Corso Educazionale GITMO

GITMO

NAPOLI

Emopatie non maligne e trapianto:

Acquired Idiopathic Aplastic Anemia: non-HSCT treatment

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Federico II University of Naples

Azienda Ospedaliera Universitaria Federico II

Integrata con il Servizio Sanitario Regionale

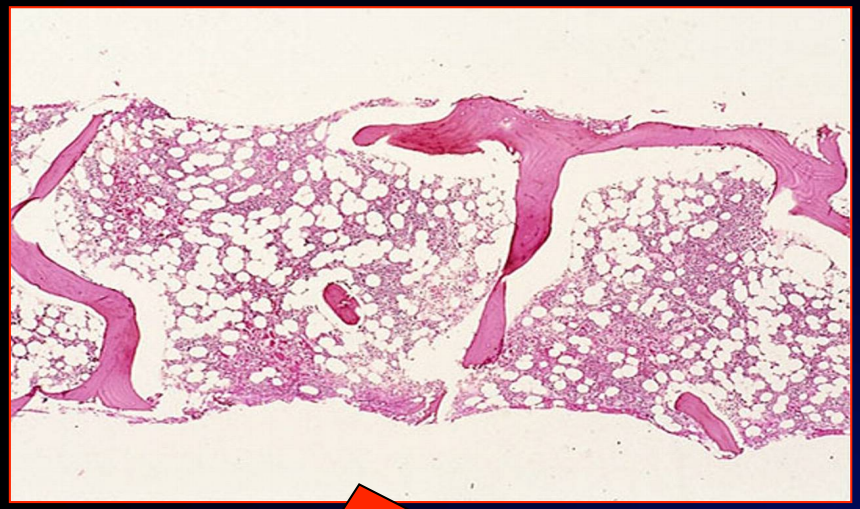
Welcome to Napoli!!!

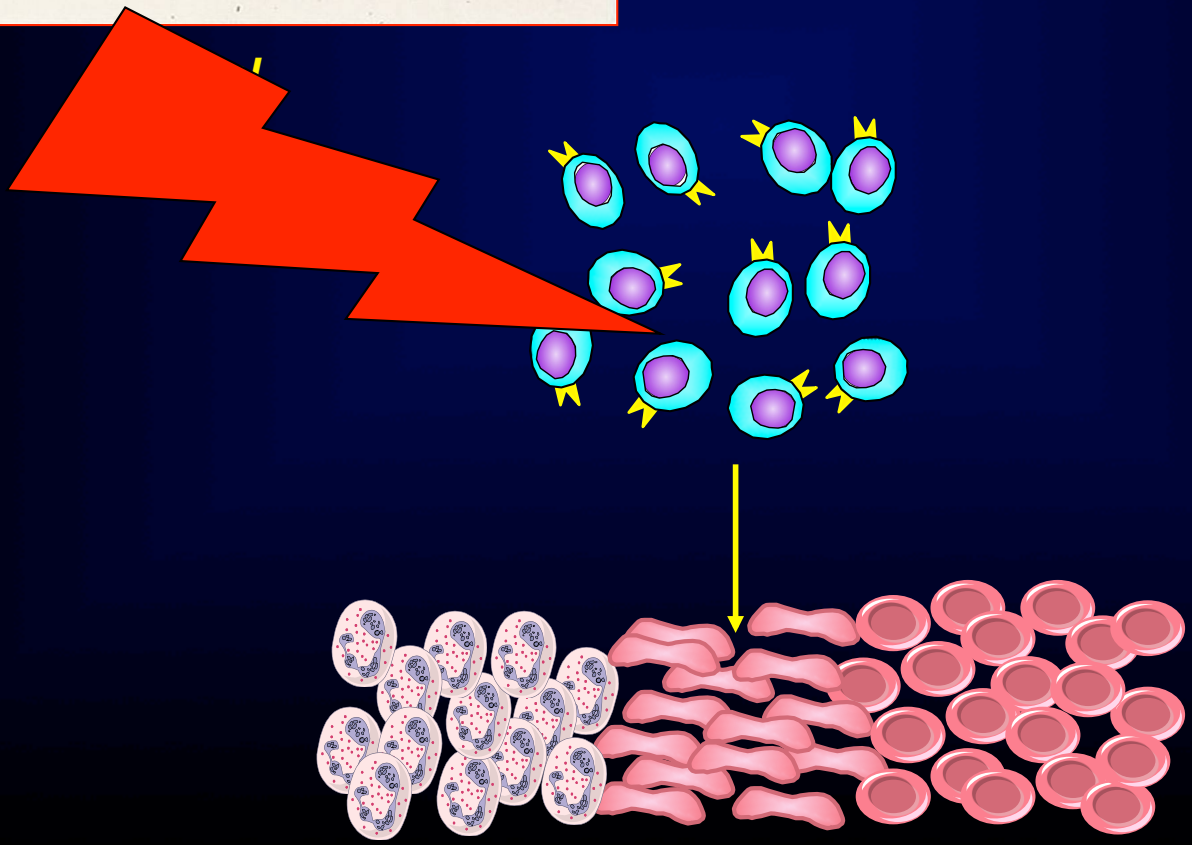
Thanks!!!

The most ancient public University in Europe
(July 5th, 1224)

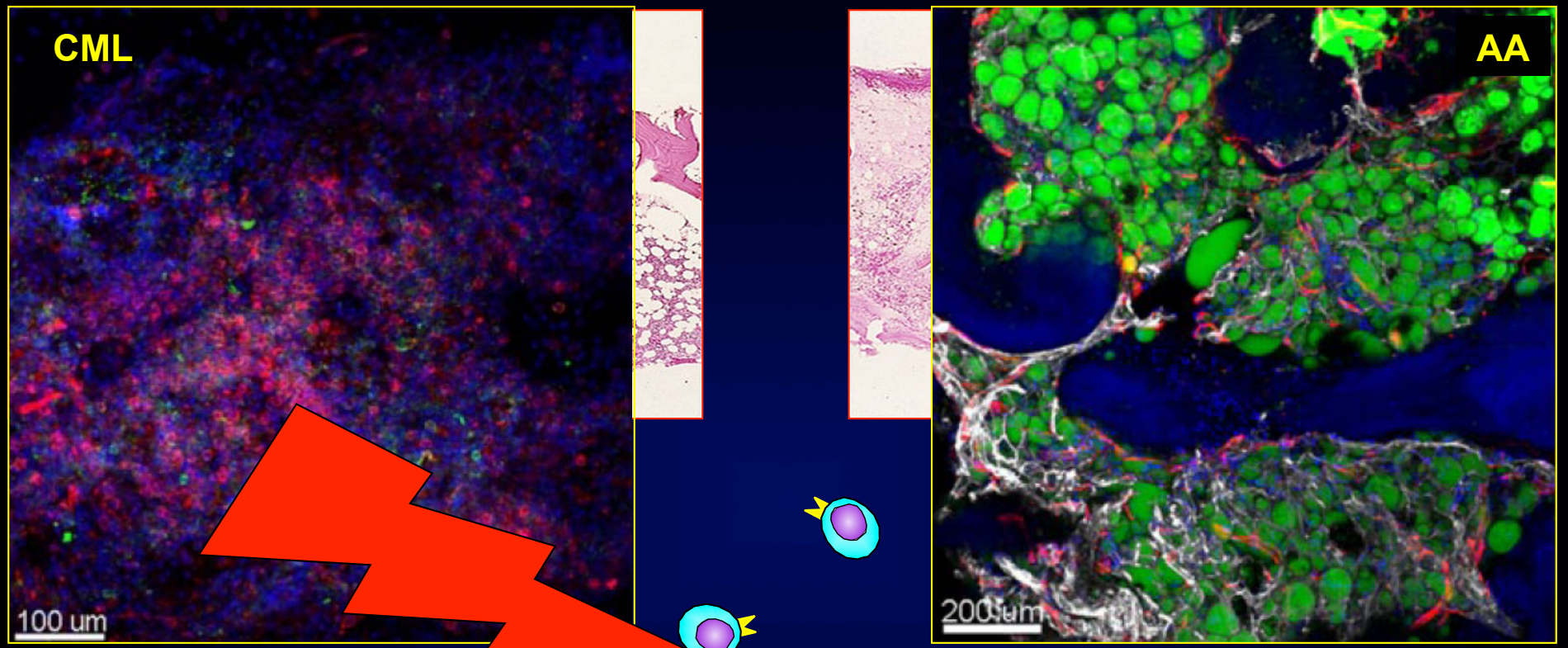
Prof. Bruno Rotoli **Prof. Carmine Selleri** **Prof. Fabrizio Pane**
Prof. Gennaro De Rosa
Dr. Serena Marotta **Dr. Francesco Grimaldi** **Dr. Patrizia Ricci**

Aplastic anemia





Aplastic anemia



Takaku et al, Blood 2010

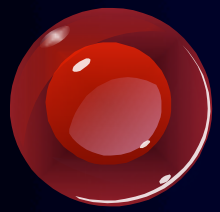
Takaku et al, Blood 2010

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

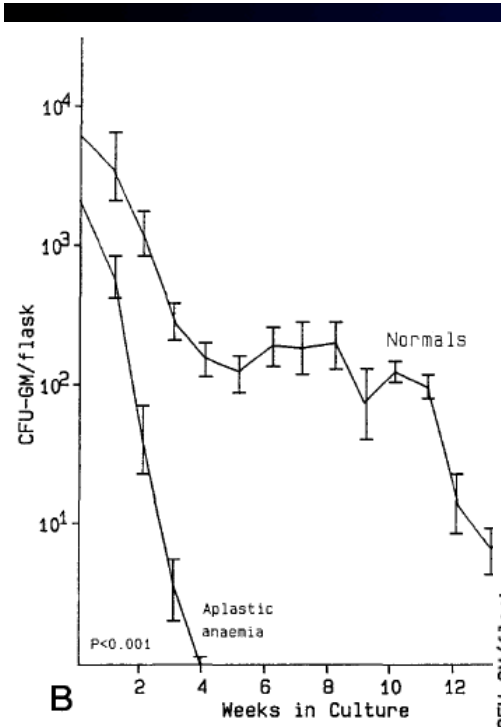
The hematopoietic defect in aplastic anemia assessed by long-t marrow culture

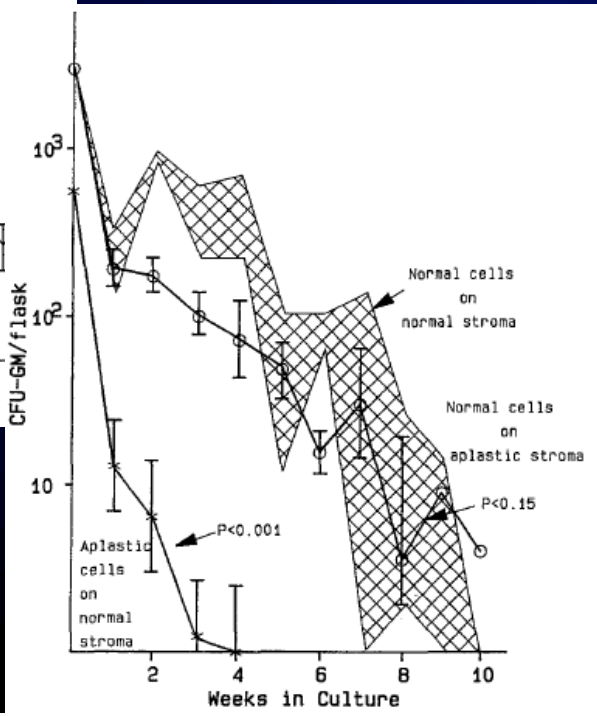
JC Marsh, J Chang, NG Testa, JM Hows and TM Dexter

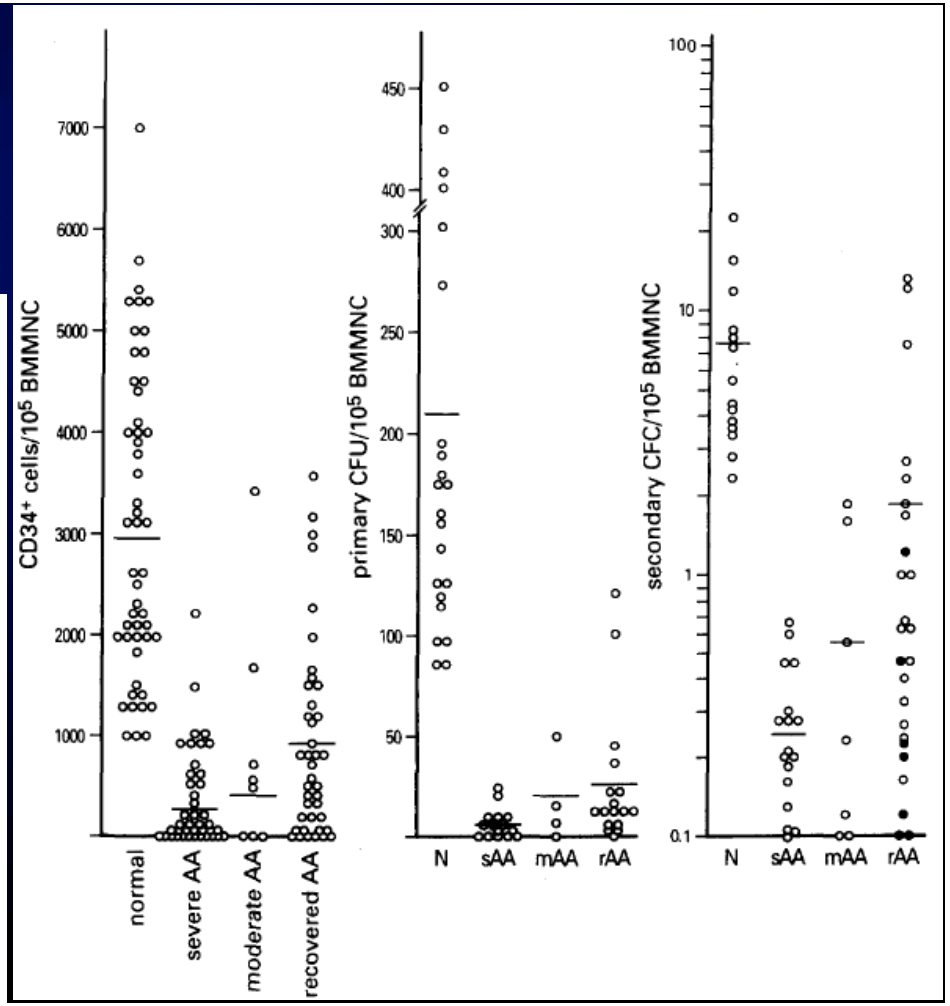
blood 1996 88: 1983-1991

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



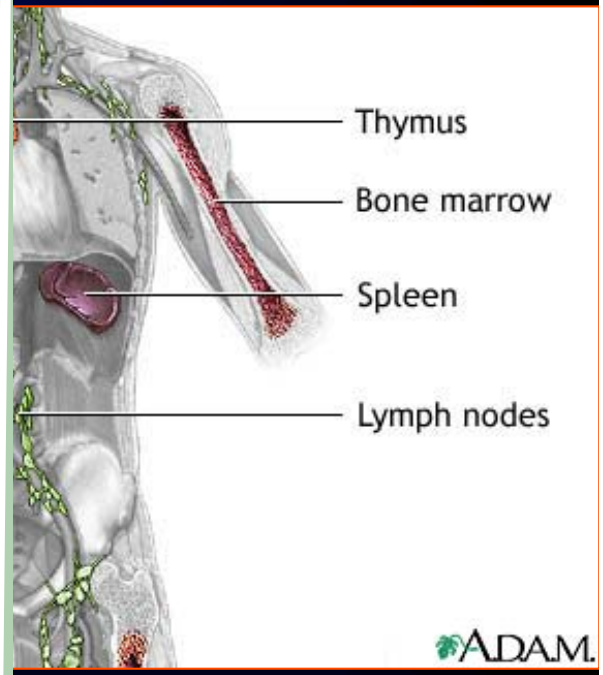




Pathophysiology of aplastic anemia

Hematopoietic stem cell

The immune system



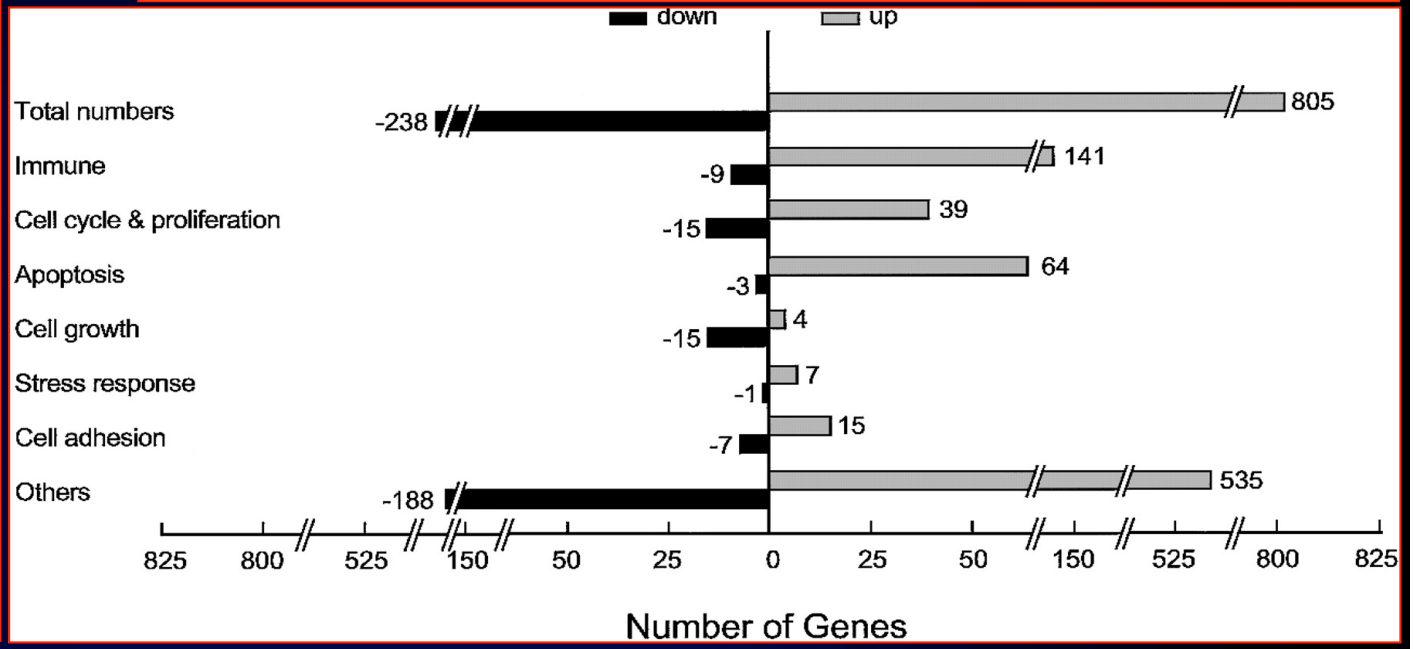
GENE EXPRESSION PROFILING IN CD34+ FROM AA PATIENTS

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."

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

THE NEW ENGLAND JOURNAL OF MEDICINE

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

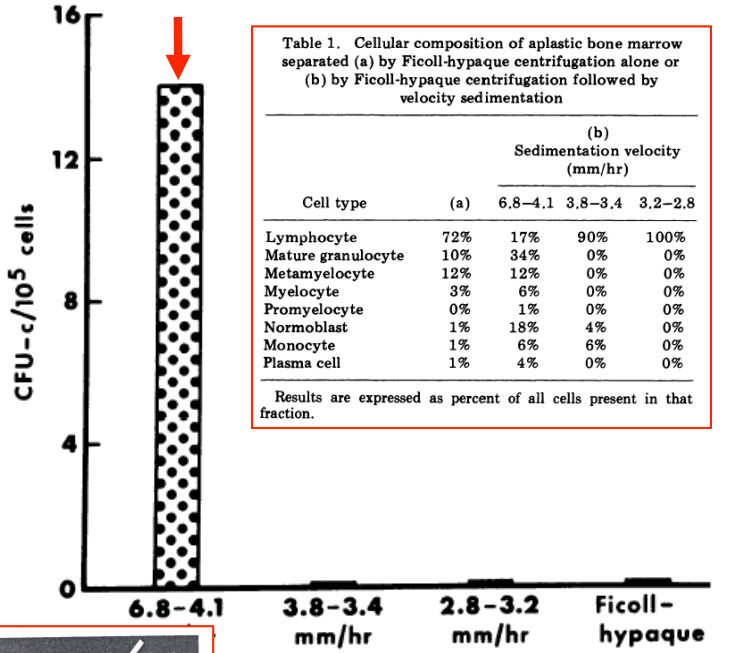
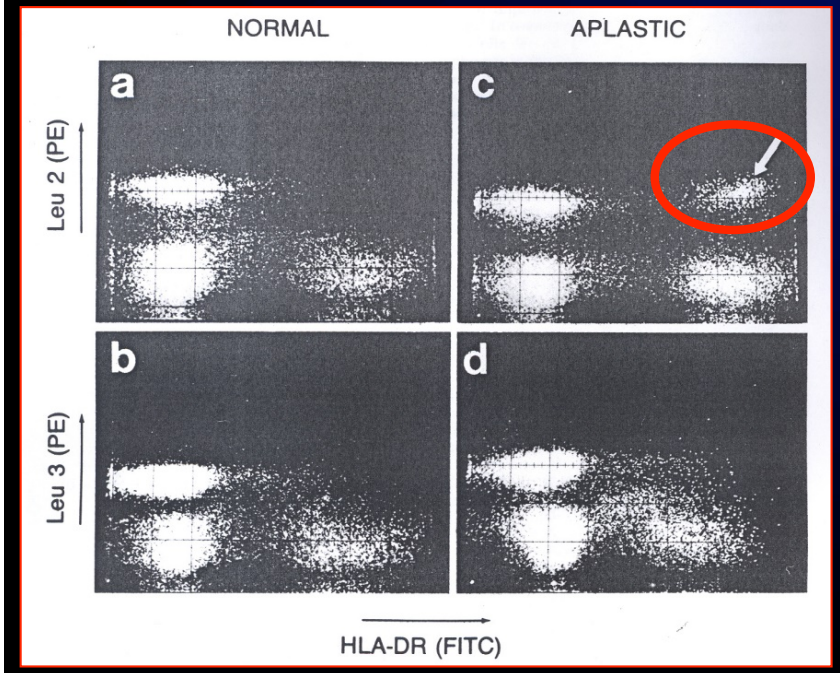


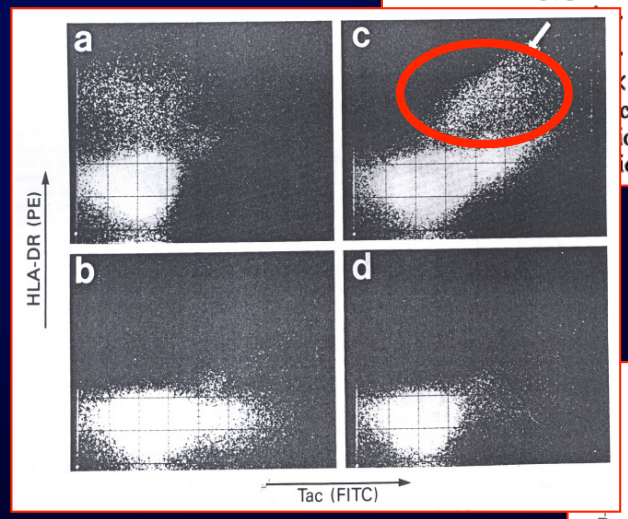
Table 1. Cellular composition of aplastic bone marrow separated (a) by Ficoll-hypaque centrifugation alone or (b) by Ficoll-hypaque centrifugation followed by velocity sedimentation

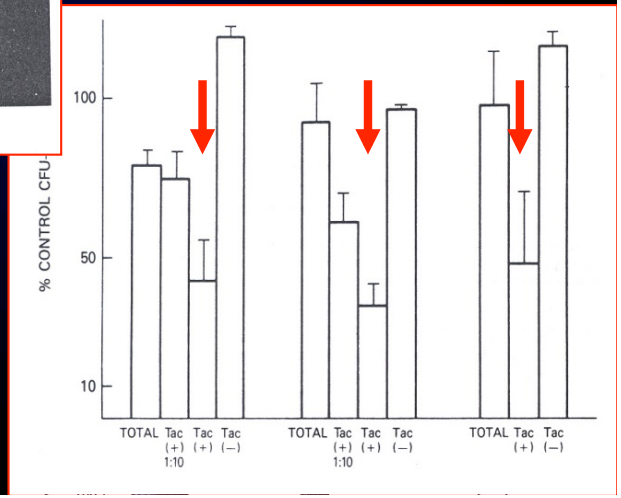
Cell type	(a)	(b) Sedimentation velocity (mm/hr) 6.8–4.1	3.8–3.4	3.2–2.8
Lymphocyte	72%	17%	90%	100%
Mature granulocyte	10%	34%	0%	0%
Metamyelocyte	12%	12%	0%	0%
Myelocyte	3%	6%	0%	0%
Promyelocyte	0%	1%	0%	0%
Normoblast	1%	18%	4%	0%
Monocyte	1%	6%	6%	0%
Plasma cell	1%	4%	0%	0%

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

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







T-cell clonality in aplastic anemia

A surrogate marker for Ag-driven immune response

Clonal Analysis of CD4⁺/CD8⁺ T Cells in a Patient with Aplastic Anemia

Ulrich Moebius,* Friedhelm Herrmann,‡ Thierry Hercend,§ and Stefan C. Meuer*
**Abteilung Angewandte Immunologie, Institut für Radiologie und Pathophysiologie, Deutsches Krebsforschungszentrum, 6900 Heidelberg, FRG, ‡Innere Medizin I, Albert Ludwig Universität, Freiburg, FRG, §Unité Biologie Cellulaire, Institute Gustave Roussy, 94800 Villejuif, France*

J. Clin. Invest. Volume 87, May 1991, 1567–1574

ELSEVIER

EXPERIMENTAL HEMATOLOGY

Experimental Hematology 23 (1995): 433

Establishment of a CD4+ T cell clone recognizing autologous hematopoietic progenitor cells from a patient with immune-mediated aplastic anemia.

Nakao S, Takamatsu H, Yachie A, Itoh T, Yamaguchi M, Ueda M, Shiobara S, Matsuda T.

