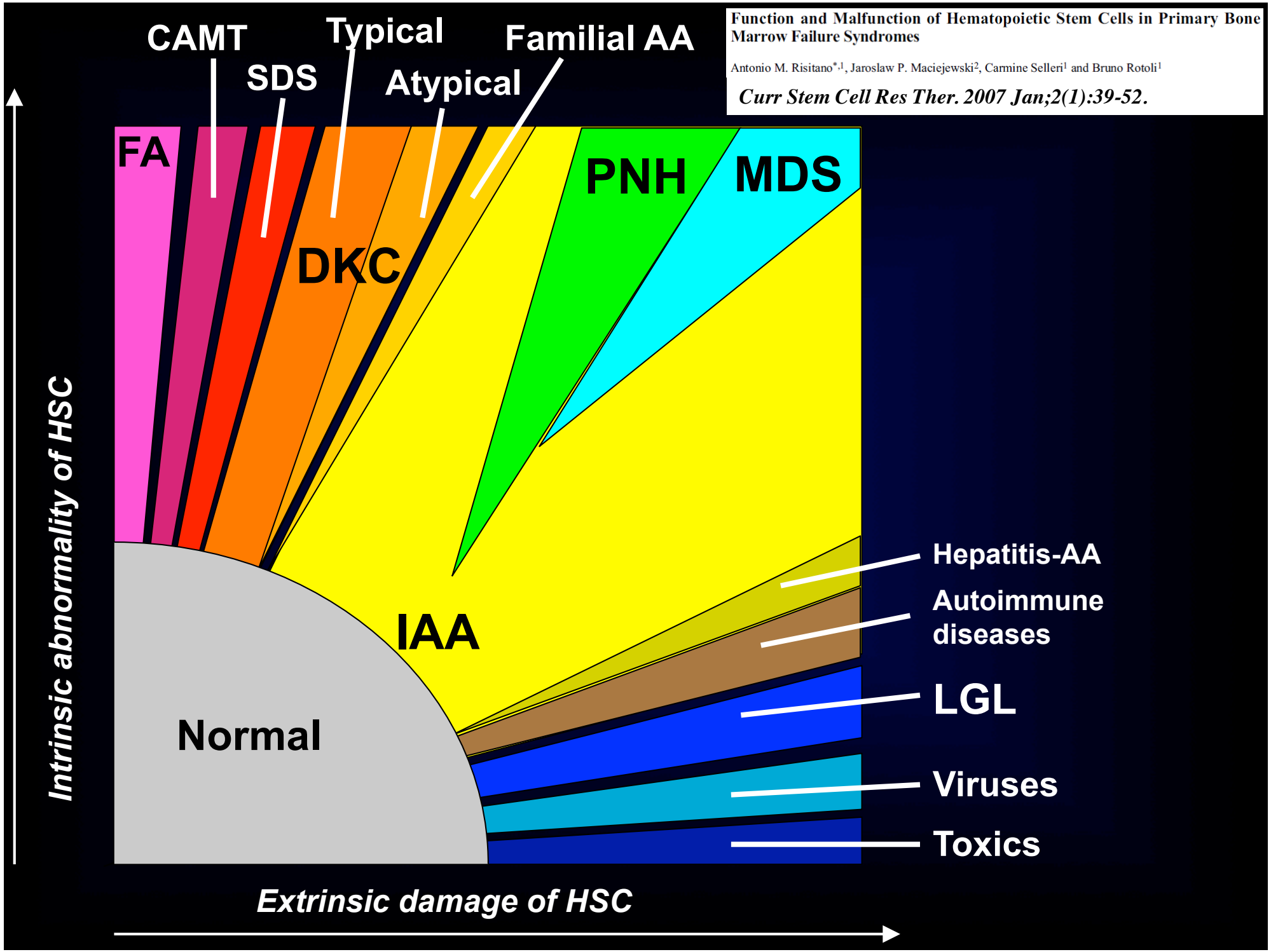
Federico II University of Naples

Azienda Ospedaliera Universitaria Federico II

Integrata con il Servizio Sanitario Regionale

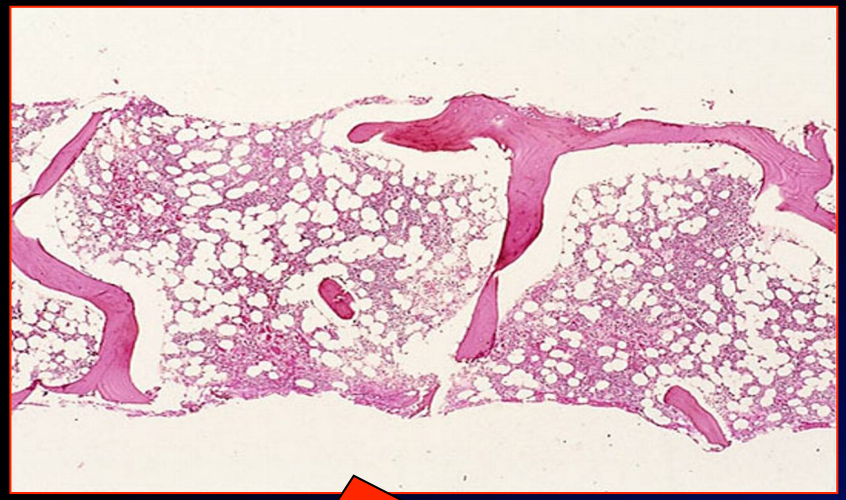


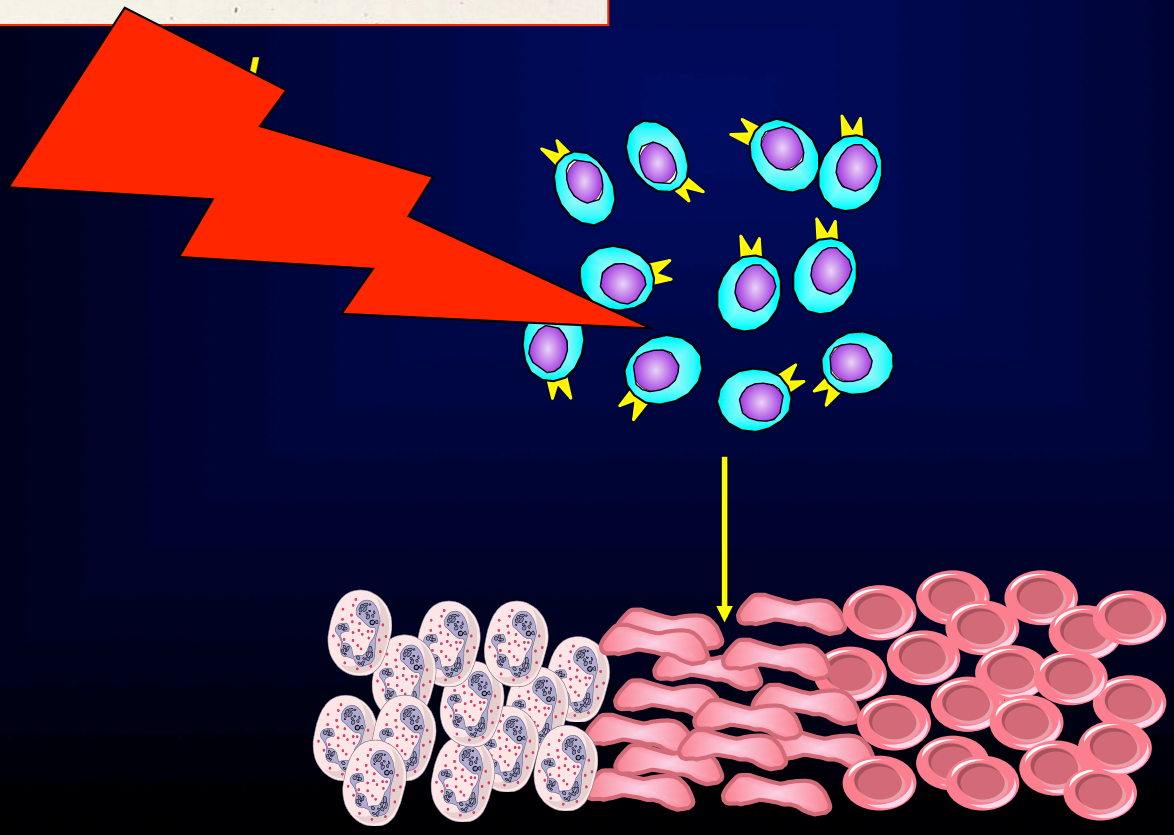
Function and Malfunction of Hematopoietic Stem Cells in Primary Bone Marrow Failure Syndromes

Antonio M. Risitano*,1, Jaroslaw P. Maciejewski2, Carmine Selleri1 and Bruno Rotoli1

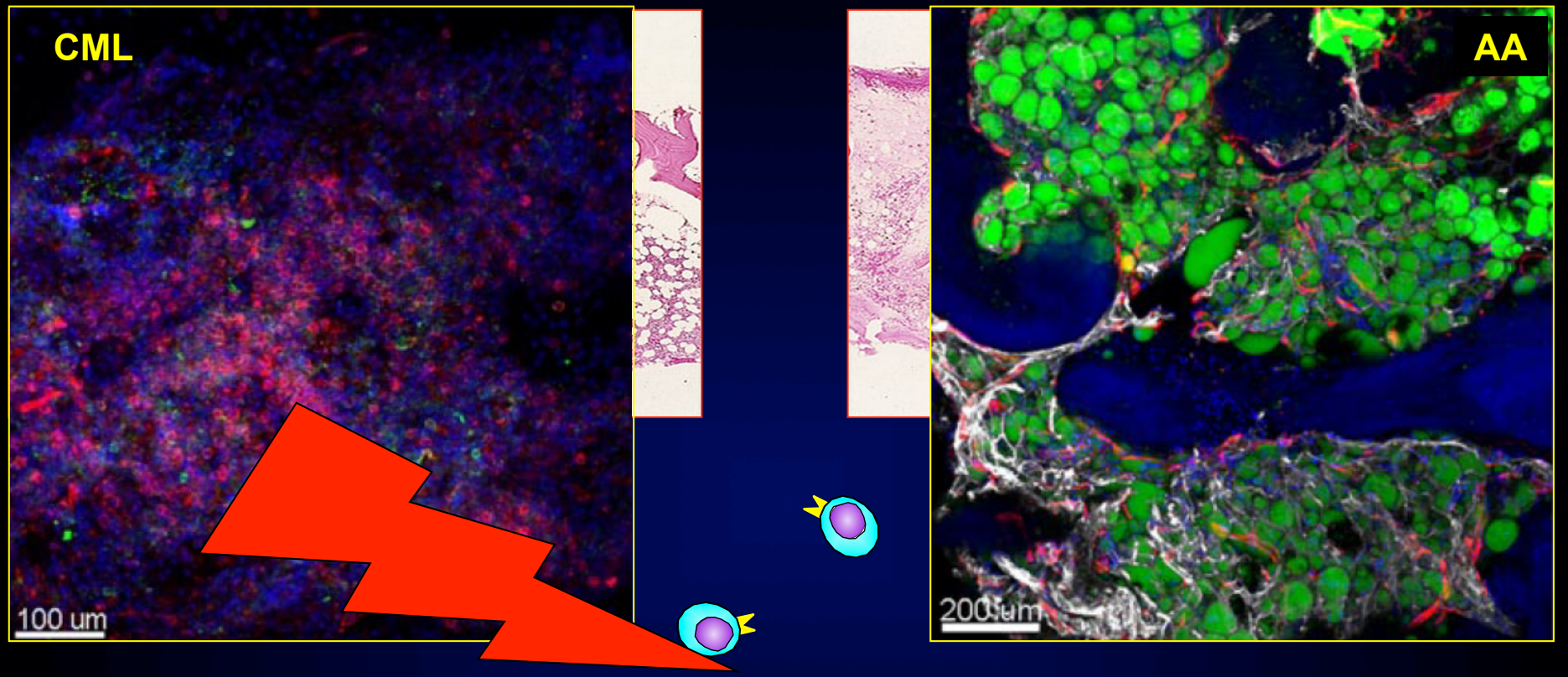
Curr Stem Cell Res Ther. 2007 Jan;2(1):39-52.

Aplastic anemia





Aplastic anemia



Takaku et al, Blood 2010

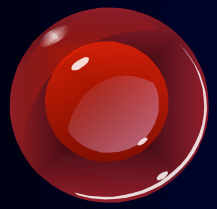
Takaku et al, Blood 2010

Contraction of stem cell pool

Cytopenia

Pathophysiology of aplastic anemia

Hematopoietic stem cell



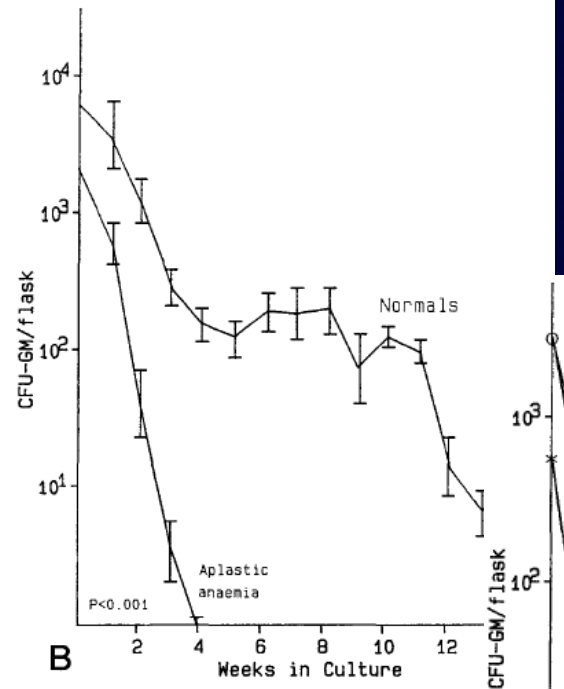
Hematopoietic stem cells in AA

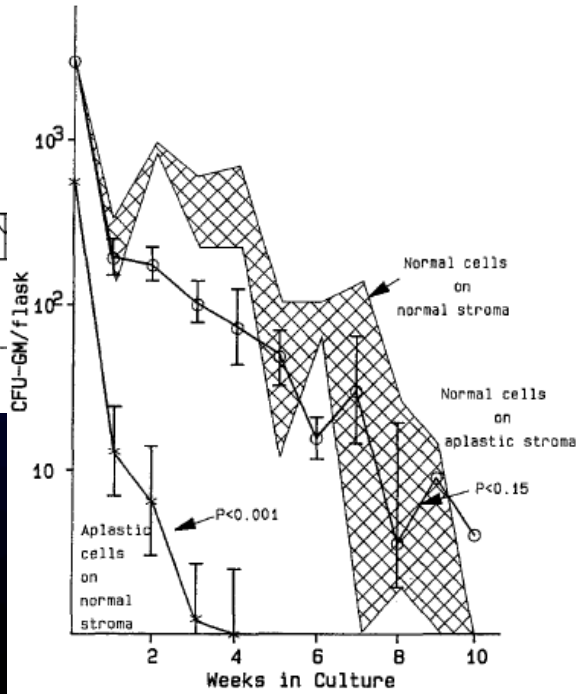
Hematopoietic progenitor cultures

blood 1990 76: 1748-1757

The hematopoietic defect in aplastic anemia assessed by long-t marrow culture

JC Marsh, J Chang, NG Testa, JM Hows and TM Dexter

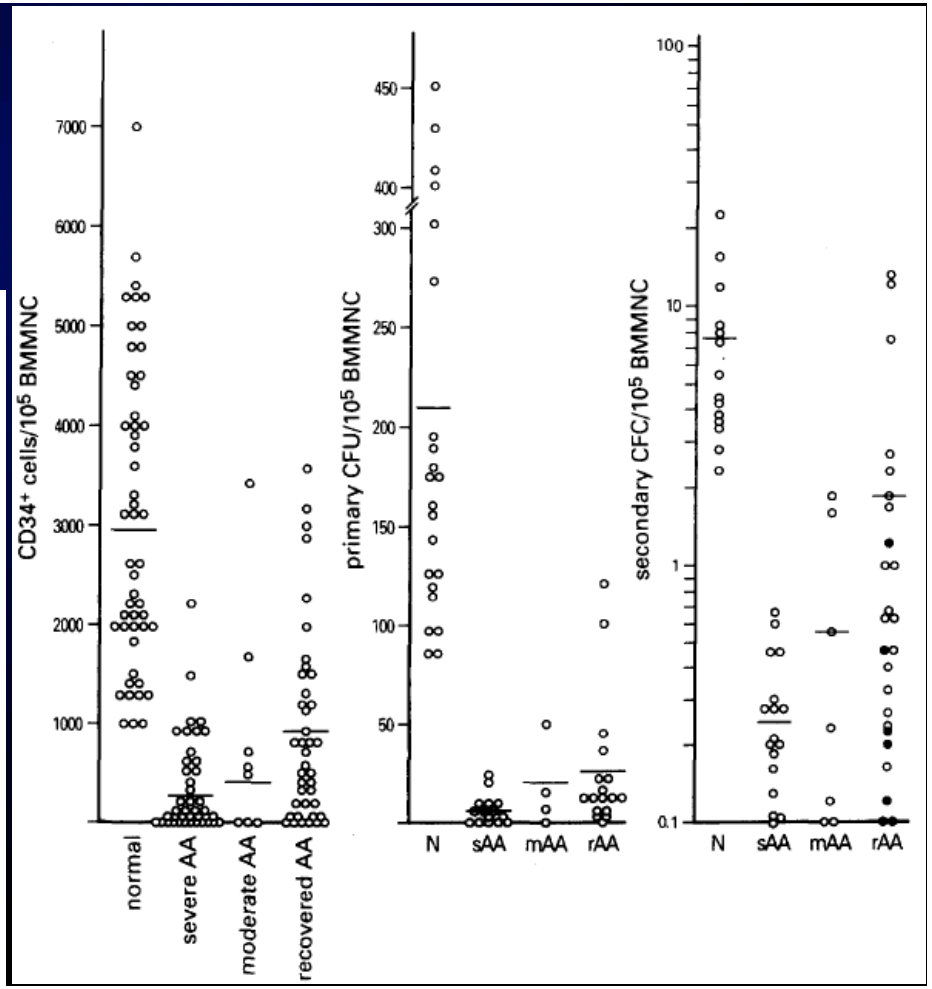




blood 1996 88: 1983-1991

A severe and consistent deficit in marrow and circulating primitive hematopoietic cells (long-term culture-initiating cells) in acquired aplastic anemia

JP Maciejewski, C Selleri, T Sato, S Anderson and NS Young



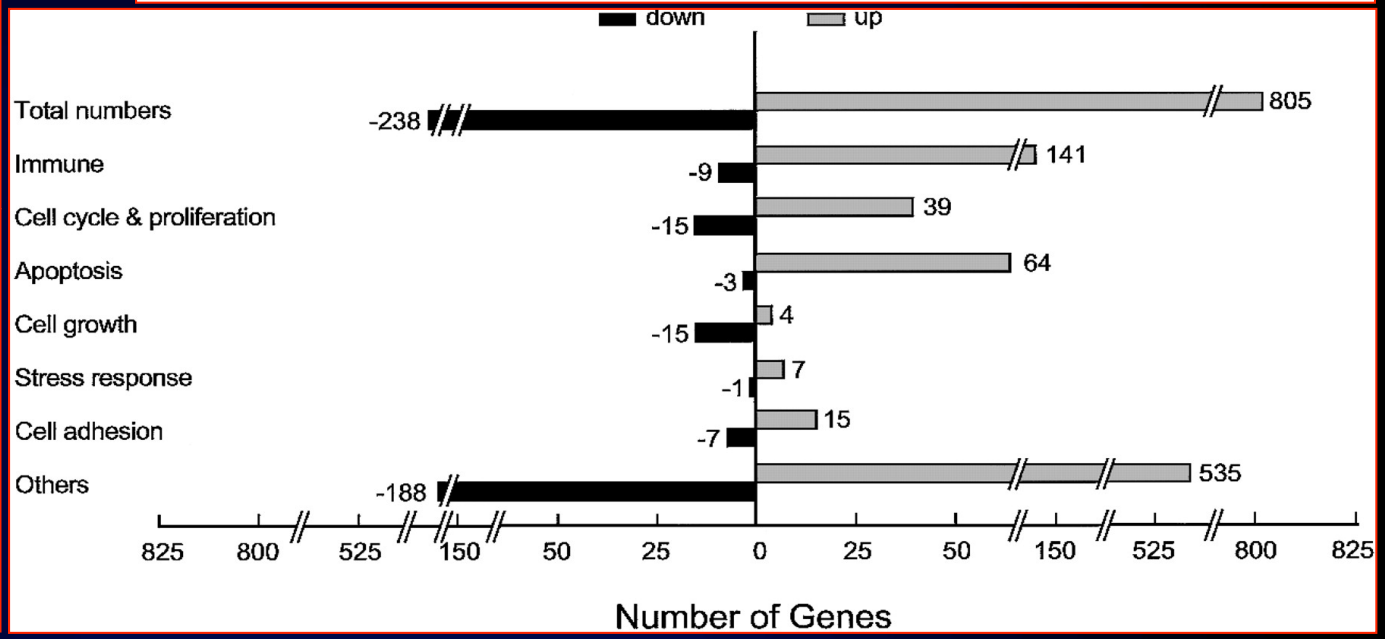
GENE EXPRESSION PROFILING IN CD34+ FROM AA PATIENTS

RED CELLS

BLOOD, 1 JANUARY 2004 • VOLUME 103, NUMBER 1

Gene expression profiling in CD34 cells to identify differences between aplastic anemia patients and healthy volunteers

Weihua Zeng, Guibin Chen, Sachiko Kajigaya, Olga Nunez, Alexandra Charrow, Eric M. Billings, and Neal S. Young



Over-expressed

- Apoptosis
- Stress response
- Cytokine/chemokine transduction
- Defense/immune response genes
- Cell cycle/proliferation inhibitors

Down-expressed

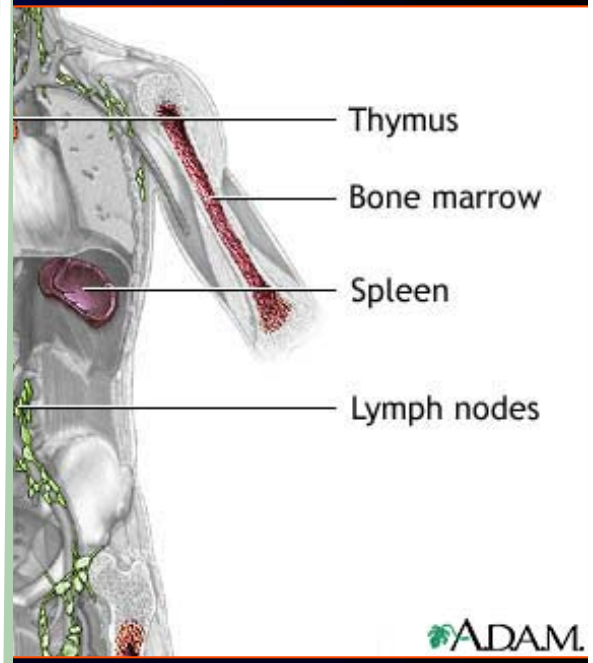
- Cell cycle/proliferation promoters

"…the transcriptome analysis of HSC in AA is consistent with the presence of stressed, immunologically activated or dying target cells rather than of an intrinsically abnormal population."