Blood, Vol 89, No 10 (May 15), 1997: pp 3691-3699

Isolation of a T-Cell Clone Showing HLA-DRB1*0405-Restricted Cytotoxicity for Hematopoietic Cells in a Patient With Aplastic Anemia

By Shinji Nakao, Akiyoshi Takami, Hideyuki Takamatsu, Weihua Zeng, Naomi Sugimori, Hiroto Yamazaki, Yuji Miura, Mikio Ueda, Shintaro Shiobara, Takeshi Yoshioka, Toshihiko Kaneshige, Masaki Yasukawa, and Tamotsu Matsuda

Changes in T-cell receptor VB repertoire in aplastic anemia: effects of different immunosuppressive regimens

Hoon Kook, Antonio M. Risitano, Weihua Zeng, Marcin Wlodarski, Craig Lottemann, Ryotaro Nakamura, John Barrett, Neal S. Young, and Jaroslaw P. Maciejewski

BLOOD, 15 MAY 2002 • VOLUME 99, NUMBER 10

Oligoclonal and polyclonal CD4 and CD8 lymphocytes in aplastic anemia and paroxysmal nocturnal hemoglobinuria measured by Vβ CDR3 spectratyping and flow cytometry

BLOOD, 1 JULY 2002 • VOLUME 100, NUMBER 1

Antonio M. Risitano, Hoon Kook, Weihua Zeng, Guibin Chen, Neal S. Young, and Jaroslaw P. Maciejewski

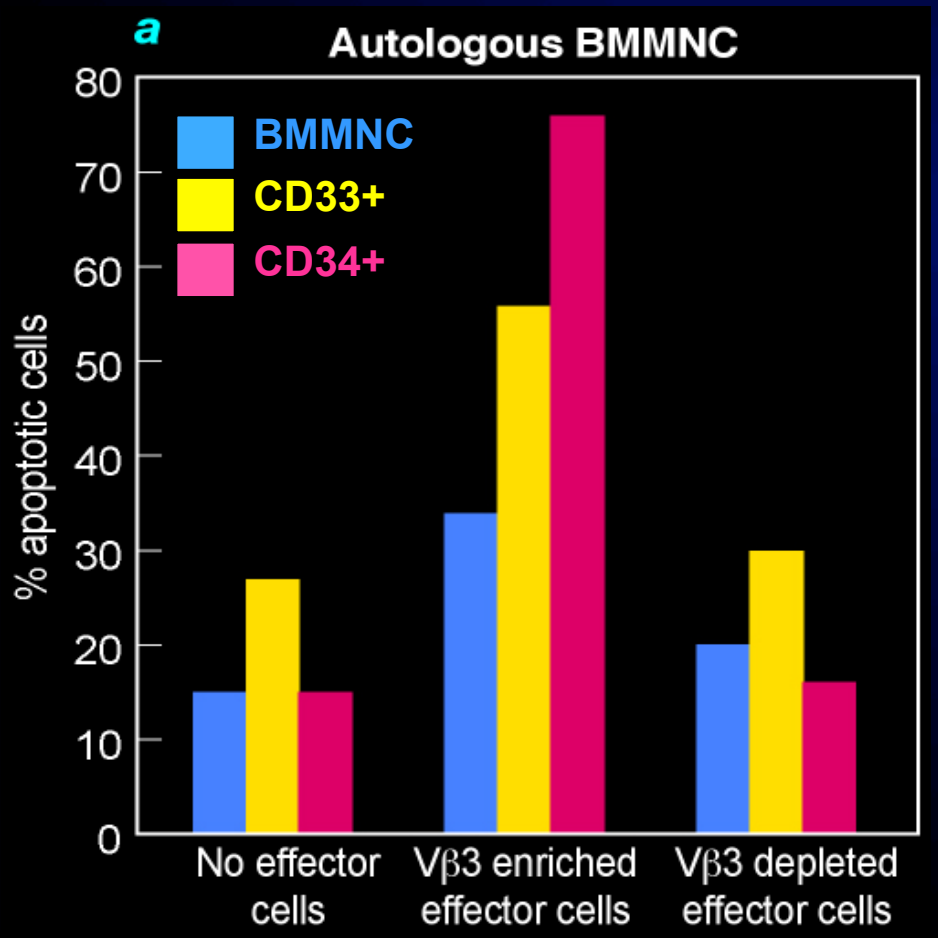
Molecular Tracking of Pathogenic Clonotypic T-cells

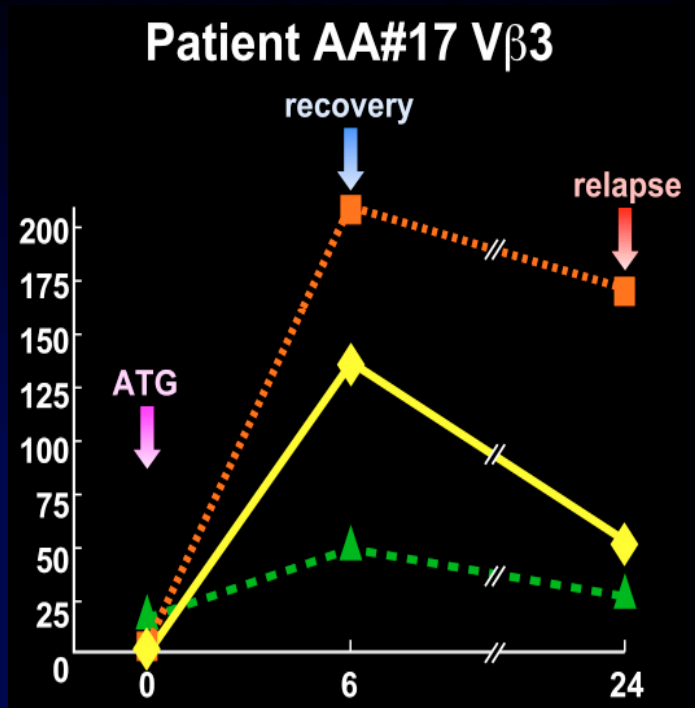
Lancet 2004; 364: 355–64

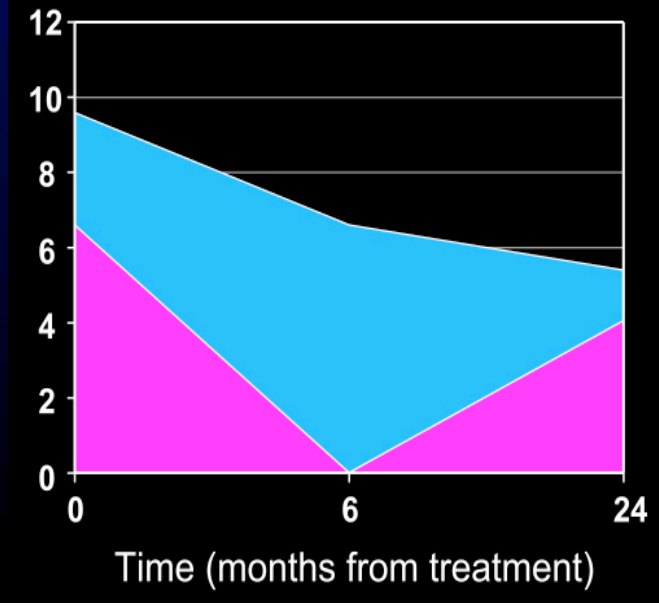
Mechanisms of Disease

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

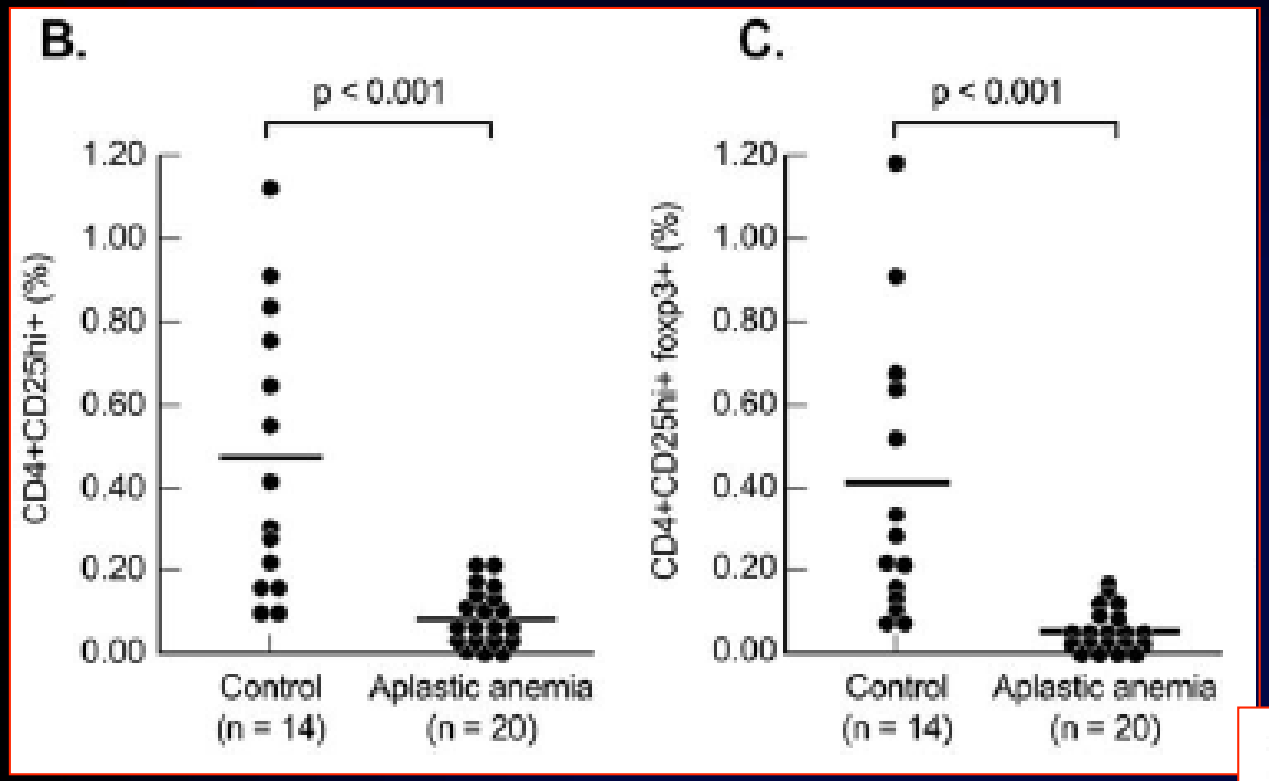


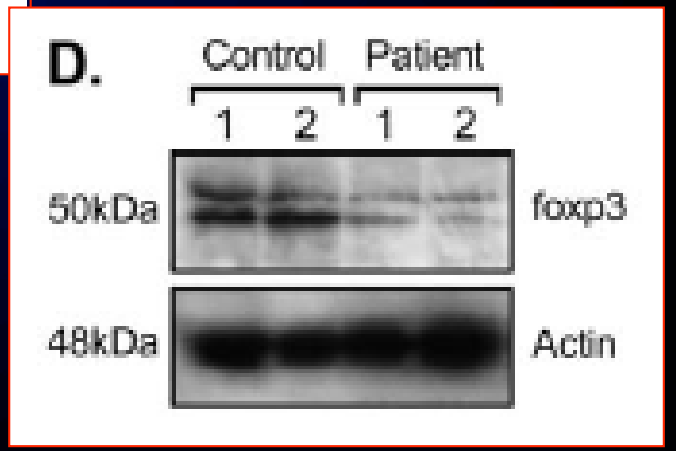




T-REGULATORY CELLS IN APLASTIC ANEMIA

Solomou et al., Blood 2007

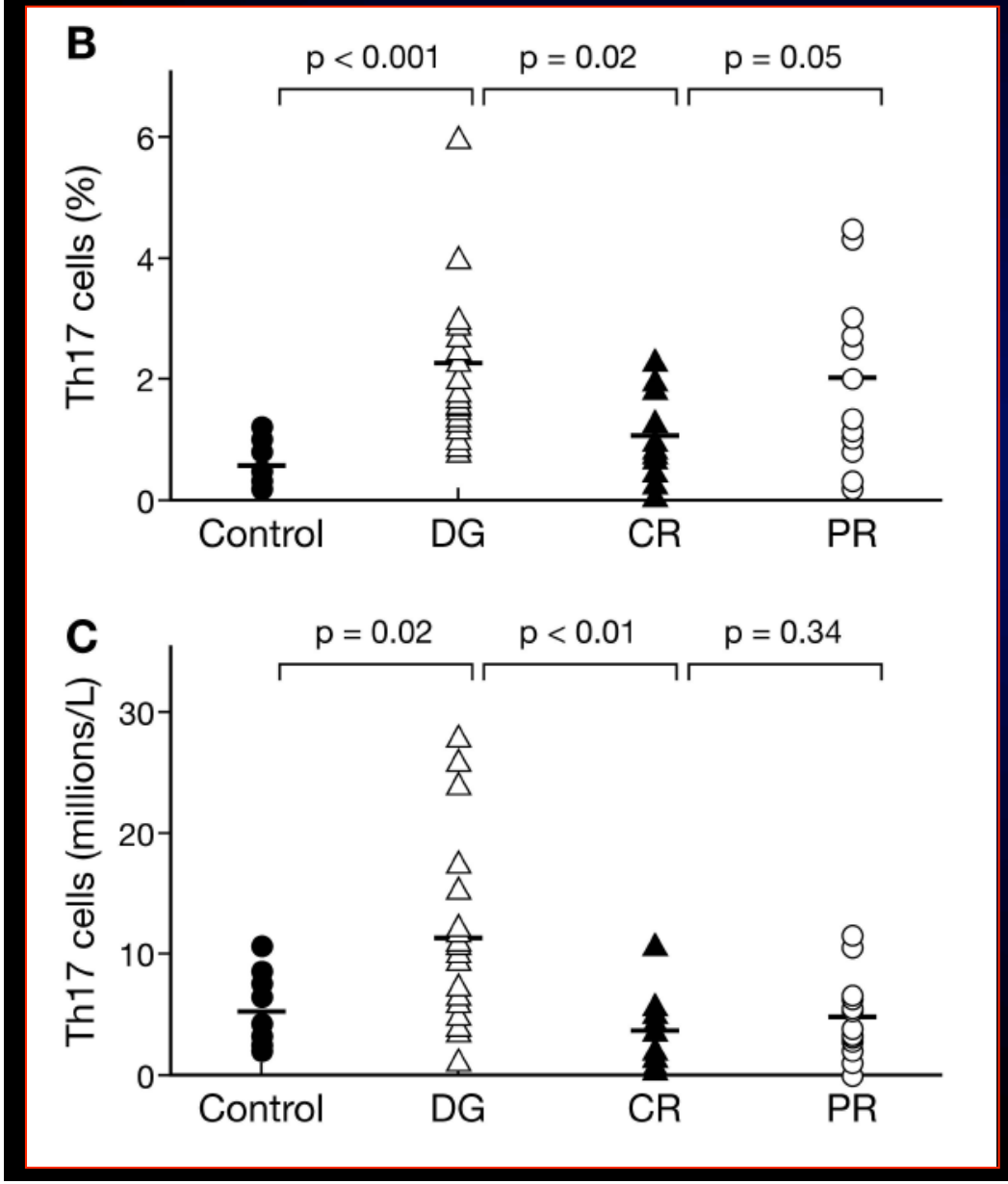


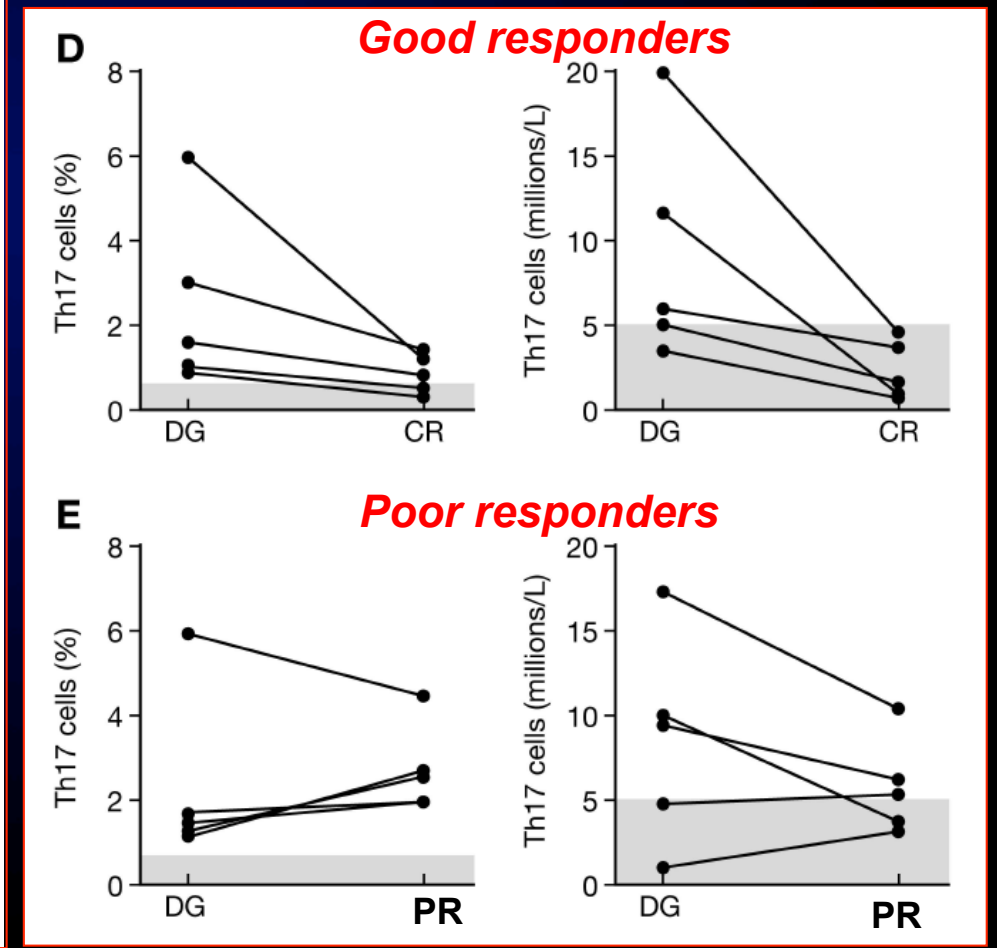


Th17 CELLS IN APLASTIC ANEMIA

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

Acquired
Idiopathic: multifactorial?

Genetic susceptibility

- Permissive SNPs
- Immunity (cytokine polymorphism, HLA)
- Stem cell self-renewal (telomerase complex)

Immune-mediated

- Auto-immune
- Virus-related
- Drug metabolites

Constitutional

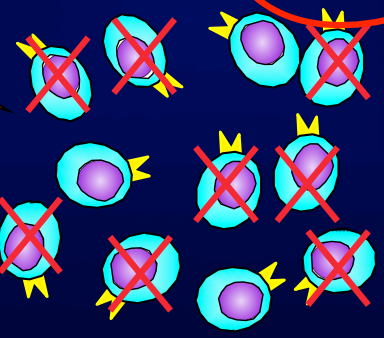
Intrinsic defect

- Fanconi's anemia
- Dyskeratosis congenita
- Shwachman-Diamond syndrome
- Congenital amegakaryocytic thrombocytopenia

Acquired

Direct toxicity

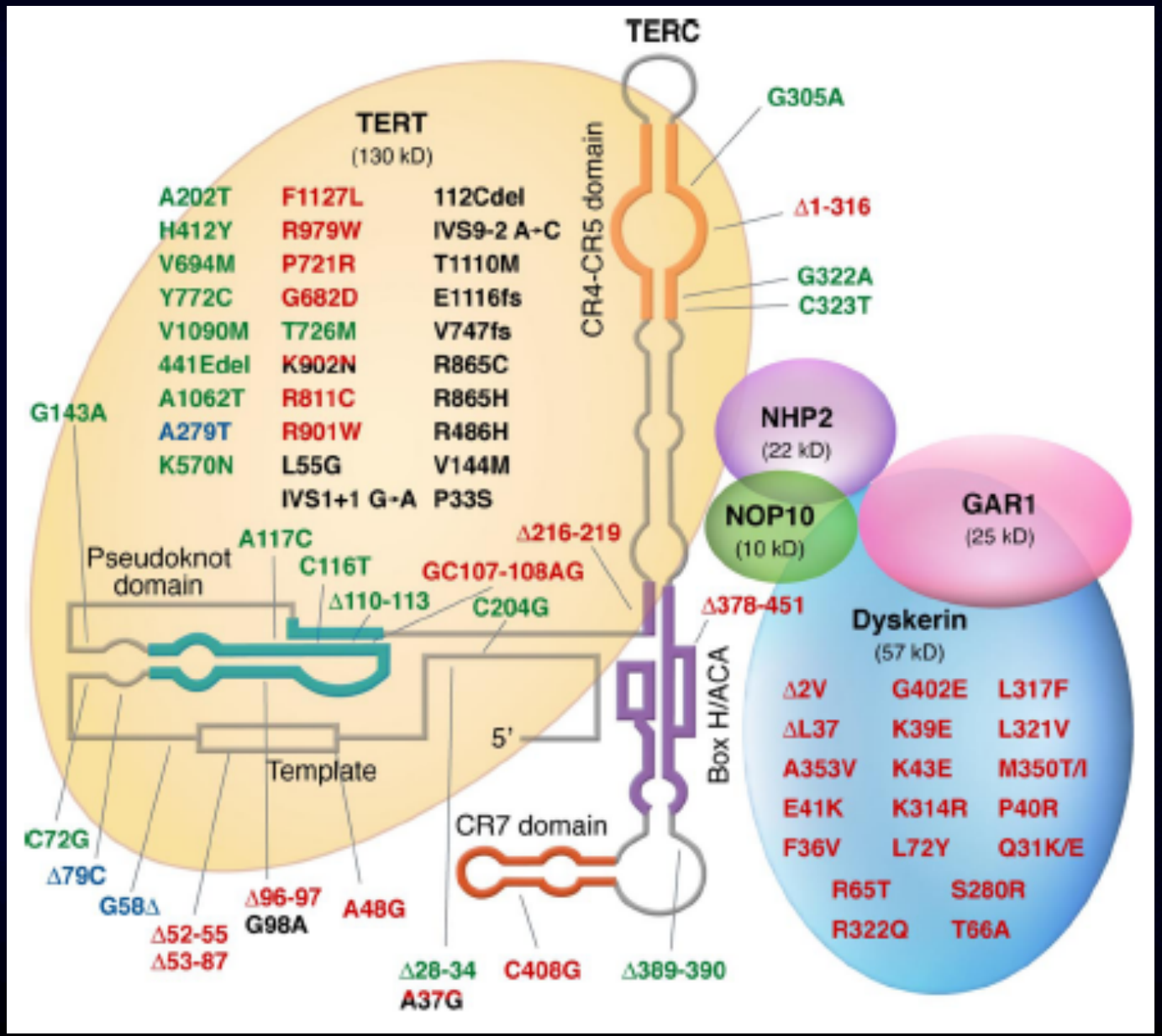
- Chemicals
- Radiation



Contraction of stem cell pool

Cytopenia

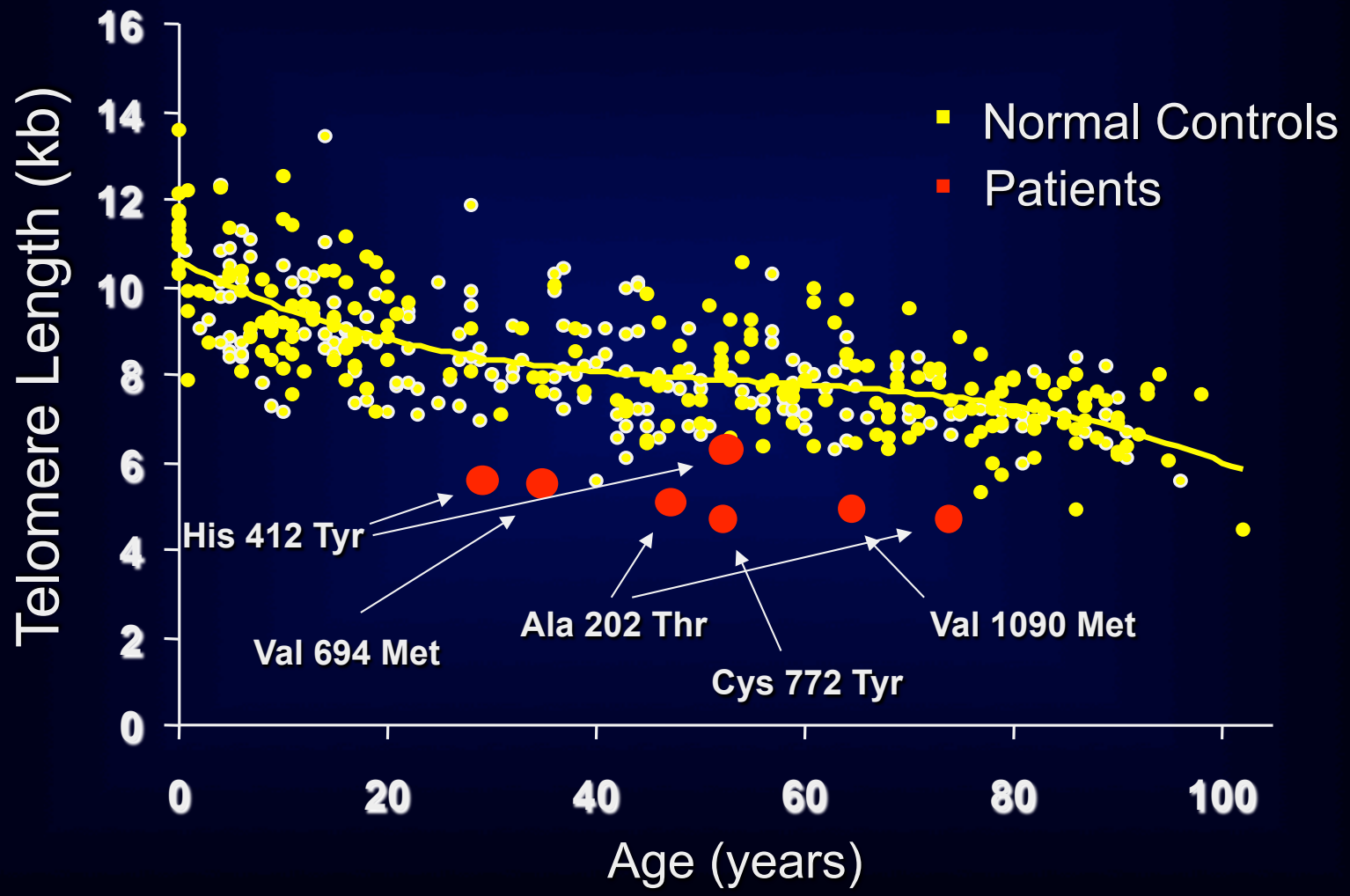
TELOMERASE REPAIR COMPLEX GENE MUTATION AND BONE MARROW FAILURE



Calado and Young, Blood 2008

TELOMERE LENGHT OF PERIPHERAL BLOOD LEUKOCYTES

Patients with TERT or TERC mutations



Calado and Young, Blood 2008

The actual meaning of somatic mutations in hematology

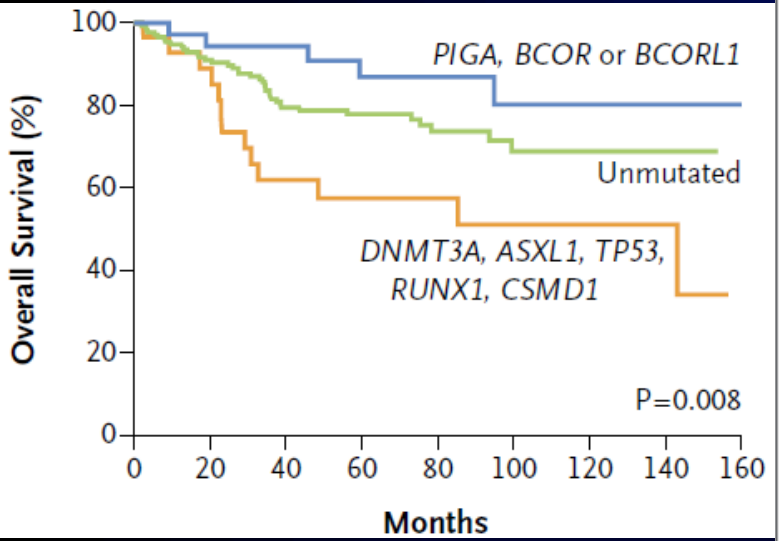
Do all mutations imply cancer (especially in marrow failure)?

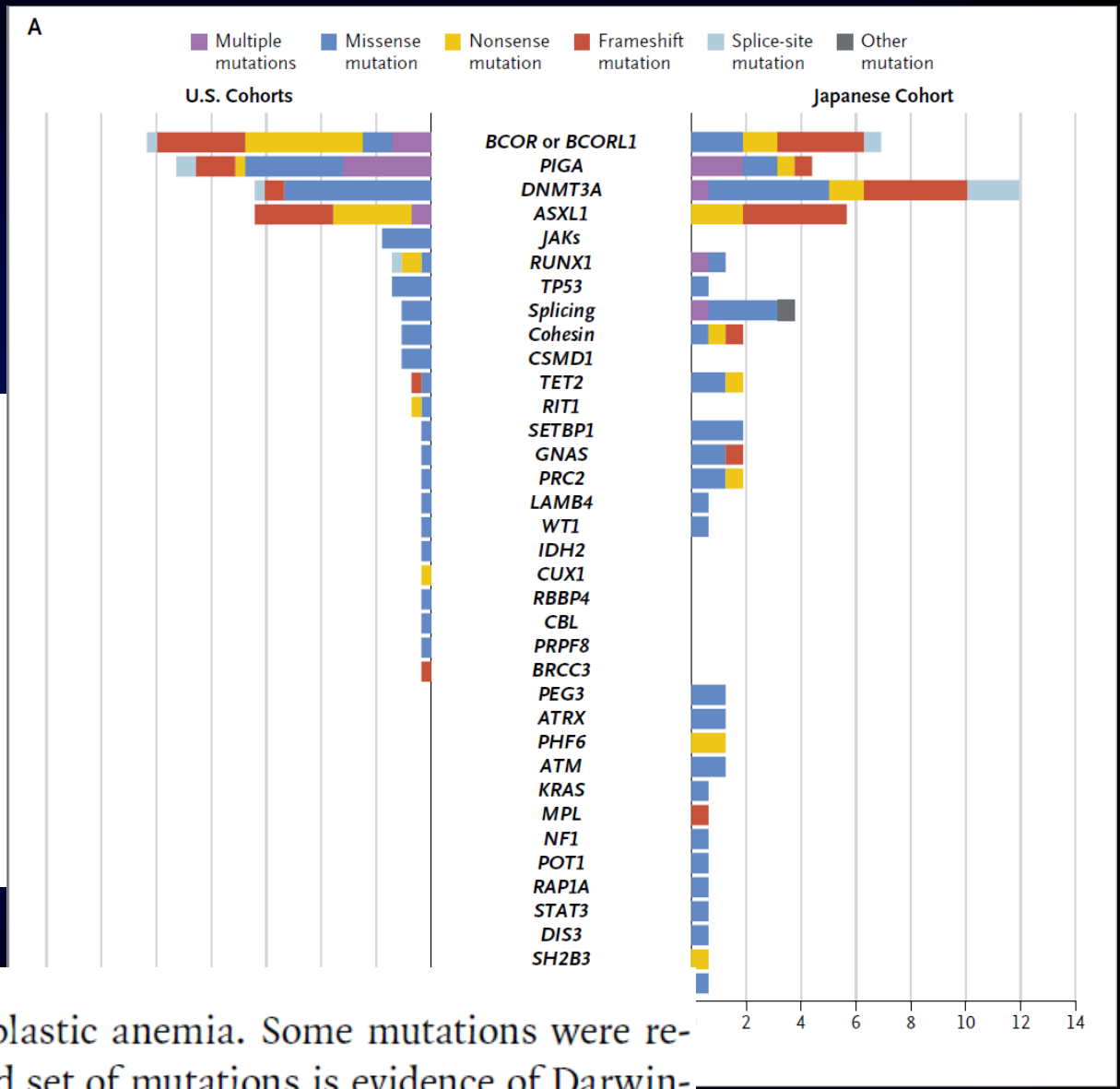
The NEW ENGLAND JOURNAL of MEDICINE

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

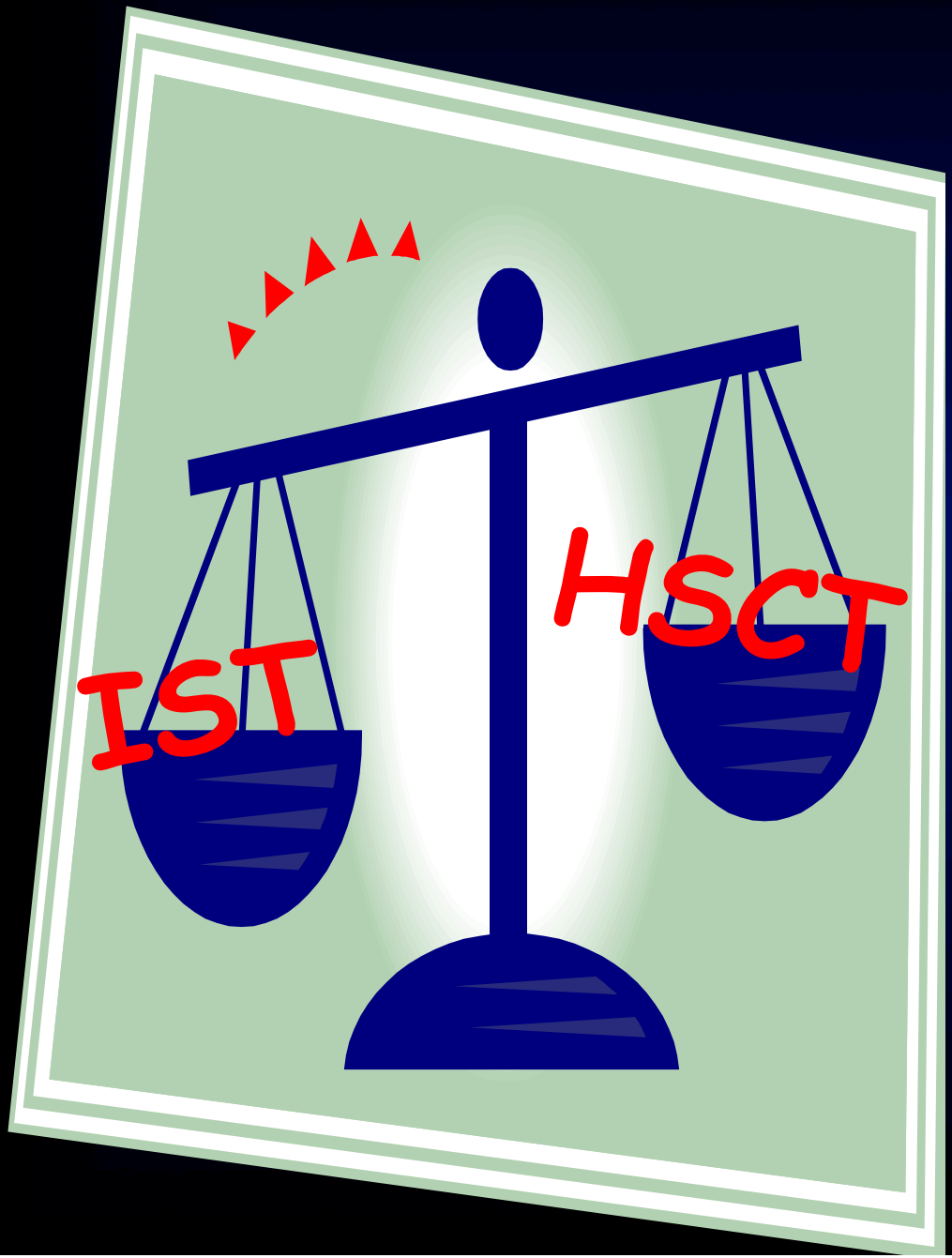




CONCLUSIONS

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.

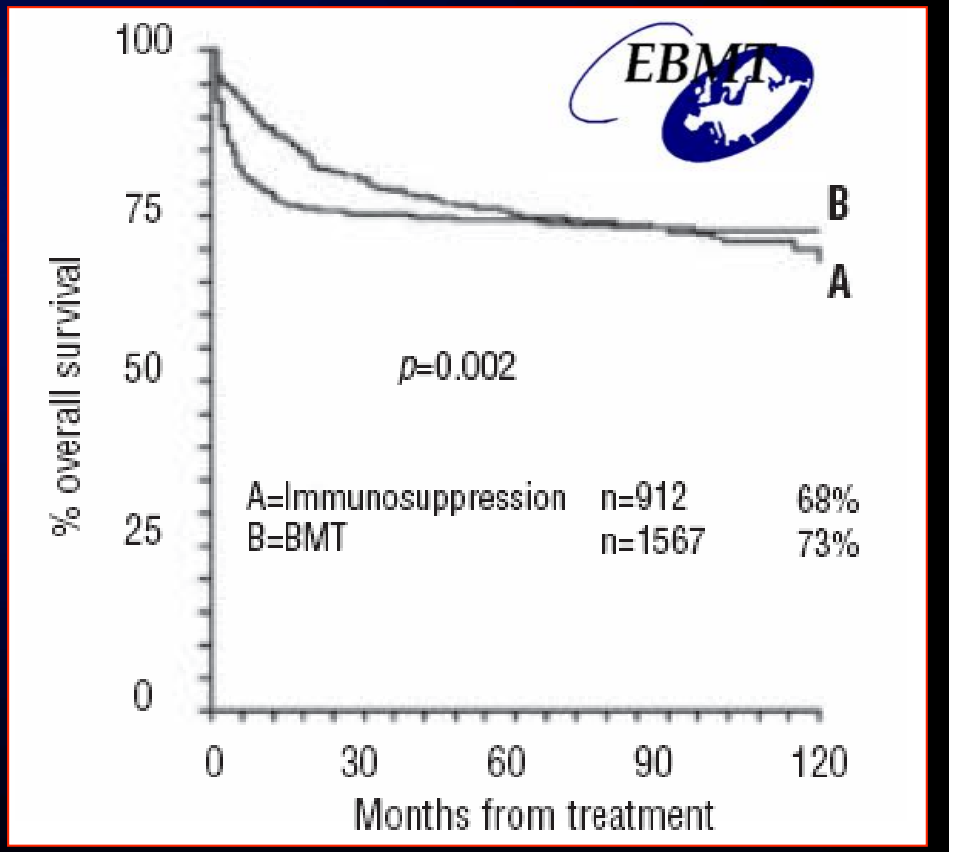
Treatment options for aplastic anemia



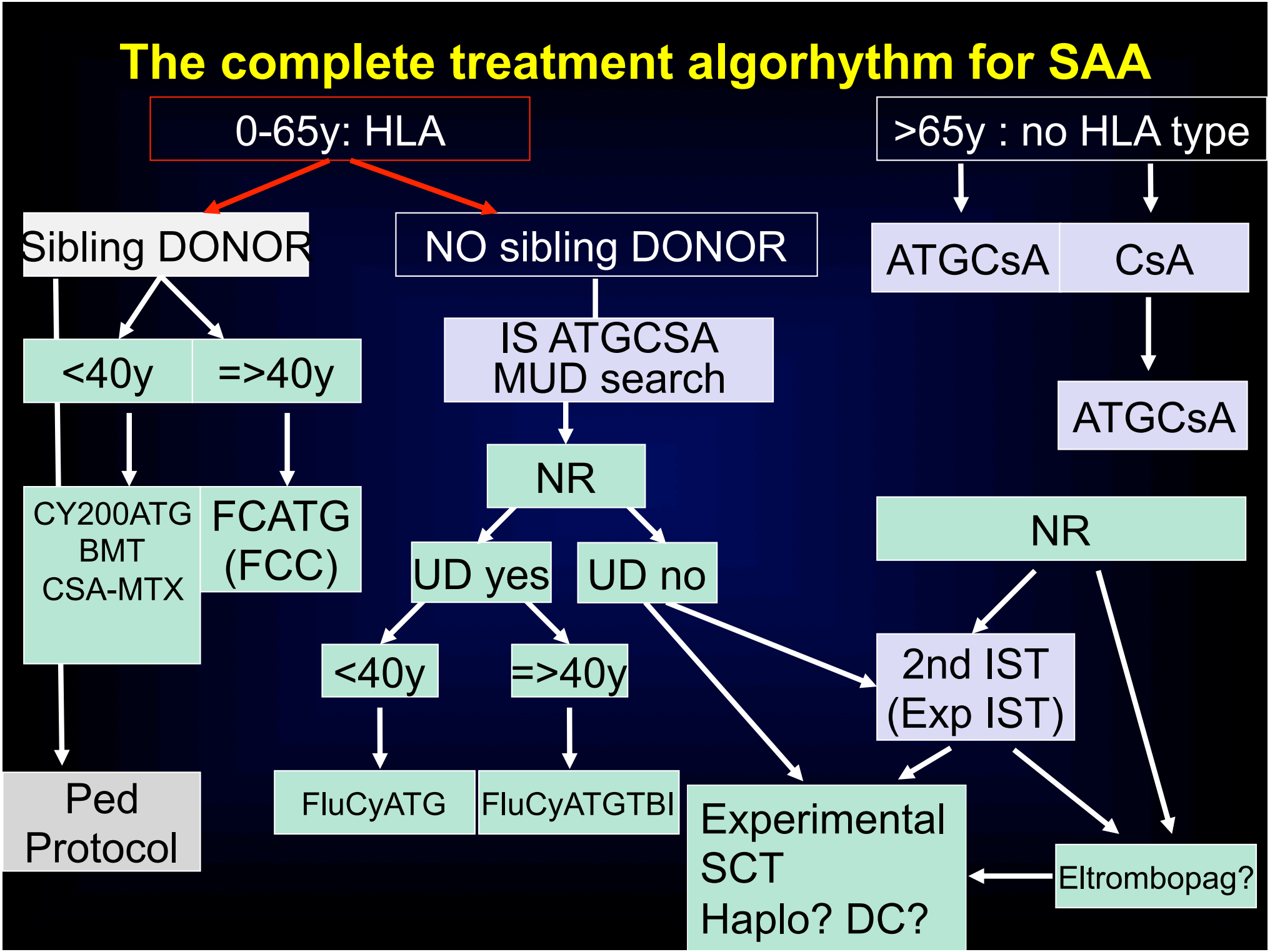
Original Article

Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation

Anna Locasciulli, Rosi Oneto, Andrea Bacigalupo, Gerard Socié, Elisabeth Korthof, Albert Bekassy, Hubert Schrezenmeier, Jakob Passweg, Monika Führer
on the Behalf of the Severe Aplastic Anemia Working Party of the European Blood and Marrow Transplant Group (SAA-WP, BMT).



Locasciulli et al, Haematologica 2007


The complete treatment algorhythm for SAA
0-65y: HLA
>65y : no HLA type
Sibling DONOR
NO sibling DONOR
ATGCsA
CsA
<40y
=>40y
IS ATGCSA
MUD search
ATGCsA
CY200ATG
BMT
CSA-MTX
FCATG
(FCC)
NR
NR
UD yes
UD no
<40y
=>40y
2nd IST
(Exp IST)
Ped
Protocol
FluCyATG
FluCyATGTBI
Experimental
SCT
Haplo? DC?
Eltrombopag?

AA and…

… supportive care

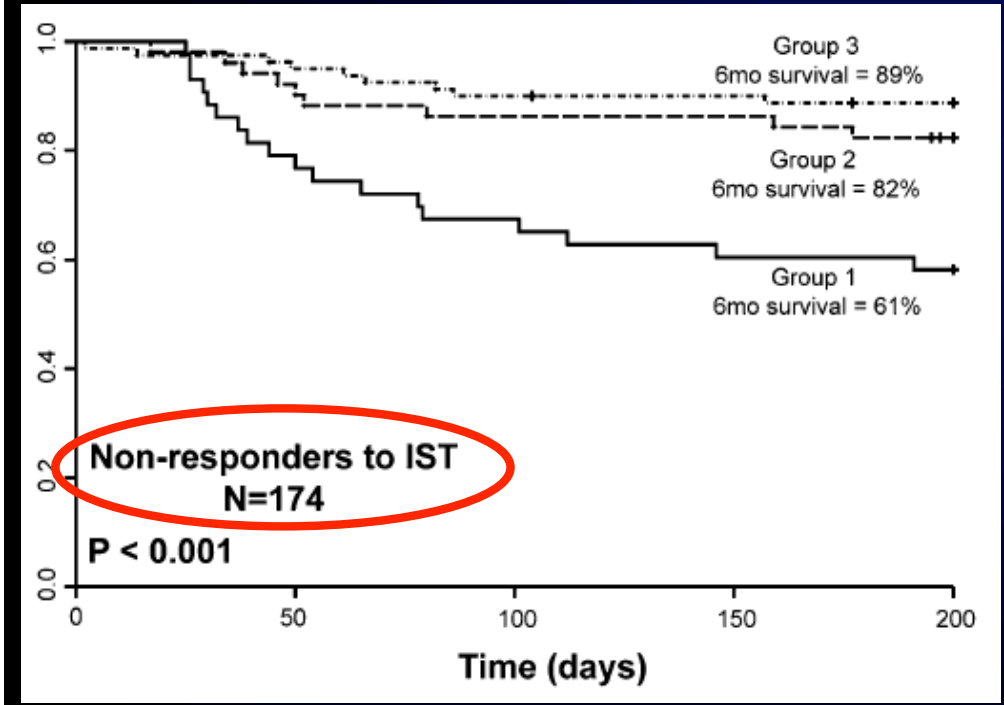
Supportive care

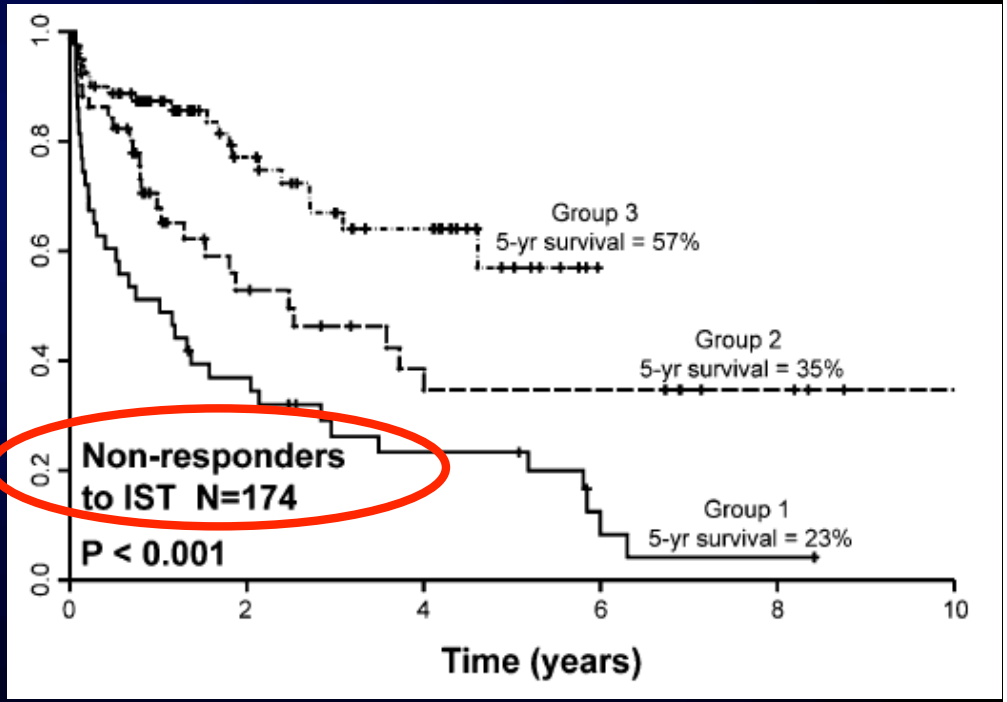
The improvement in anti-infectious management

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



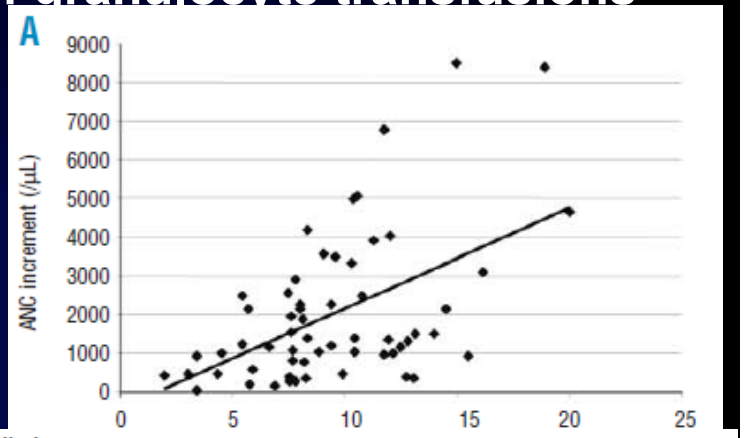


The most relevant breakthrough in AA treatment was the anti-infectious supportive care: keeping AA patients alive until they recover (IST or SCT)

Supportive care
Granulocyte transfusions

NIH retrospective analysis (1997-2007) on the use of granulocyte transfusions in life-threatening infectious complications in AA

- ✓32 AA patients (9/32 with anti-HLA alloantibodies)
- ✓Median transfusions n=9 (range 2-43); mean granulocyte 6.8±2.3x10^{10} cells
- ✓Daily or alternate day schedule
- ✓OS 58% (correlating with hematological recovery)
- ✓Response with anti-HLA-Ab



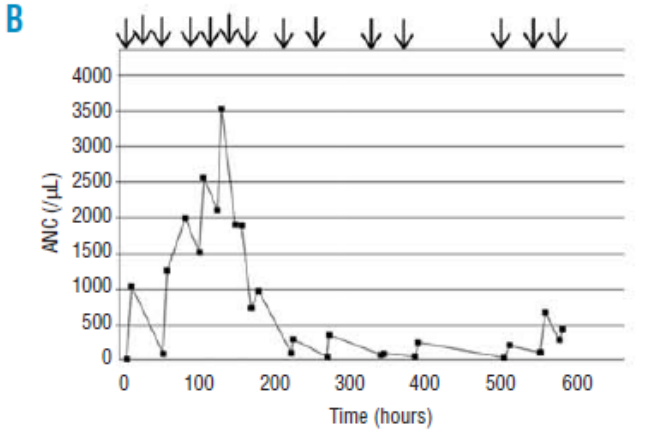


Table 3A. Response at day 7 and day 30 versus survival to hospital discharge.

	Day7 response: N. of patients (%)	Survival to discharge	Day30 response: N. of patients (%)	Survival to discharge	Long-term follow-up
All patients					
Complete response	10 (30%)	9/10 (90%)	11 (33%)	11/11 (100%)	3 in CR(median 4 yr); 2 retreated for SAA relapses over 5 yr ; 1 with low platelets, hepatitis C recurrence 2 yr post-HSCT; 2 at 1 yr post HSCT; 1 died 3 months post-HSCT; 1 died in relapse 1 yr post ATG therapy
Partial response	10 (30%)	6/10 (60%)	9 (27%)	7/9 (78%)	2 in CR (5, 8 yr); 2 in CR 1 yr post-HSCT; 1 umbilical HSCT one yr after SAA diagnosis
Stable disease	6 (18%)	3/6 (50%)	1 (3%)	0/1	Not applicable
Progressive disease	7 (21%)	1/7 (14%)	12 (36%)	1/12 (8%)	Death 16 months post HSCT from GVHD, sepsis
Overall				19/33=58%	
Patients with invasive fungal infection					**Organism involved**
Complete response	6 (33%)	5/6 (83%)	6 (33%)	6/6 (100%)	2 *Aspergillus*, 1 *Zygomycete*, 1 *Bipolaris*, 2 *Alternaria/Fusarium*
Partial response	4 (22%)	2/4 (50%)	3 (17%)	2/3 (67%)	*Aspergillus, Alternaria; Hyphomycete*
Stable disease	3 (17%)	1/3 (33%)	0	not applicable	
Progressive disease	5 (28%)	0/5 (0)	9 (50%)	0/9 (0)	6 *Aspergillus*, 1 *Fusarium*, 1 *Paecilomyces*, 1 *Alternaria*
Overall				8/18=44%	

CR: complete response; ATG: antithymocyte globulin; GVHD: graft-versus-host disease.