Pathophysiology of aplastic anemia

Hematopoietic stem cell

The immune system



Proc. Natl. Acad. Sci. USA
Vol. 73, No. 8, pp. 2890–2894, August 1976
Medical Sciences

Aplastic anemia: Presence in human bone marrow of cells that suppress myelopoiesis*

(thymus-derived lymphocytes/suppressor cells/differentiation)

WALT A. KAGAN, JOÃO A. ASCENSÃO, RAJENDRA N. PAHWA, JOHN A. HANSEN, GIDEON GOLDSTEIN, ELISA B. VALERA, GENEVIEVE S. INCEFY, MALCOLM A. S. MOORE, AND ROBERT A. GOOD

CIRCULATING ACTIVATED SUPPRESSOR T LYMPHOCYTES IN APLASTIC ANEMIA

N.C. Zoumbos, P. Gascon, J.Y. Djeu, S.R. Trost, and N.S. Young

Volume 312 January 31, 1985 Number 5

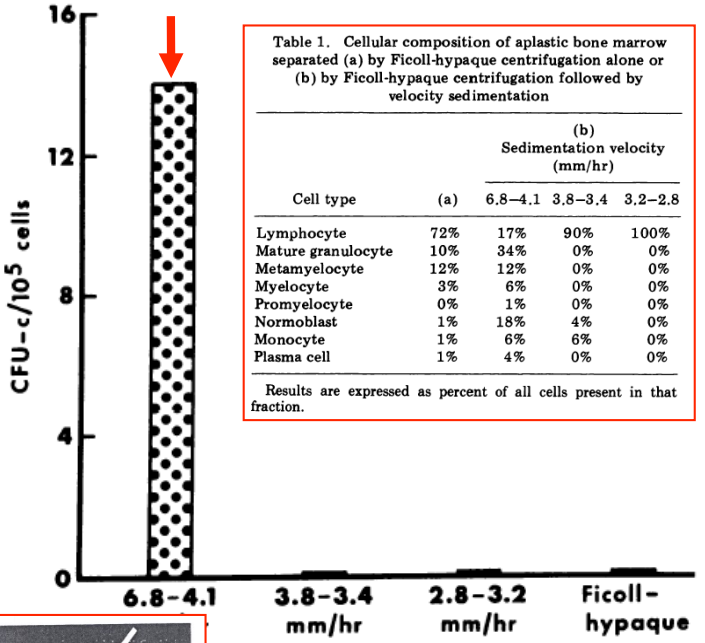
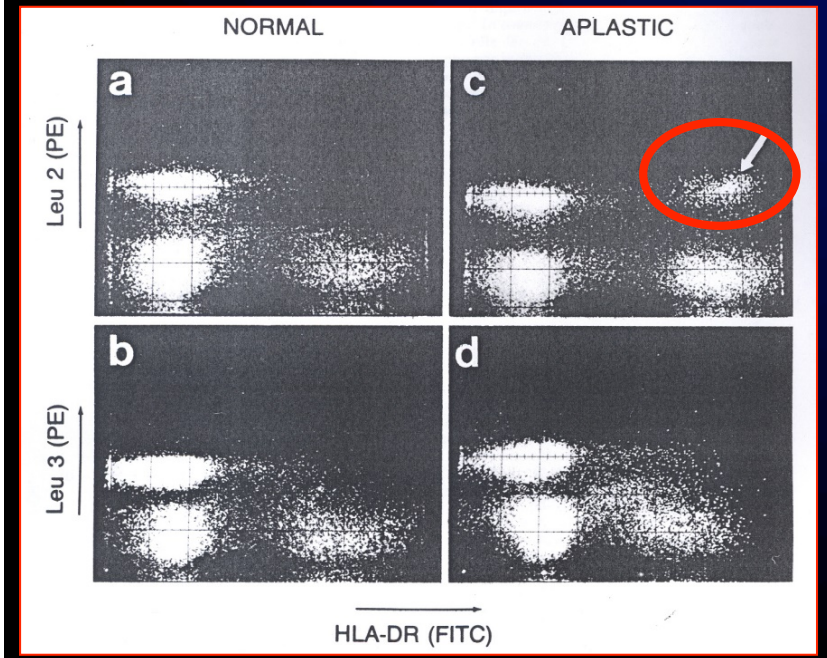


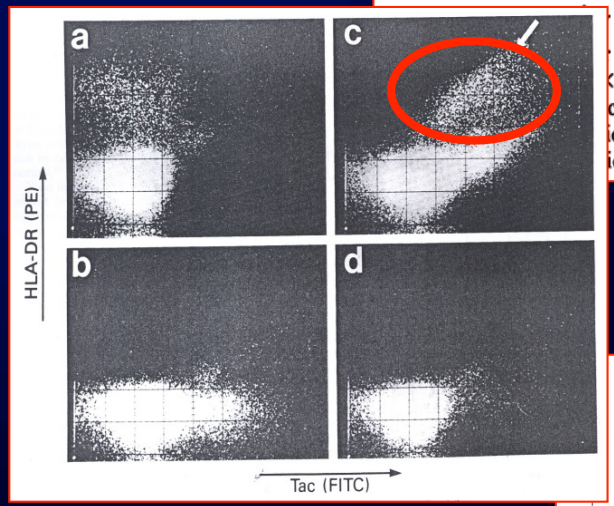
Table 1. Cellular composition of aplastic bone marrow separated (a) by Ficoll-hypaque centrifugation alone or (b) by Ficoll-hypaque centrifugation followed by velocity sedimentation

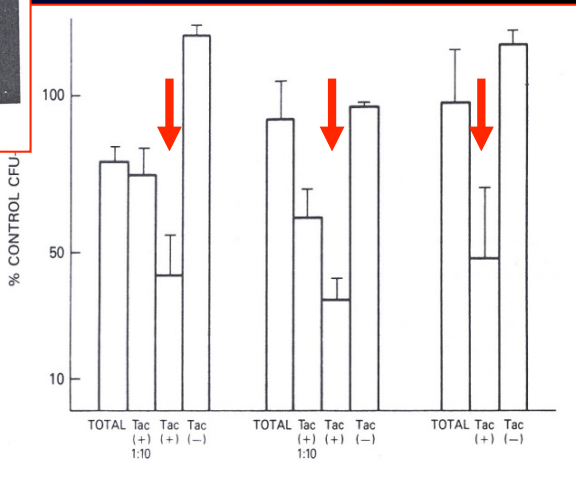
Cell type	(a)	(b) Sedimentation velocity (mm/hr) 6.8–4.1	3.8–3.4	3.2–2.8
Lymphocyte	72%	17%	90%	100%
Mature granulocyte	10%	34%	0%	0%
Metamyelocyte	12%	12%	0%	0%
Myelocyte	3%	6%	0%	0%
Promyelocyte	0%	1%	0%	0%
Normoblast	1%	18%	4%	0%
Monocyte	1%	6%	6%	0%
Plasma cell	1%	4%	0%	0%

Results are expressed as percent of all cells present in that fraction.

...of granulocyte-monocyte colonies (CFU-c) after ...10^5 cells per ml in soft agar of marrow from a ...c anemia. Cells were separated by either Ficoll-...ion alone or by Ficoll-hypaque centrifugation and ...ion.







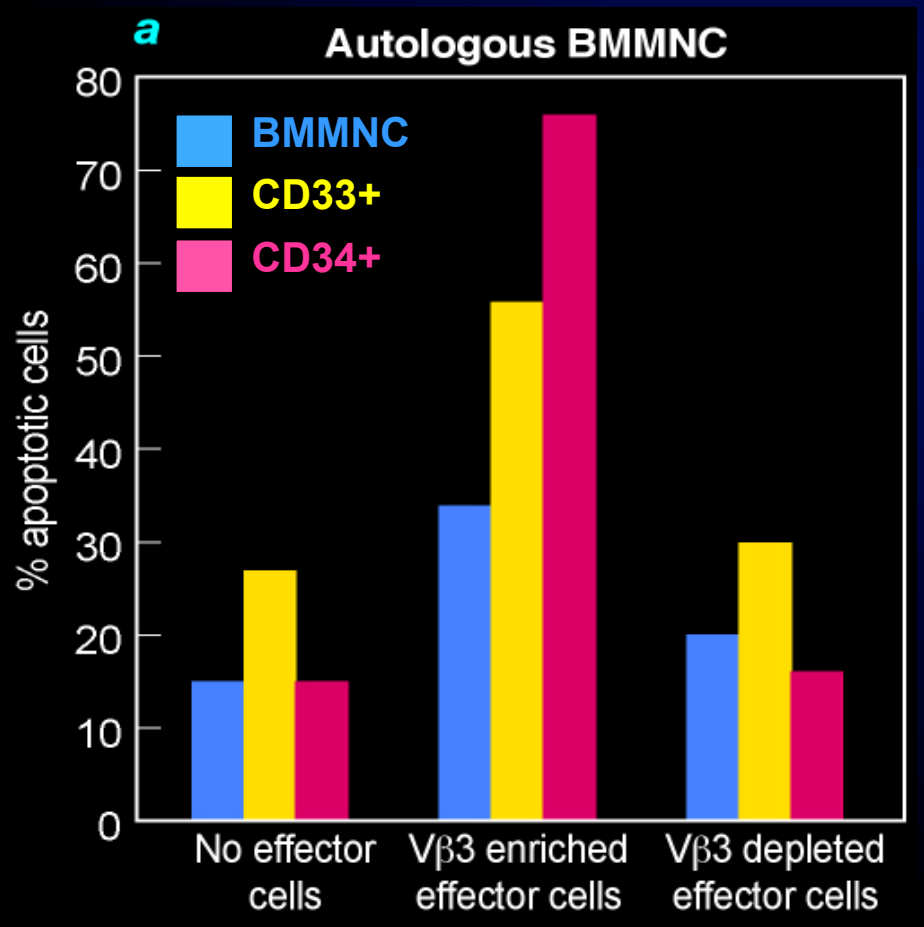
Molecular Tracking of Pathogenic Clonotypic T-cells

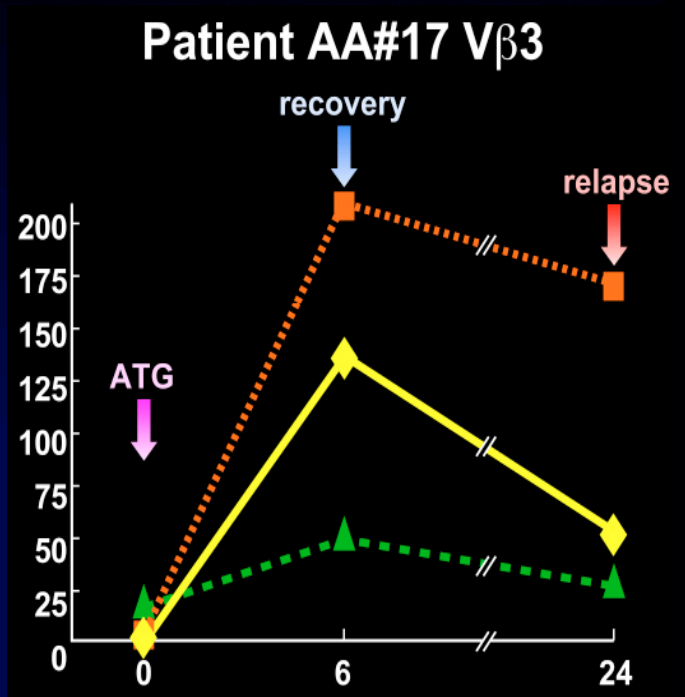
Lancet 2004; 364: 355–64

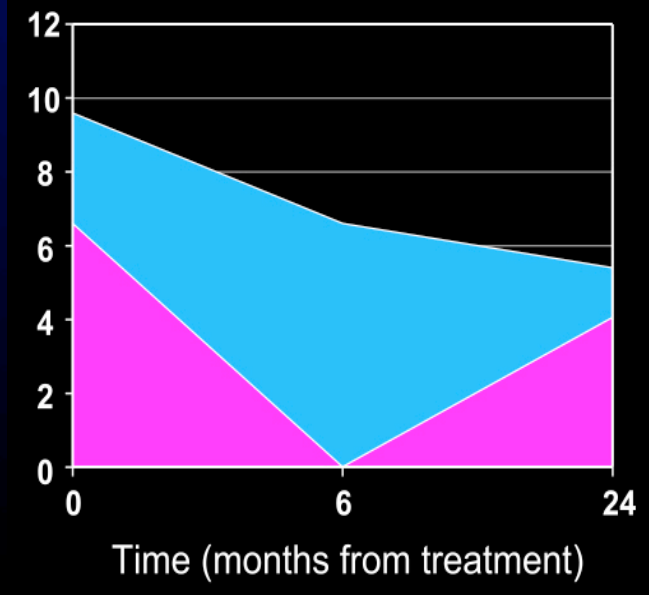
Mechanisms of Disease

In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR β-CDR3 sequencing

Antonio M Risitano, Jaroslaw P Maciejewski, Spencer Green, Magdalena Plasilova, Weihua Zeng, Neal S Young

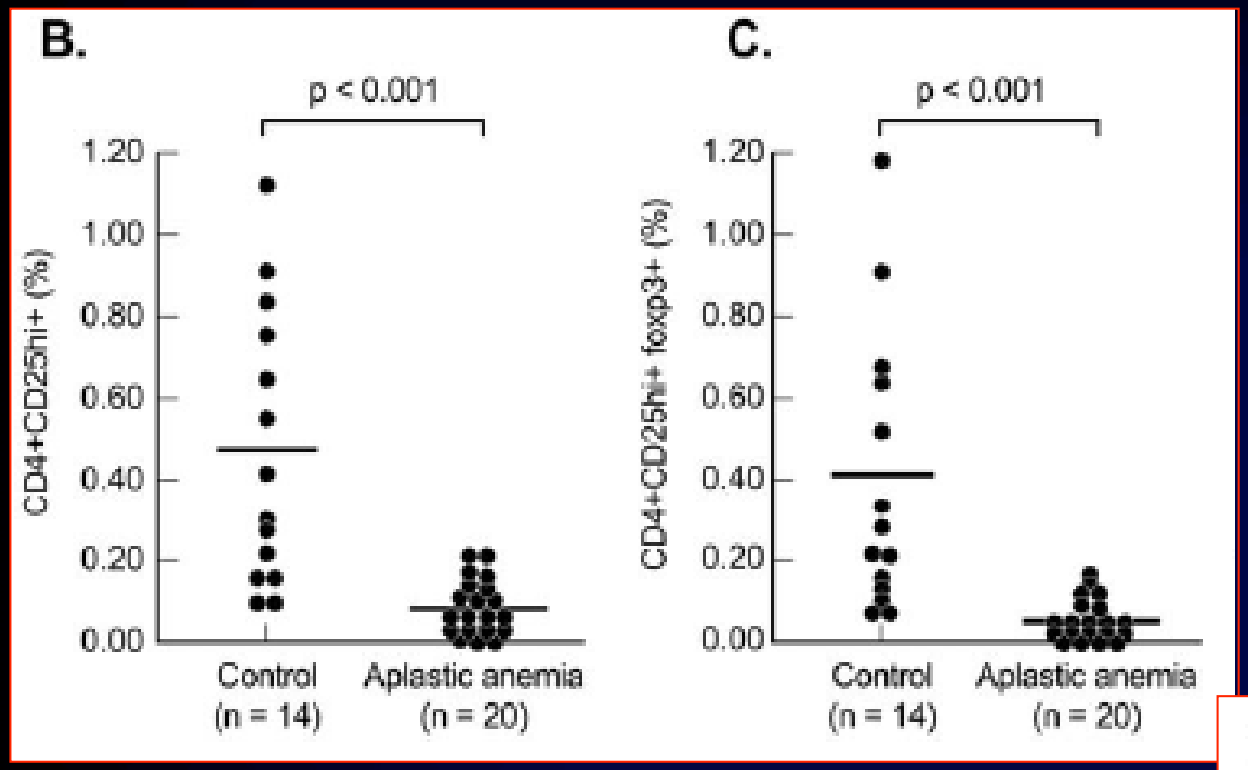


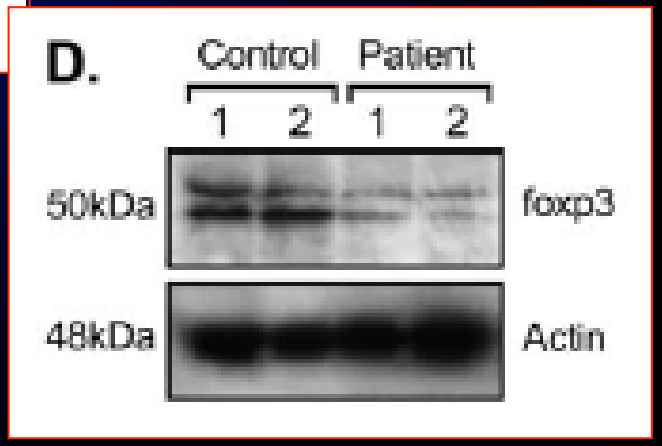




T-REGULATORY CELLS IN APLASTIC ANEMIA

Solomou et al., Blood 2007

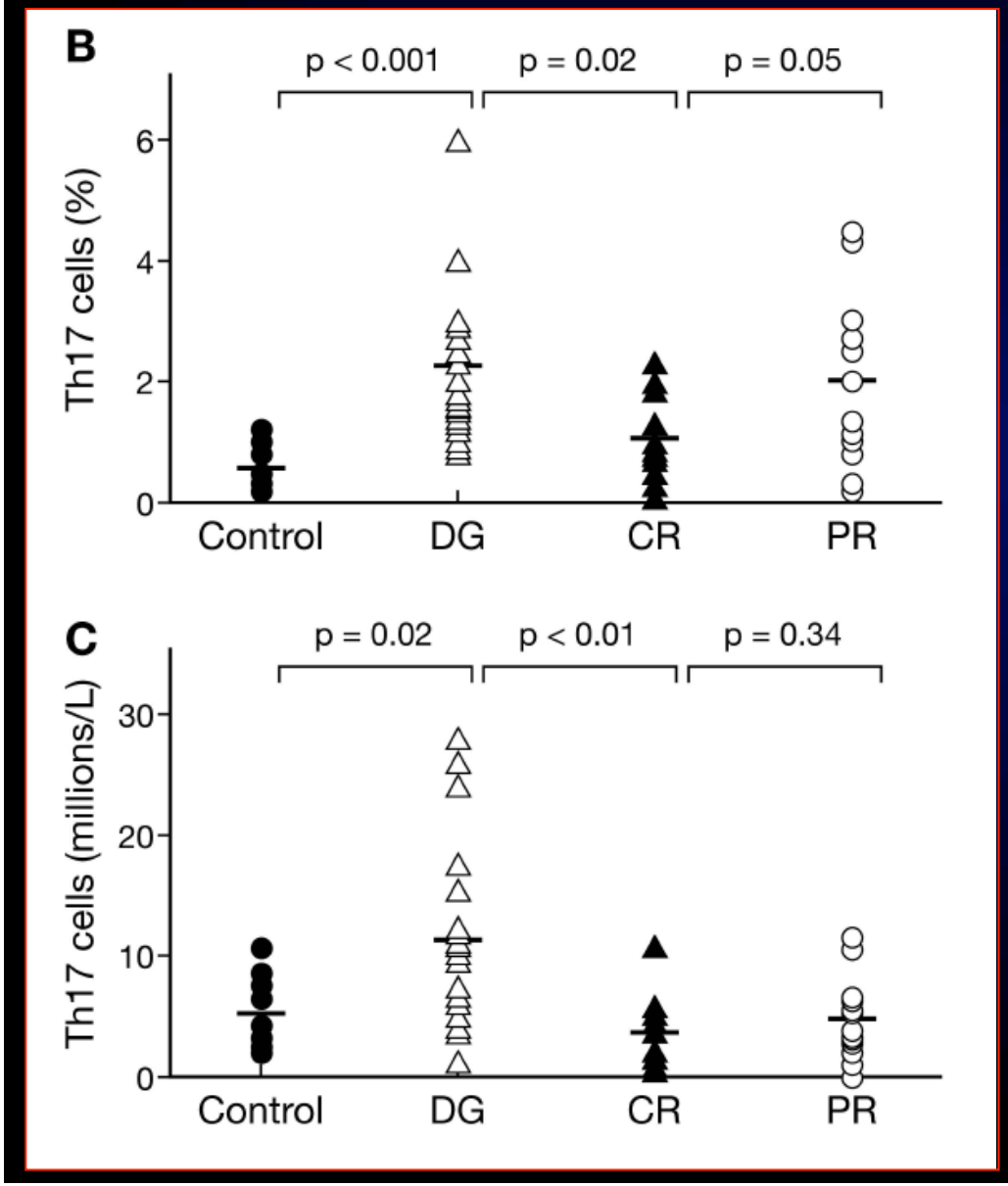


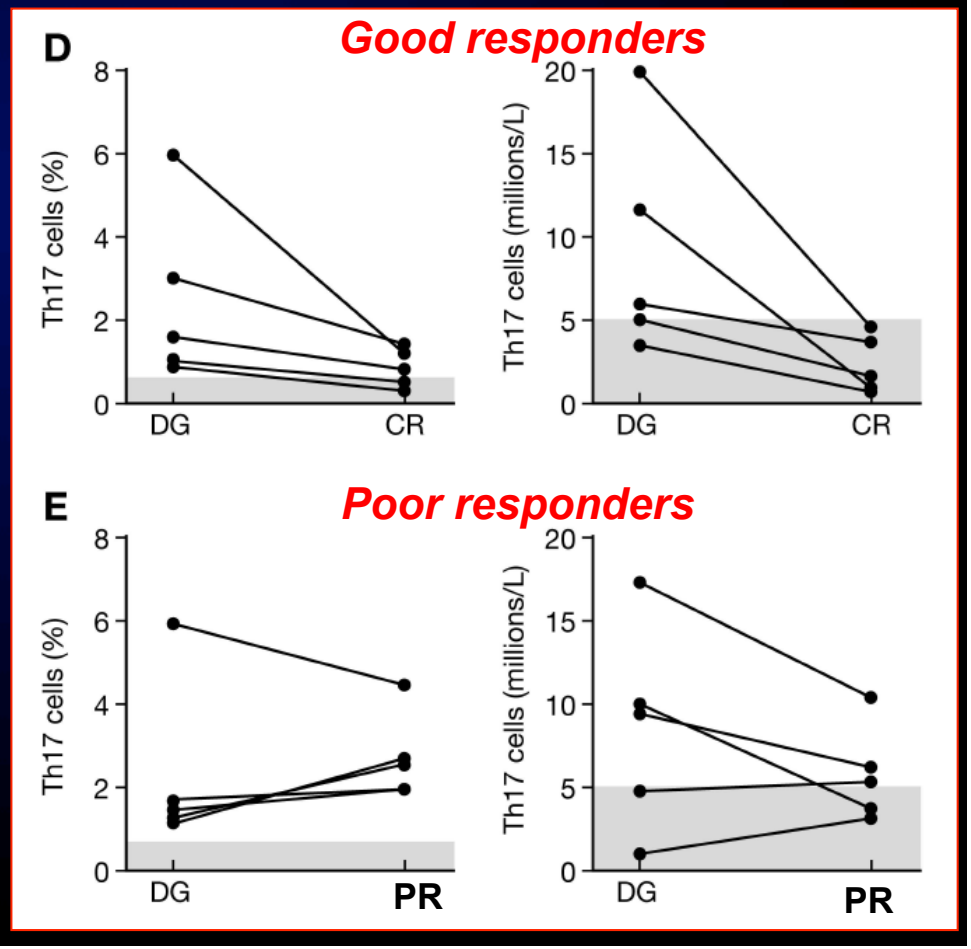


Th17 CELLS IN APLASTIC ANEMIA

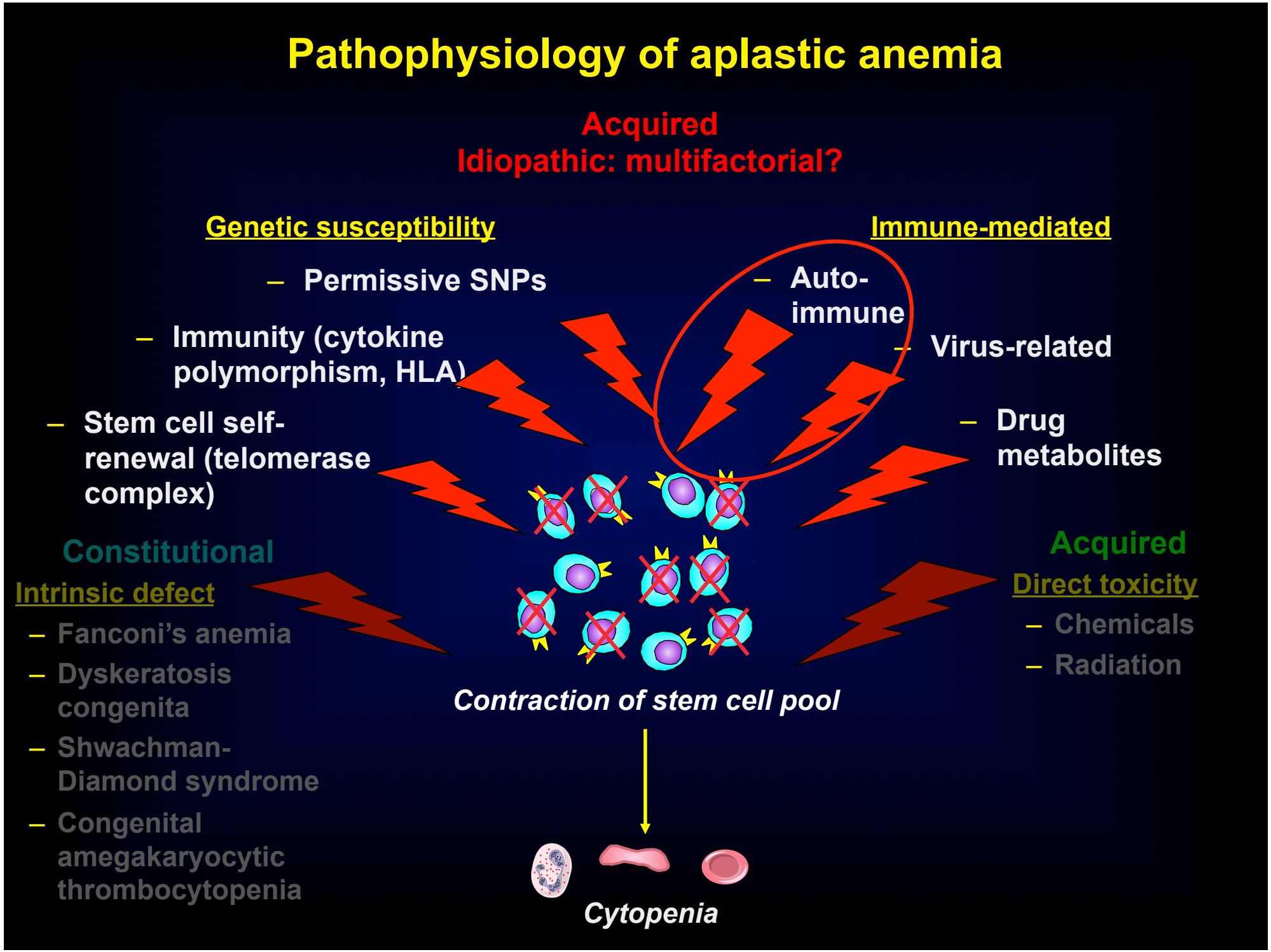
Peffault De Latour et al., Blood 2010 First Edition

- *Increased in AA patients*
- *Correlate with disease status*
- *Normalize after treatment in good responders only*





Pathophysiology of aplastic anemia



The actual meaning of somatic mutations in hematology

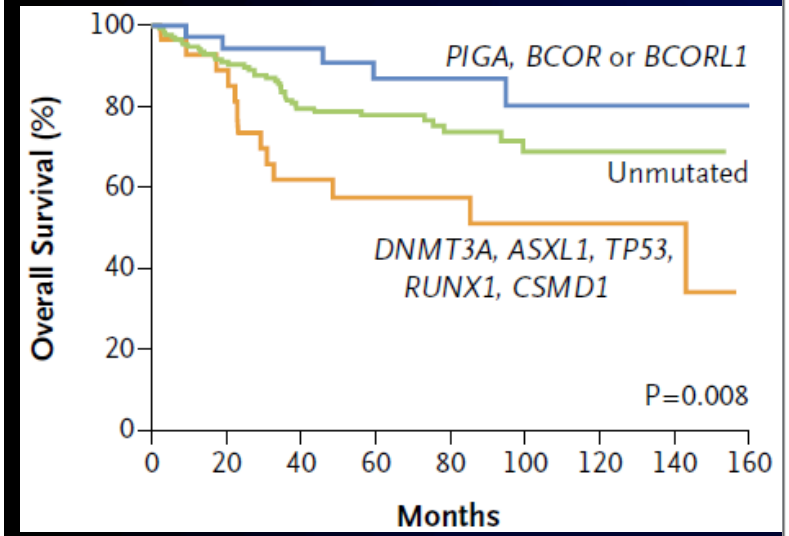
Do all mutations imply cancer (especially in marrow failure)?