Quillen et al Haematologica 2011

Supportive care

The management of iron overload: deferasirox (1)

- ✓EPIC study (deferasirox): a total of 1174 patients (AA n=116)
- ✓Significant reduction of ferritine and LPI (but normal at baseline)

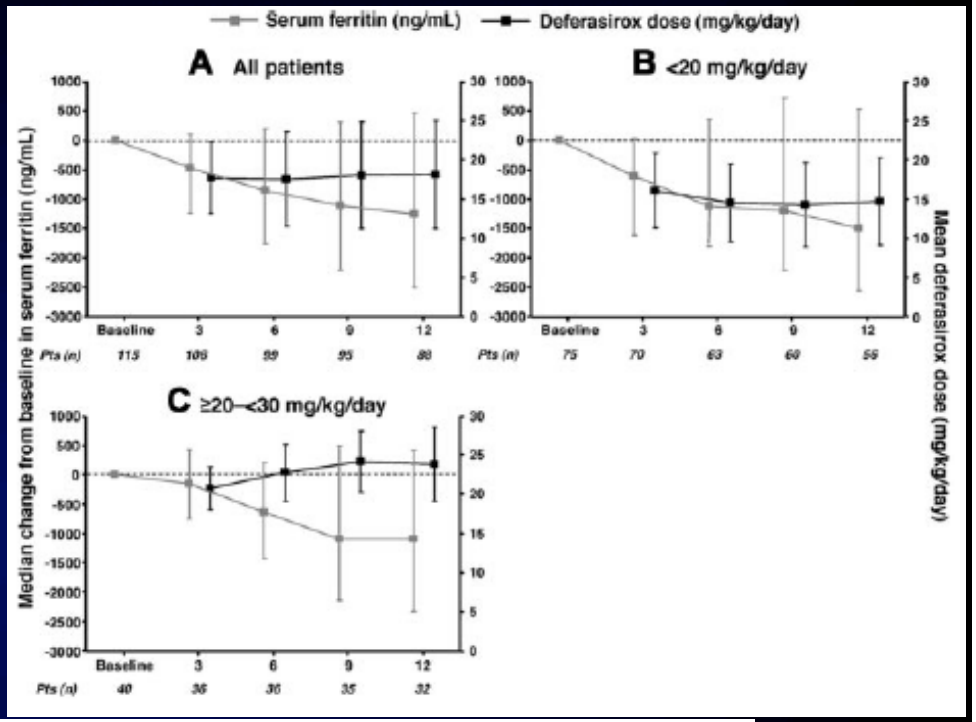


Table 3. Most common (> 5% overall) drug-related AEs by dose group

	< 20 mg/kg/d (n = 75), no. (%)	≥ 20-< 30 mg/kg/d (n = 41), no. (%)	All (n = 116), no. (%)
Nausea	19 (25.3)	7 (17.1)	26 (22.4)
Diarrhea	10 (13.3)	8 (19.5)	18 (15.5)
Rash	9 (12.0)	4 (9.8)	13 (11.2)
Vomiting	9 (12.0)	1 (2.4)	10 (8.6)
Dyspepsia	8 (10.7)	1 (2.4)	9 (7.8)
Abdominal pain	5 (6.7)	2 (4.9)	7 (6.0)
Upper abdominal pain	4 (5.3)	3 (7.3)	7 (6.0)
Anorexia	6 (8.0)	1 (2.4)	7 (6.0)

Table 2. Change from baseline in median serum ferritin by average actual dose

	Average actual deferasirox dose		
	< 20 mg/kg/d (n = 75)	≥ 20-< 30 mg/kg/d (n = 41)	All (n = 116)
Baseline serum ferritin, ng/mL	3263 (908-18 635)	3238 (1129-25 346)	3254 (908-25 346)
Serum ferritin at 1 y, ng/mL	1819 (212-14 509)	2191 (87-17 233)	1854 (87-17 233)
Absolute change in serum ferritin, ng/mL*	−970 (−11 753 to −7883)	−884 (−15 704 to −13 894)	−964 (−15 704 to −13 894)
P	< .001	< .278	< .001
Mean iron intake ± SD, mg/kg/d	0.21 ± 0.18	0.31 ± 0.20	0.25 ± 0.19

Deferasirox (at 20 mg/kg) results in effective iron chelation in AA patients, with minimal side effects (no drug-related cytopenia)

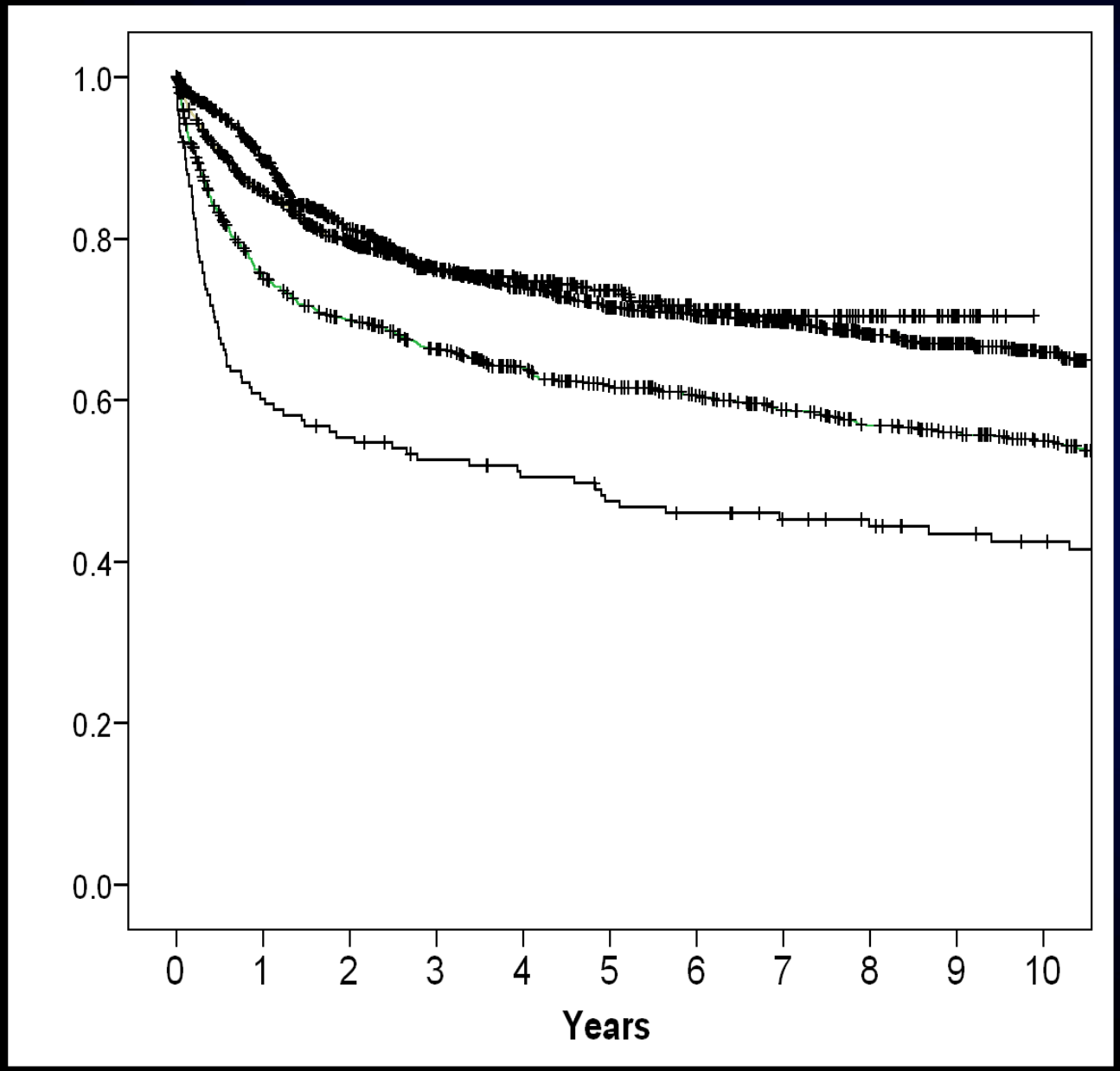
Lee et al Blood 2010, Cappellini et al Haematologica 2010

AA and…

… immunosuppressive 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

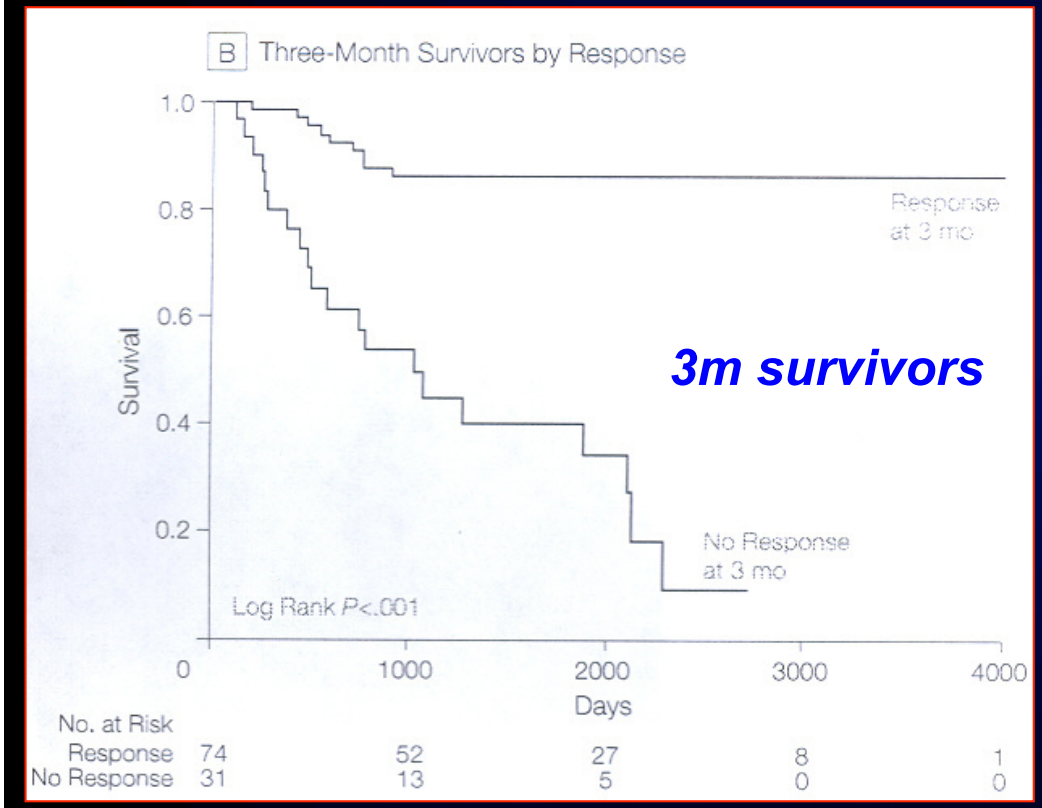
Antithymocyte Globulin and Cyclosporine for Severe Aplastic Anemia

Association Between Hematologic Response and Long-term Outcome

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

n=112

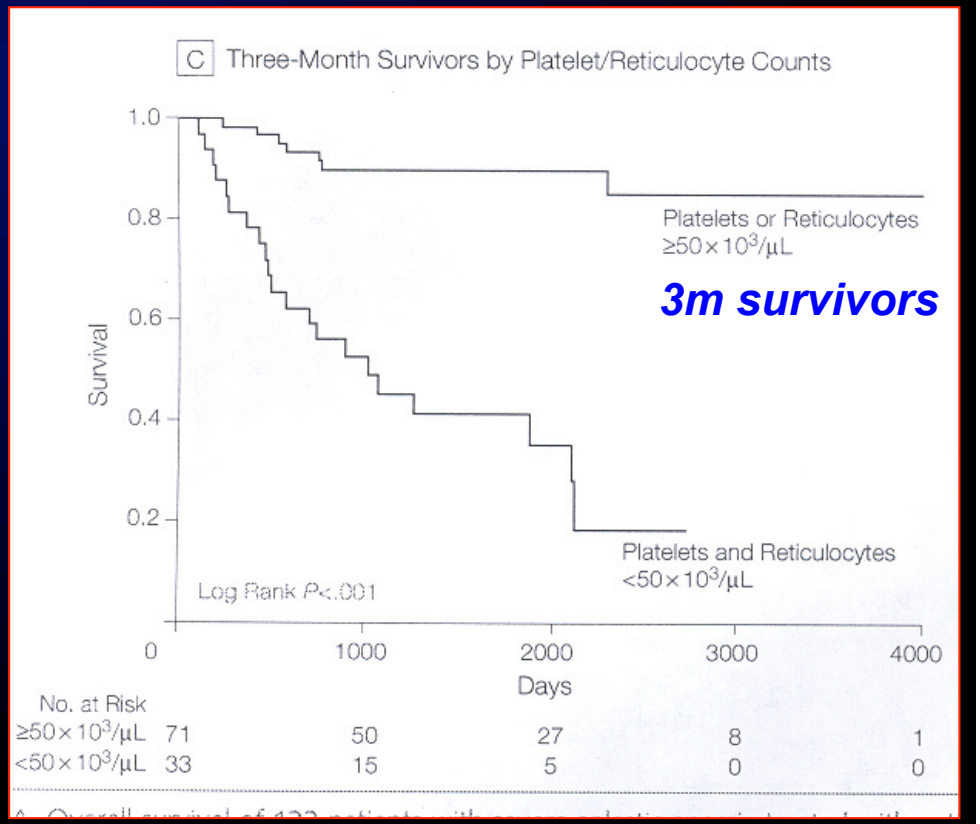
hATG x 4 (40mg/kg) + CsA x 6 m



OS 55% @7y;

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

3m survivors



3m survivors

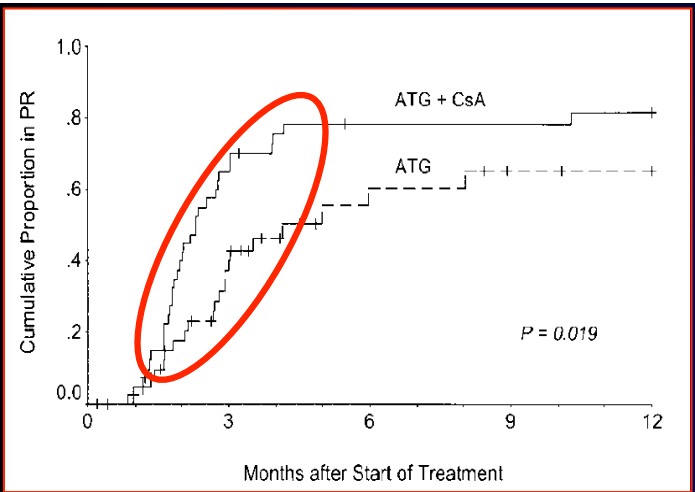
Hematological response is the main predictor for outcome

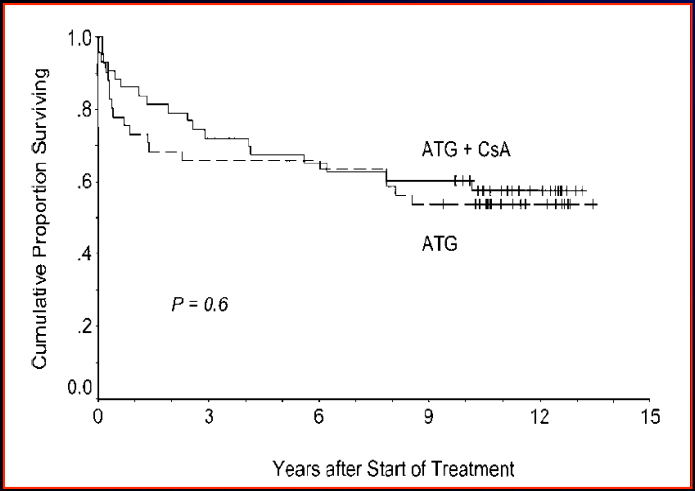
IMPROVING ATG-BASED IMMUNOSUPPRESSION

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 ***NEJM 1991***

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

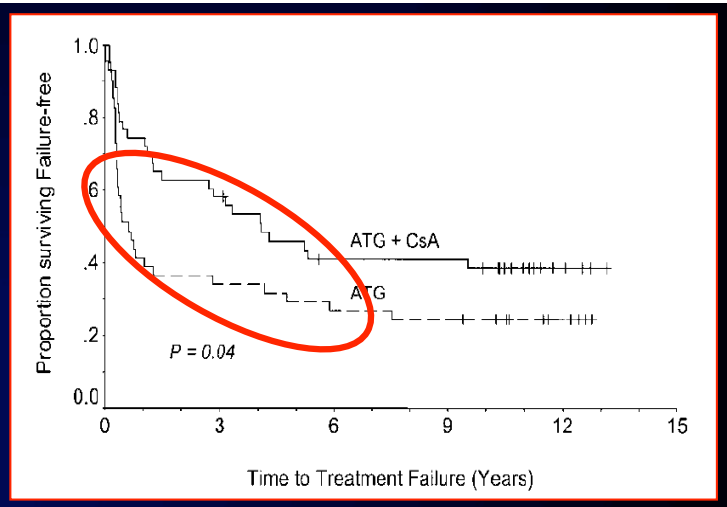


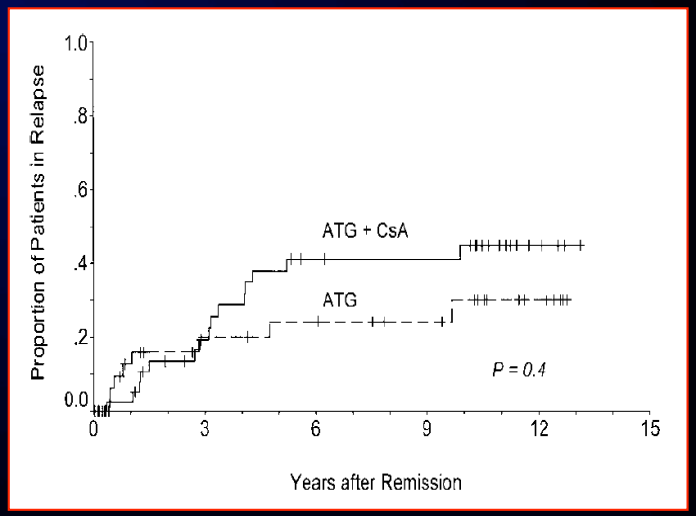


✓ **CyA speed hematological response without affecting survival**

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 ***Blood 2003***

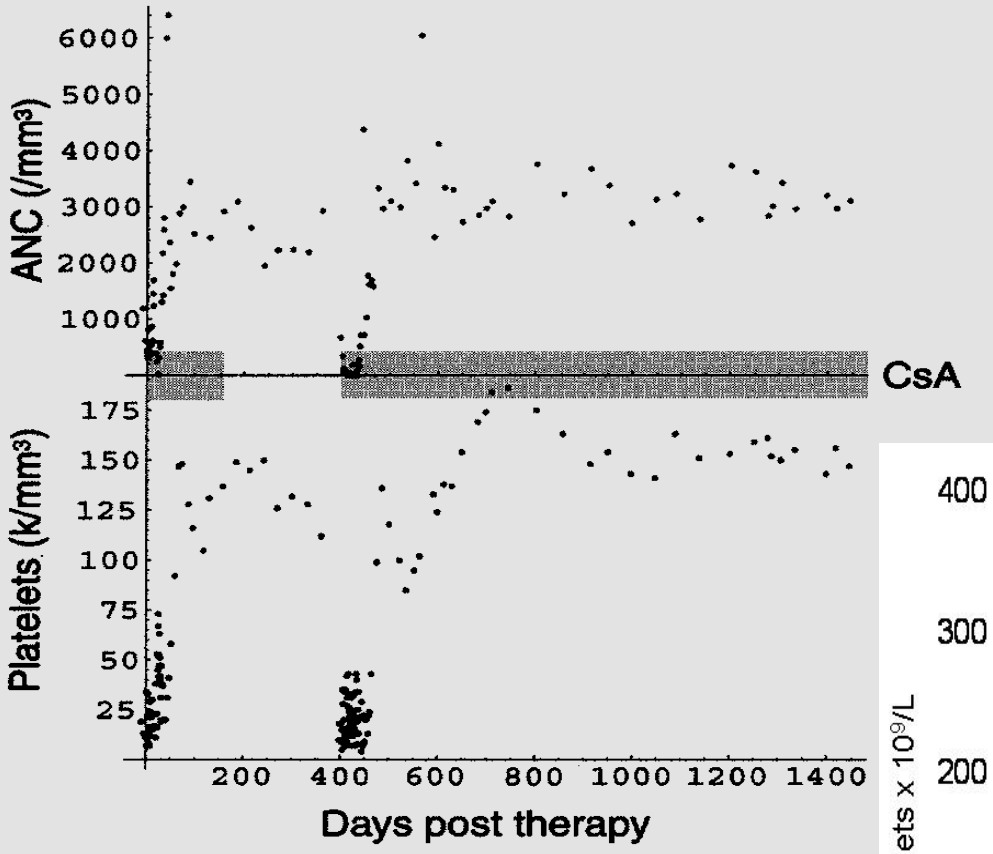




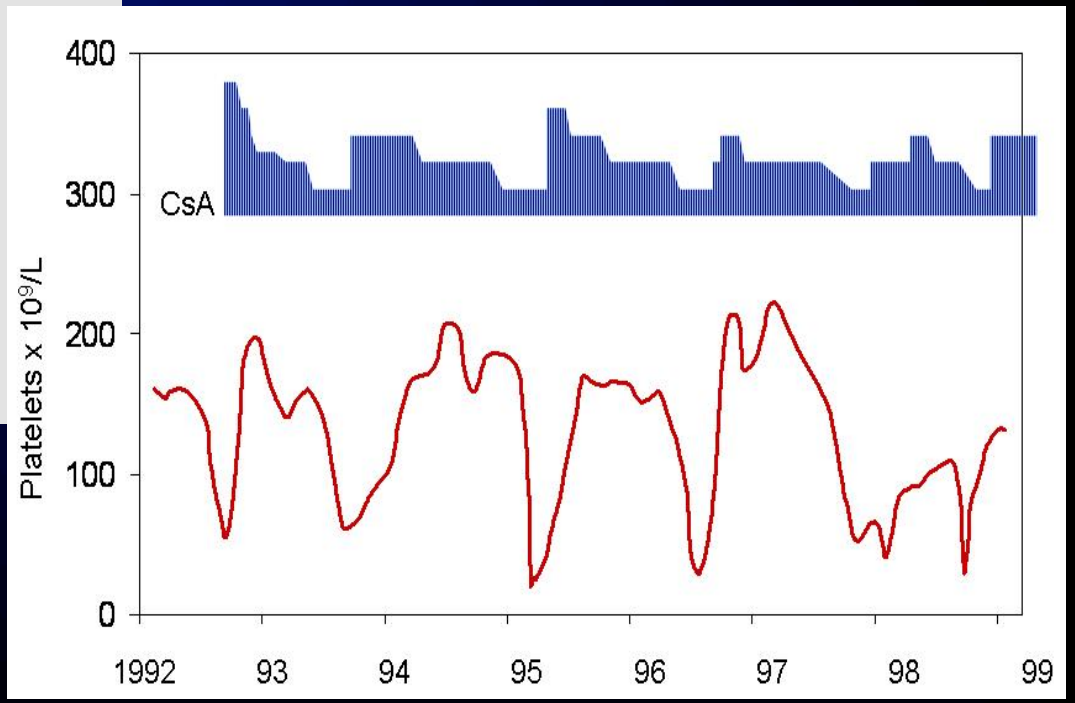
✓ **CyA reduces early treatment failure but not long-term relapse rate**

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

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!!!