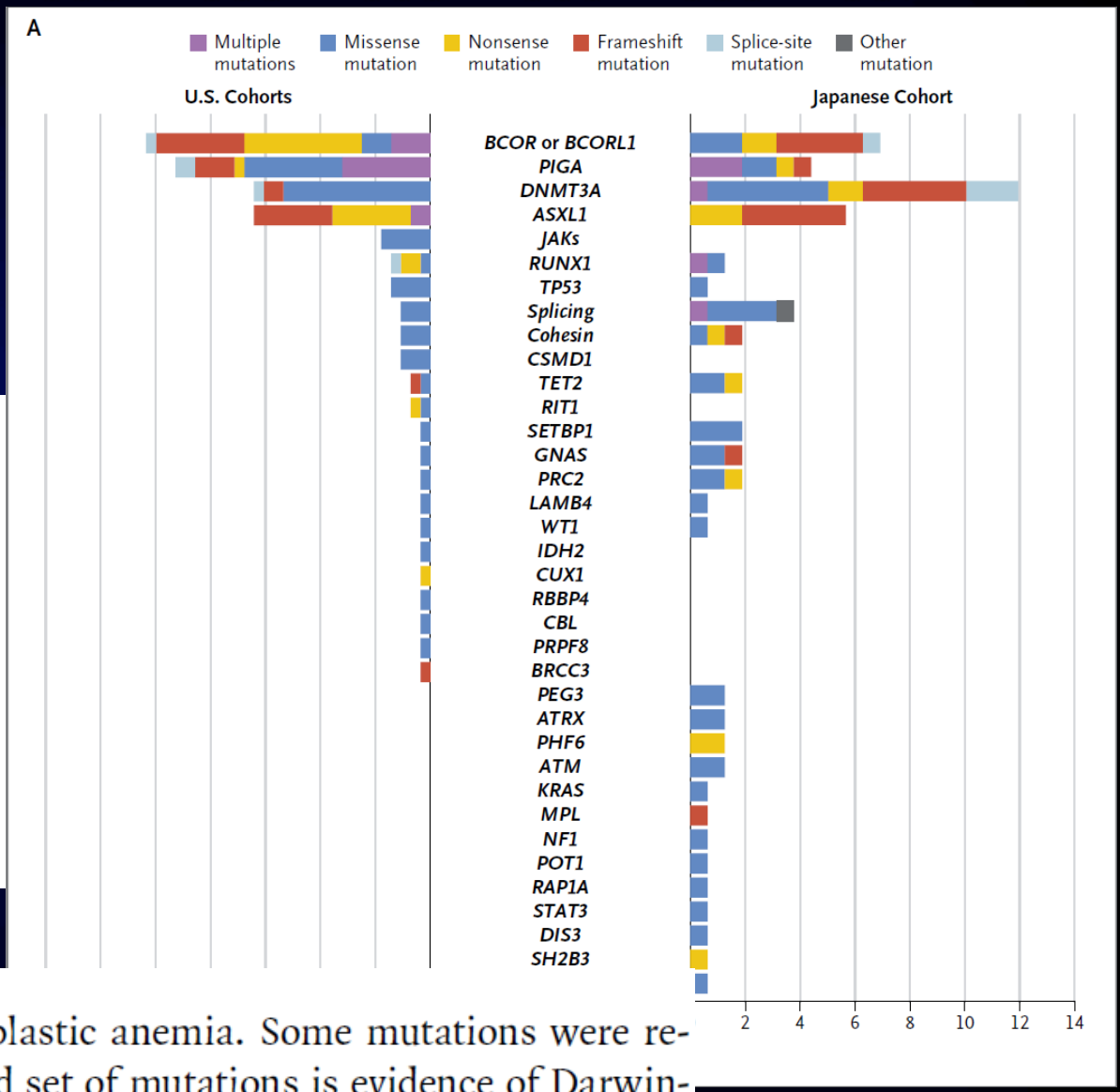
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Somatic Mutations and Clonal Hematopoiesis in Aplastic Anemia

T. Yoshizato, B. Dumitriu, K. Hosokawa, H. Makishima, K. Yoshida, D. Townsley, A. Sato-Otsubo, Y. Sato, D. Liu, H. Suzuki, C.O. Wu, Y. Shiraishi, M.J. Clemente, K. Kataoka, Y. Shiozawa, Y. Okuno, K. Chiba, H. Tanaka, Y. Nagata, T. Katagiri, A. Kon, M. Sanada, P. Scheinberg, S. Miyano, J.P. Maciejewski, S. Nakao, N.S. Young, and S. Ogawa

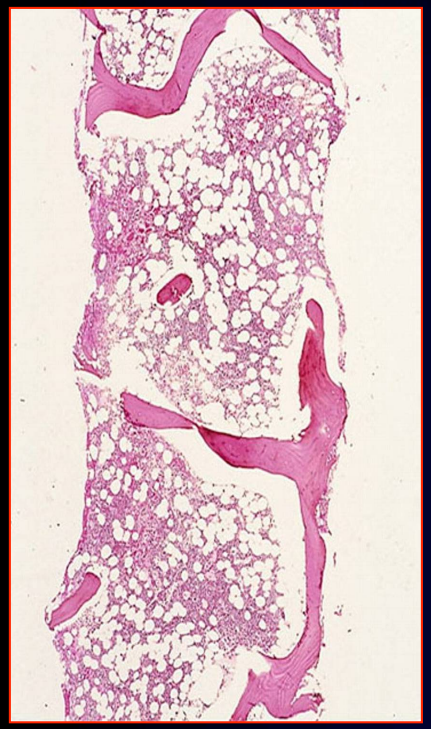




CONCLUSIONS

Clonal hematopoiesis was prevalent in aplastic anemia. Some mutations were related to clinical outcomes. A highly biased set of mutations is evidence of Darwinian selection in the failed bone marrow environment. The pattern of somatic clones in individual patients over time was variable and frequently unpredictable.

Bone Marrow Failure and PNH



AA +
PNH clone(s)

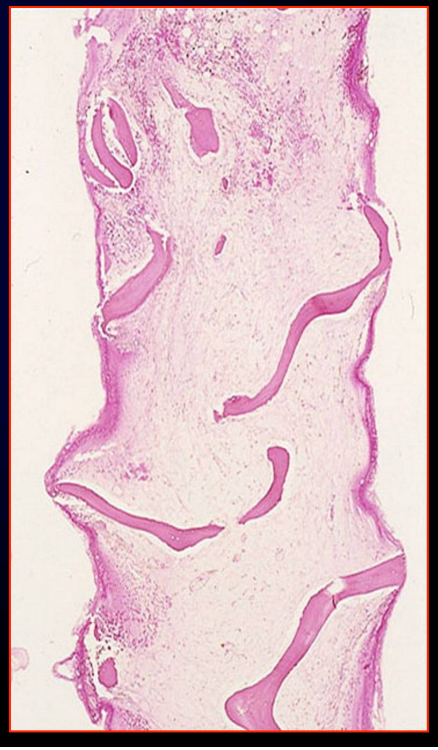
PNH

Hemolysis

Cytopenia

Aplastic Anemia

Aplastic anemia

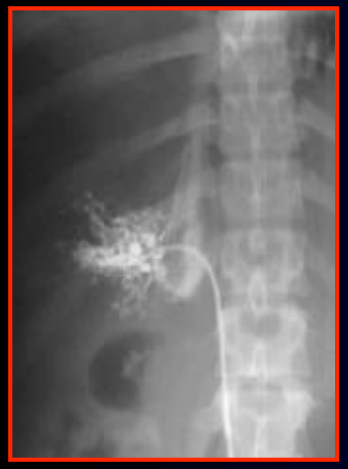


THE CLINICAL TRIAD OF PNH

EPIDEMIOLOGY: rare disease (1-5 per million/year)

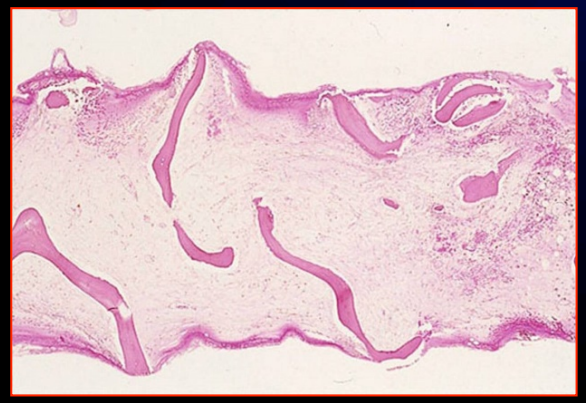
1. Chronic hemolytic anemia with paroxistic crises

Intravascular hemolysis, complement mediated



2. Propensity to thromboembolisms

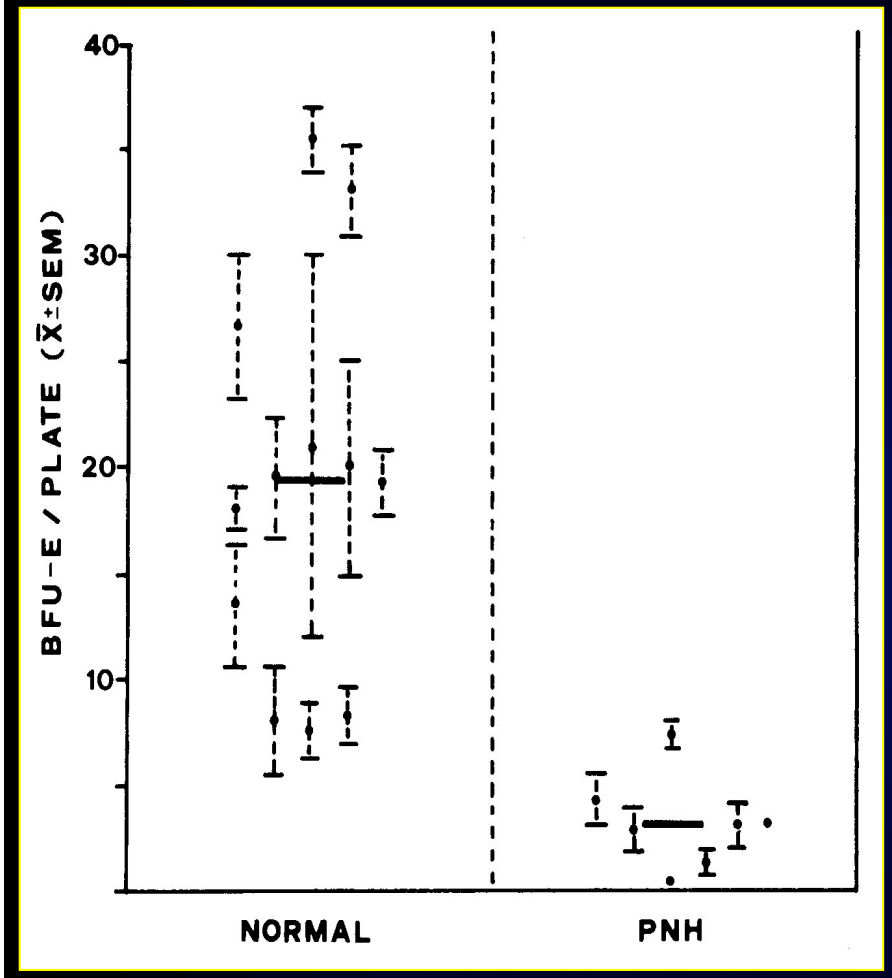
Often at unusual site, especially veins
(cerebral veins, hepatic veins, splenic vein)



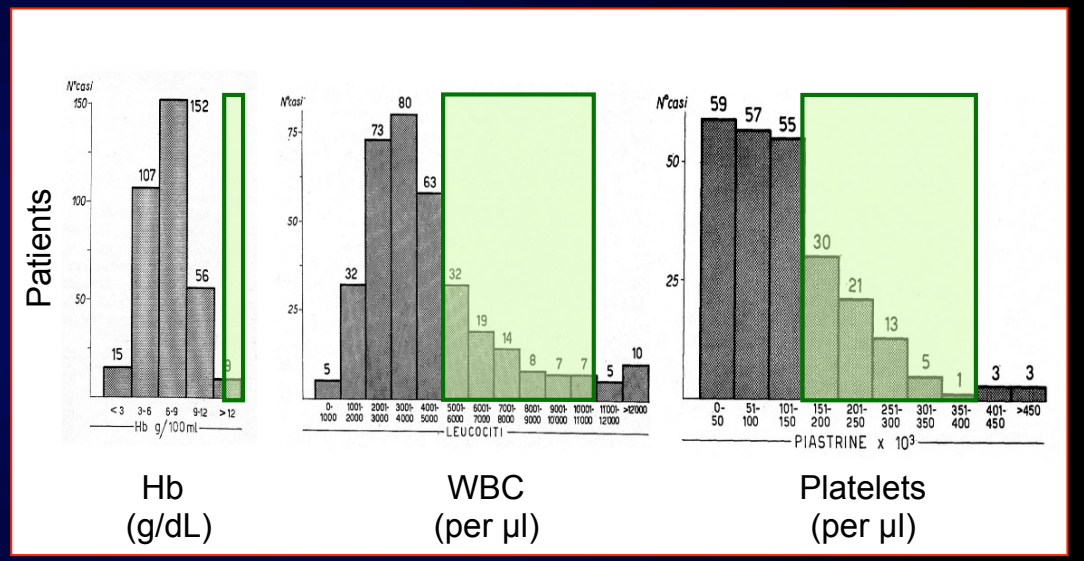
3. Variable cytopenia

Stigmata of marrow failure, possible
overlapping with aplastic anemia (AA/PNH)

Evidence of marrow failure in PNH

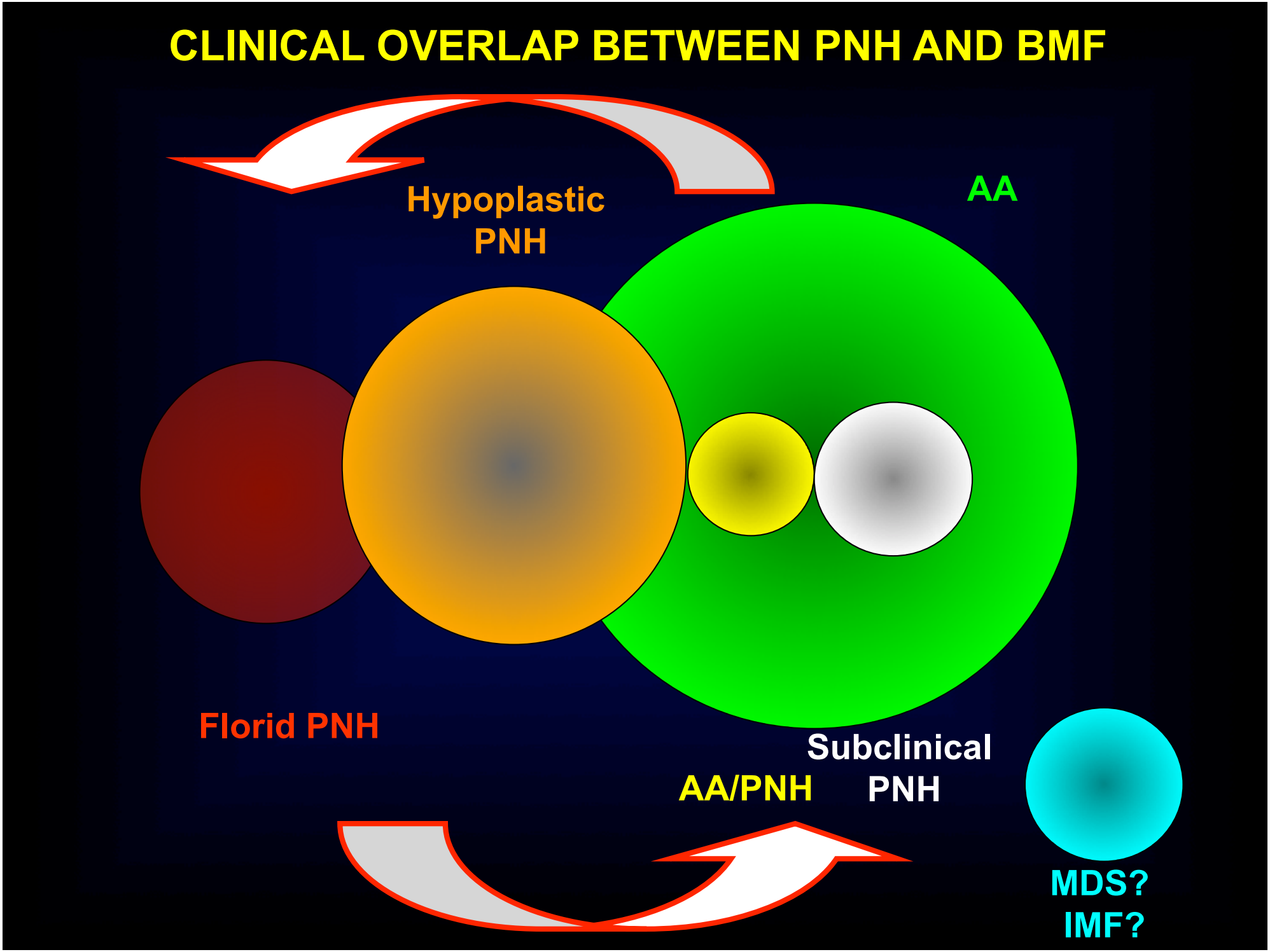


Rotoli et al, Blood 1982



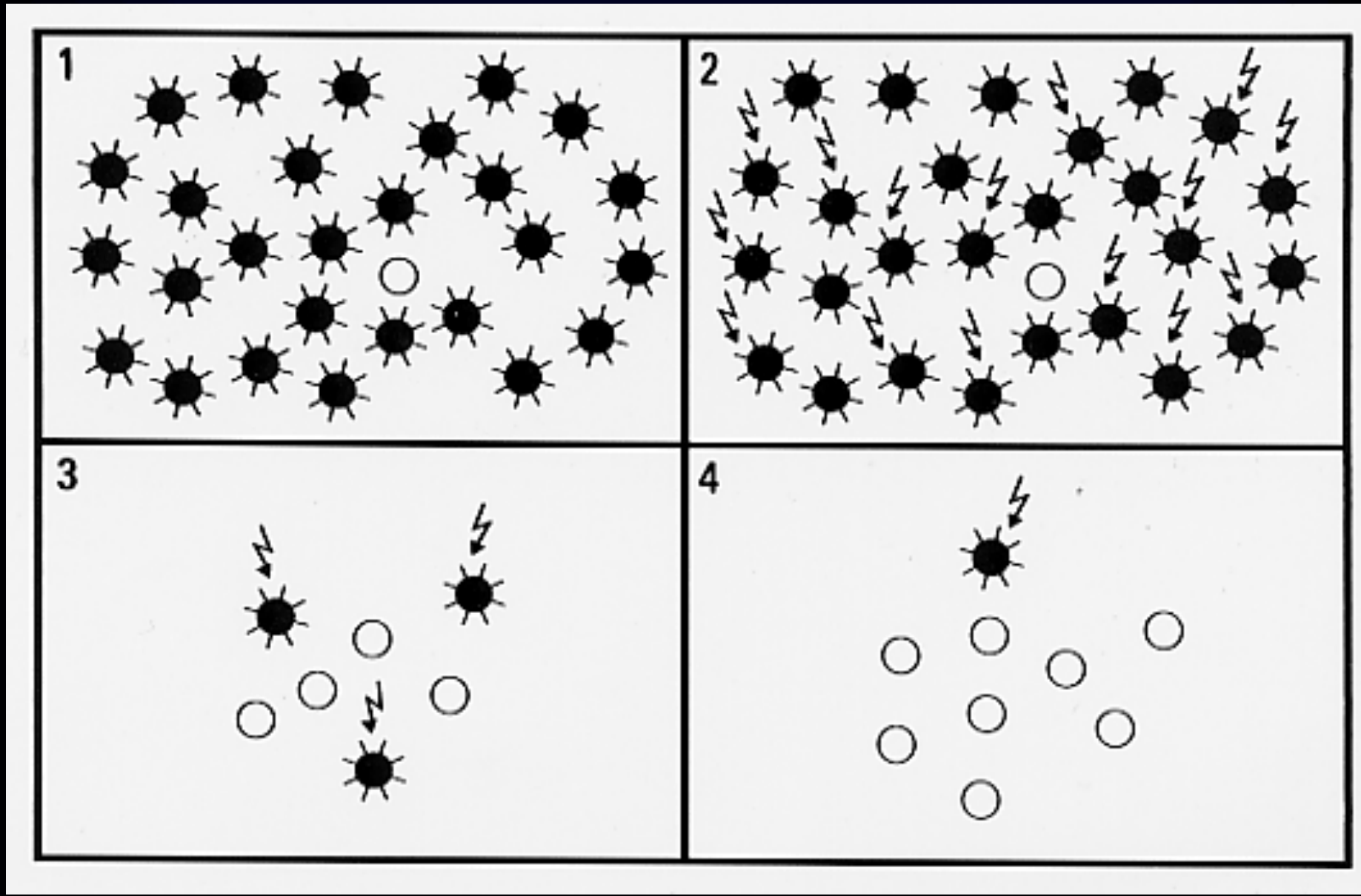
CNR, 1973

CLINICAL OVERLAP BETWEEN PNH AND BMF

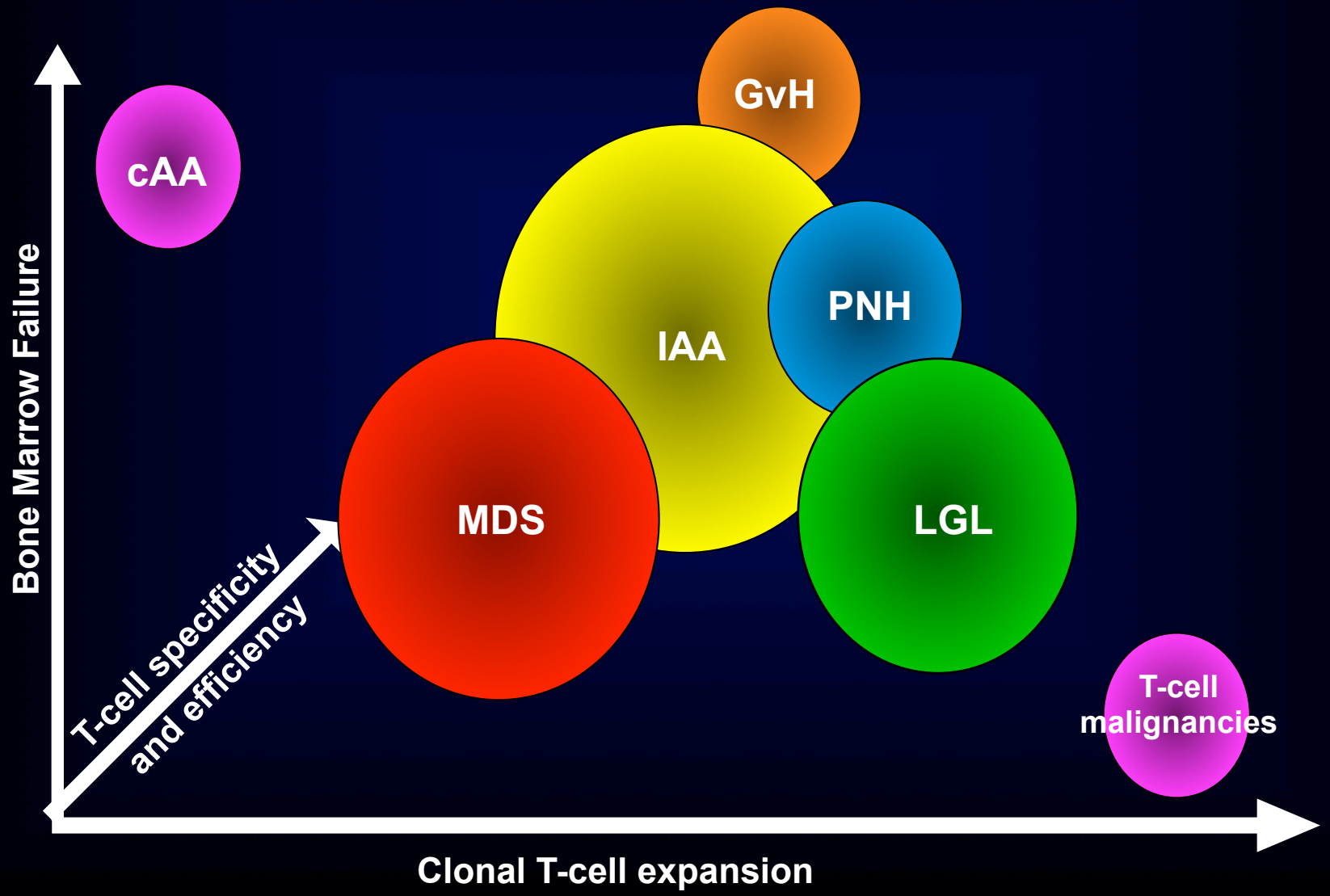


PATHOPHYSIOLOGY OF PNH

The dual hypothesis (Rotoli and Luzzatto, Baillieres Clin Haematol 1989)



IMMUNE RESPONSE AND BONE MARROW FAILURE SYNDROMES

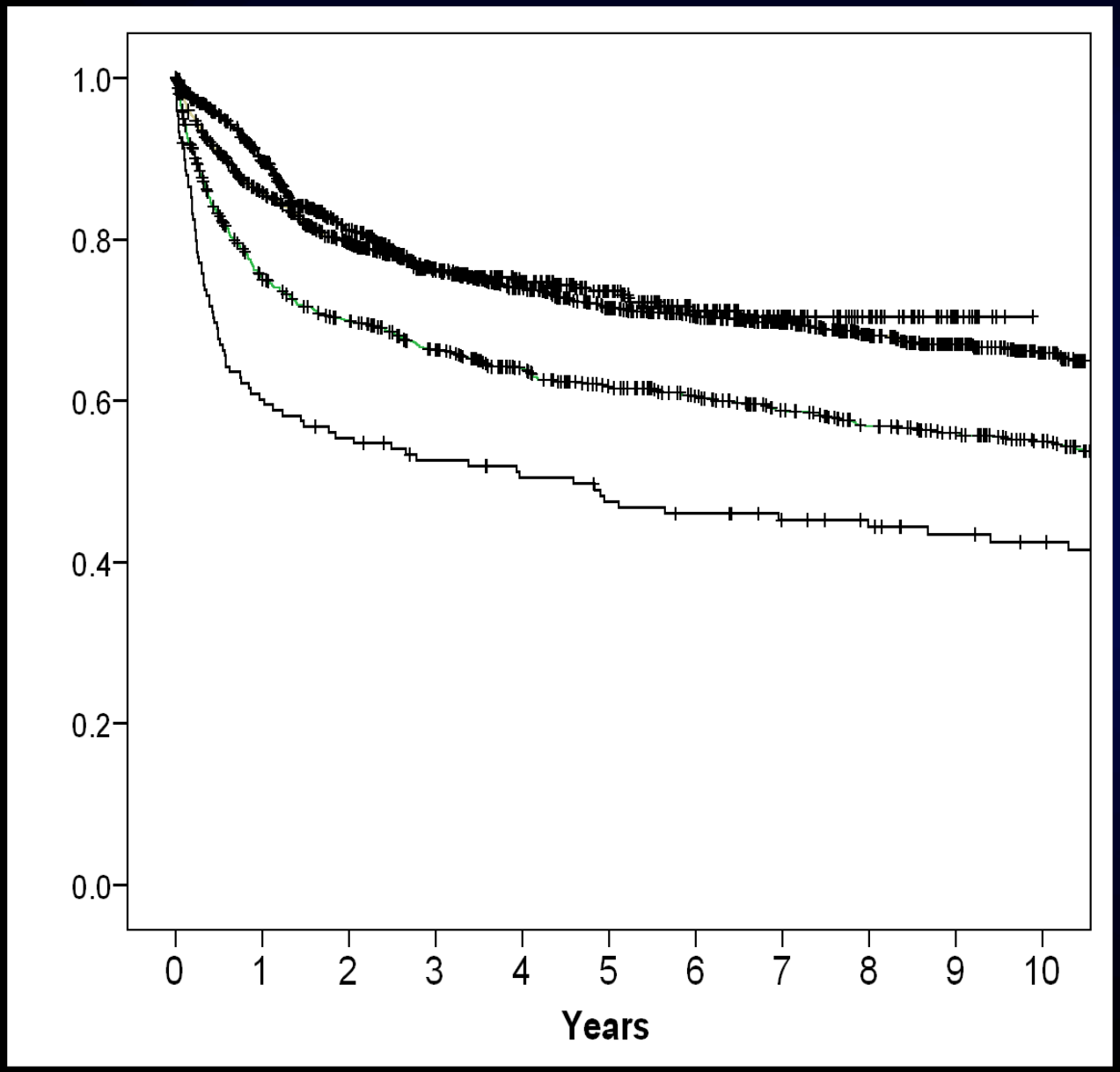


AA and…

… the non-transplant treatment

OUTCOME OF IMMUNOSUPPRESSION FOR SAA

Improvement over the years



EBMT Database

N=3202

2000-10
1990-00

1980-90

1975-80

Survival improved with years, mostly due to:

- ✓ ***Better supportive therapy***
- ✓ ***Better salvage treatment (SCT)***

Courtesy of Jakob Passweg

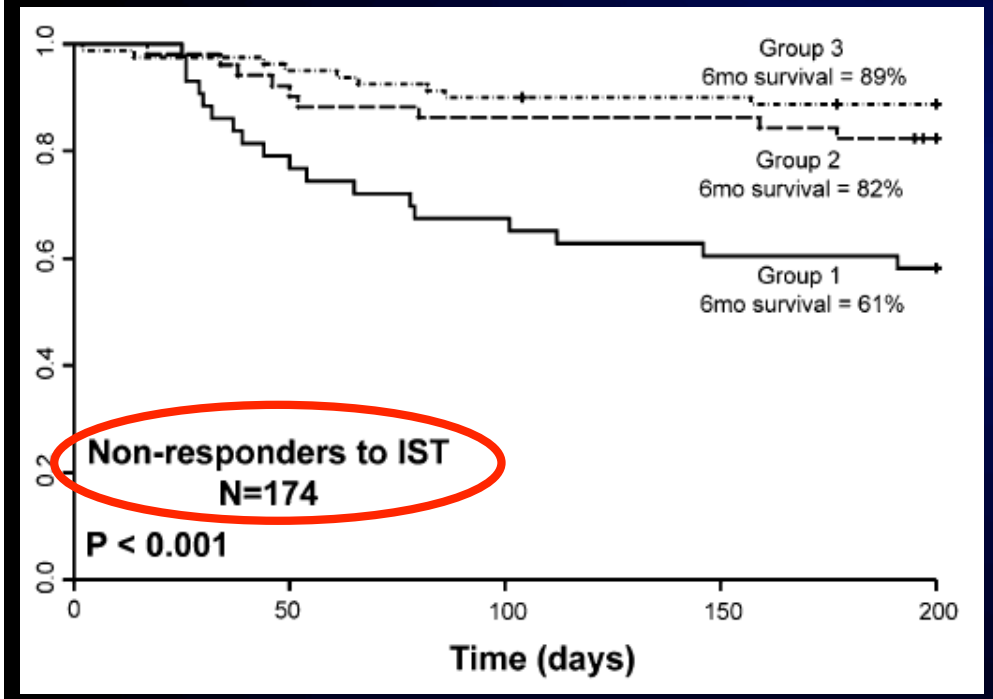
Supportive care

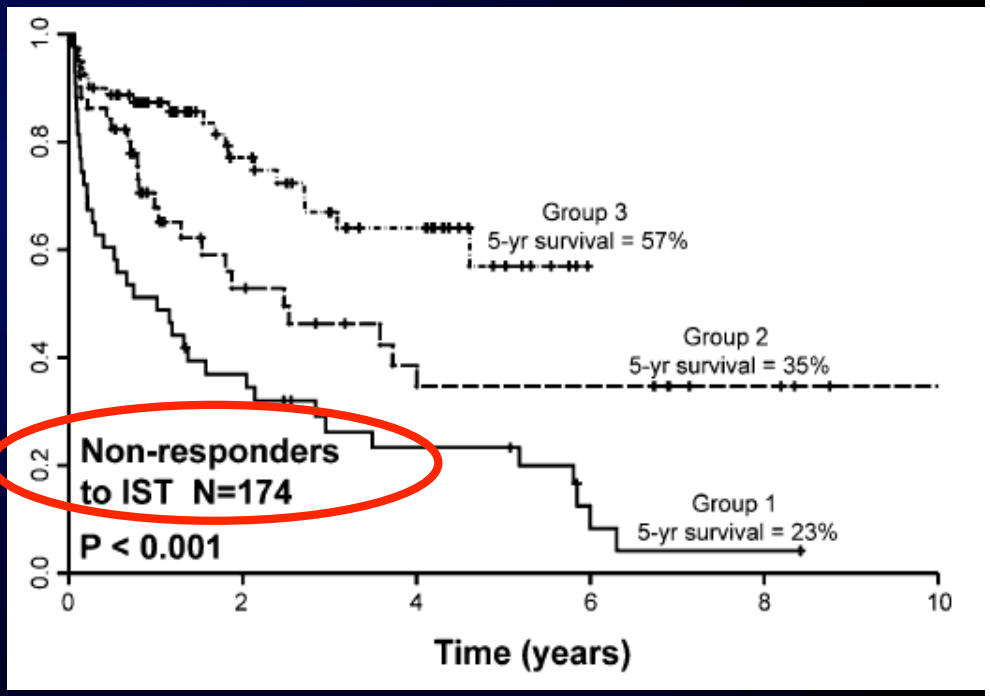
The improvement in anti-infectious management

CID 2011

- ✓n=420 (174 non-responders)
- ✓Infection-related mortality from 37% to 11%
- ✓Incidence of IFIs from 49% to 8%

Group 1: 12/1989-10/1986
Group 2: 11/1986-10/2002
Group 3: 11/2002-04/2008





The most relevant breakthrough in AA treatment was the anti-infectious supportive care: keeping AA patients alive until they recover (IST or SCT)

IMPROVING ATG-BASED IMMUNOSUPPRESSION

The benefit of combining ATG and cyclosporine A

Treatment of aplastic anemia with antilymphocyte globulin and methylprednisolone with or without cyclosporine. The German Aplastic Anemia Study Group

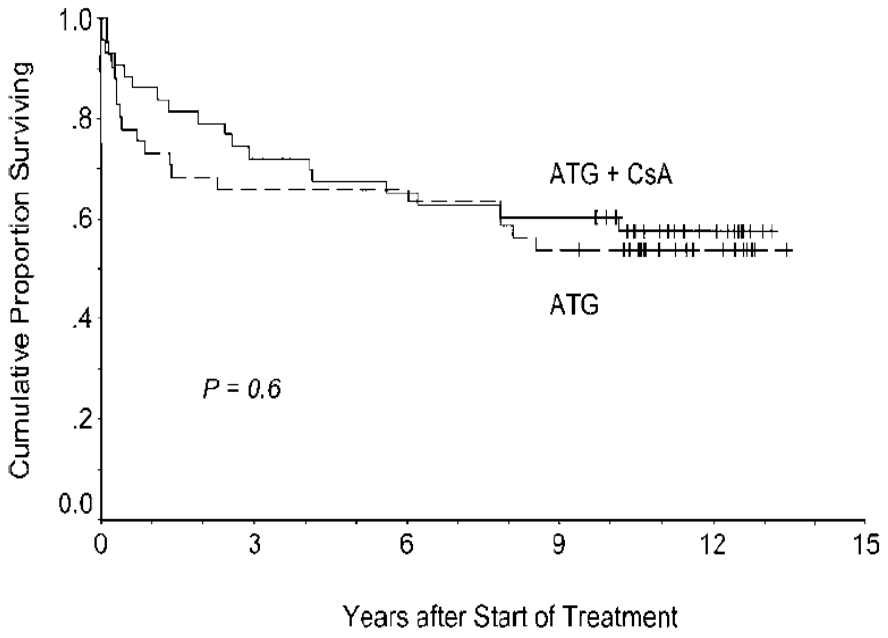
N Frickhofen, JP Kaltwasser, H Schrezenmeier, A Raghavachar, HG Vogt, F Herrmann, M Freund, P Meusers, A Salama, and H Heimpel

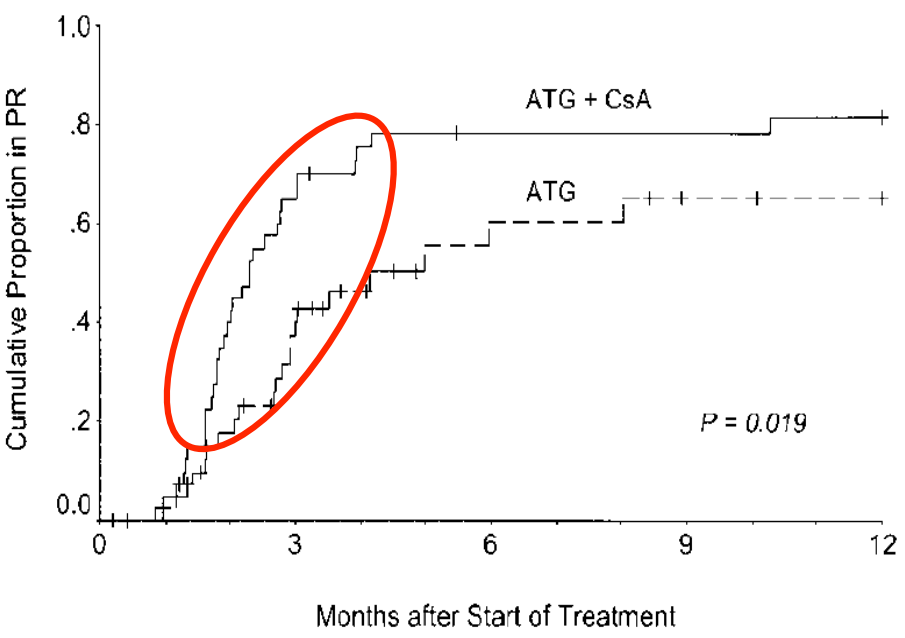
1991

Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia

Norbert Frickhofen, Hermann Heimpel, Joachim P. Kaltwasser, and Hubert Schrezenmeier, for the German Aplastic Anemia Study Group

2003

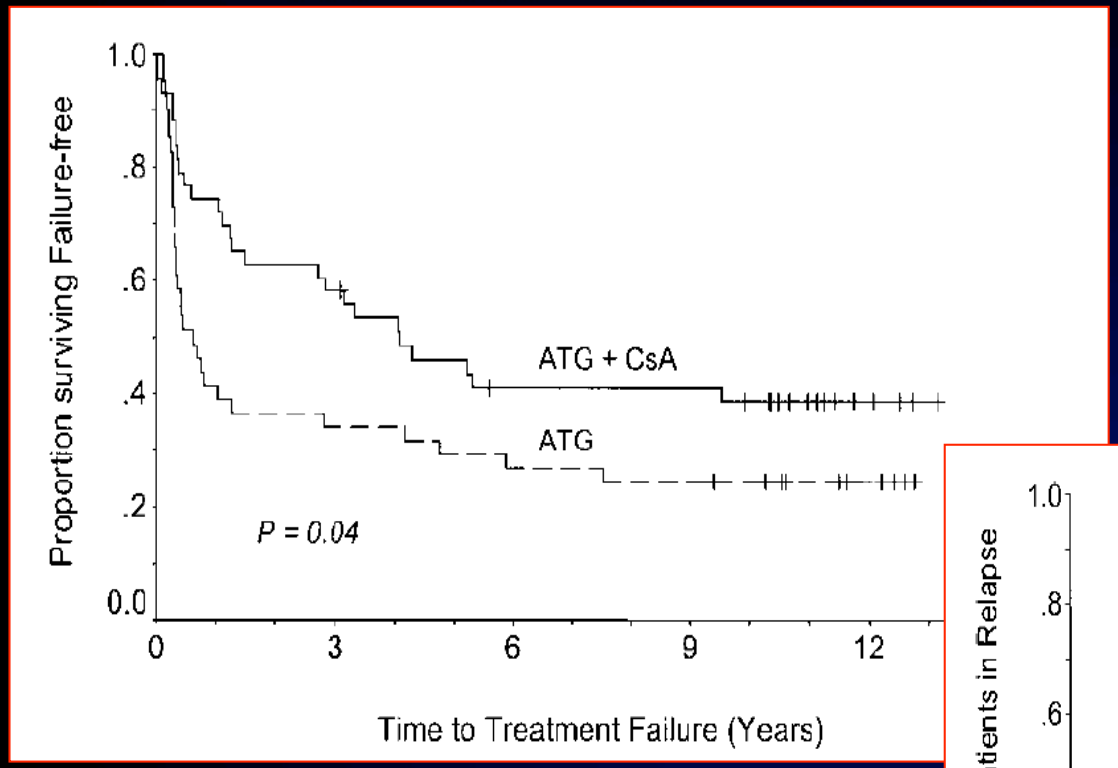


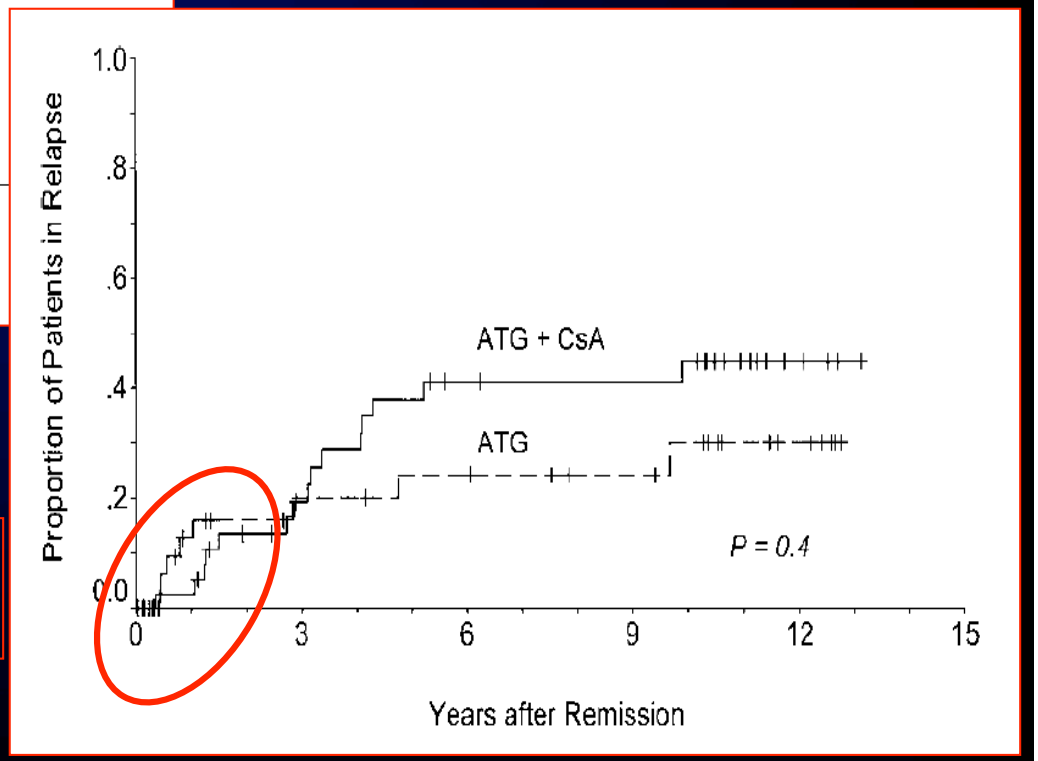


✓ **CyA speed hematological response without affecting survival**

IMPROVING ATG-BASED IMMUNOSUPPRESSION

The benefit of combining ATG and cyclosporine A





✓ **CyA reduces early treatment failure but not long-term relapse rate**

Frickhofen et al, Blood 2003

2003

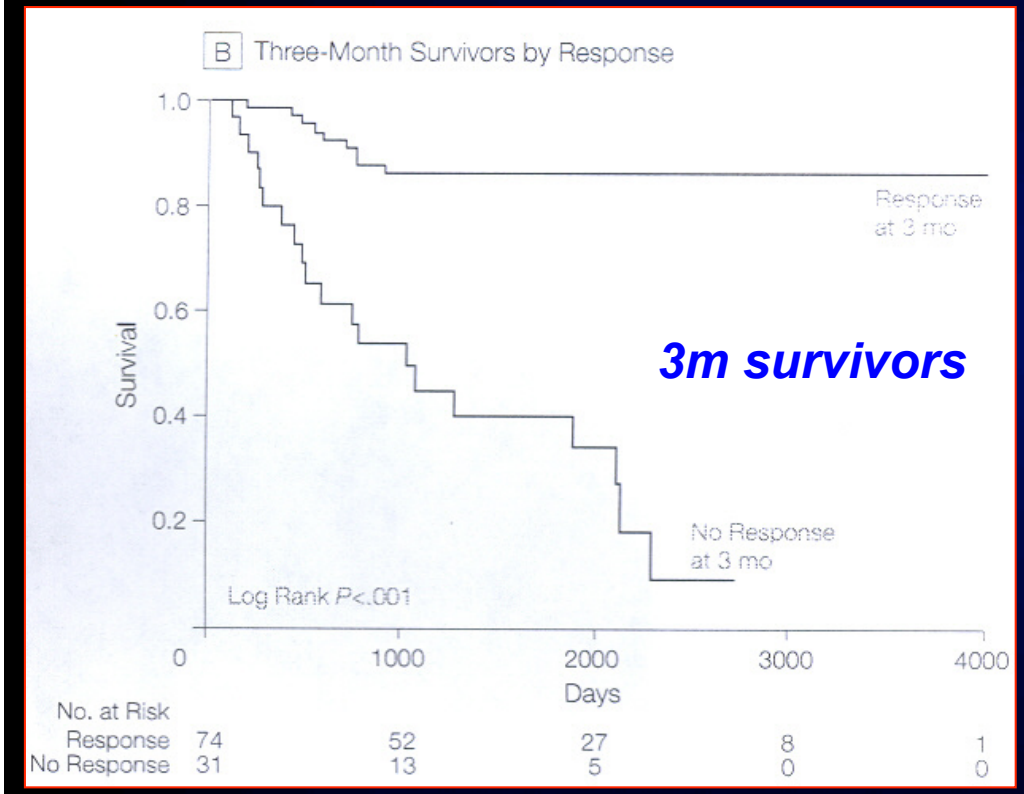
Antithymocyte Globulin and Cyclosporine for Severe Aplastic Anemia

Association Between Hematologic Response and Long-term Outcome

Stephen Rosenfeld, MD
Dean Follmann, PhD
Olga Nunez, RN
Neal S. Young, MD

n=112

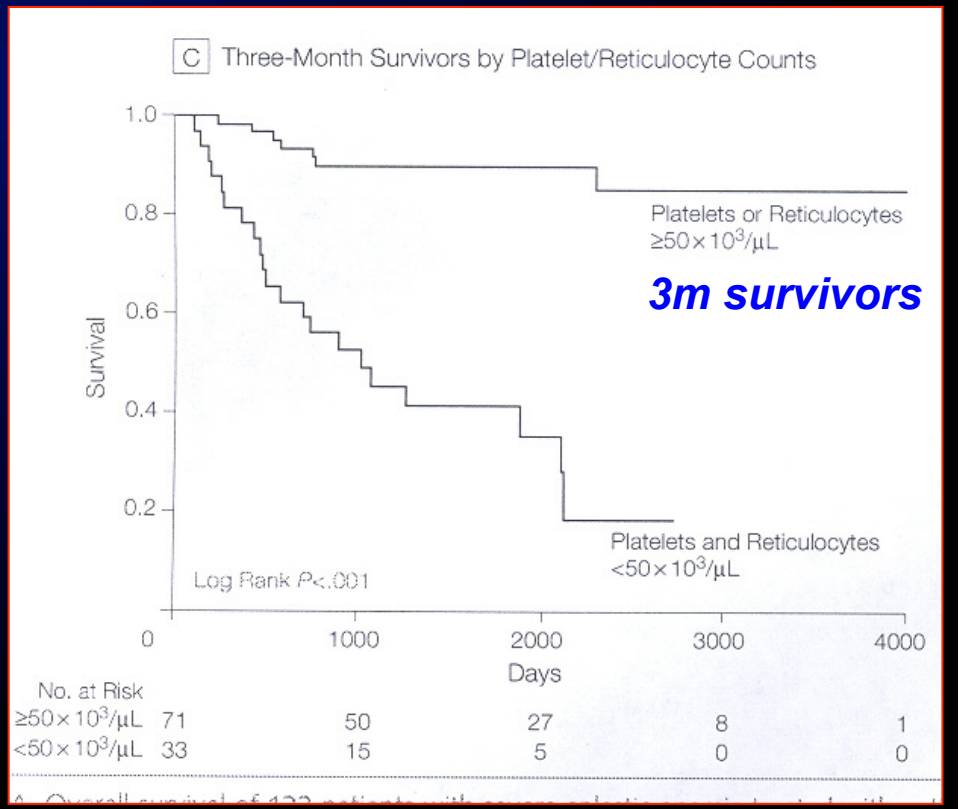
hATG x 4 (40mg/kg) + CsA x 6 m



OS 55% @7y;

OR 60% @ 3m, 61% @ 6m, 58% @ 1y

3m survivors



3m survivors

Hematological response is the main predictor for outcome

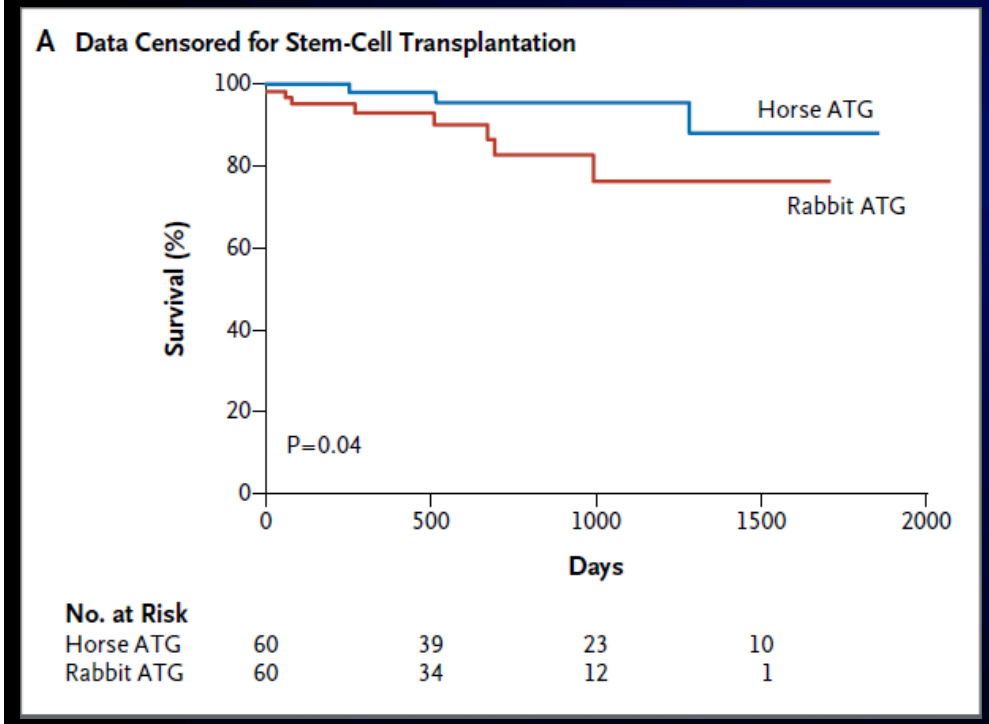
NEJM

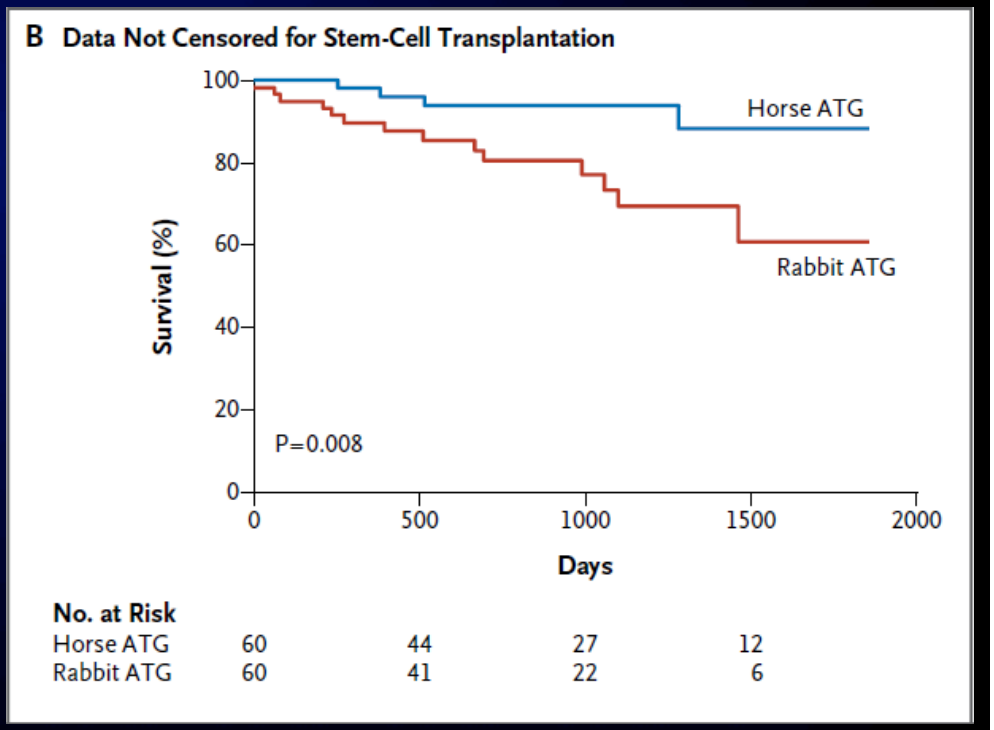
NEJM 2011

Horse versus Rabbit Antithymocyte Globulin in Acquired Aplastic Anemia

Phillip Scheinberg, M.D., Olga Nunez, R.N., B.S.N., Barbara Weinstein, R.N., Priscila Scheinberg, M.S., Angélique Biancotto, Ph.D., Colin O. Wu, Ph.D., and Neal S. Young, M.D.