Aplastic Anemia: Management of Adult Patients

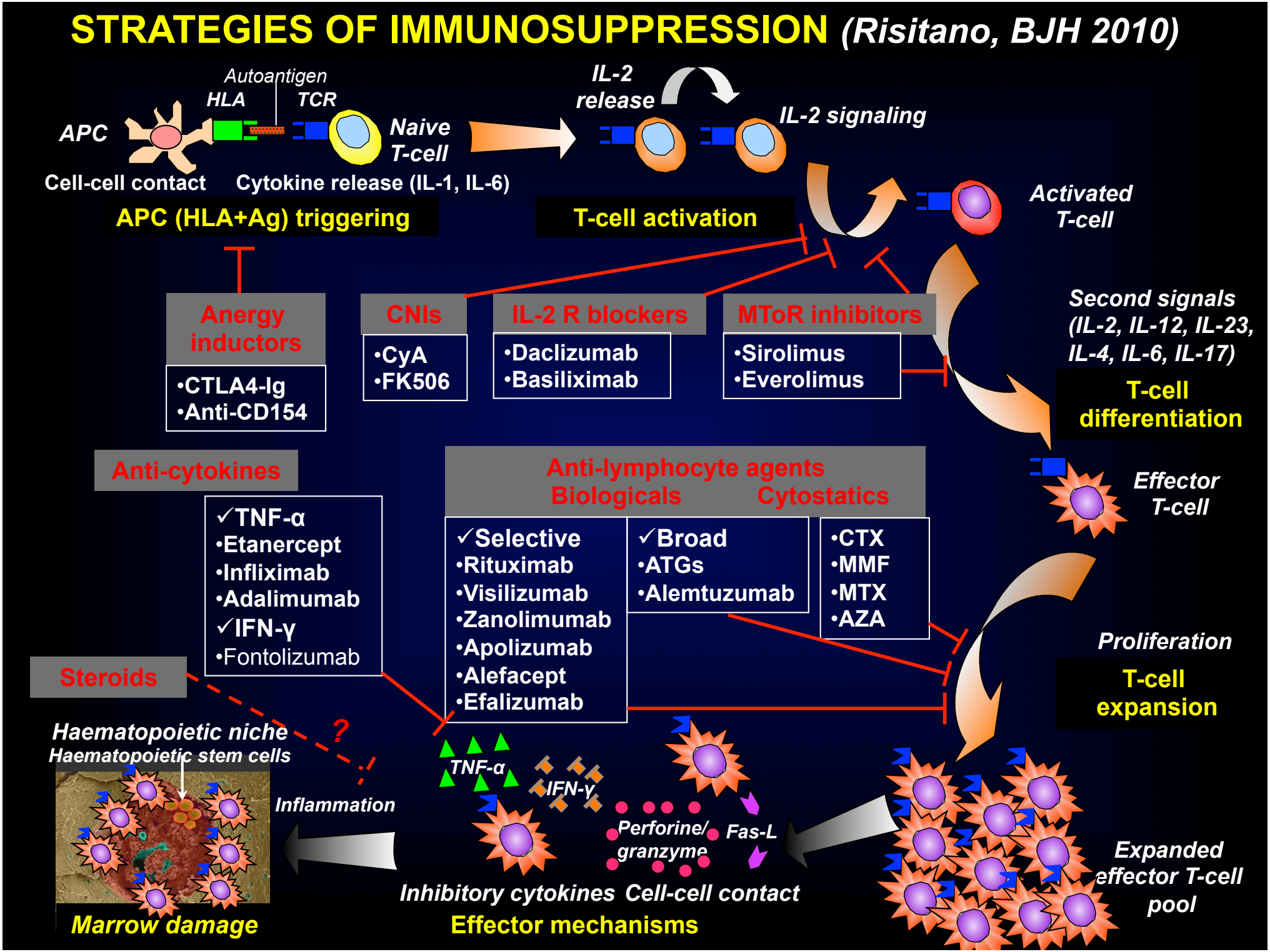
Jaroslaw P. Maciejewski and Antonio M. Risitano

REASONS FOR TREATMENT FAILURE

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

Improve front line immunosuppressive therapies

STRATEGIES OF IMMUNOSUPPRESSION *(Risitano, BJH 2010)*



Improving IST for AA: chronicle of a failure

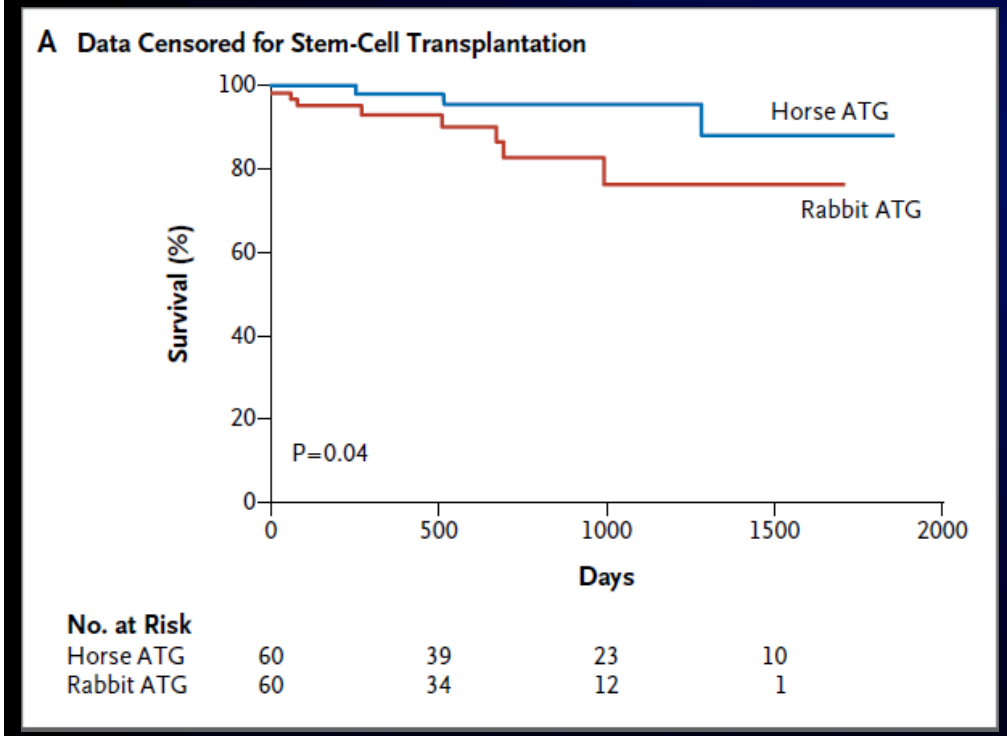
NEJM

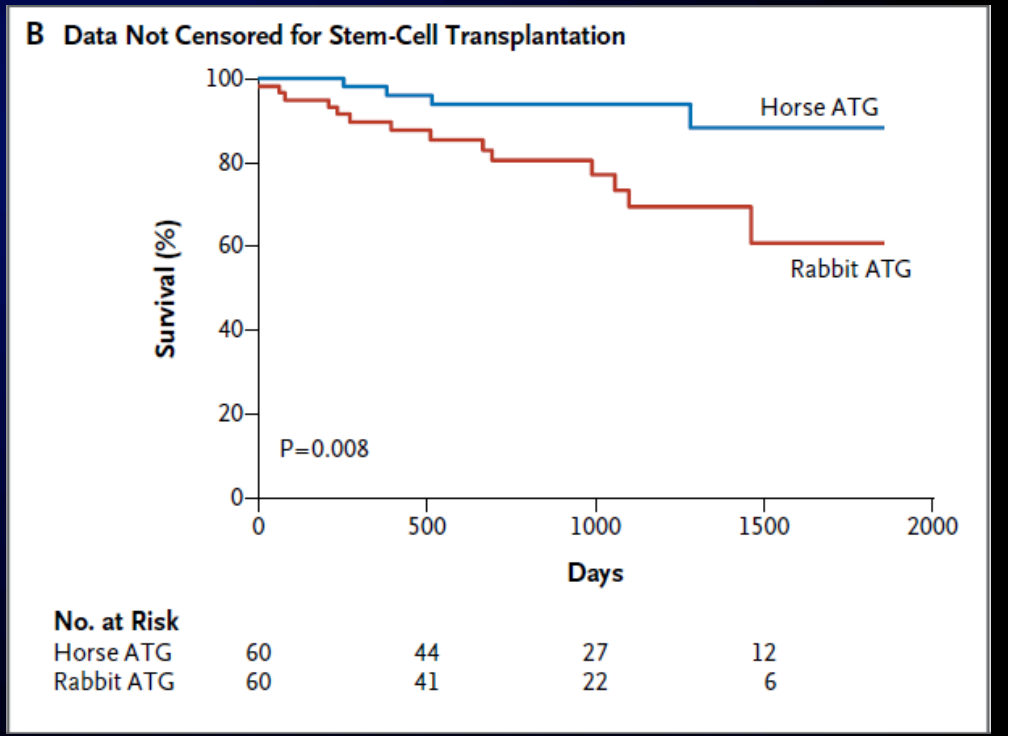
NEJM 2011

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)*





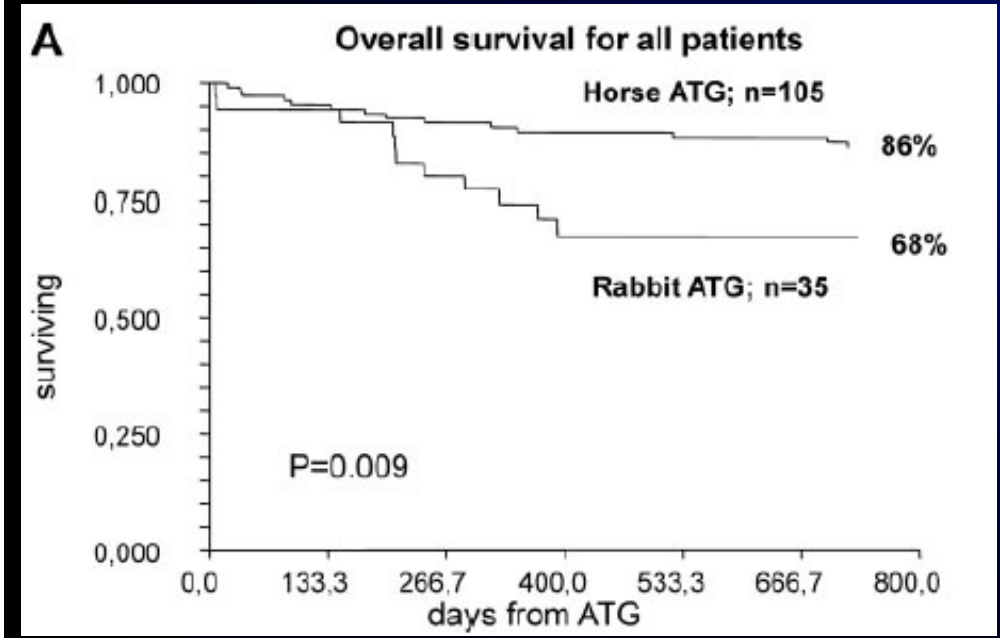
rATG is inferior to hATG in first line treatment of SAA, as indicated by hematological response and survival

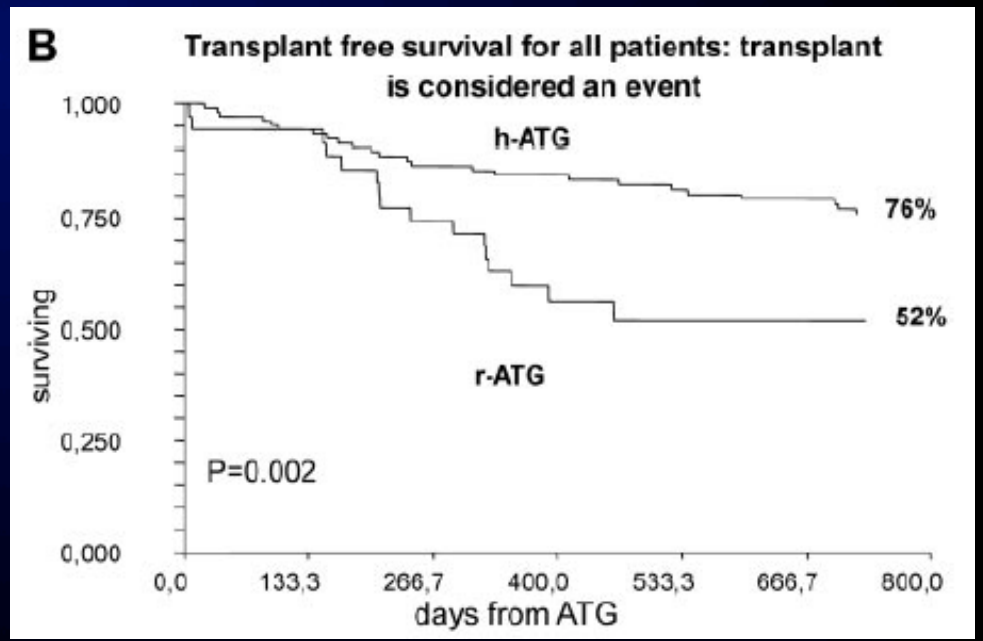
Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party

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

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

CYCLOPHOSPHAMIDE FOR TREATMENT OF SAA

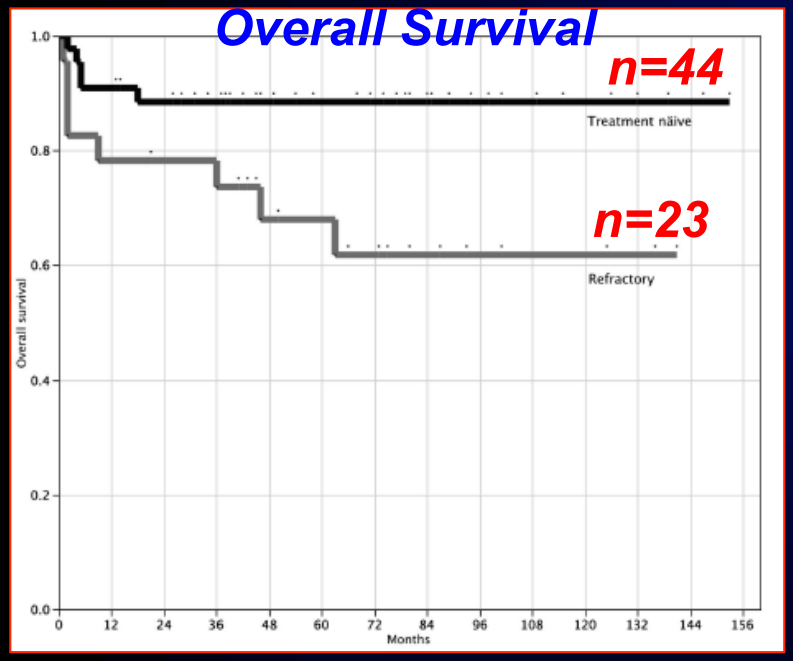
The Johns Hopkins experience

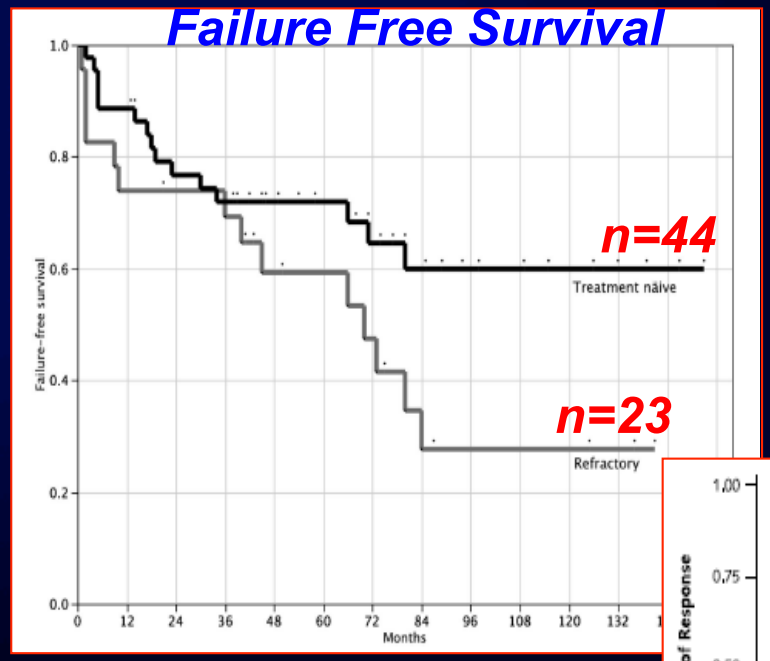
High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up

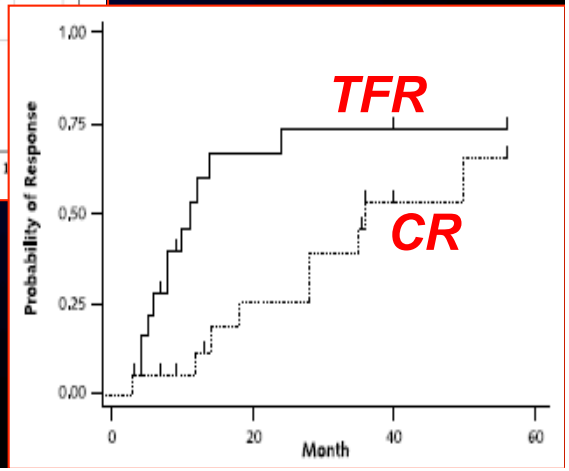
Robert A. Brodsky,[1,2] Allen R. Chen,[2] Donna Dorr,[1] Ephraim J. Fuchs,[2] Carol Ann Huff,[2] Leo Luznik,[2] B. Douglas Smith,[2] William H. Matsui,[2] Steven N. Goodman,[2] Richard F. Ambinder,[2] and Richard J. Jones[2]

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*







- ✓*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

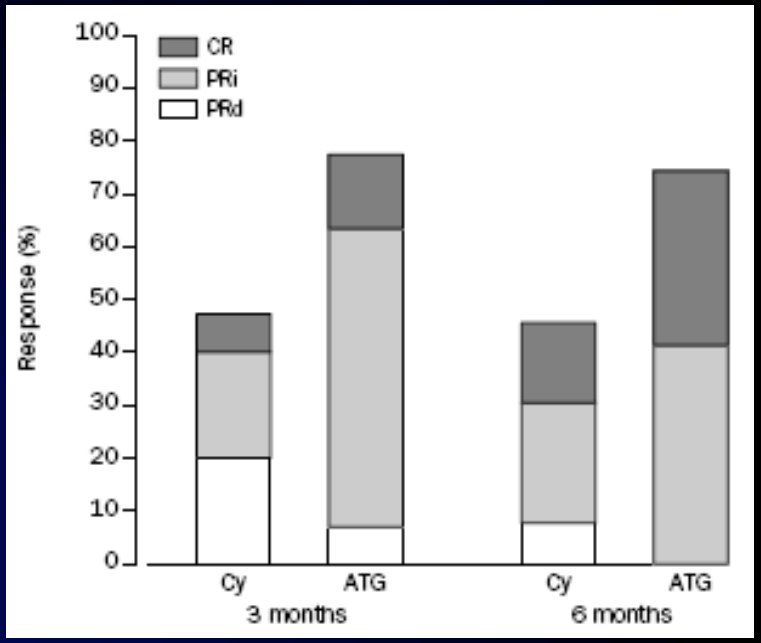
High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial

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**

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]

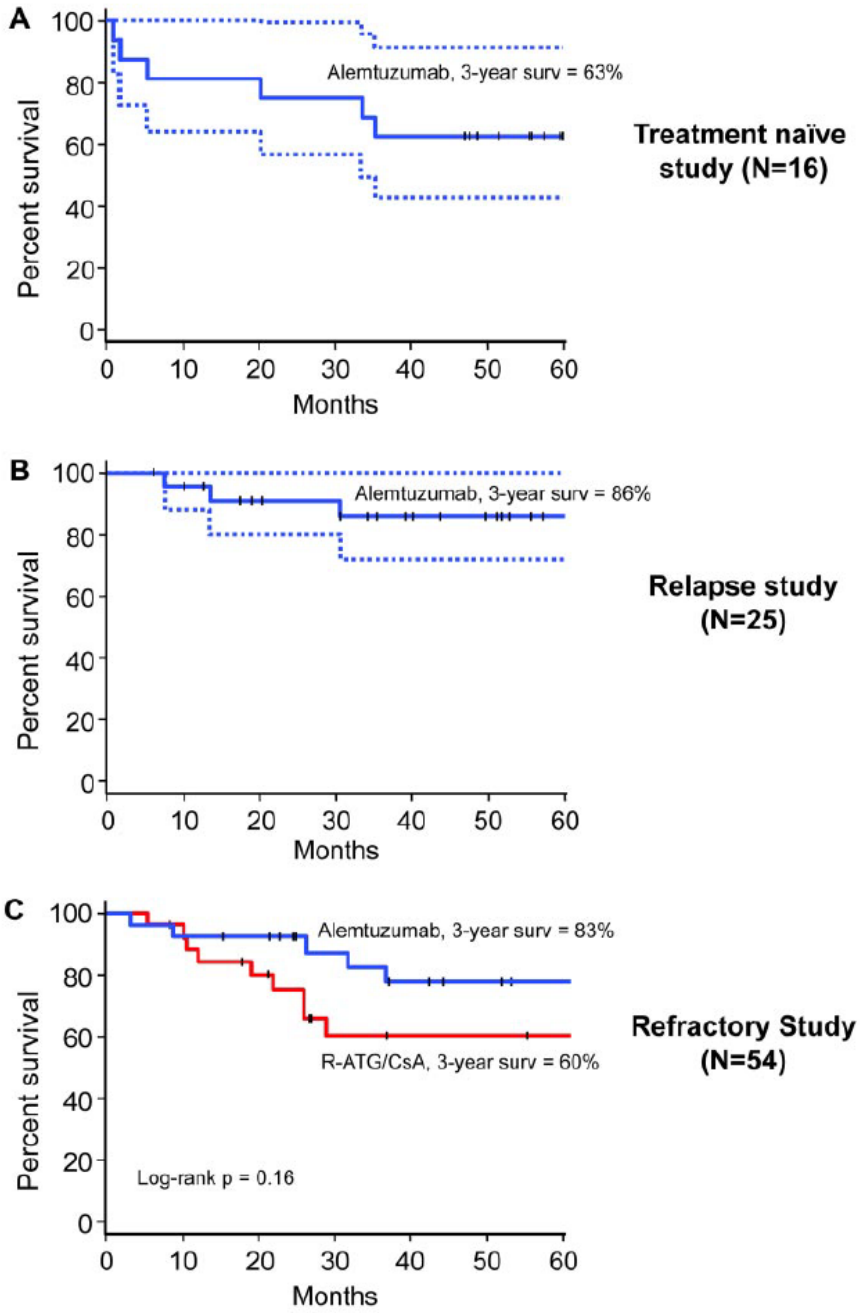
Blood 2012

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

?

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

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

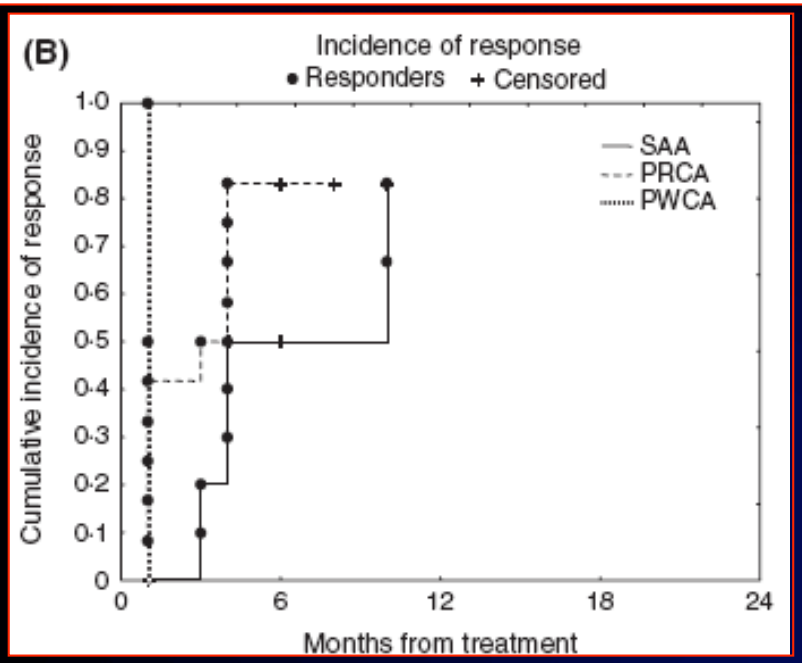


bjh short report

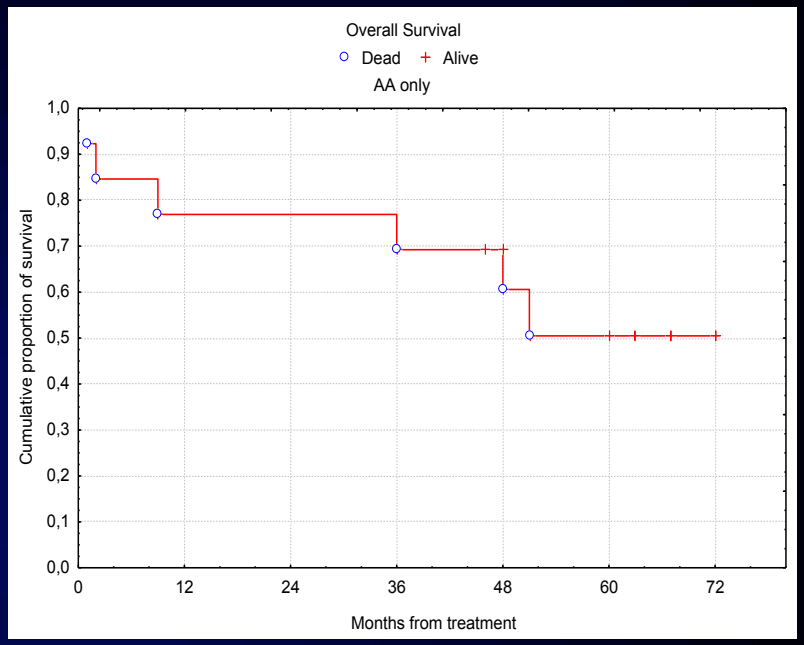
Risitano et al, 2010

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

- ✓ *Phase II prospective study*
- ✓ *Alemtuzumab s.c. (73-103 mg in 5 days)*
- ✓ *N=28: AA=13, PRCA=13, PWCA=2 (1st and higher)*



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



Best Hematological Response

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

13 AA

- ✓ 4 out 13 in current remission (3 CR, 1 VGPR)
- ✓ 4 late failures: 2 clonal evolution (non-responders), 2 refractory relapses

- ✓ 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%)

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. **L… of a…**
   - ✓
   - ✓

3. **A… e f…**
   - ✓ 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

Alternative IST: a narrow path but not a dead end

CD26-antiCD26

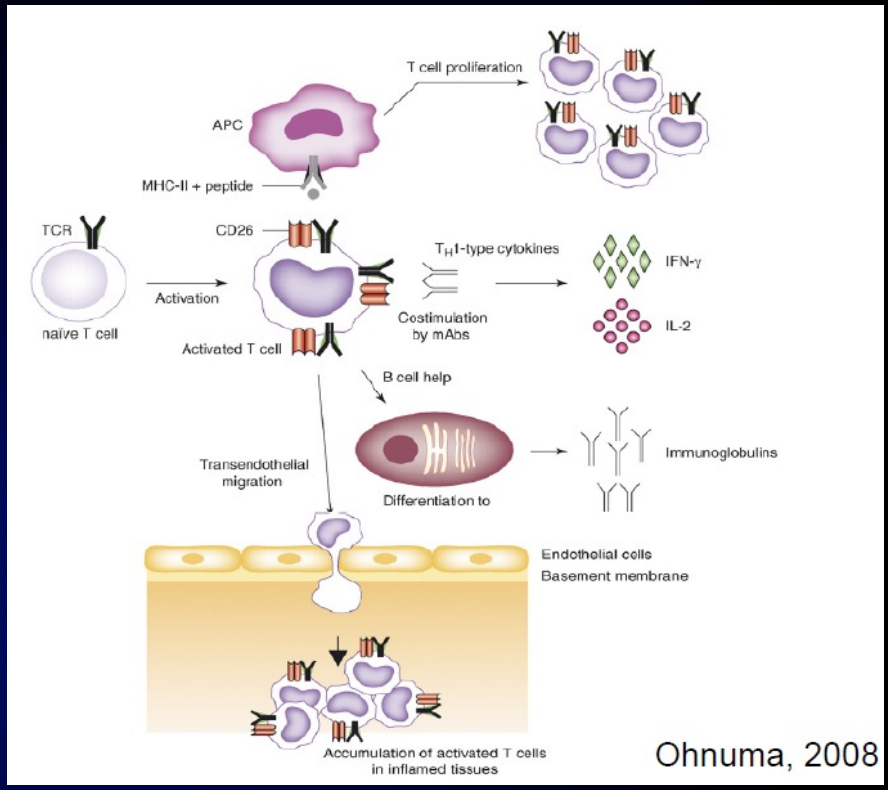
A possible role in auto-immune diseases?