- ✓ ***Phase III prospective randomized study, first-line treatment***
- ✓ ***hATG + CyA (n=60) vs rATG + CyA (n=60)***
- ✓ ***OR @ 6m 68% vs 37% (p<0.001)***





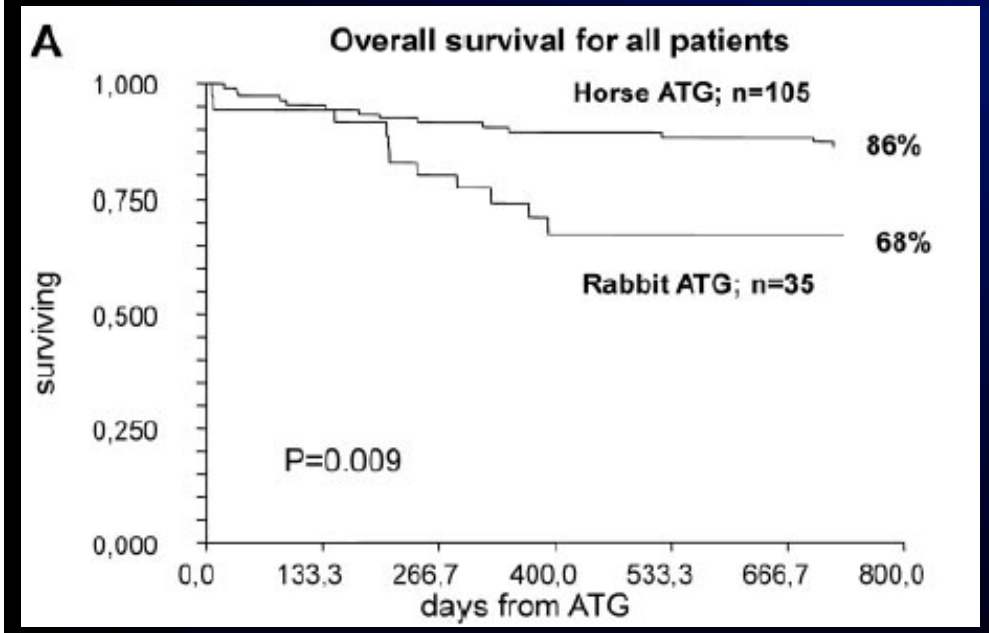
rATG is inferior to hATG in first line treatment of SAA, as indicated by hematological response and survival

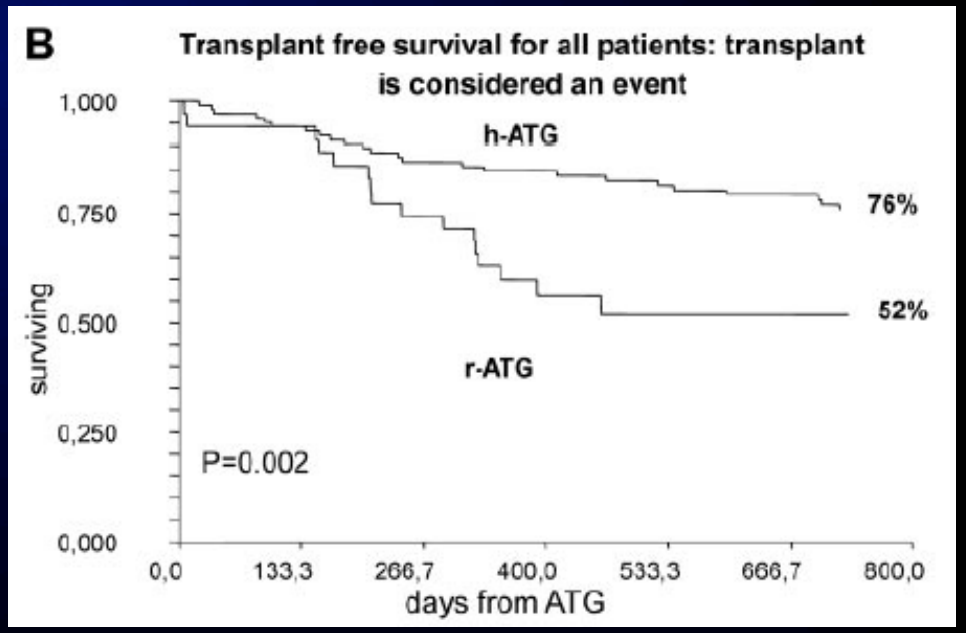
Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party

Judith C. Marsh,[1] Andrea Bacigalupo,[2] Hubert Schrezenmeier,[3] Andre Tichelli,[4] Antonio M. Risitano,[5] Jakob R. Passweg,[4] Sally B. Killick,[6] Alan J. Warren,[7] Theodora Foukaneli,[7] Mahmoud Aljurf,[8] H. A. Al-Zahrani,[8] Philip Schafhausen,[9] Alexander Roth,[10] Anke Franzke,[11] Tim H. Brummendorf,[12] Carlo Dufour,[13] Rosi Oneto,[14] Philip Sedgwick,[15] Alain Barrois,[16] Shahram Kordasti,[1] Modupe O. Elebute,[1] Ghulam J. Mufti,[1] and Gerard Socie,[17] on behalf of the European Blood and Marrow Transplant Group Severe Aplastic Anaemia Working Party

Blood 2012

- ✓ ***Phase II pilot study rATG + CyA (n=35)***
- ✓ ***Retrospective matched comparison (pair-matched) with hATG + CyA (n=105)***
- ✓ ***Pilot rATG + CyA study: OR 40% @ 6m (CR 3%, PR 37%)***





rATG is inferior to hATG in first line treatment of SAA, as indicated by hematological response and survival

REASONS FOR BAD OUTCOME IN SAA

- ✓ **Primary failures**
  - Refractoriness (about a third: predicting factors and early identification)
  - Partial responses
- ✓ **Secondary failures**
  - CyA-dependent responses
  - Relapses
  - Recurrent diseases
- ✓ **Late failures**
  - Clonal evolution
  - Secondary malignancies

Many AA patients are not cured by IST!!!

Antithymocyte Globulin and Cyclosporine for Severe Aplastic Anemia

Association Between Hematologic Response and Long-term Outcome

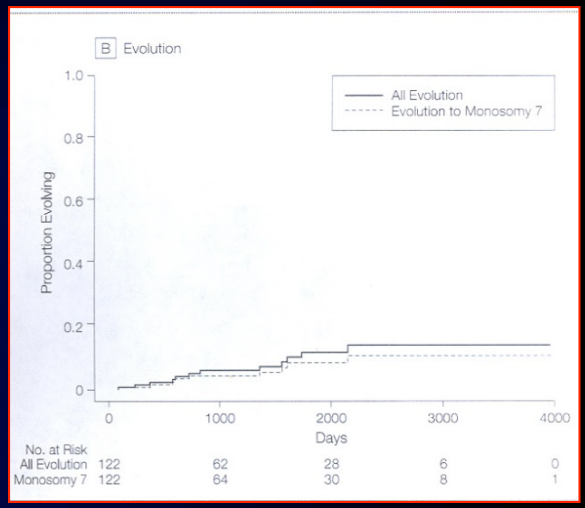
Stephen Rosenfeld, MD
Dean Follmann, PhD
Olga Nunez, RN
Neal S. Young, MD

n=112

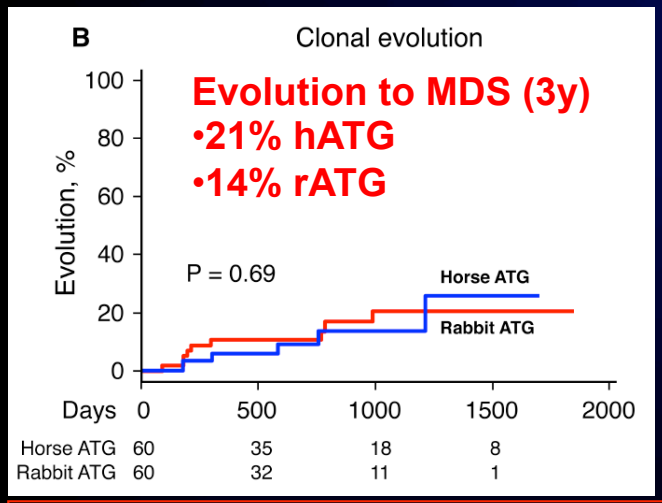
hATG x 4 (40mg/kg)
+ CsA x 6 m

Clonal evolution (3y)

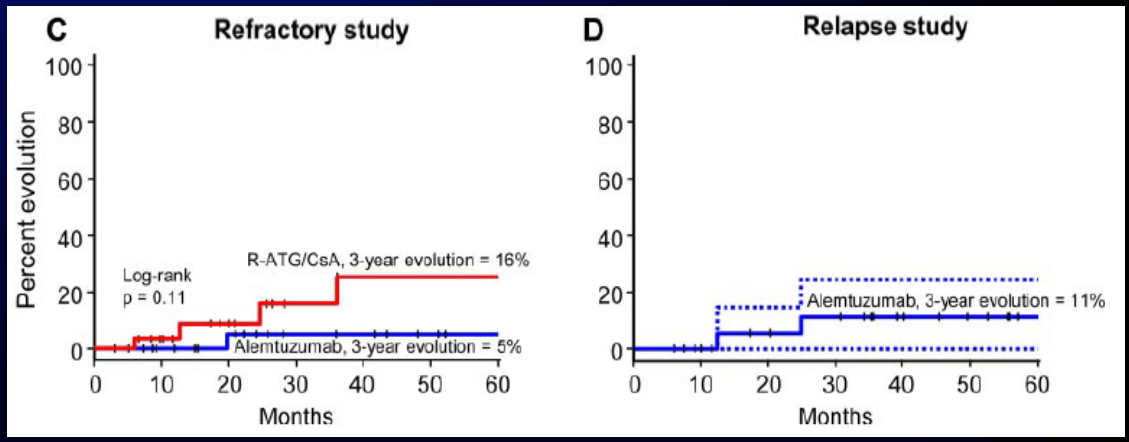
- **11% MDS (especially 7-)**
- **10% PNH**



NEJM 2011



Blood 2012



In all recent studies, the incidence of clonal evolution is about 10%, regardless the specific treatment

Aplastic Anemia: Management of Adult Patients

Jaroslaw P. Maciejewski and Antonio M. Risitano

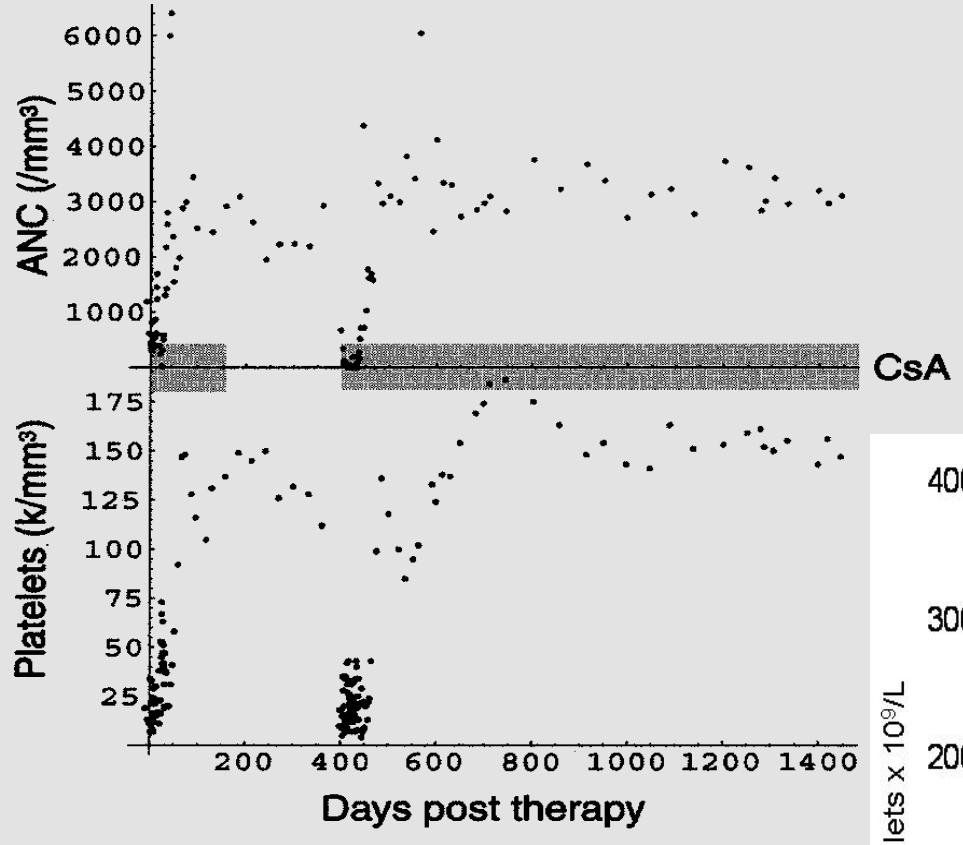
REASONS FOR TREATMENT FAILURE

- Pathophysiology other than immune-mediated
- Irreversible stem cell deficit
- **Insufficient immunosuppression**

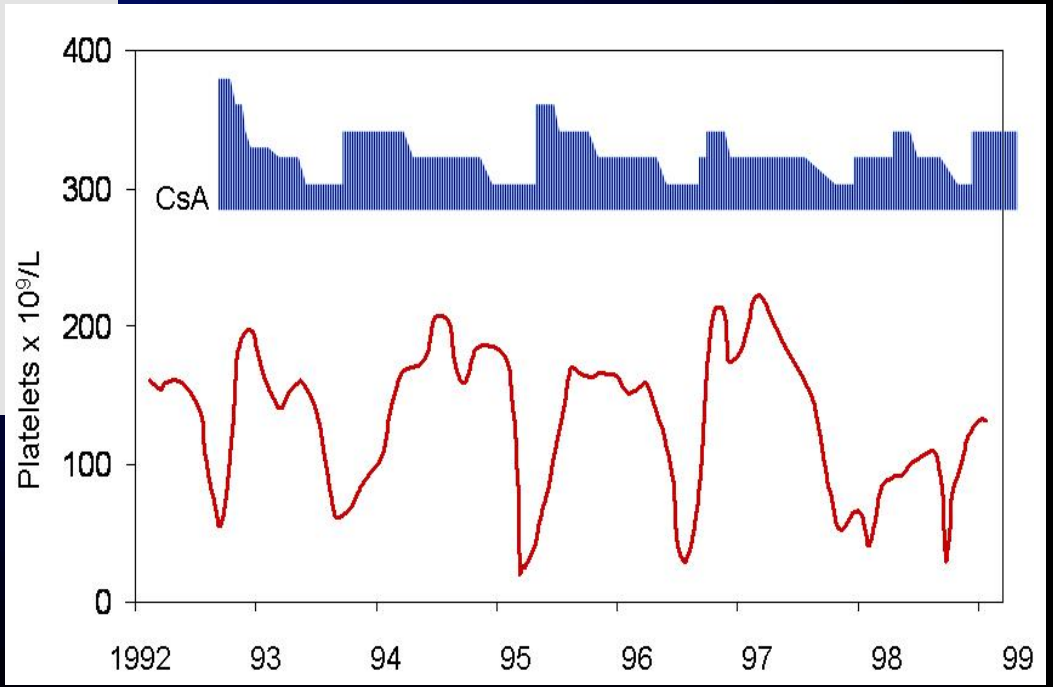
Improve immunosuppressive therapies

RELAPSES AFTER IST

The role of maintenance CyA therapy



Maintenance CyA is required to sustain blood counts after initial response to IST

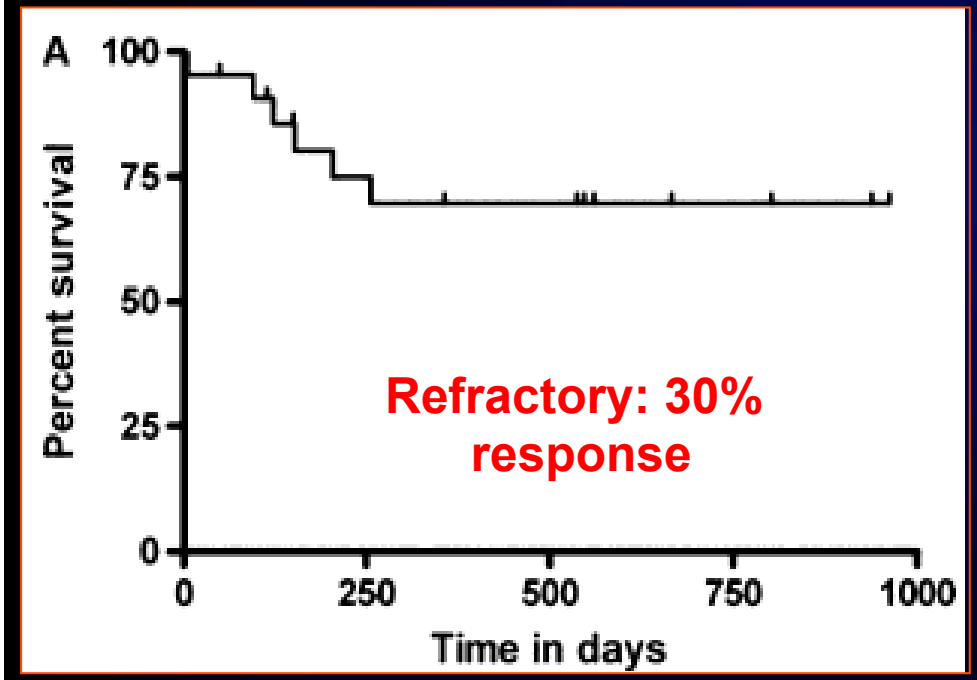


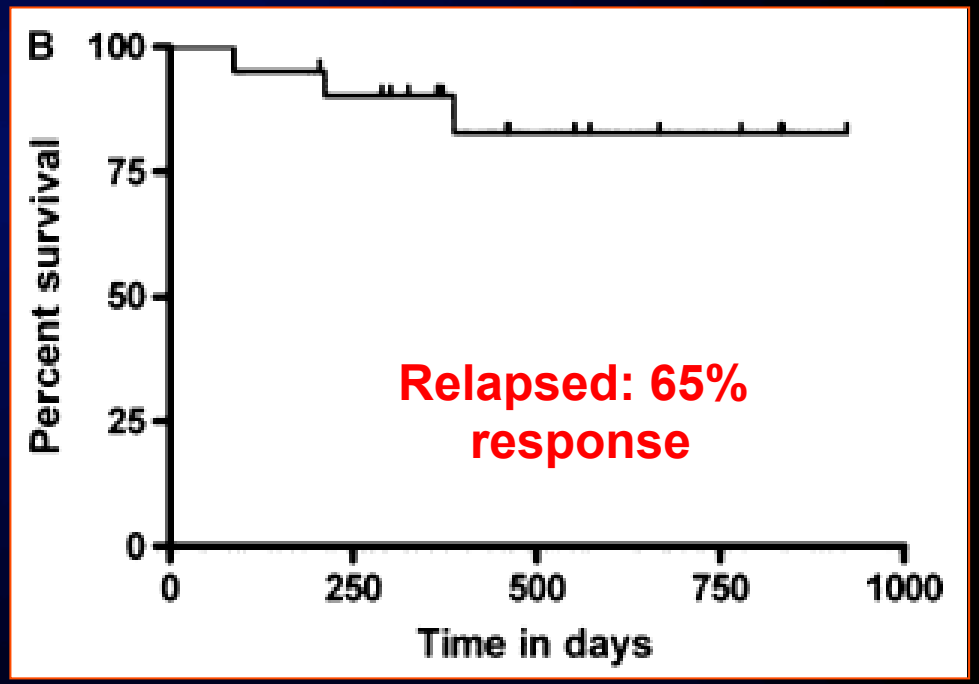
Frickhofen N. Blood. 2003 (101). 1236-1242

bjh research paper

Retreatment with rabbit anti-thymocyte globulin and ciclosporin for patients with relapsed or refractory severe aplastic anaemia

Scheinberg Br J Haematol. 2006

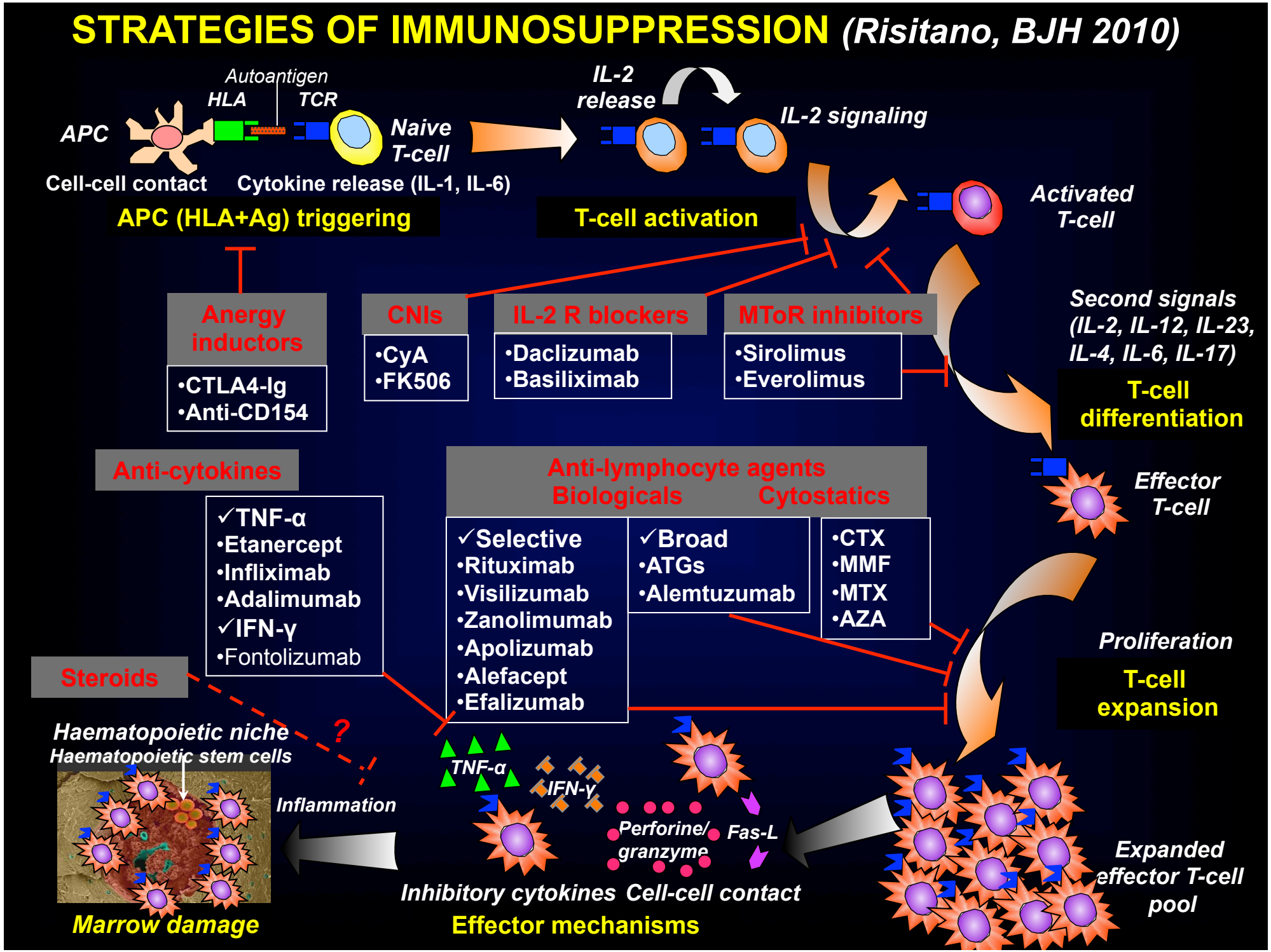




- ✓Retreatment by rATG is more effective in relapsed than in refractory patients
- ✓OS not affected due to salvage therapy

Improving IST: intensification by a third drug

STRATEGIES OF IMMUNOSUPPRESSION *(Risitano, BJH 2010)*



bjh research paper

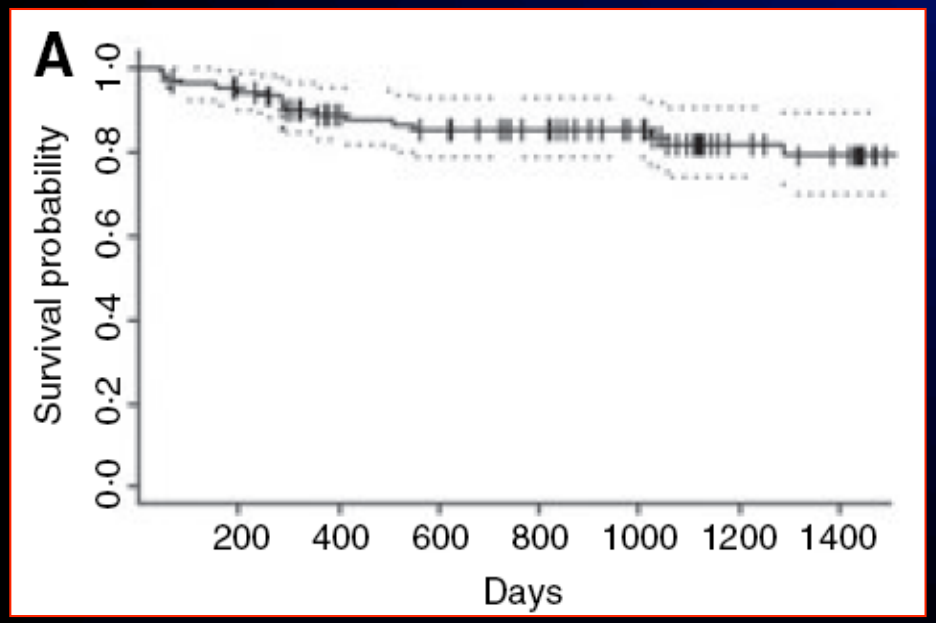
Treatment of severe aplastic anaemia with combined immunosuppression: anti-thymocyte globulin, ciclosporin and mycophenolate mofetil

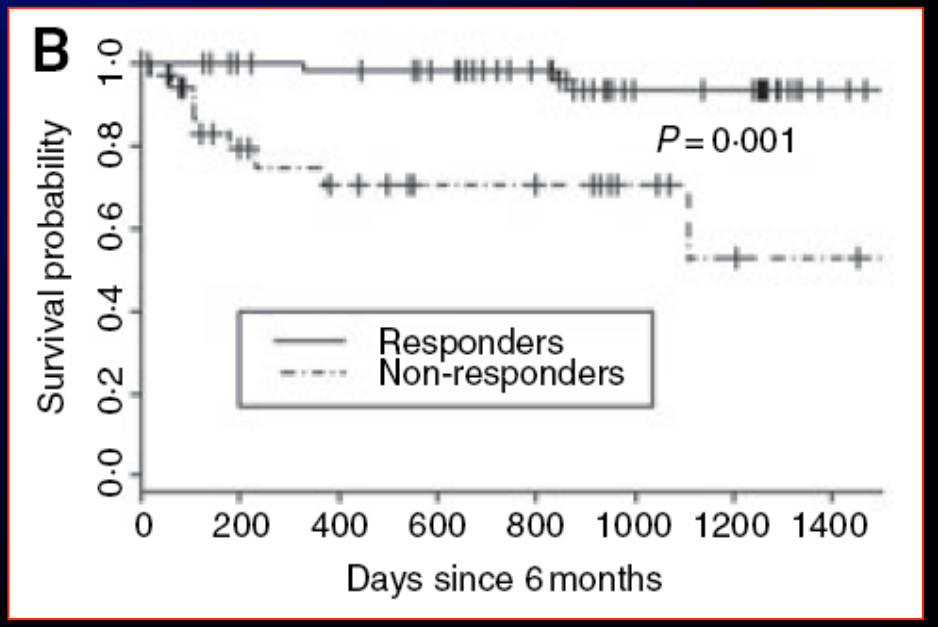
 British Journal of Haematology, 133, 606–611

Phillip Scheinberg,[1] Olga Nunez,[1] Colin Wu[2] and Neal S. Young[1]

n=104 (38% vSAA)
hATG+CsA+MMF

Overall response 3m 56% (14CR + 43PR)
Overall response 6m 62% (16CR + 48PR)





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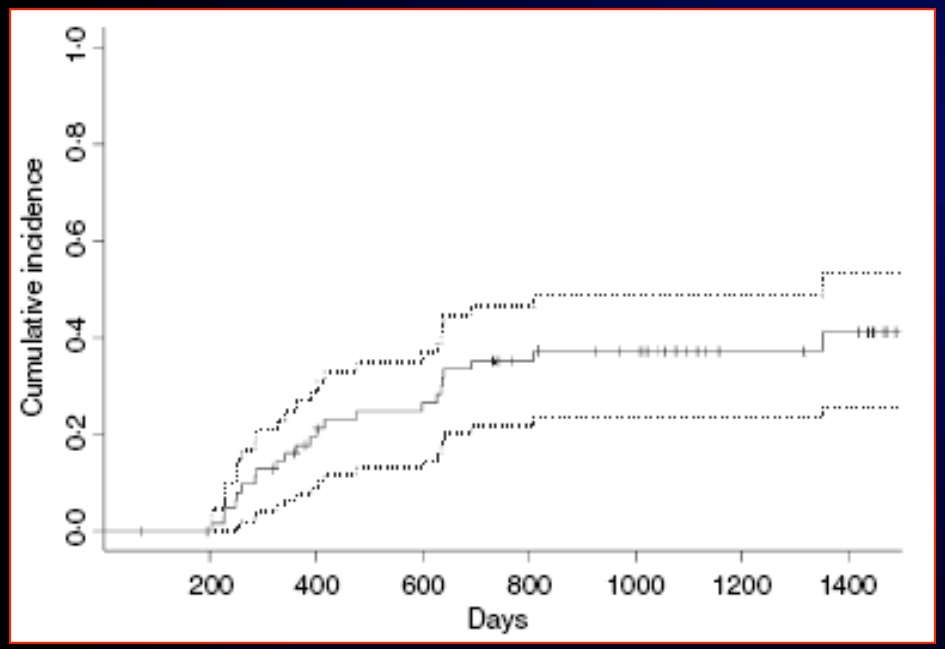
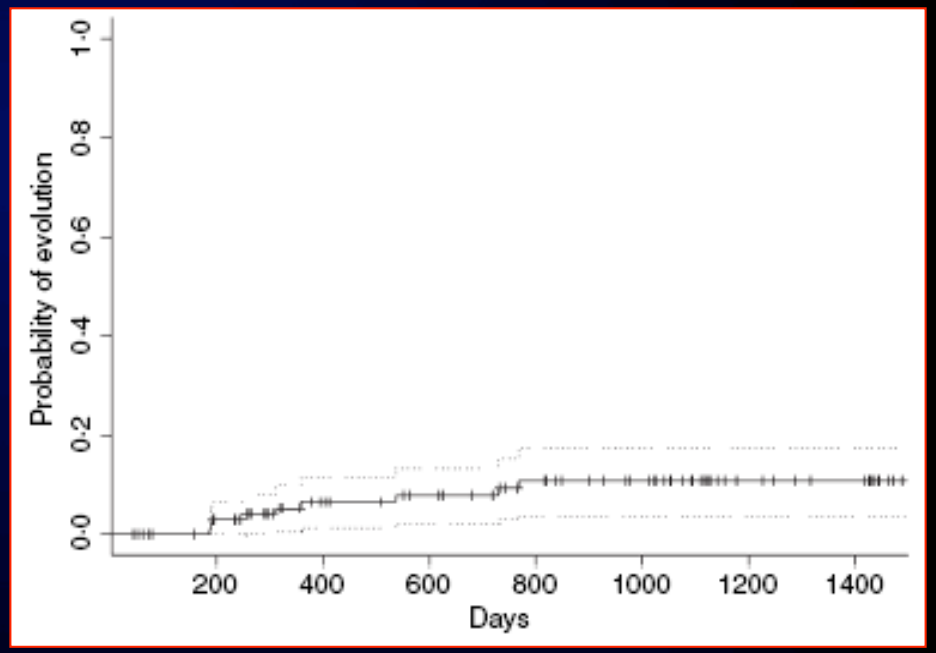


Relapse



Clonal evolution

Sirolimus (Rapamune®)

Original Article

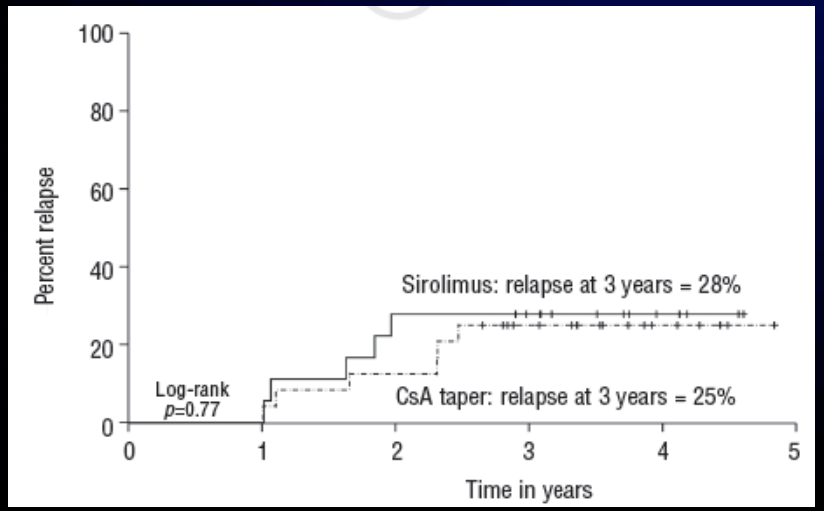
Treatment of severe aplastic anemia with a combination of horse antithymocyte globulin and cyclosporine, with or without sirolimus: a prospective randomized study

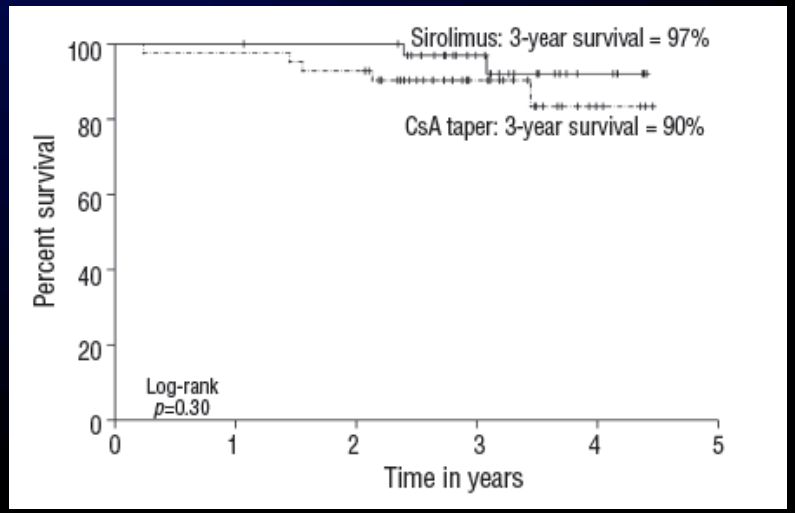
Phillip Scheinberg,[1] Colin O. Wu,[2] Olga Nunez,[1] Priscila Scheinberg,[1] Carol Boss,[1] Elaine M. Sloand,[1] and Neal S. Young[1]

haematologica | 2009; 94(3)

Table 3. Response to the immunosuppressive regimens.

	3 months		6 months		Total response
	CR (%)	PR (%)	CR (%)	PR (%)	CR + PR (%)
h-ATG/CsA	3 (7)	21 (50)	5 (12)	21 (50)	26 (62%)
h-ATG/CsA/sirolimus	0	13 (37)	0	18 (51)	18 (51%)





Improving IST: alternative regimens (not ATG-based)

CYCLOPHOSPHAMIDE FOR TREATMENT OF SAA

The Johns Hopkins experience

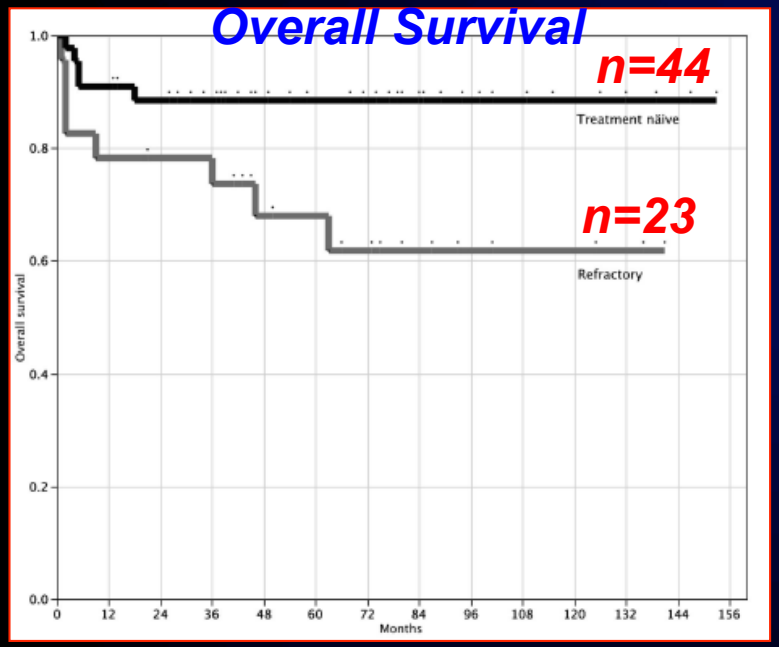
High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up

Robert A. Brodsky,[1,2] Allen R. Chen,[2] Donna Dorr,[1] Ephraim J. Fuchs,[2] Carol Ann Huff,[2] Leo Luznik,[2] B. Douglas Smith,[2] William H. Matsui,[2] Steven N. Goodman,[2] Richard F. Ambinder,[2] and Richard J. Jones[2]

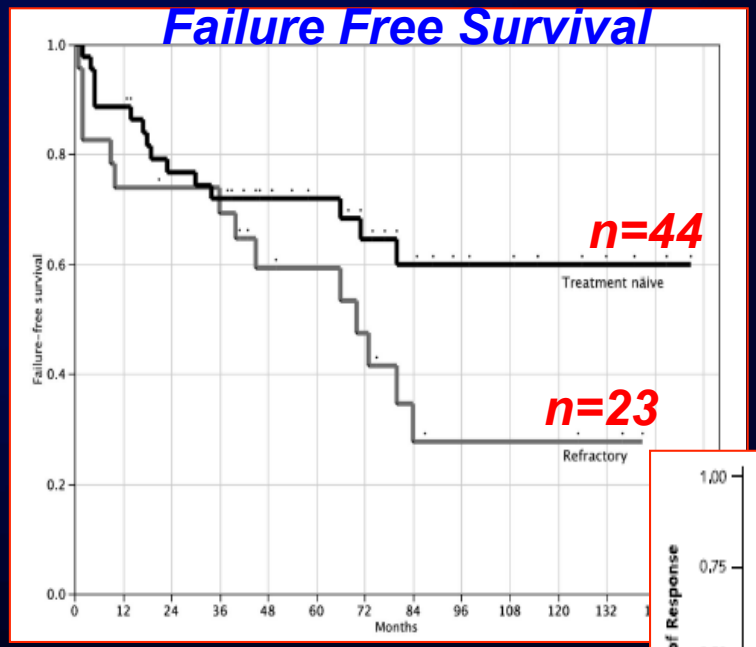
BLOOD, 18 MARCH 2010 • VOLUME 115, NUMBER 11

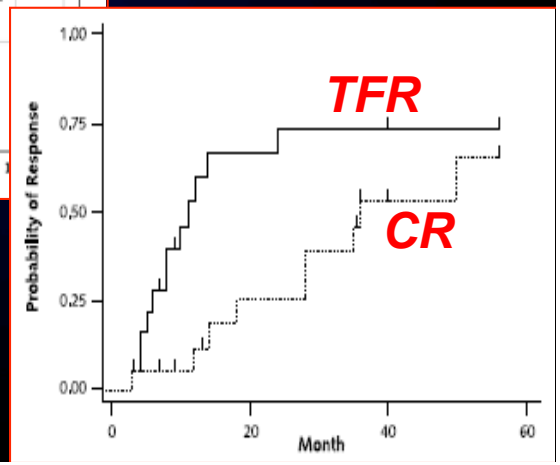
- ✓*N=67 (44 naive, 23 refractory); 50 mg/kg/day for 4 days (total 200 mg)*
- ✓*OR 71% in naive, 48% in refractory patients*
- ✓*OS and FFS 88% and 58% in naive patients, 62% and 27% in refractory patients*

Overall Survival



Failure Free Survival





- ✓*CI of fungal infections: 21% (naive) and 39% (refractory)*
- ✓*Slower but more robust and durable responses*
- ✓*No clonal evolution*

CYCLOPHOSPHAMIDE FOR TREATMENT OF SAA

NIH randomized trial

ARTICLES Lancet 2000; 356: 1554–59

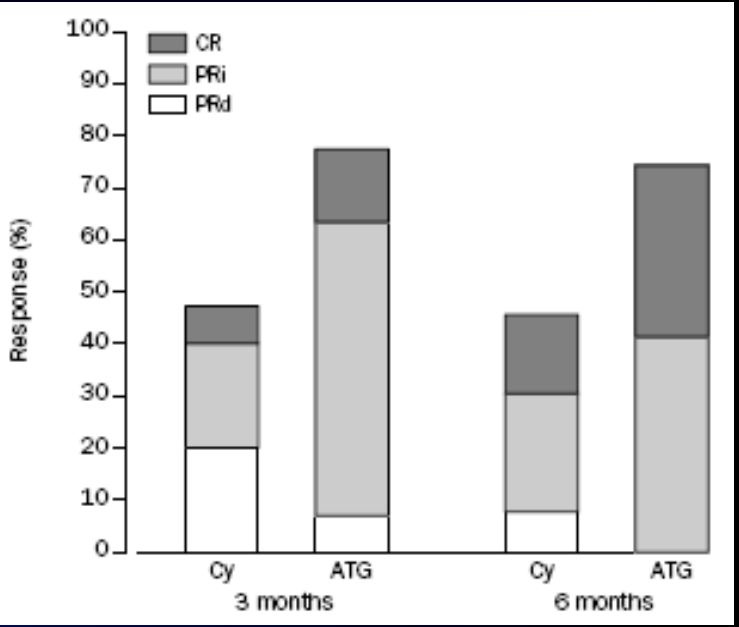
High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial

John F Tisdale, Daniel E Dunn, Nancy Geller, Michelle Plante, Olga Nunez, Cynthia E Dunbar, A John Barrett, Thomas J Walsh, Stephen J Rosenfeld, Neal S Young

n=31

ATG+CsA vs CTX+CsA

Early termination due to increased toxicity in the CTX arm (3 early deaths because of infections, plus additional cases rescued by granulocyte transfusions)



Late complications following treatment for severe aplastic anemia (SAA) with high-dose cyclophosphamide (Cy): follow-up of a randomized trial

John F. Tisdale, Jaroslaw P. Maciejewski, Olga Nuñez, Stephen J. Rosenfeld, and Neal S. Young

BLOOD, 15 DECEMBER 2002 • VOLUME 100, NUMBER 13

Table 1. Results at median follow-up of 38 months

	ATG/CSA (%)	Cy/CSA (%)
Overall response	13/16 (81)	8/15 (53)
CR	10 (63)	6 (40)
PRi	3 (18)	2 (13)
Relapse	6/13 (46)	2/8 (25)
Cytogenetic evolution	2/14 (14)	1/12 (8)

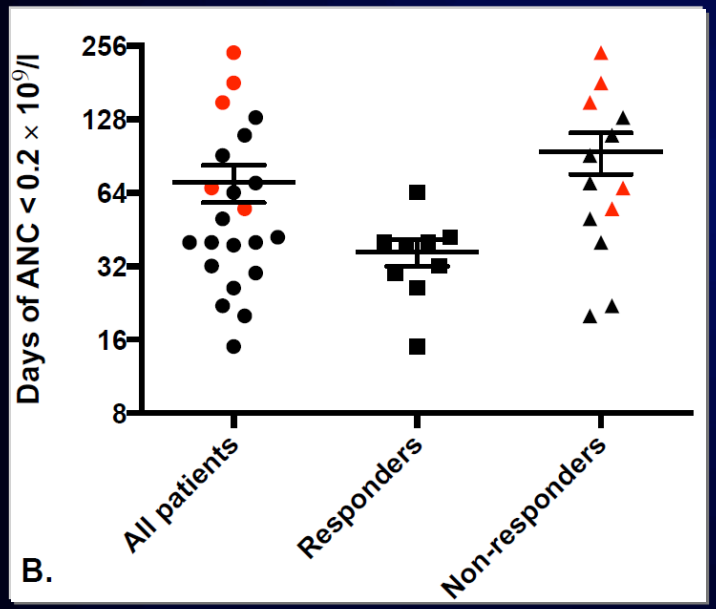
Long-term analysis (median 38m):

- **No difference in response**
- **No prevention of late complication of SAA/SAA treatment**

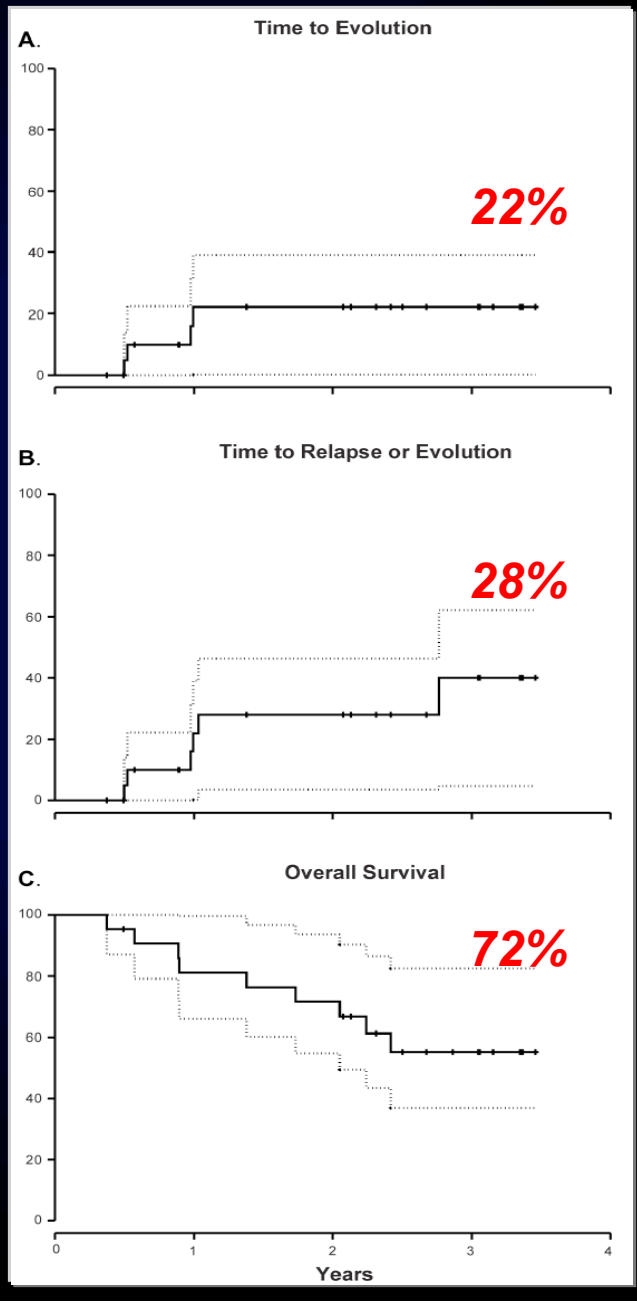
Moderate-dose cyclophospamide plus CsA for AA

The NIH experience (Scheinberg et al, Blood 2014 in press)

- ✓CTX 30 mg/kg x 4 dd (total dose 120 mg) + CsA
- ✓N=22, all naive (2010-2012)
- ✓OR 9/22 (41%)
- ✓Severe and long-lasting neutropenia



- ✓Confirmed IFI n=6;
- ✓Early termination due to unacceptable toxicity
- ✓No reason to further investigate this regimen



Activity of alemtuzumab monotherapy in treatment-naive, relapsed, and refractory severe acquired aplastic anemia

Phillip Scheinberg,[1] Olga Nunez,[1] Barbara Weinstein,[1] Priscila Scheinberg,[1] Colin O. Wu,[2] and Neal S. Young[1]

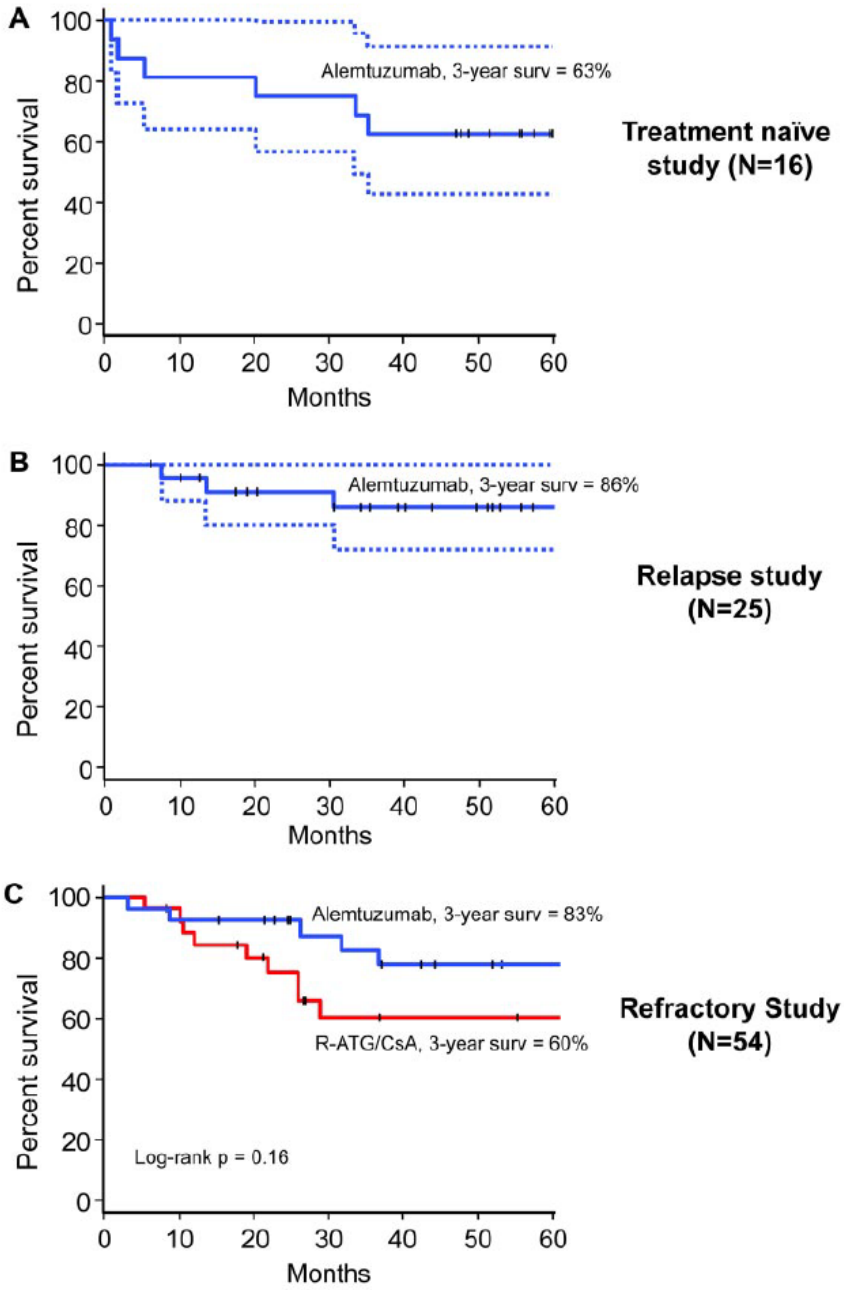
Blood 2012

Response	Treatment-naive study (n = 16) Alemtuzumab (95% CI)
3-mo	19% (0-40)
6-mo	19% (0-40)

?