✓CD26 (T cell activation antigen) or dipeptidyl peptidase-4 (DPP4) or adenosine deaminase complexing protein 2 (ADCP 2)

✓CD26 is involved in T-cell activation, proliferation and tissue migration

✓Inhibition of CD26 seems to prevent immune-mediated tissue damage by impairint T-cell migration (mouse model)

- experimental autoimmune encephalitis
- pancreatic islands transplantation
- lung transplantation



Ohnuma, 2008

BEGEDINA®

An anti-CD26 mAb

✓Murine mAb

✓Under investigation for GvHD

✓Encouraging data in a phase I-II single-institution study (Bacigalupo et al)

✓Large multi-center registrative study already planned

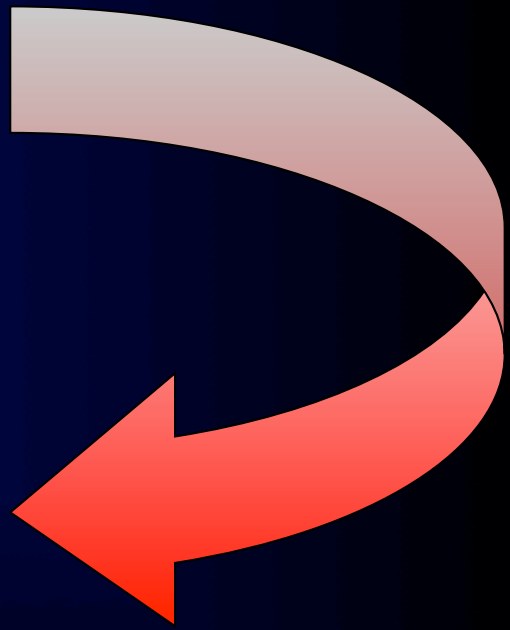
✓Any room for its use in AA???

Aplastic Anemia: Management of Adult Patients

Jaroslaw P. Maciejewski and Antonio M. Risitano

REASONS FOR TREATMENT FAILURE

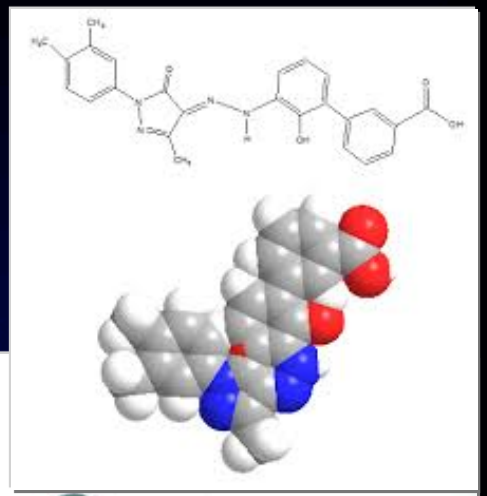
- Pathophysiology other than immune-mediated
- Irreversible stem cell deficit
- Insufficient immunosuppression

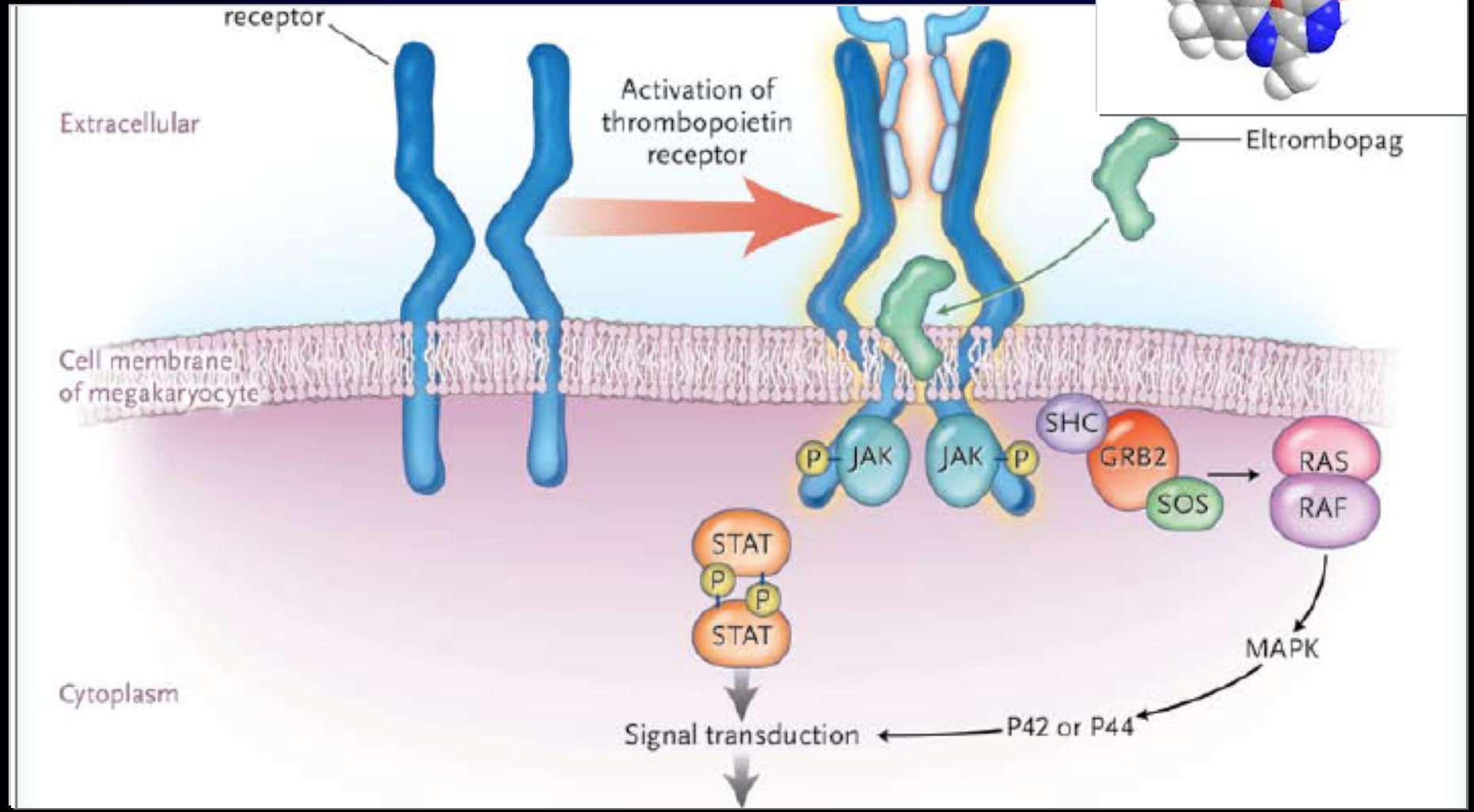


Eltrombopag???

ELTROMBOPAG

A Tpo-mimetic agent





ELTROMBOPAG IN REFRACTORY SAA

The status of art

NEJM

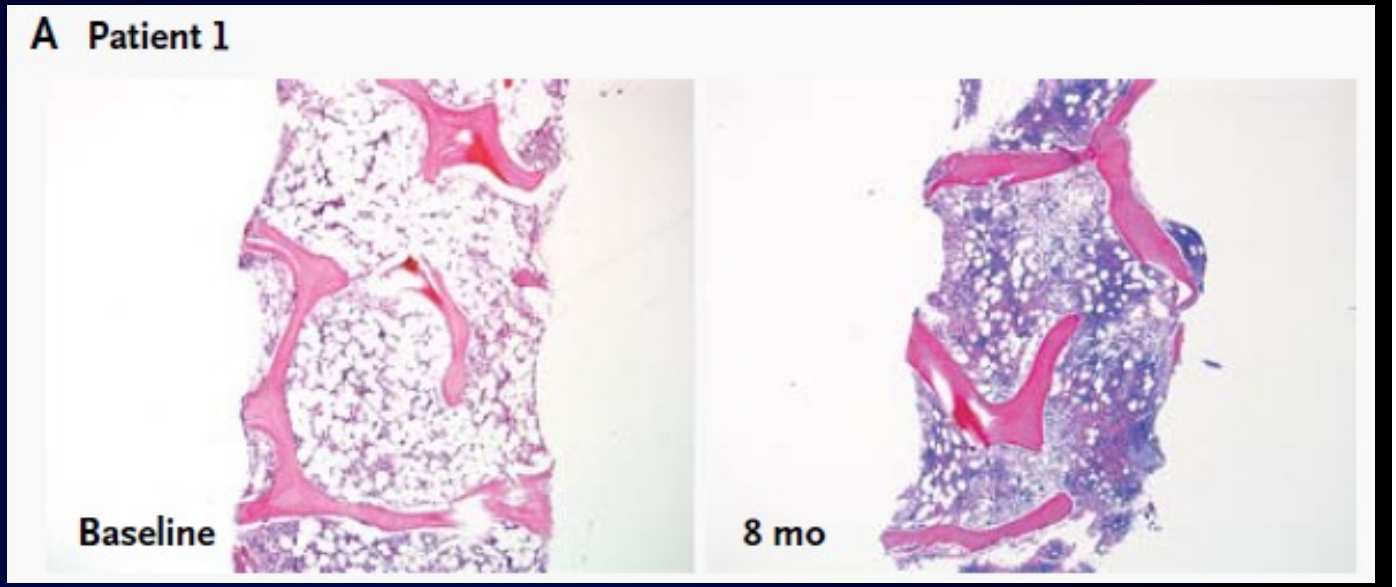
Eltrombopag and Improved Hematopoiesis in Refractory Aplastic Anemia

Phase II study

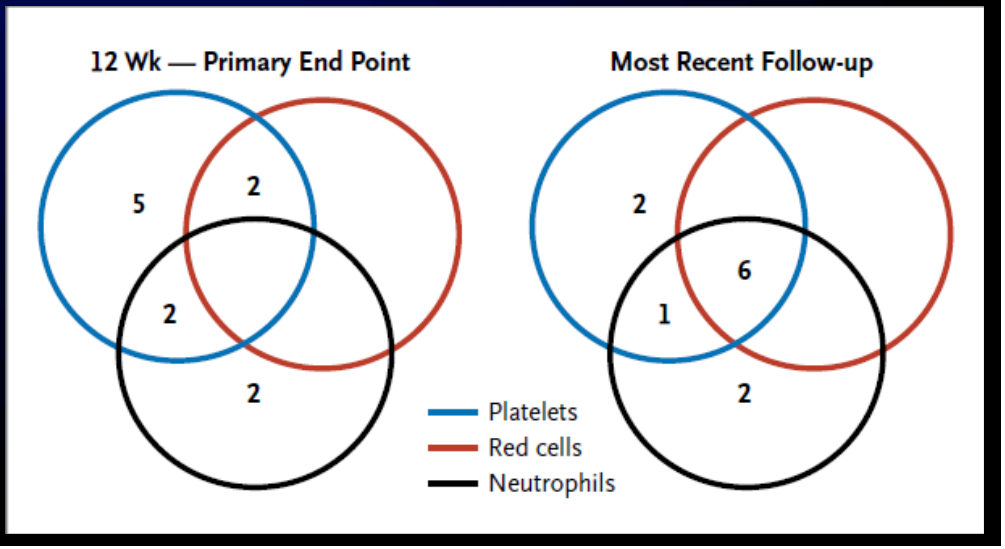
n=25

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 (liver?), no fibrosis

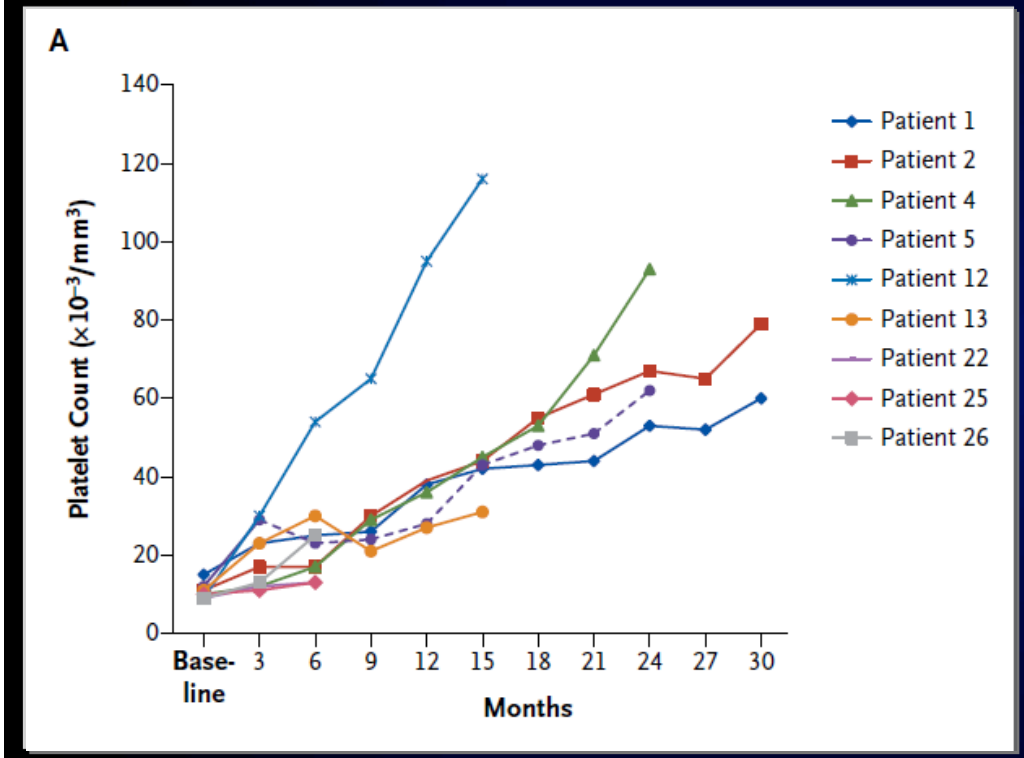


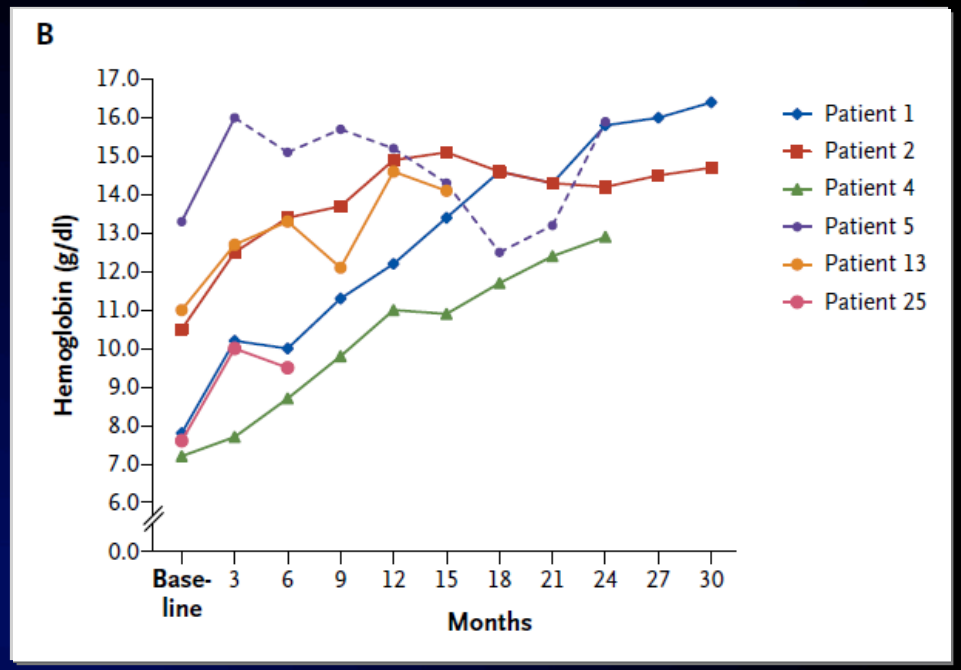
ELTROMBOPAG IN REFRACTORY SAA

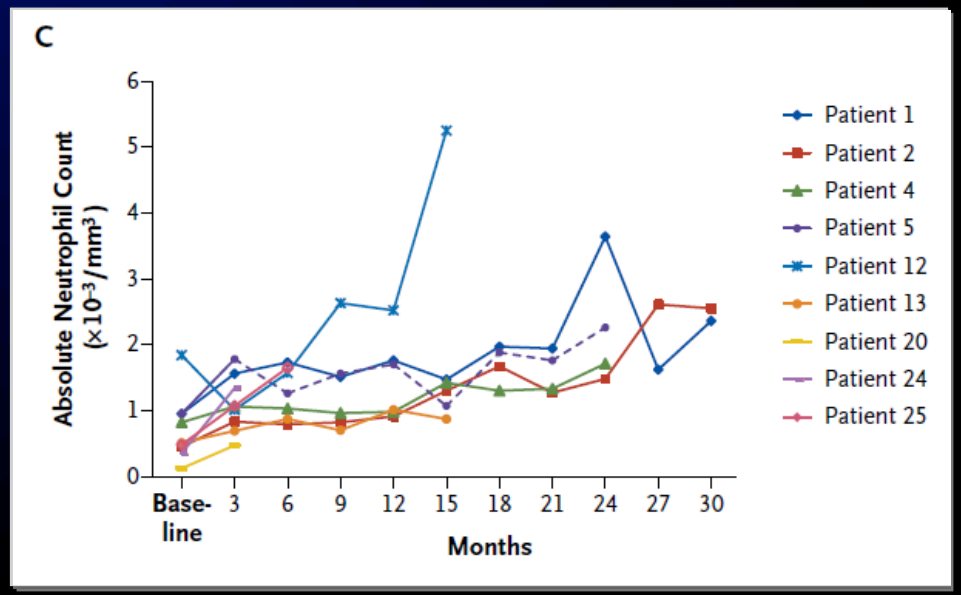
The status of art

NEJM

Eltrombopag and Improved Hematopoiesis in Refractory Aplastic Anemia







- ✓ Out 11 responders
  - ✓ 7 still on eltrombopag, showing further improvement
  - ✓ 4 discontinued (2 ANC responders and 2 toxicities)

ELTROMBOPAG IN REFRACTORY SAA

The risk of clonal evolution

BLOOD, 20 MARCH 2014 •

VOLUME 123, NUMBER 12

Regular Article

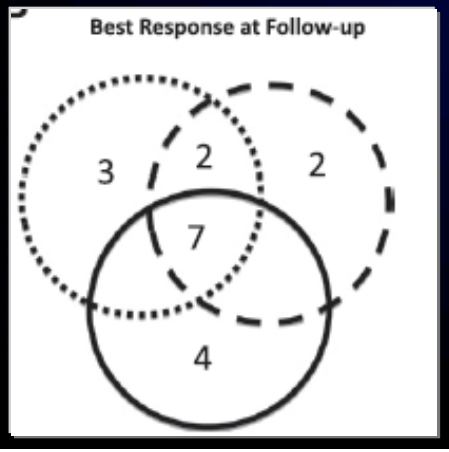
CLINICAL TRIALS AND OBSERVATIONS

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]

- ✓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 REFRACTORY SAA

Kinetics of blood count recovery

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

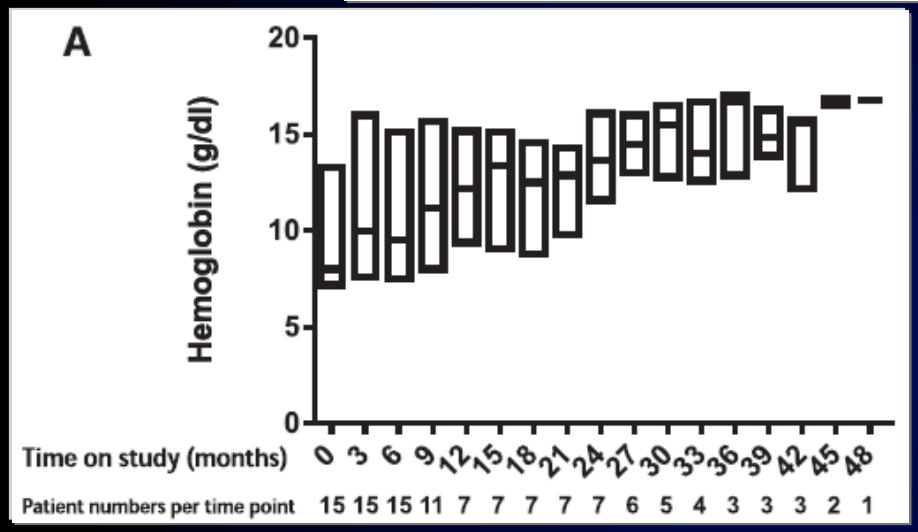
Ronan Desmond,[1] Danielle M. Townsley,[1] Bogdan Dumitriu,[1] Matthew J. Olnes,[2] Phillip Scheinberg,[3] Margaret Bevans,[4] Ankur R. Parikh,[1] Kinneret Broder,[1] Katherine R. Calvo,[5] Colin O. Wu,[6] Neal S. Young,[1] and Cynthia E. Dunbar[1]

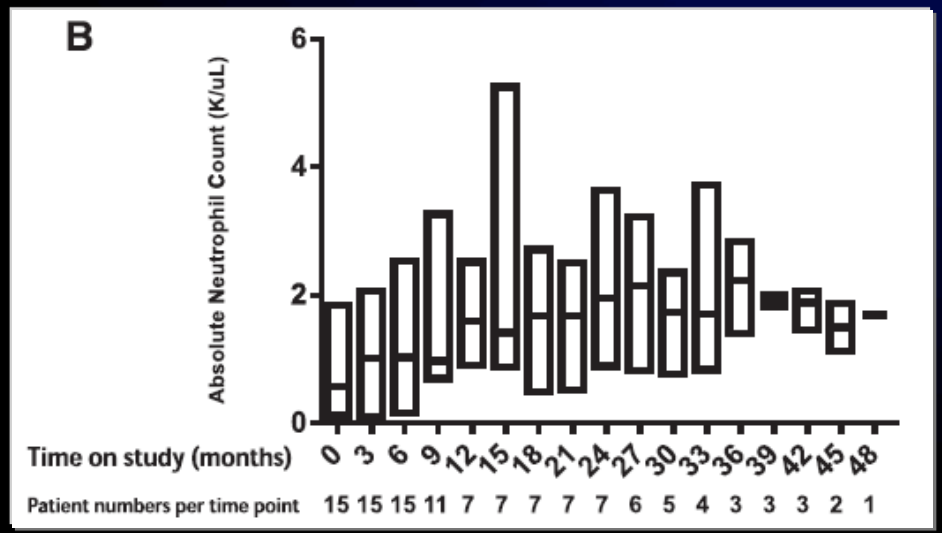
BLOOD, 20 MARCH 2014 •

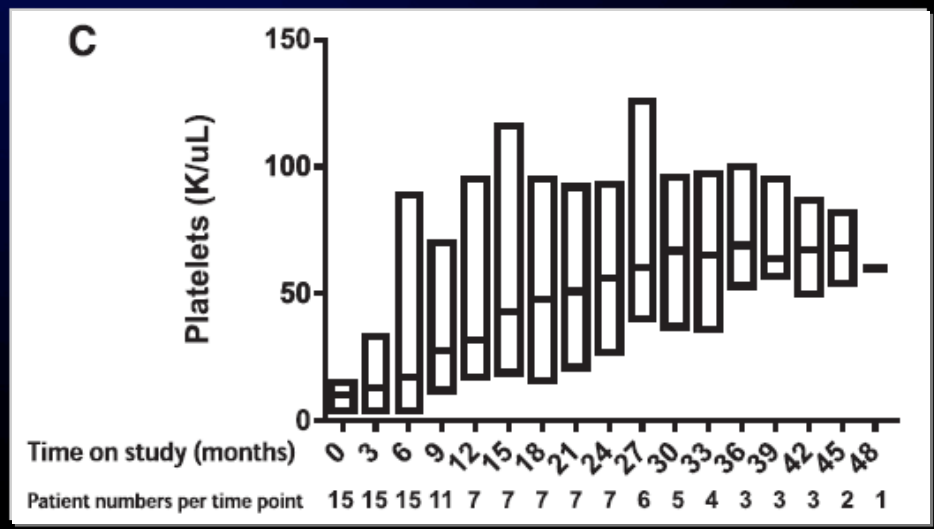
VOLUME 123, NUMBER 12

Key Points

- Eltrombopag promotes hematopoiesis in patients with severe aplastic anemia by stimulating stem and progenitor cells.
- Eltrombopag can be discontinued safely in robust responders with maintenance of hematopoiesis.







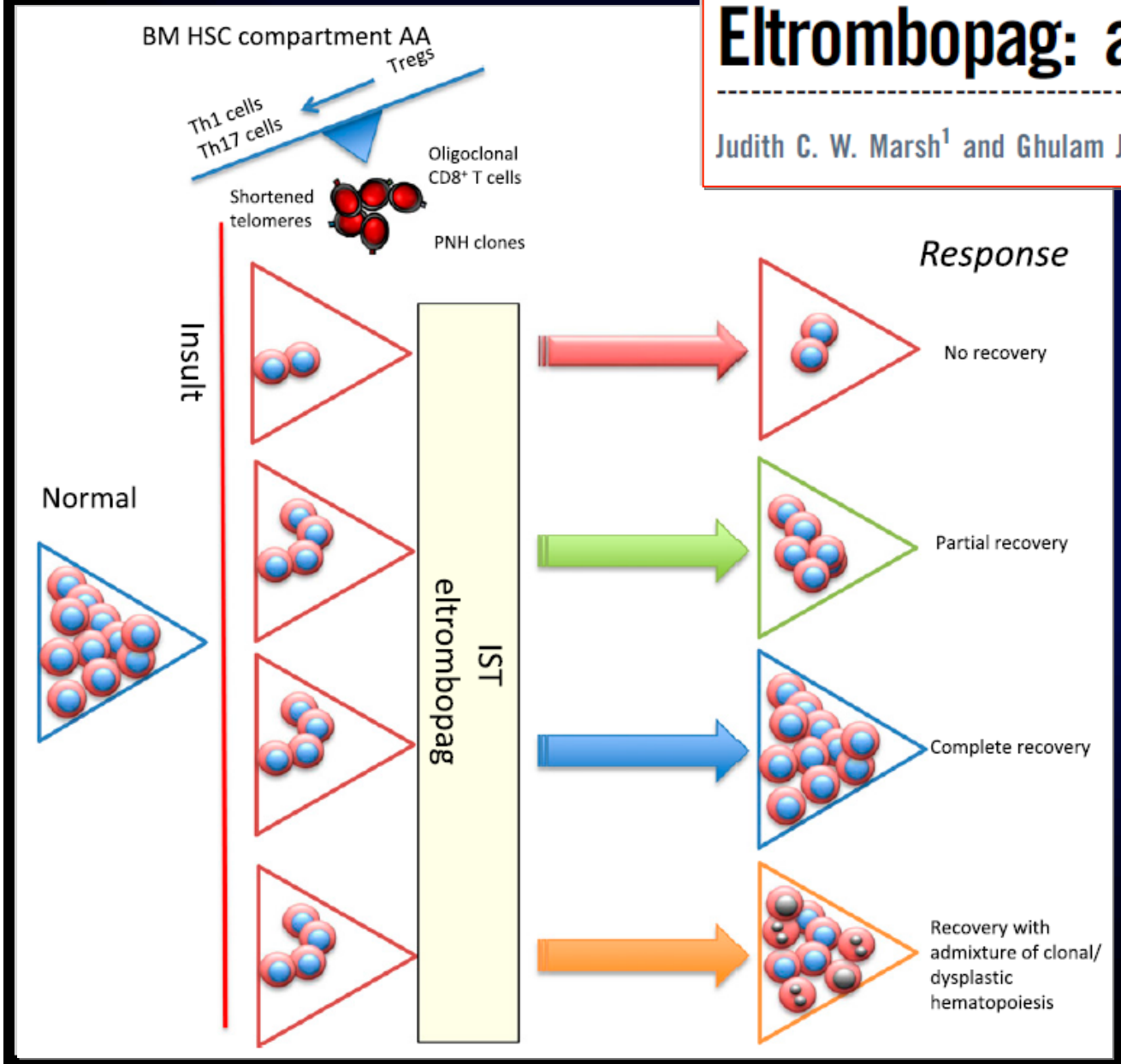
ELTROMBOPAG IN REFRACTORY SAA

The risk of clonal evolution

Comment on Desmond et al, page 1818

Eltrombopag: a stem cell cookie?

Judith C. W. Marsh[1] and Ghulam J. Mufti[1] [1]KING'S COLLEGE LONDON



2003

Antithymocyte Globulin and Cyclosporine for Severe Aplastic Anemia

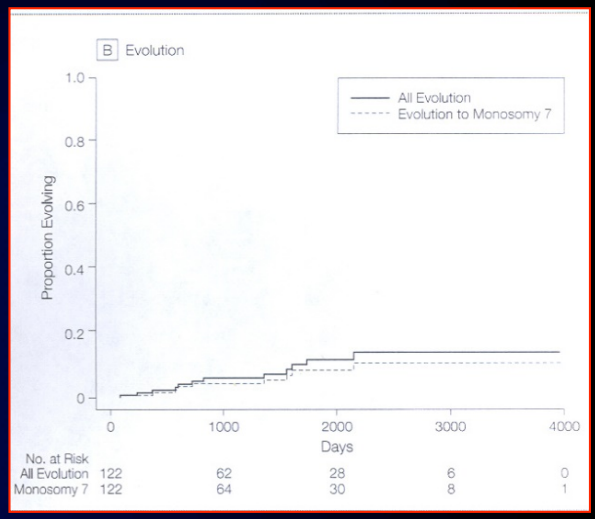
Association Between Hematologic Response and Long-term Outcome

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

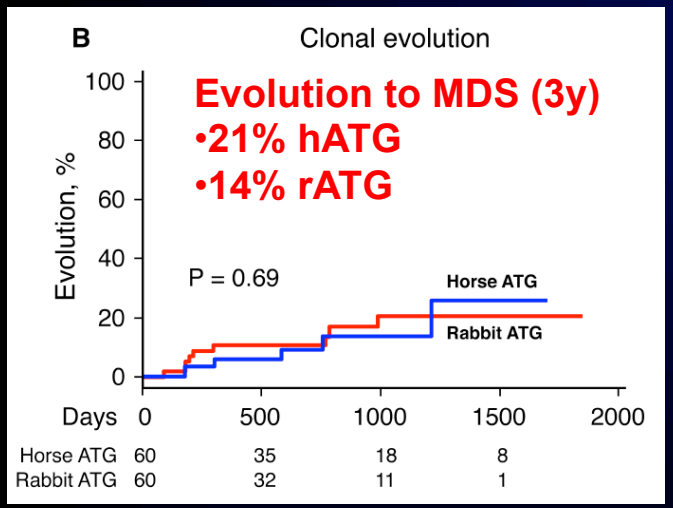
n=112

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

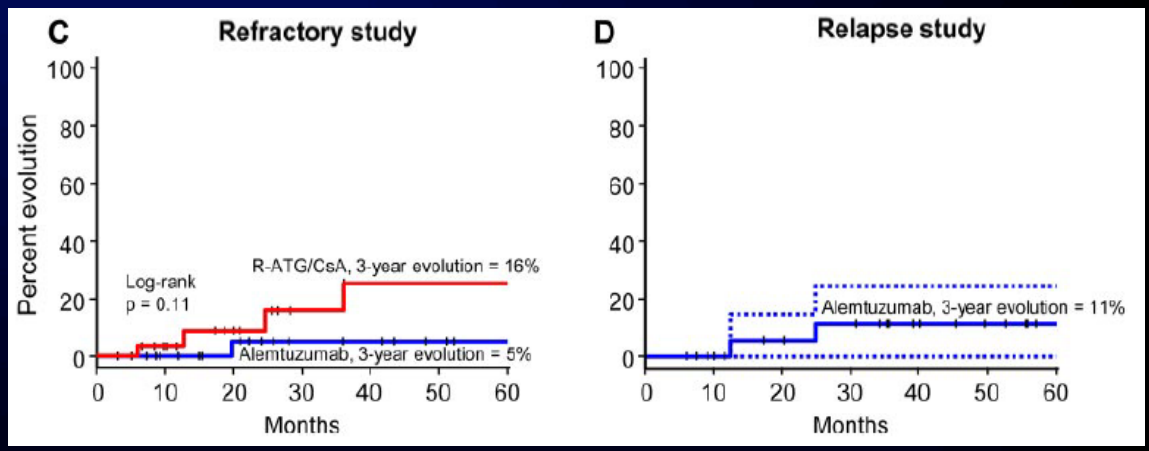
Clonal evolution (3y)
- **11% MDS (especially 7-)**
- **10% PNH**



NEJM 2011



Blood 2012



In all recent studies, the incidence of clonal evolution is about 10-15%, regardless the specific treatment

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)

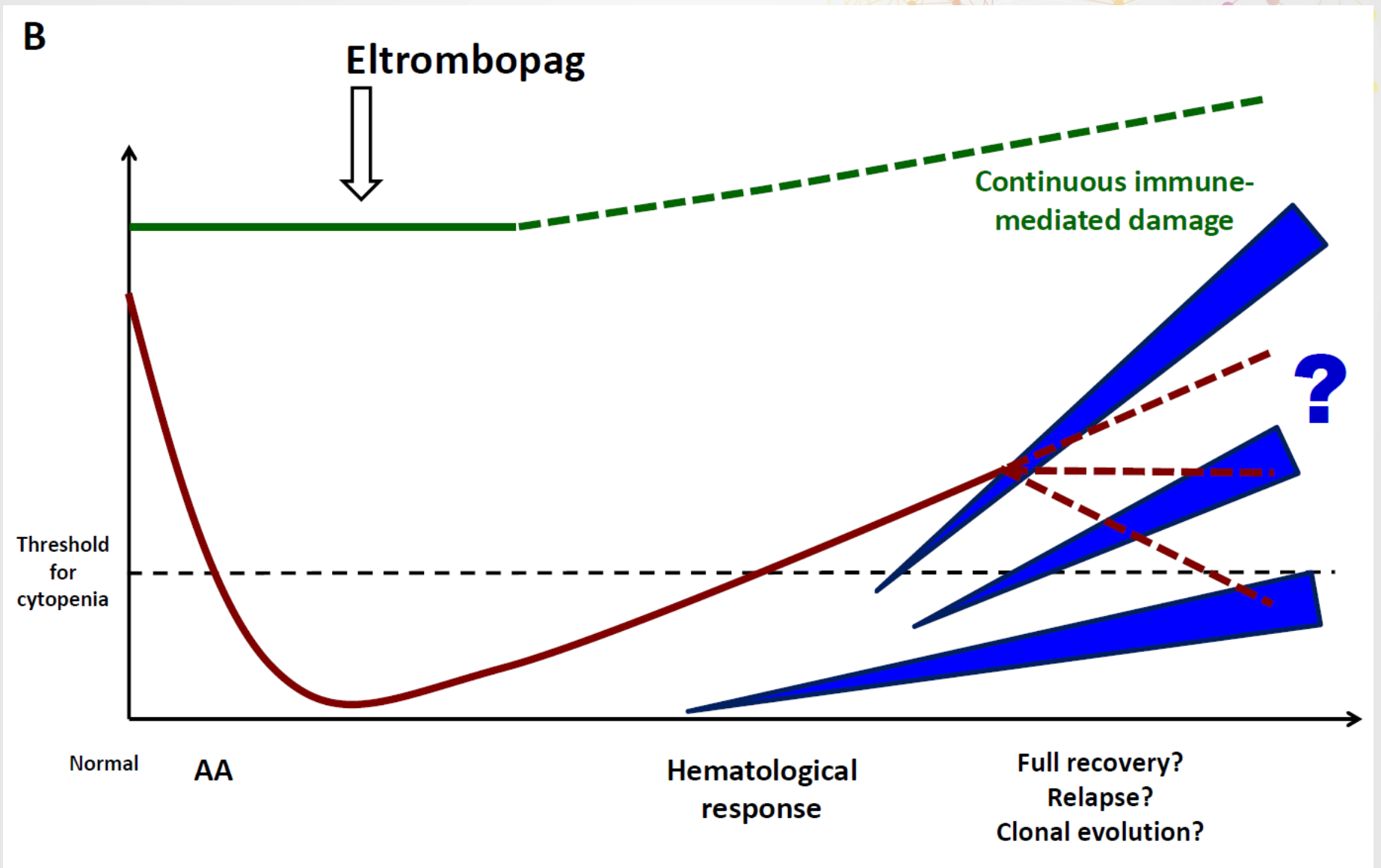
- **Chronic ITP:** Initiate PROMACTA at 50 mg once daily for most patients. Reduce initial dose in patients with hepatic impairment and/or patients of East Asian ancestry. Adjust to maintain platelet count greater than or equal to 50 x 10^9/L. Do not exceed 75 mg per day. (2.1)
- **Chronic Hepatitis 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)

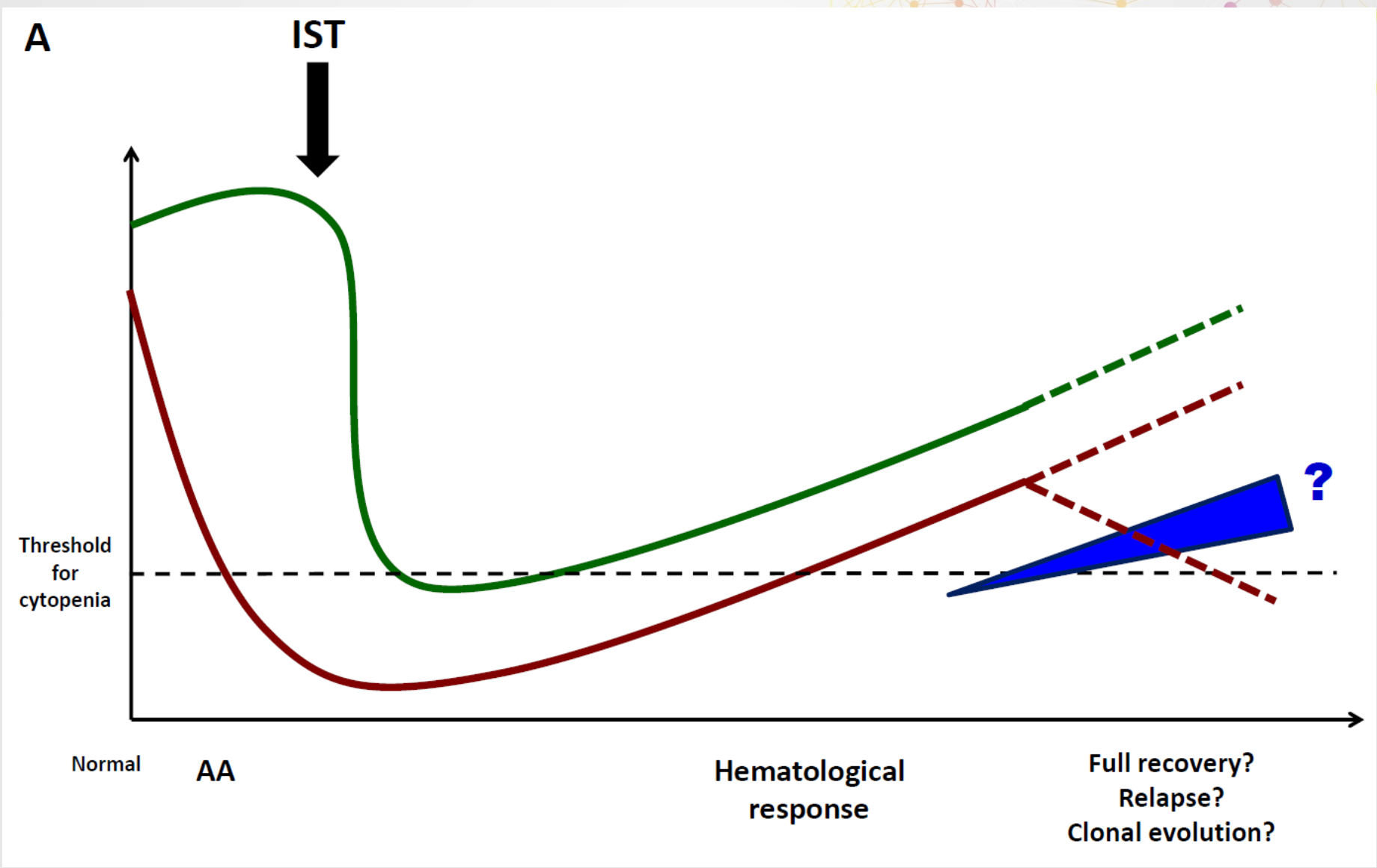
Hematopoiesis, immunity and (clonal) evolution

Risitano and Peffault De Latour, in press



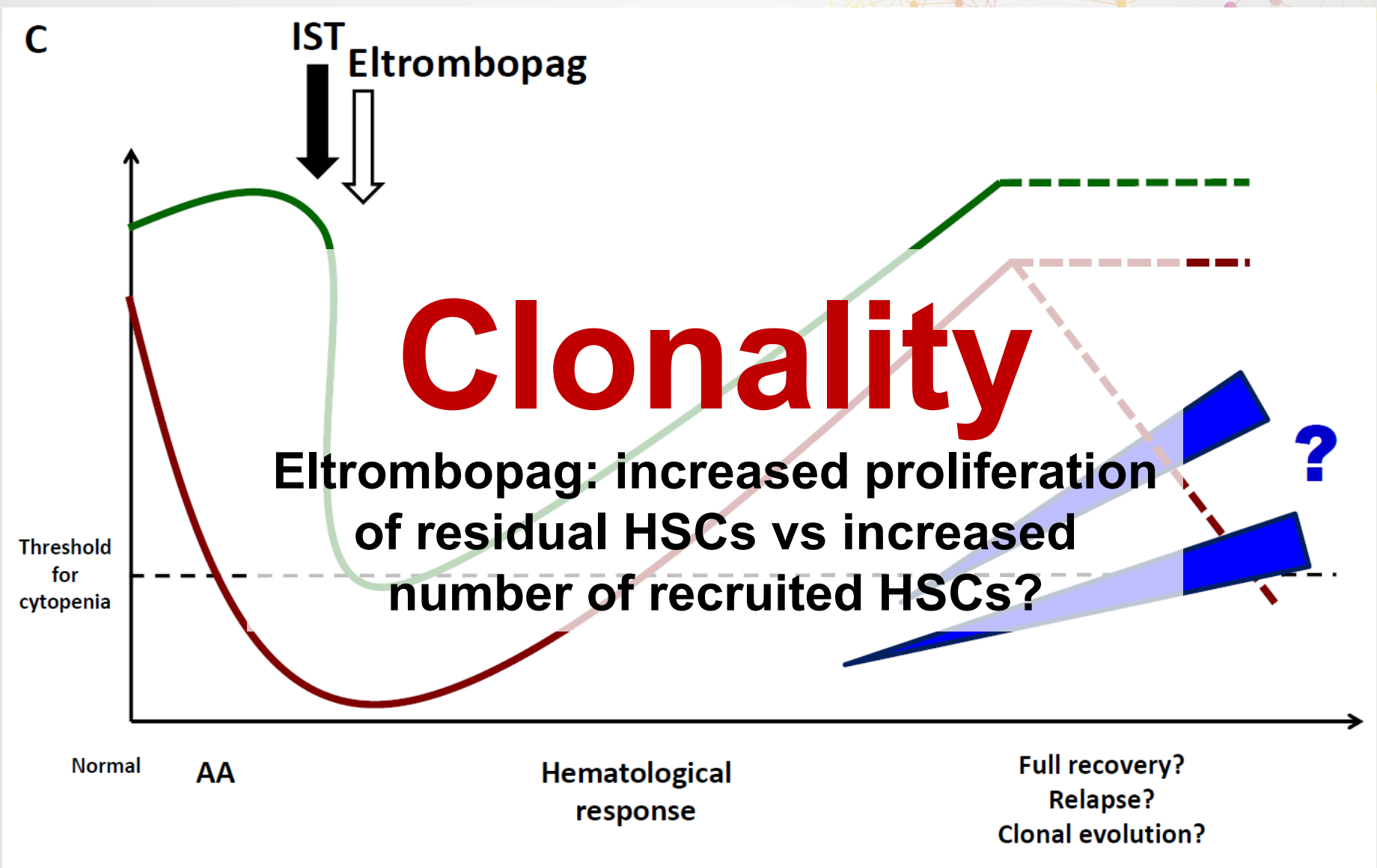
Hematopoiesis, immunity and (clonal) evolution

Risitano and Peffault De Latour, in press



Hematopoiesis, immunity and (clonal) evolution

Risitano and Peffault De Latour, in press



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

Trial Protocol

Efficacy and Safety of Eltrombopag in Patients with Acquired Moderate Aplastic Anemia (EMAA) who are treated with Ciclosporin A

Type of trial:

This is a prospective, randomized, placebo-controlled, double-blind multicenter study.

Patient numbers: 116 evaluable patients (58 each group)

Treatment:

Patients are randomized to receive either
Cyclosporine + Eltrombopag or Cyclosporine + placebo

Eltrombopag (or Placebo) starting dose: of 150 mg orally per day

Option of dose modification regarding to response

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

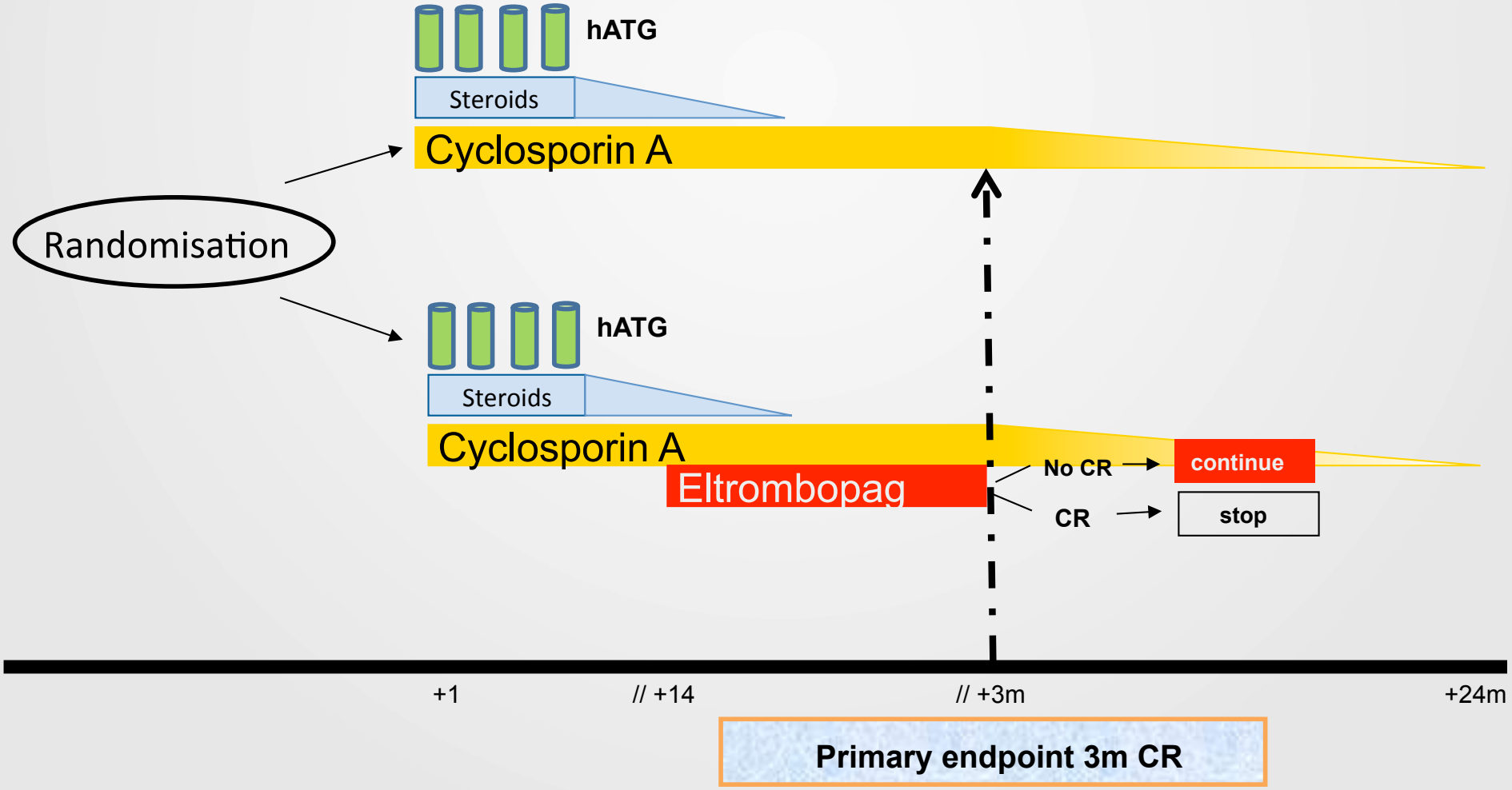
Statistical design

- ✓**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

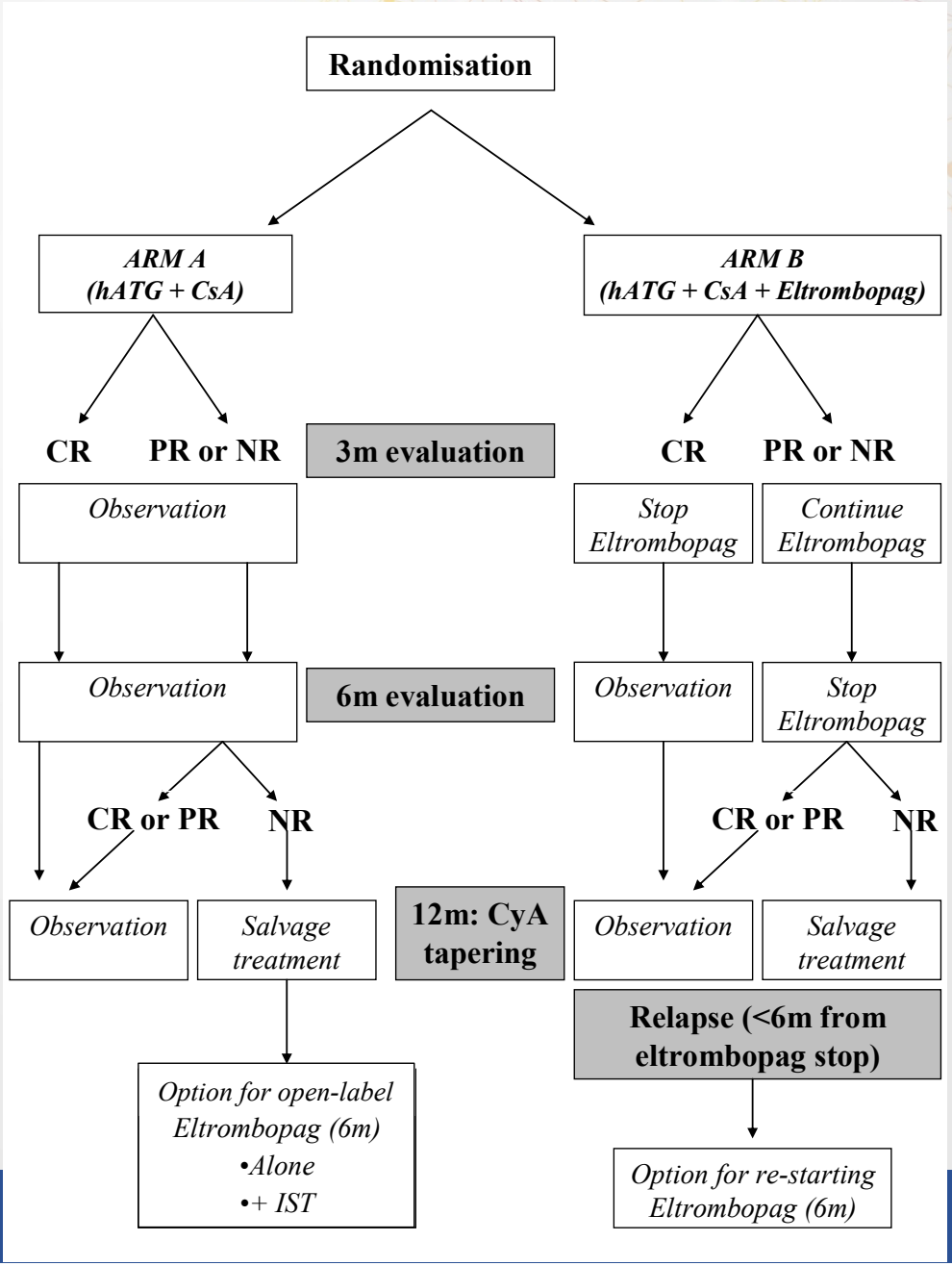
3 month evaluation:
primary endpoint

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



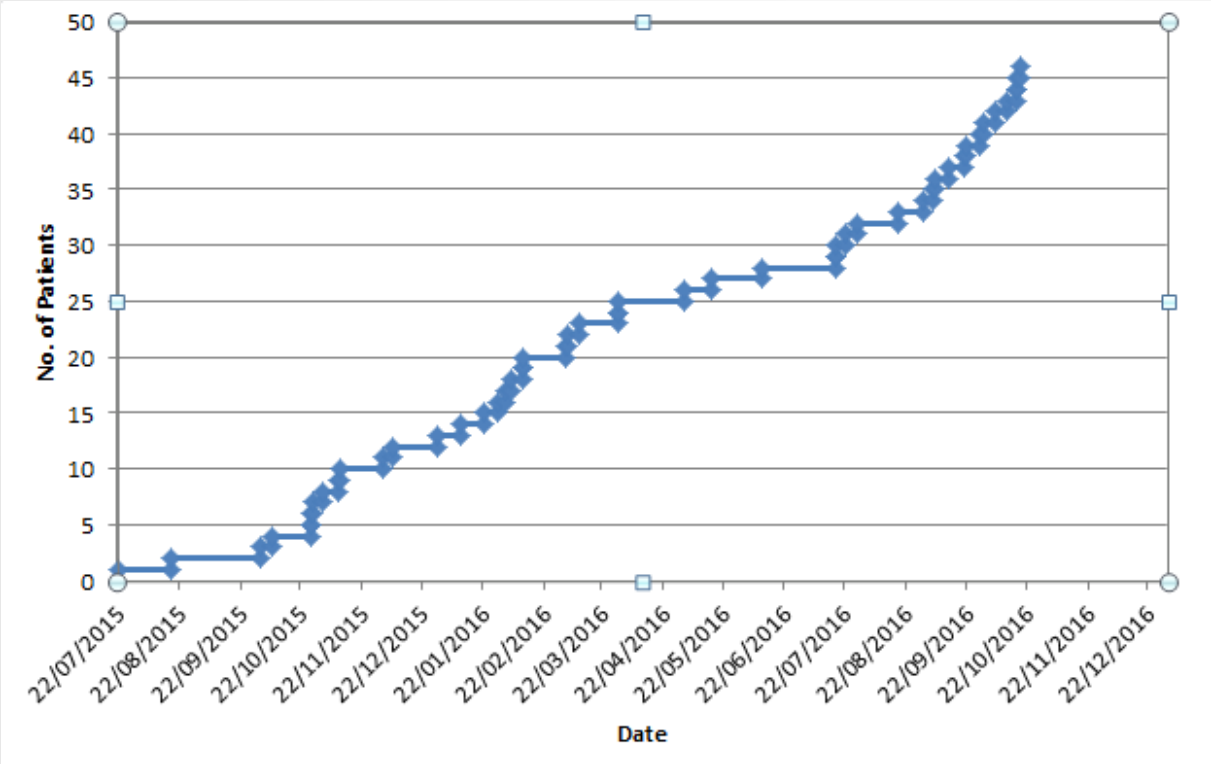
RACE trial – **participating sites**

Country	# sites
France	6 (6 open) +2
Germany	5 (on hold)
Italy	6 (2 open) +3
Netherlands	4 (3 open)
Spain	5 (1 open)
Switzerland	1 (0 open) +1
United Kingdom	5
Total	32 (up to 40)

Brazil	Back up site?