Response	Relapse study (n = 25) Alemtuzumab (95% CI)
3-mo	48% (27-69)
6-mo	56% (35-77)

Response	Refractory study (n = 54) Rabbit ATG (95% CI)	Alemtuzumab (95% CI)
3-mo	19% (3-34)	19% (3-34)
6-mo	33% (14-52)	37% (18-57)



bjh short report

Risitano et al, 2010

Alemtuzumab is safe and effective as immunosuppressive treatment for aplastic anaemia and single-lineage marrow failure: a pilot study and a survey from the EBMT WPSAA

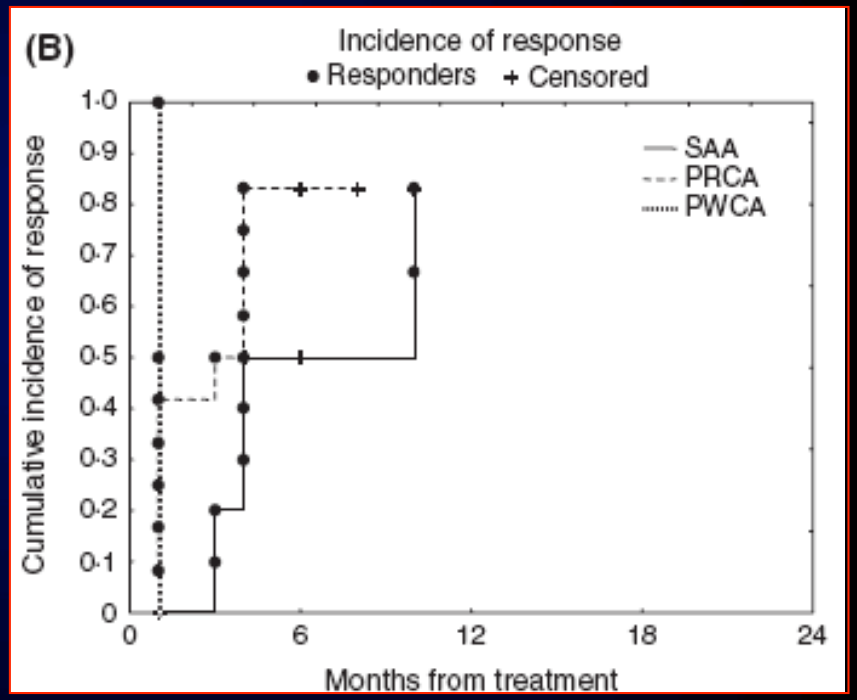
EBMT

✓*Phase II prospective study with s.c. alemtuzumab (73-103 mg in 5 days)*

✓*N=28 (AA=13, PRCA=13, PWCA=2); first line and salvage*

Best Hematological Response

	n	CR	PR	OR
SAA	13	5	4°	69%
PRCA	13	8	3	85%
PWCA	2	2	0	100%



✓s.c. alemtuzumab is feasible and safe (no increased infectious morbidity)

✓Remarkably effective, especially in single lineage marrow failures

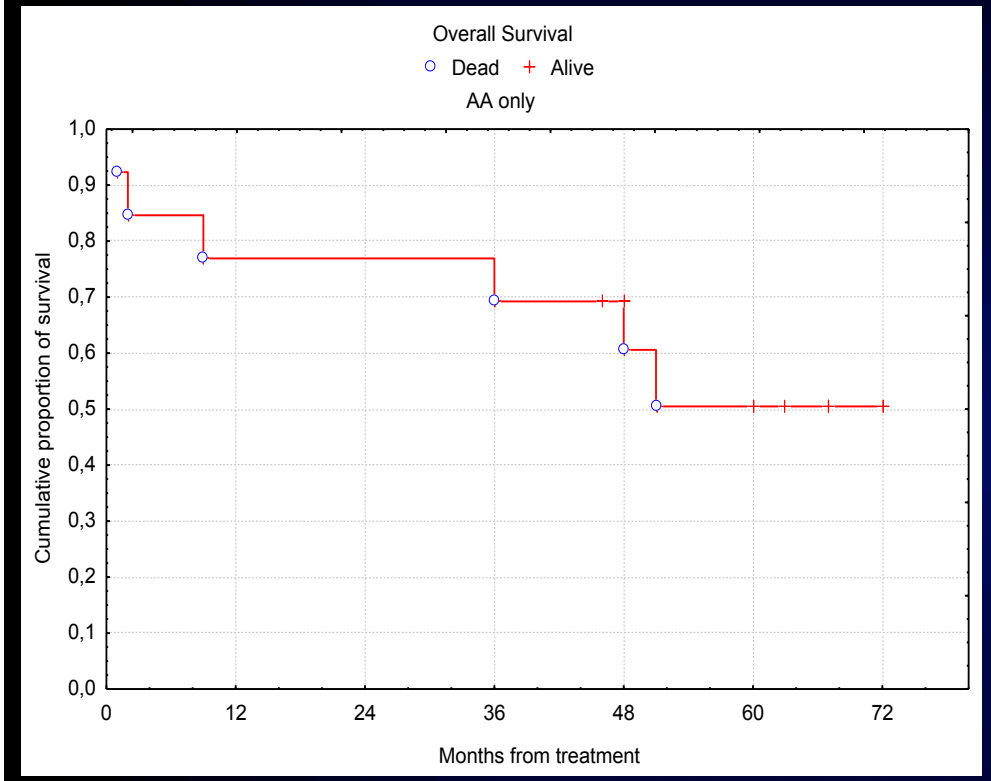
✓Frequent relapses (maintenance IS or retreatment needed)

✓Late failures due to refractory relapses (15%) or clonal evolution (15%)

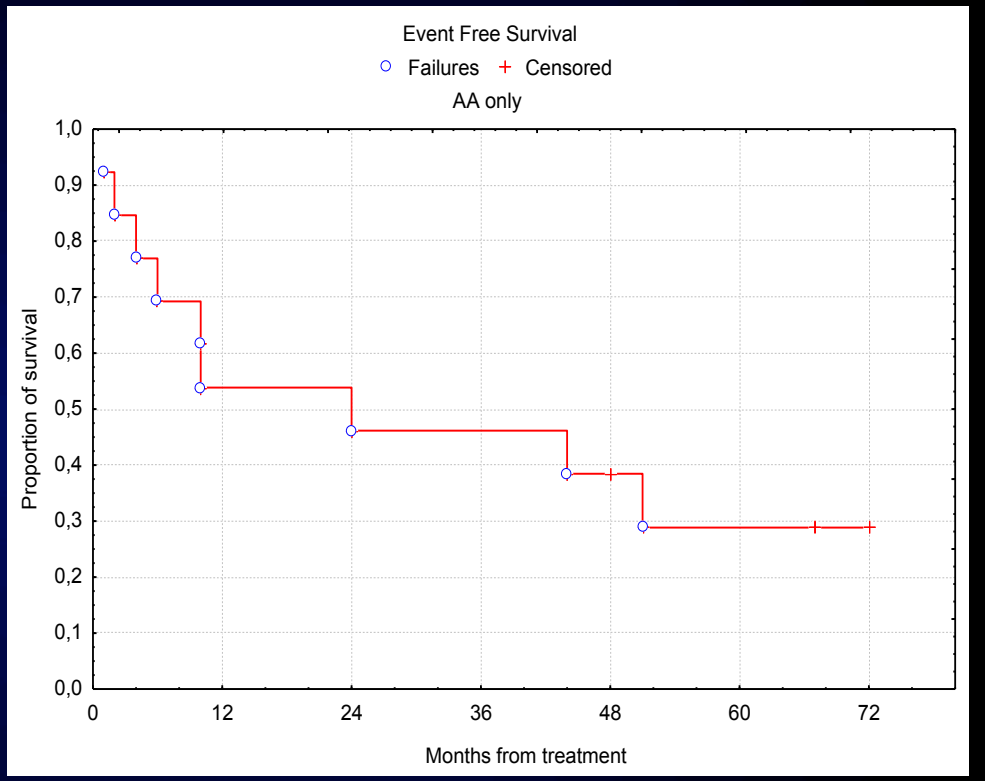
Alemtuzumab for marrow failure syndromes

Long-term follow up (median 4 years, March 2014)

Overall Survival



Event Free Survival



Long-term outcome (AA only)

- ✓ 4 out 13 in current remission (3 CR, 1 VGPR)
- ✓ Late failures: 2 clonal evolution (non-responders), 2 refractory relapses
- ✓ No late infectious complications

The lesson from alternative IST for AA

Take home messages

1. **Different IS agents are biologically active as IST for AA**
   - ✓ Different ATG preparations, cyclophosphamide, alemtuzumab
   - ✓ Other (novel) agents seems failing in demonstrating any benefit (mostly as third drug)
2. **Lymphocyte depletion remains the most likely mechanisms of action of IST**
   - ✓ But the equation more profound lymphocyte depletion = better clinical response has been proven wrong
   - ✓ Effect on specific lymphocyte subsets?
3. **Attempts to improve non-transplant treatment for AA may be focused on other mechanism of action**
   - ✓ Targeted IST agents may have a role in specific phase of AA treatment (i.e. induction or maintenance) or in combination with some standard agents (i.e., synergism)
   - ✓ Non-IST agent may play a more relevant role: eltrombopag

Aplastic Anemia: Management of Adult Patients

Jaroslaw P. Maciejewski and Antonio M. Risitano

REASONS FOR TREATMENT FAILURE

- Pathophysiology other than immune-mediated
- Irreversible stem cell deficit
- Insufficient immunosuppression

Eltrombopag???

ELTROMBOPAG IN SAA

The status of art

NEJM

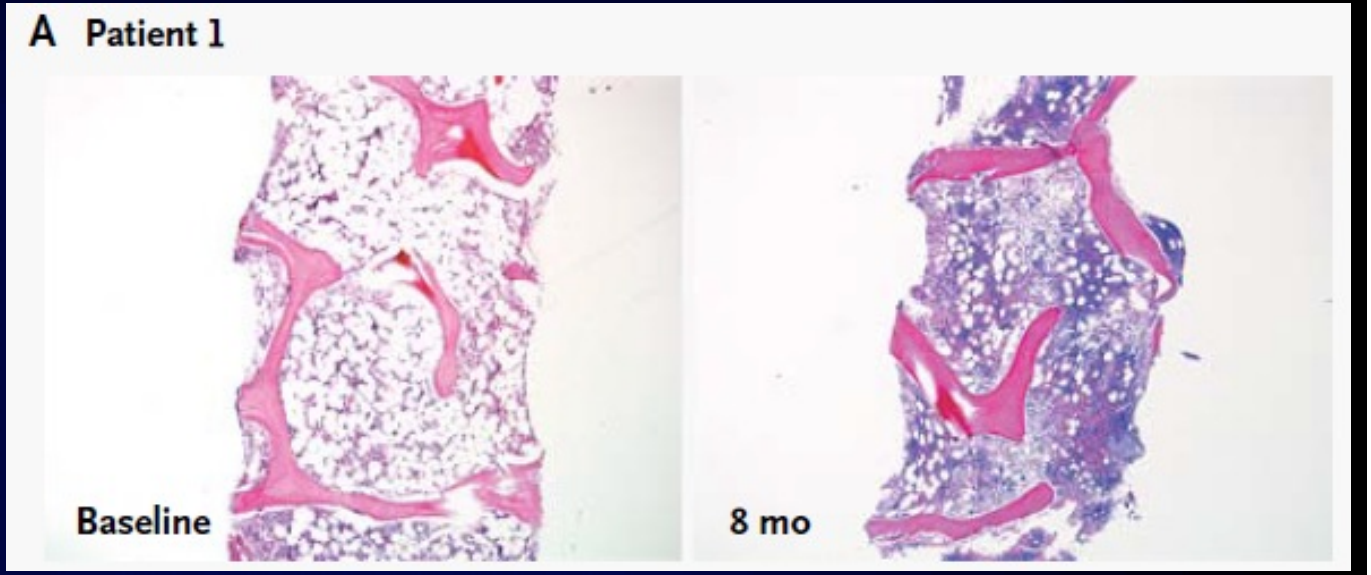
Eltrombopag and Improved Hematopoiesis in Refractory Aplastic Anemia

Phase II study

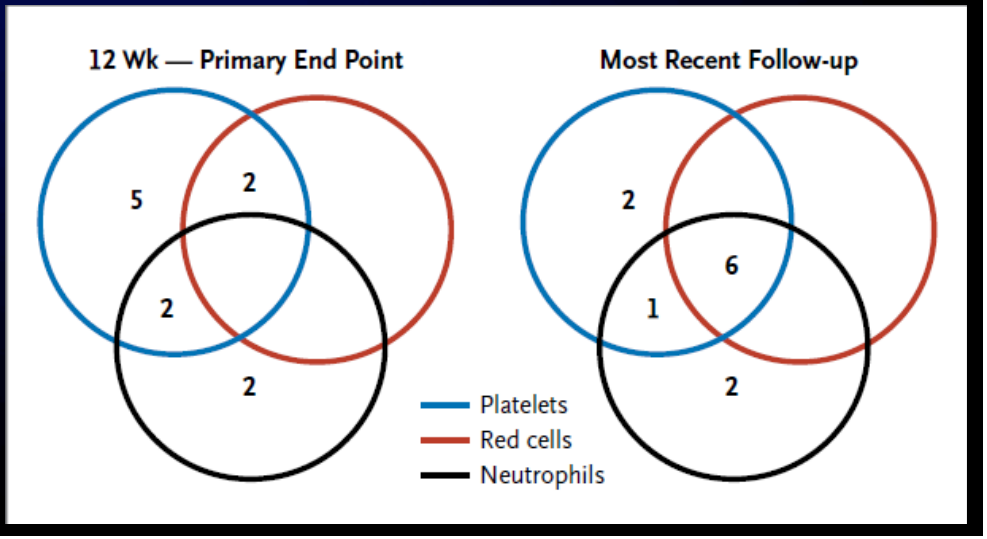
n=25

Refractory SAA

Eltrombopag 50-150 mg, orally, for 12 weeks



- ✓44% hematological response (at least 1 lineage)
  - ✓ Plt response 36%
  - ✓ Hb response 24%
  - ✓ ANC response 36%
- ✓Increased marrow cellularity (resp.)
- ✓Minimal toxicity, no fibrosis



ELTROMBOPAG IN SAA

The risk of clonal evolution

Regular Article

CLINICAL TRIALS AND OBSERVATIONS

CME Article

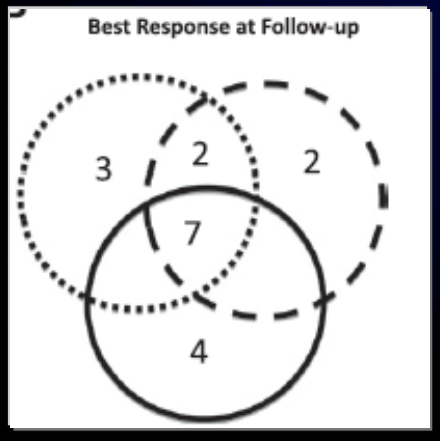
Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug

Ronan Desmond,[1] Danielle M. Townsley,[1] Bogdan Dumitriu,[1] Matthew J. Olnes,[2] Phillip Scheinberg,[3] Margaret Bevans,[4] Ankur R. Parikh,[1] Kinneret Broder,[1] Katherine R. Calvo,[5] Colin O. Wu,[6] Neal S. Young,[1] and Cynthia E. Dunbar[1]

BLOOD, 20 MARCH 2014 •

VOLUME 123, NUMBER 12

- ✓**Additional 18 patients (n=43), OR 17/43 (40%)**
- ✓**Long-term follow up**
  - ✓ **Eltrombopag discontinued in 5 robust VGPR, with sustained response**
- ✓**Clonal evolution in 8/43 (18%), mostly in non-responders (6/8); no RAEB/AML**
  - ***NR: 7-/del(7) [n=5], +8 [n=1]***
  - ***R: del(13) [n=2]***



Age (y)	Response	CGH (SNP-based) Baseline	At evolution	Time on eltrombopag (mo)	Dysplasia	Outcome
60	NR	46XY[20]	−7[20]	3	N	Died of progressive cytopenias
18	NR	46XX[6]	+8[9]/46XX[11]	3	N	Transplanted successfully
20	NR	46XY[20]	−7[5]t(1;16) [3]/46XY[12]	3	N	Transplanted successfully
67	R	46XY[20]	del(13)[19]/46XY[1]	13	Mild dyserythropoeisis	Transplanted
41	NR	46XY[20]	+21[3]/46XY[17] −7[2]/46XY[19]	3 6	Mild dyserythropoeisis	Awaiting transplant
66	R	46XY[20]	46XY del13q[2]/46XY[18]	9	N	Under observation
23	NR	46XY[20]	−7[5],XY[15]	3	N	Transplanted successfully
17	NR	No metaphases	+1,der(1;7) [4]/46XY[16]	3	N	Transplanted successfully

ELTROMBOPAG IN SAA

The status of art

U.S. Food and Drug Administration

Protecting and Promoting *Your* Health

FDA Approvals > Medscape Medical News

FDA OKs Eltrombopag (Promacta) for Severe Aplastic Anemia

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use PROMACTA safely and effectively. See full prescribing information for PROMACTA.

PROMACTA (eltrombopag) tablets, for oral use
Initial U.S. Approval: 2008

WARNING: RISK FOR HEPATIC DECOMPENSATION IN PATIENTS WITH CHRONIC HEPATITIS C

See full prescribing information for complete boxed warning

In patients with chronic hepatitis C, PROMACTA in combination with interferon and ribavirin may increase the risk of hepatic decompensation. (5.1)

- **Chronic ITP:** Initiate PROMACTA at 50 mg once daily for most patients. Reduce initial dose in patients with hepatic impairment and/or patients of East Asian ancestry. Adjust to maintain platelet count greater than or equal to 50 x 10^9/L. Do not exceed 75 mg per day. (2.1)
- **Chronic Hepatitis C-associated Thrombocytopenia:** Initiate PROMACTA at 25 mg once daily for all patients. Adjust to achieve target platelet count required to initiate antiviral therapy. Do not exceed a daily dose of 100 mg. (2.2)
- **Severe Aplastic Anemia:** Initiate PROMACTA at 50 mg once daily for most patients. Reduce initial dose in patients with hepatic impairment or patients of East Asian ancestry. Adjust to maintain platelet count greater than 50 x 10^9/L. Do not exceed 150 mg per day. (2.3)

---------- DOSAGE FORMS AND STRENGTHS ----------

12.5-mg, 25-mg, 50-mg, 75-mg, and 100-mg tablets. (3)

EBMT studies for AA

	moderate AA (EMAA)	vSAA / SAA (RACE)
Primary objective	PR + CR at 6 months	CR at 3 months
Inclusion criteria	- age ≥ 18 years - Treatment requiring MAA (transfusion dependency or ANC < 1G/l or Thrombo < 30G/l or Hb < 8,5g/dl & Reti < 60G/l)	- age ≥ 15 years - SAA/ vSAA - No primary allo-SCT
Treatment	**CsA + Eltrombopag** versus CsA + Placebo	**hATG (ATGAM) + CsA + Eltrombopag** versus h ATG + CsA
Eltrombopag Dosage	150 mg (225 mg)	150 mg
Design	Placebo controlled	Open lable
Patient number	2 x 58	2 x 100
Sponsor	University hospital Ulm	EBMT

THE EMAA trial

Eltrombopag in moderate Aplastic Anemia (MAA) and Supportive Care in Aplastic Anemia

Britta Höchsmann &
Hubert Schrezenmeier

Institute of Clinical Transfusion Medicine and Immunogenetics Ulm
German Red Cross Blood Donor Services Baden-Wuerttemberg - Hessia
& Institute of Transfusion Medicine, University Hospital of Ulm

THE RACE trial

A prospective Randomized multicenter study comparing horse Antithymocyte globuline (hATG) + Cyclosporine A (CsA) ± Eltrombopag as front-line therapy for severe aplastic anemia patients.

PRINCIPAL INVESTIGATORS

Regis Peffault de Latour (Paris)

Antonio M Risitano (Naples)

A prospective Randomized multicenter study comparing horse Antithymocyte globuline (hATG) + Cyclosporine A (CsA) with or without Eltrombopag as front-line therapy for severe aplastic anemia patients – RACE STUDY(1)

RACE Trial

11 March 2016

Working party	Principal investigators	Trial Coordinator
SAA-WP	**Antonio M Risitano / Regis Peffault de Latour**	**Marleen van Os**

To investigate whether **Eltrombopag** (Revolade, GSK) added to standard immune-suppressive treatment, CsA + hATG (ATGAM, Pfizer) increases the rate of early complete response in untreated AA patients*

* Patients will be stratified by age and disease severity

Participating countries

THE EBMT RACE STUDY

Study design

- ✓An **EBMT Severe Aplastic Anemia Working Party study** (approved by the CTO), entirely funded by Novartis and Pfizer
- ✓Aim of the study: to improve the current standard treatment for SAA
  - ✓ To improve the **robustness of hematological response** of SAA patients receiving IST
- ✓Prospective, open label, phase III randomized study
  - ✓ Control arm: horse ATG (40 mg/kg x 4dd, iv) + cyclosporine (5 mg/kg, os)
  - ✓ Investigational arm: horse ATG + cyclosporine + eltrombopag (150 mg/die, os)
- ✓Type B trial, because eltrombopag may theoretically result in a somewhat higher risk (mostly clonal evolution) in comparison to standard medical care
- ✓Participating centers: 30 sites from 7 EU Countries (France, Italy, UK, Germany, Spain, Netherlands, Switzerland)

THE EBMT RACE STUDY

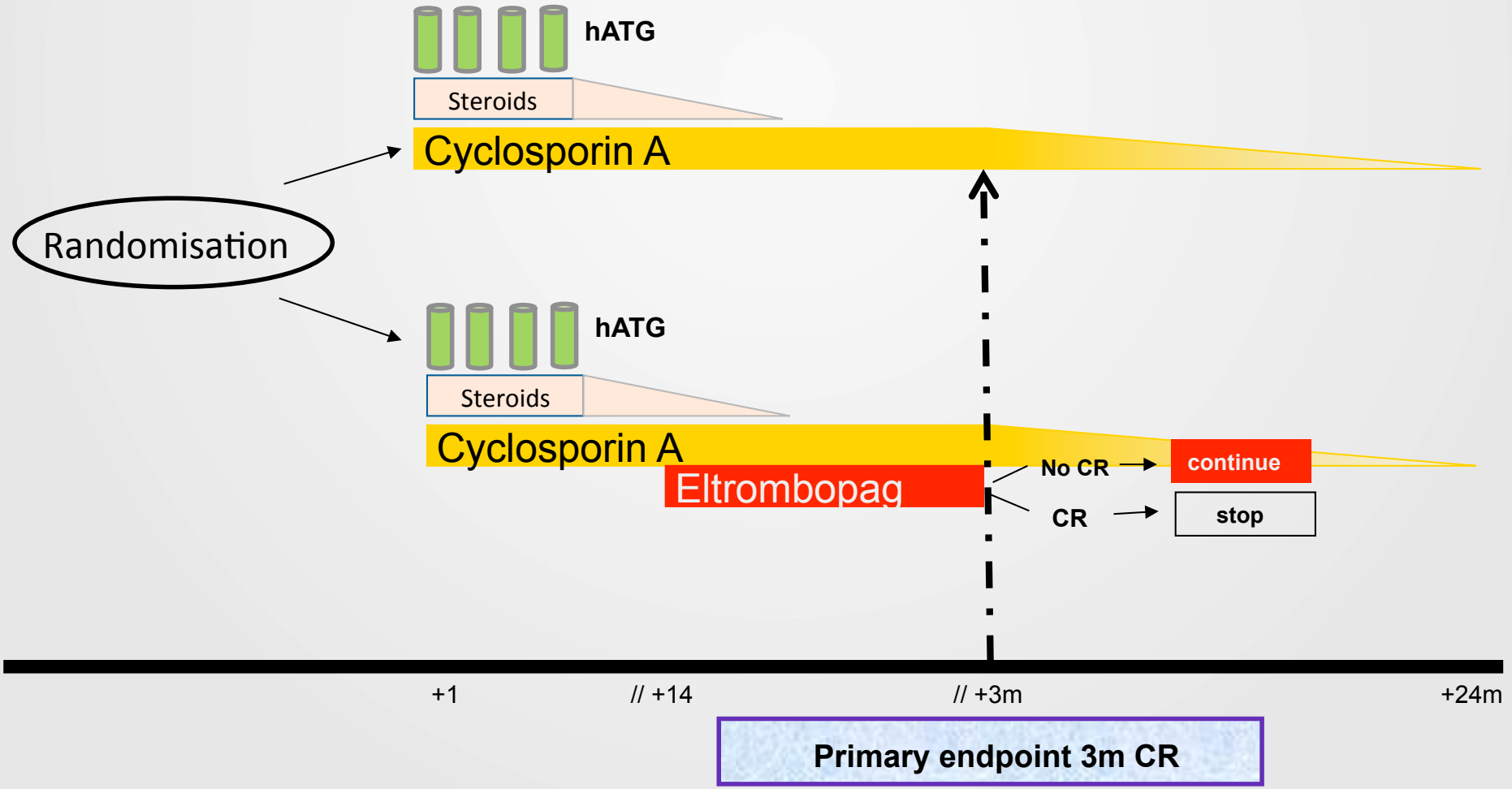
Statistical design

- ✓**Superiority study**
- ✓**Sample size calculation**
  - ✓ Aiming to increase the 3m CR rate from 7% *(Scheinberg, Haematologica 2010)* to 21% *(current NIH data)*
  - ✓ Sample size to reject the null hypothesis at 5% significance level (alpha-error) and with 80% power (two-sided test) is n=96 patients for treatment arm
  - ✓ Sample size increased by 4% to compensate for possibly not evaluable patients: **total number of 200 patients (100 each arm)**
- ✓**Randomization**
  - ✓ **1:1 randomization, including a stratified block design**
  - ✓ Stratification according to:
    - **Disease severity:**
      - Severe aplastic anemia (SAA)
      - Very severe aplastic anemia (VSAA: SAA plus ANC <200/µL)
    - **Age:**
      - >=15 and <40 year old
      - >=40 year old
- ✓**No stopping rules (study continuation led to discretion of the DMSB)**
- ✓**No interim analysis**

RACE STUDY (2)

SAA-WP

TREATMENT Scheme



THE EBMT RACE STUDY

Study flow-chart

Initial treatment

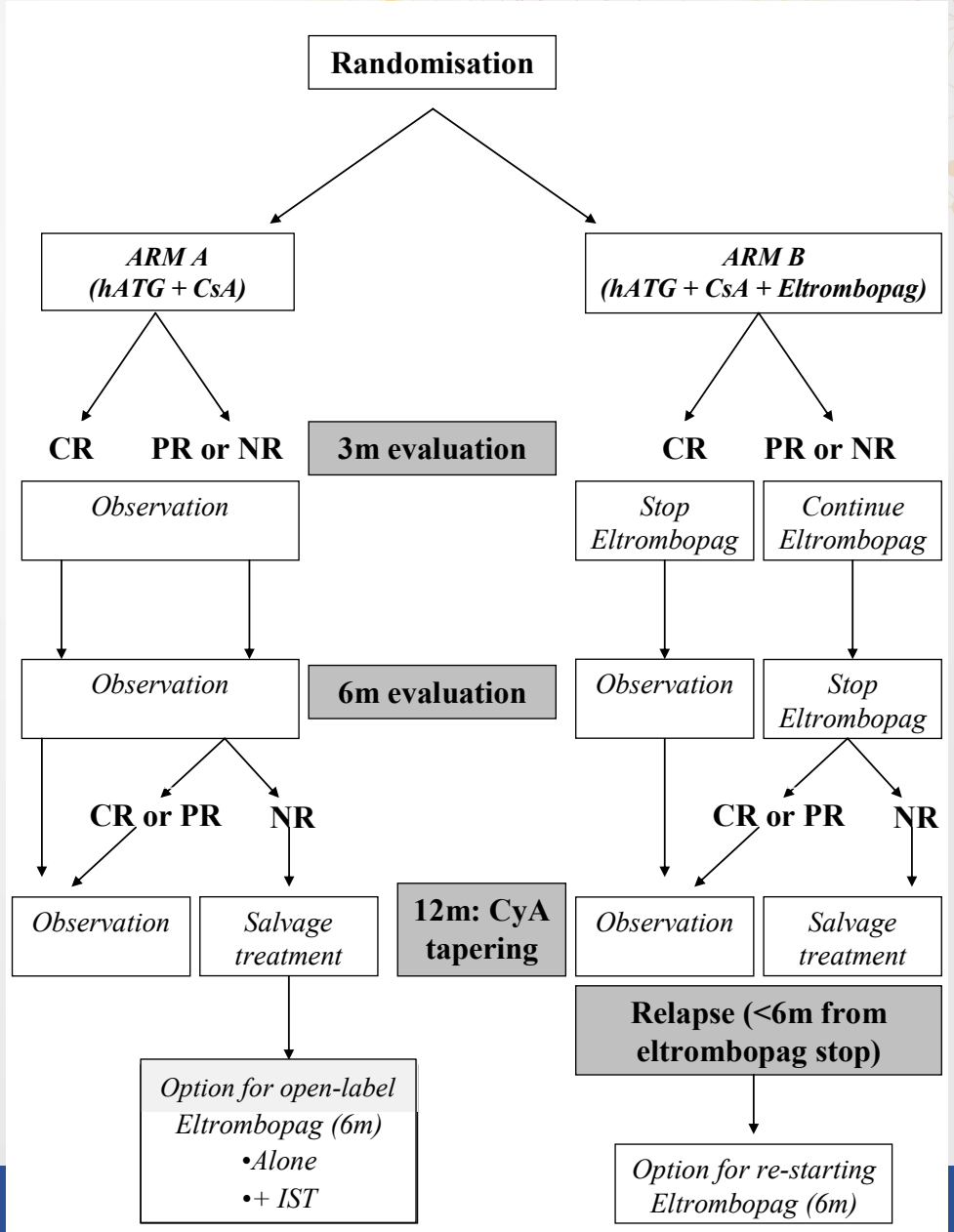
3 month evaluation:
primary endpoint

6 month evaluation:
stop eltrombopag
Possible cross-over (standard arm only)

12 month evaluation:

Relapse: possible eltrombopag re-starting (investigational arm only)

24 month evaluation: end of the study

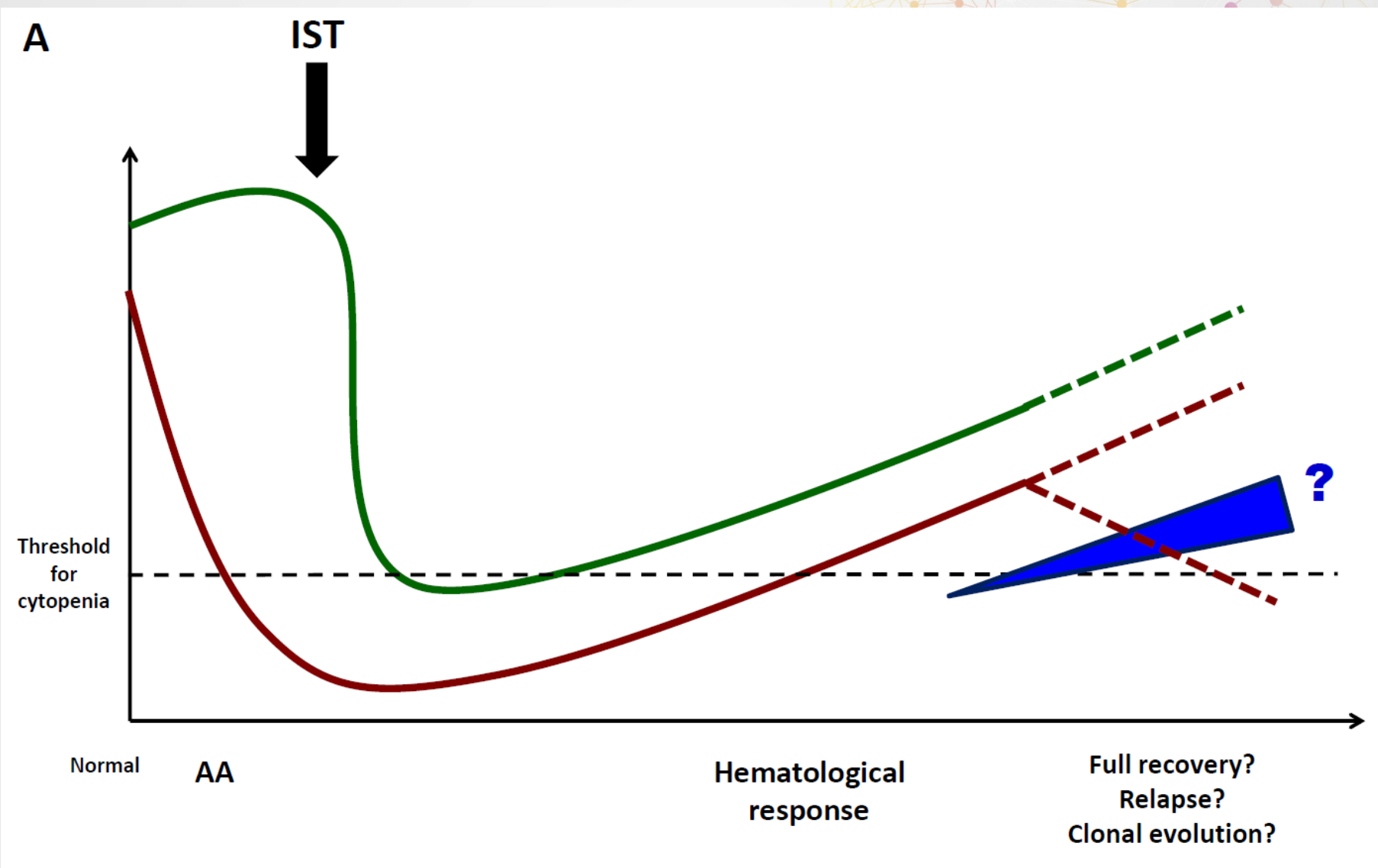


RACE trial – **participating sites**

Country	# sites
France	6
Germany	5
Italy	6
Netherlands	4
Spain	5
Switzerland	1
United Kingdom	5
Total	32

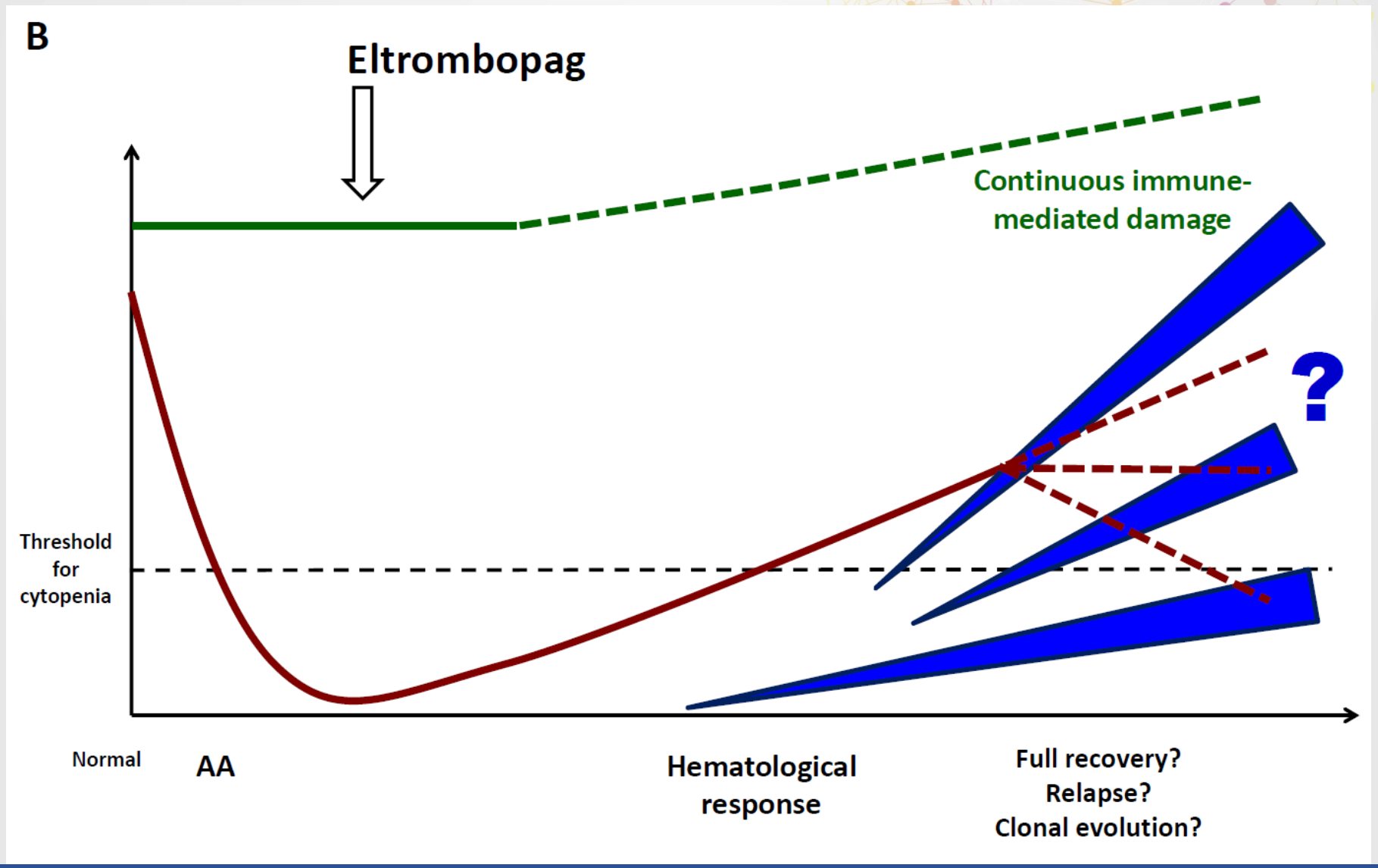
Hematopoiesis, immunity and (clonal) evolution

Risitano and Peffault De Latour, in press



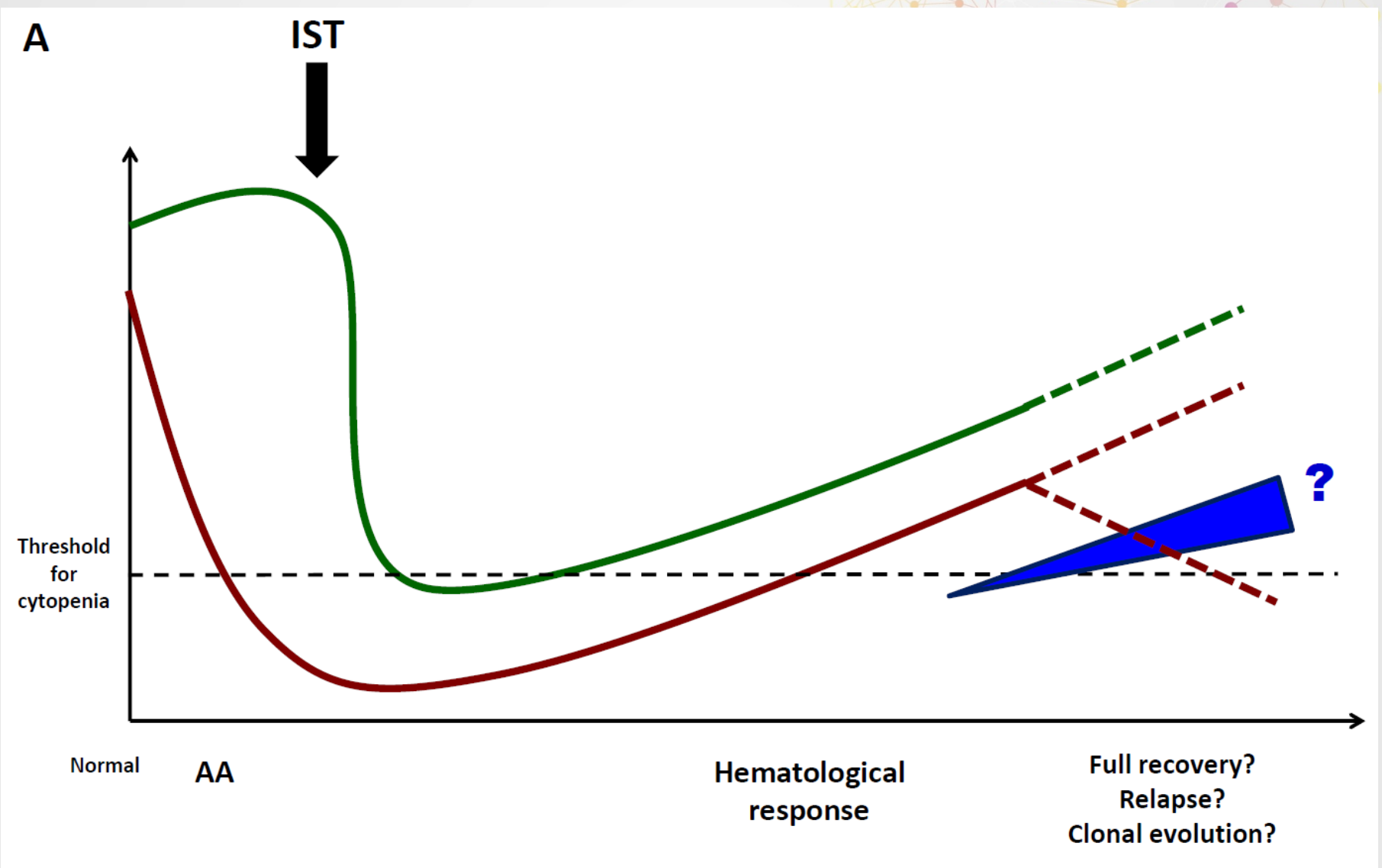
Hematopoiesis, immunity and (clonal) evolution

Risitano and Peffault De Latour, in press



Hematopoiesis, immunity and (clonal) evolution

Risitano and Peffault De Latour, in press



Hematopoiesis, immunity and (clonal) evolution

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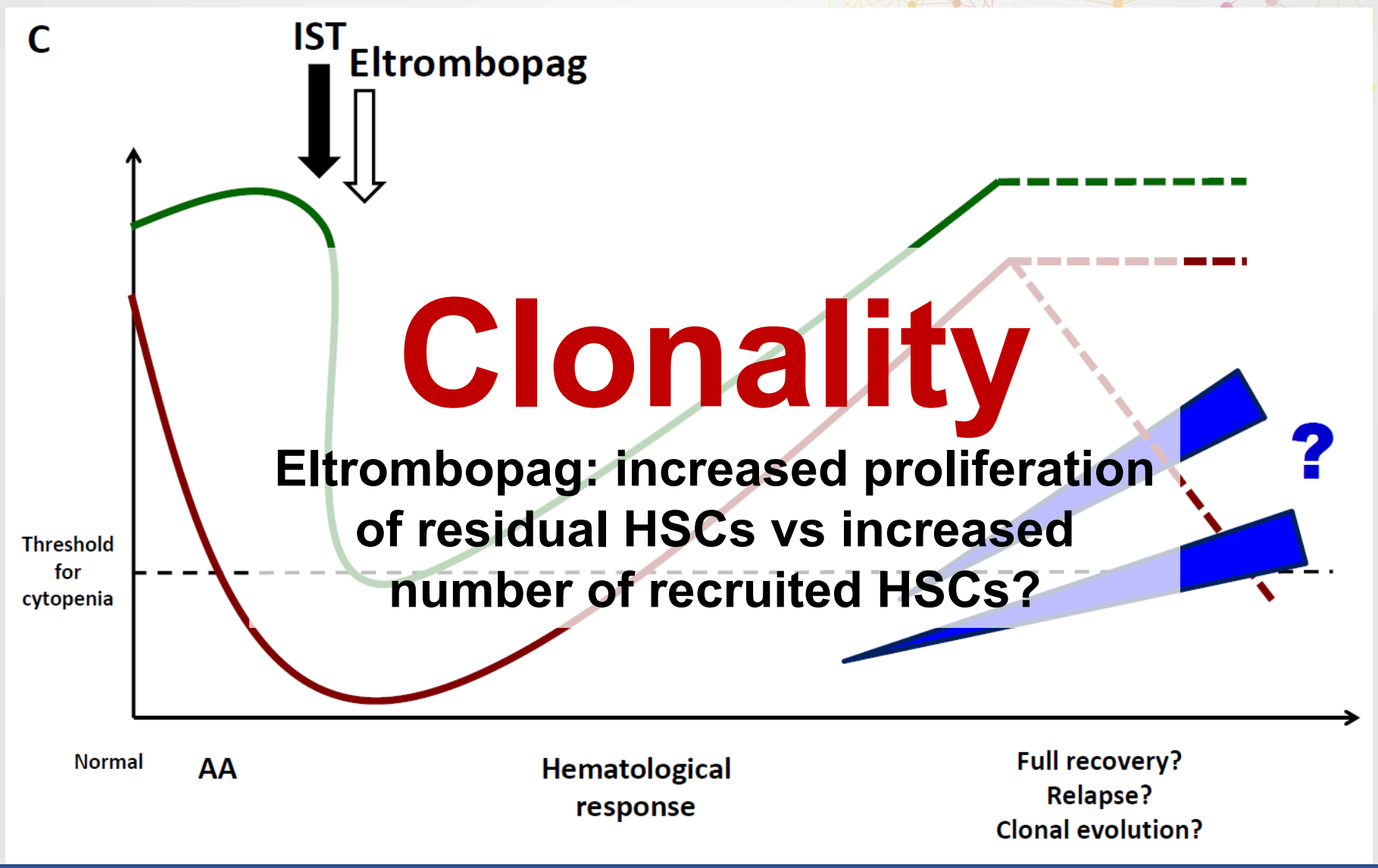

RACE trial – **ancillary biological study (King's College)**

Regular Article

MYELOID NEOPLASIA

Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome

Austin G. Kulasekararaj,[1,2] Jie Jiang,[1,2] Alexander E. Smith,[1,2] Azim M. Mohamedali,[1,2] Syed Mian,[1] Shreyans Gandhi,[2] Joop Gaken,[1] Barbara Czepulkowski,[2] Judith C. W. Marsh,[1,2] and Ghulam J. Mufti[1,2]

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Table 3. Details of all the somatic mutations in the study

UPN	Gene	Mutant allele burden (%)	Variant class	Nucleotide and protein change	Constitutional DNA
2*	*ASXL1*	30	Frameshift insertion	c.1927_1928insG:p.G643fs	Skin
2*	*DNMT3A*	42	Nonsynonymous SNV	c.C1540G:p.L514V	Skin
2*	*ERBB2*	44	Nonsynonymous SNV	c.G922A:p.V308M	Skin
5*	*TET2*	5	Stopgain SNV	c.C3100T:p.Q1034X	Skin
6*	*ASXL1*	38	Stopgain SNV	c.C2242T:p.Q748X	Buccal
10*	*SRSF2*	43	Nonsynonymous SNV	c.C284T:p.P95L	Buccal
16*	*ASXL1*	23	Frameshift insertion	c.2469_2470insAG:p.L823fs	Skin
18*	*DNMT3A*	31	Nonsynonymous SNV	c.C2644T:p.R882C	Skin
19*	*IKZF1*	14	Nonsynonymous SNV	c.C640G:p.H214D	Skin
21*	*BCOR*	5	Stopgain SNV	c.C526T:p.Q176X	Buccal
29*	*ASXL1*	41	Stopgain SNV	c.G4068A:p.W1356X	Skin
33*	*BCOR*	68	Stopgain SNV	c.G4832A:p.W1611X	Skin
40*	*ASXL1*	31	Nonframeshift deletion	c.2894_2896del:p.965_966del	Buccal
46*	*MPL*	10	Nonsynonymous SNV	c.G1544T:p.W515L	Buccal
64	*DNMT3A*	47	Nonsynonymous SNV	c.C2644T:p.R882C	Skin
66	*ASXL1*	37	Frameshift deletion	c.2433delT:p.N811fs	Skin
67	*U2AF1*	19	Nonsynonymous SNV	c.C101A:p.S34Y	Skin
69	*ASXL1*	34	Stopgain SNV	c.C2077T:p.R693X	Buccal
70	*ASXL1*	2	Stopgain SNV	c.G2026T:p.E676X	Buccal
70	*BCOR*	14	Stopgain SNV	c.T912G:p.Y304X	Buccal
73	*BCOR*	6	Frameshift insertion	c.4834_4835insC:p.L1612fs	Skin
79	*ASXL1*	36	Stopgain SNV	c.G2026T:p.E676X	Buccal
81	*ASXL1*	3	Stopgain SNV	c.T2324G:p.L775X	Skin
88	*ASXL1*	7	Frameshift deletion	c.2126delC:p.A709fs	Skin
93	*DNMT3A*	8	Stopgain SNV	C2311T:p.R771X	Skin
94	*BCOR*	30	Splice site	splice site c.3052-2A>G	Skin
97	*DNMT3A*	7	Nonsynonymous SNV	c.C2644T:p.R882C	Buccal
107	*ASXL1*	30	Stopgain SNV	c.T2468G:p.L823X	Buccal
129	*DNMT3A*	5	Nonsynonymous SNV	c.G2207A:p.R736H	Skin
130	*DNMT3A*	5	Nonsynonymous SNV	c.G2645A:p.R882H	Skin
140	*BCOR*	5	Frameshift deletion	c.4760delC:p.P1587fs	Buccal
142	*DNMT3A*	1.5	Nonsynonymous SNV	c.C2644T:p.R882C	Buccal

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APLASTIC ANEMIA

Differential diagnosis with hypoplastic MDS

Characteristics	AA	hypoplastic MDS
dyserythropoiesis	sometimes	yes
abnormal neutrophil	no	yes
dysplastic megakaryocytes	no	yes
fibrosis	no	occasional
increased blasts	no	Sometimes (ALIPS)
CD34+ cells in BM	< 1.0%	sometimes increased
clonality	possible	sometimes
splenomegaly	absent	occasional

Bennett et al. Sem Hemato 2000;37:15-29

Bennett & Orazi. Haematologica 2009 Feb; 94(2):264-843-70

Hama A et al. Rinsho Ketsueki 2011 Aug ;52(8) :653-8

IMPROVING IMMUNOSUPPRSSIVE TREATMENT FOR AA

The history of a failure

1. **No benefit from the addition of a third drug over the hATG-CsA platform**
   - ✓ Mycophenolate mofetil (randomized NIH trial)
   - ✓ Rapamicine (open-label NIH trial)
2. **No benefit from using non-hATG based regimens**
   - ✓ Rabbit ATG (NIH, EBMT, etc)
   - ✓ Alemtuzumab (NIH, Naples)
   - ✓ Cyclophosphamide (John Hopkins, NIH)
3. **Novel immunosuppressive strategies**
   - ✓ Anti-cytokine mAbs (TNF, IFN, IL2/IL23, etc)
   - ✓ Daclizumab (anti-IL2R), alefacept (anti-LFA-3), efalizumab (anti-LFA-1)
   - ✓ Mesenchimal stem cells
   - ✓ Anti-CD26 (Begedina®): in development for aGvHD

Scheinberg et al BJH 2006; Scheinberg et al Haematologica 2009; Risitano et al BJH 2009; Scheinberg et al NEJM 2011; Marsh et al Blood 2013; Scheinberg et al Blood 2012