Patient recruitment *(October 10, 2016)*

- 46 patients, 16 sites open (10 sites recruiting) out of 27 sites (not DE)



Patient recruitment is excellent for the number of sites

Delays are in site opening (contracts and regulatory hurdles) – improving

- Target of 50 patients (milestone Novartis) by end Oct – on track (September 14 patients, October 10 so far)

ELTROMBOPAG ADDED TO STANDARD IMMUNOSUPPRESSION AS FIRST TREATMENT IN APLASTIC ANEMIA

Danielle Townsley, MD

Bogdan Dumitriu, MD, Phillip Scheinberg, MD, Ronan Desmond, MD, FRCPath, Xingmin Feng, PhD, Olga Rios, RN, Barbara Weinstein, RN, Janet Valdez, PA-C, Thomas Winkler, MD, Marie Desierto, BS, Harshraj Leuva, MBBS, Colin Wu, PhD, Katherine R. Calvo, MD, PhD, Andre Larochelle, MD, PhD, Cynthia E. Dunbar, MD and Neal S. Young, MD

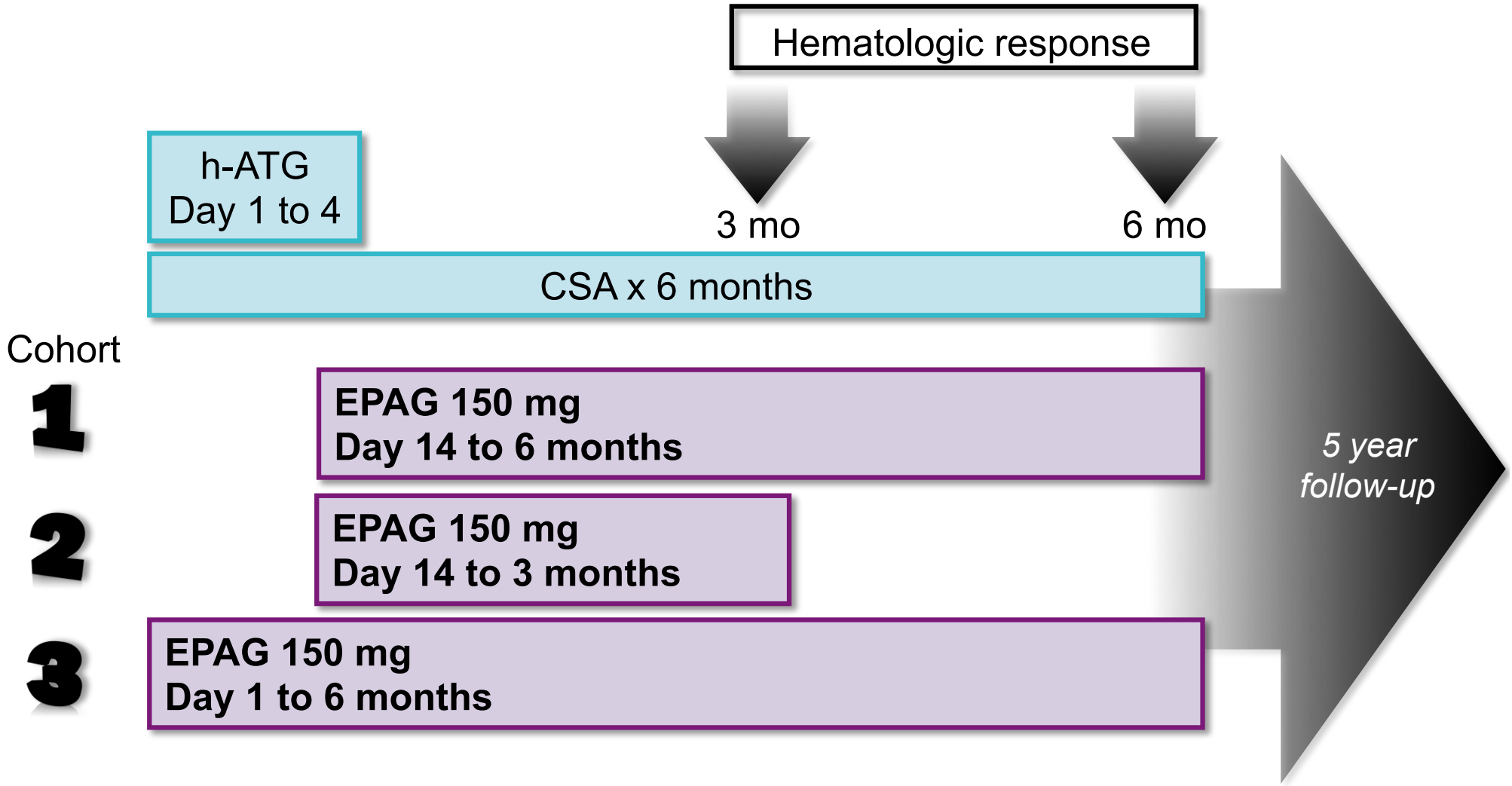
National Heart, Lung, and Blood Institute

American Society for Hematology 2015 Annual Meeting

December 8, 2015

NIH National Heart, Lung, and Blood Institute

STUDY DESIGN
ELTROMBOPAG ADDED TO IST



clinicaltrials.gov NCT01623167

NIH National Heart, Lung, and Blood Institute

RESPONSE RATES

	Cohort 1 N=30	Cohort 2 N=31	Cohort 3 N=31	All Cohorts N=92
	N (%)	N (%)	N (%)	**N (%)**
3 months				**86/92**
OR	23 (77)	24 (77)	23/25 (92)	**70 (81)**
CR	5 (17)	8 (26)	11/25 (44)	**24 (28)**
6 months				**81/92**
OR	24 (80)	27 (87)	19/20 (95)	**70 (86)**
CR	10 (33)	8 (26)	12/20 (60)	**30 (37)**

RESPONSE RATES

	Cohort 1 N=30	Cohort 2 N=31	Cohort 3 N=31	All Co N=	Historic rates N=388*
	N (%)	N (%)	N (%)		
3 months				**86/92**	
OR	23 (77)	24 (77)	**23/25 (92)**	**81%**	**60%**
CR	5 (17)	8 (26)	**11/25 (44)**	**28%**	**8%**
6 months				**81/92**	
OR	24 (80)	27 (87)	**19/20 (95)**	**86%**	**63%**
CR	10 (33)	8 (26)	**12/20 (60)**	**37%**	**12%**

* IST only (hATG and CsA)

Scheinberg & Young. *Blood* 2012

NIH National Heart, Lung, and Blood Institute

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]

[1]Department of Haematological Medicine, King's College London School of Medicine, London, United Kingdom; and [2]Department of Haematology, King's College Hospital, London, United Kingdom

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

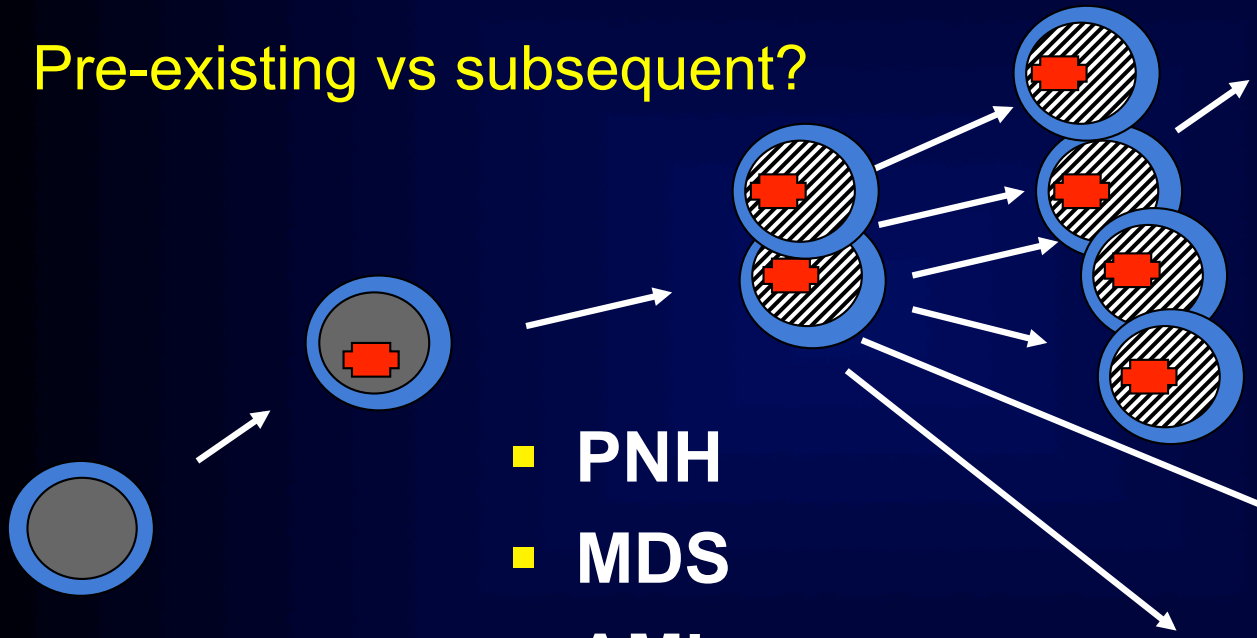
UPN	Gene	Mutant allele burden (%)	Variant class	Nucleotide and protein change	Constitutional DNA
2*	*ASXL1*	30	Frameshift insertion	c.1927_1928insG:p.G643fs	Skin
2*	*DNMT3A*	42	Nonsynonymous SNV	c.C1540G:p.L514V	Skin
2*	*ERBB2*	44	Nonsynonymous SNV	c.G922A:p.V308M	Skin
5*	*TET2*	5	Stopgain SNV	c.C3100T:p.Q1034X	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

CLONAL EVOLUTION

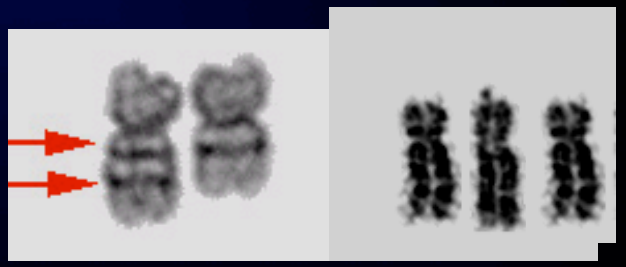
A matter of definition

Consider **oligoclonal** hematopoiesis in AA due to HSC reduction

Pre-existing vs subsequent?



- **PNH**
- **MDS**
- **AML**



7 **8**

Fixation of neutral mutation (founder effect) vs true clonal complication

Clonal evolution

vs

evolution of clones

DISMAL vs BENIGN CLONAL EVOLUTION

2016

2017

5 luglio 1984

16 gennaio 2017

SOMATIC MUTATION IN HSC

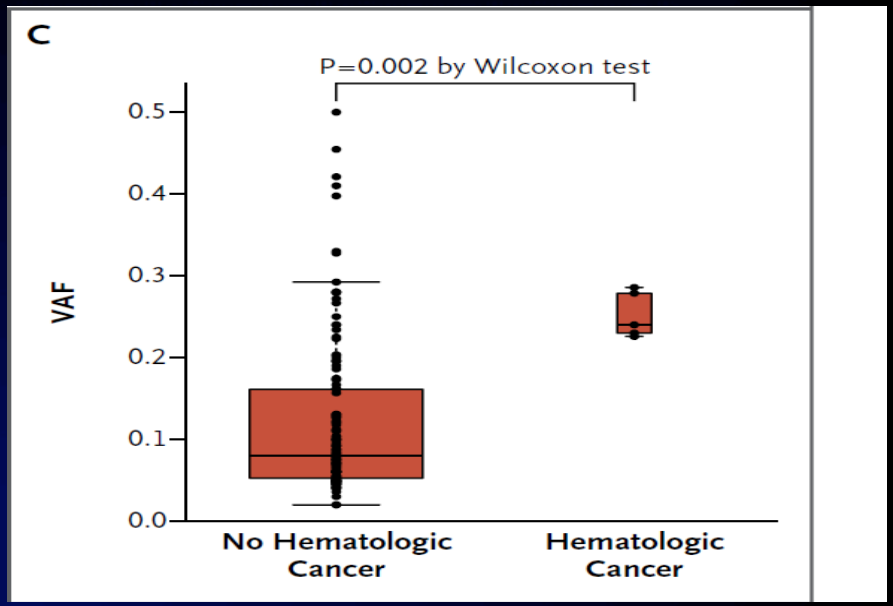
The lesson from ageing

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes

- ✓ **17,182 individuals unselected for hematologic phenotypes**
- ✓ **detectable mutations in 746 persons (4.3%)**
- ✓ **Most common variants in three genes: DNMT3A, TET2, and ASXL1**
- ✓ **The presence of a somatic mutation was associated with increased risk:**
  - hematologic cancer (hazard ratio, 11.1; 95% CI 3.9-32.6)
  - all-cause mortality (HR 1.4; 95% CI 1.1-1.8)
  - incident coronary heart disease (HR 2.0; 95% CI 1.2-3.4)
  - ischemic stroke (HR 2.6; 95% CI 1.4-4.8)



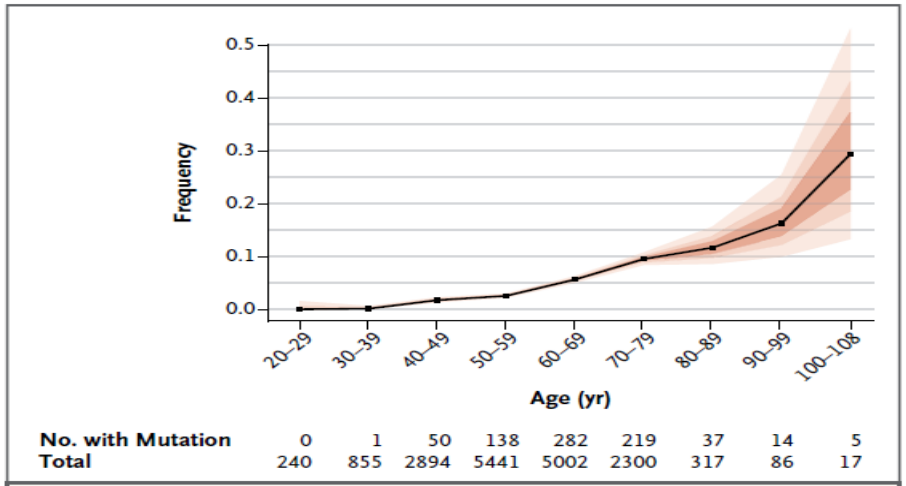
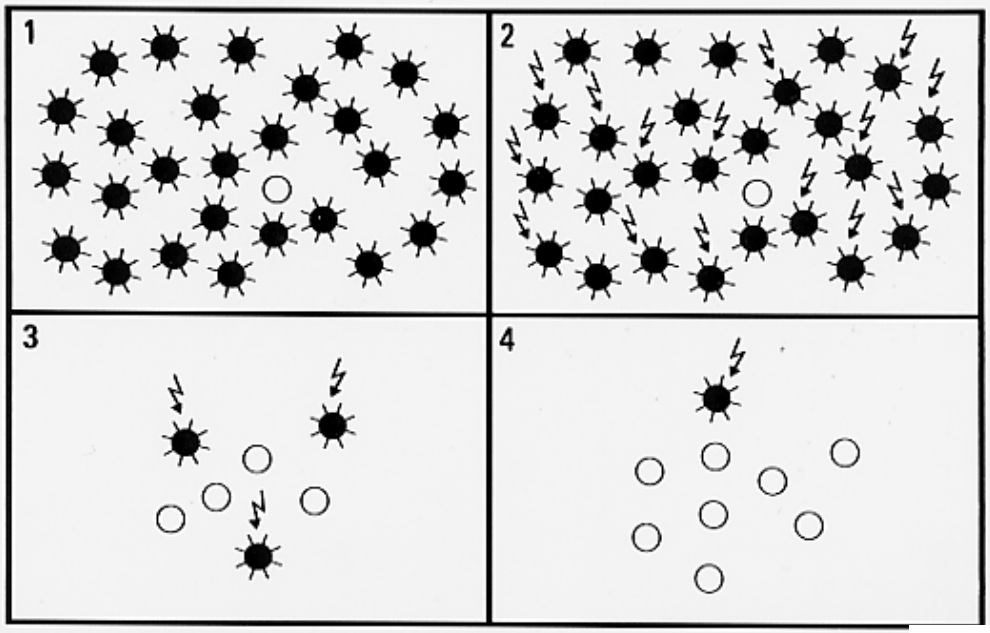


Figure 1. Prevalence of Somatic Mutations, According to Age.
Colored bands, in increasingly lighter shades, represent the 50th, 75th, and 95th percentiles.

Siddhartha Jaiswal *et al*, Dec 2014

THE DUAL PATHOPHYSIOLOGY OF PNH

Rotoli and Luzzatto, Baillieres Clin Haematol 1989; Cell, 1997



Bruno Rotoli 1937-2009

Cell, Vol. 88, 1–4, January 10, 1997, Copyright ©1997 by Cell Press

Somatic Mutations in Paroxysmal Nocturnal Hemoglobinuria: A Blessing in Disguise?

Lucio Luzzatto, Monica Bessler, and Bruno Rotoli*

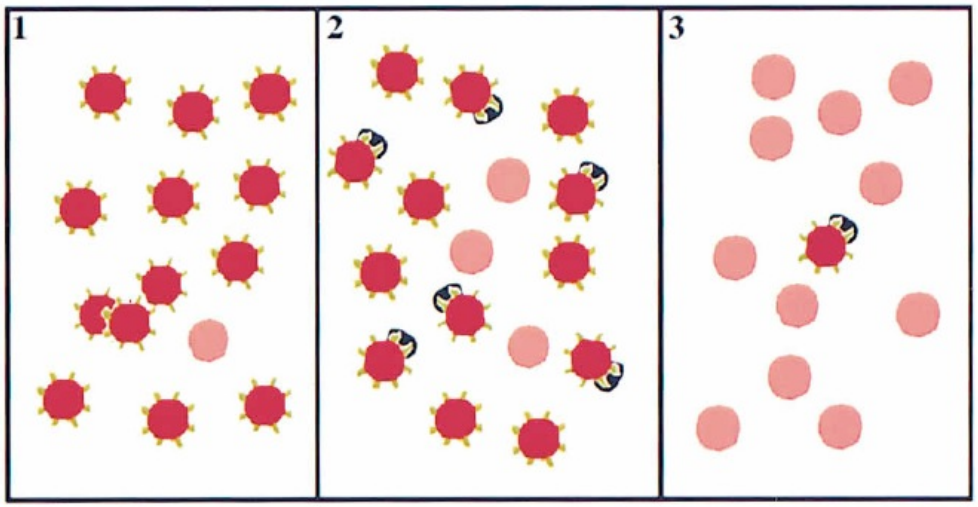
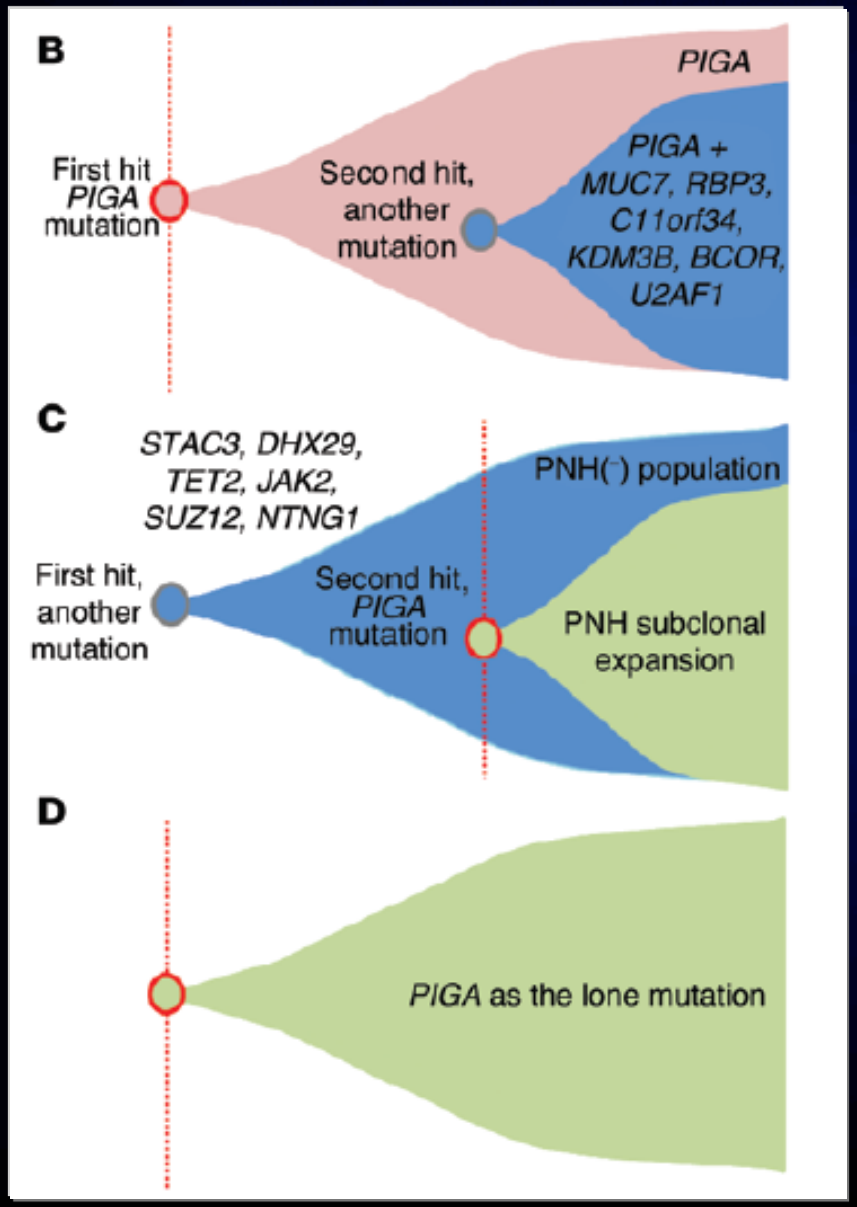
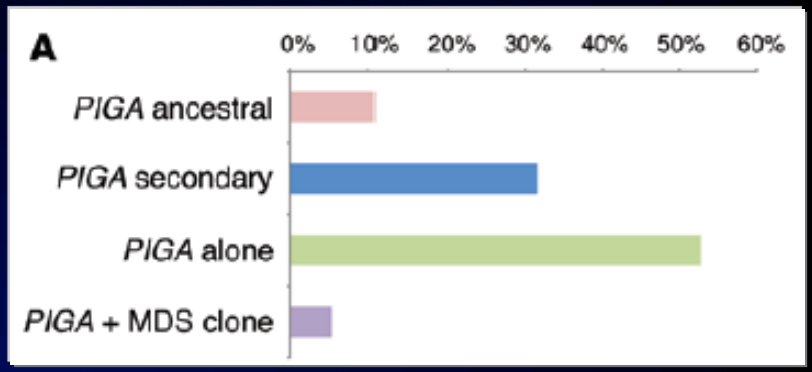


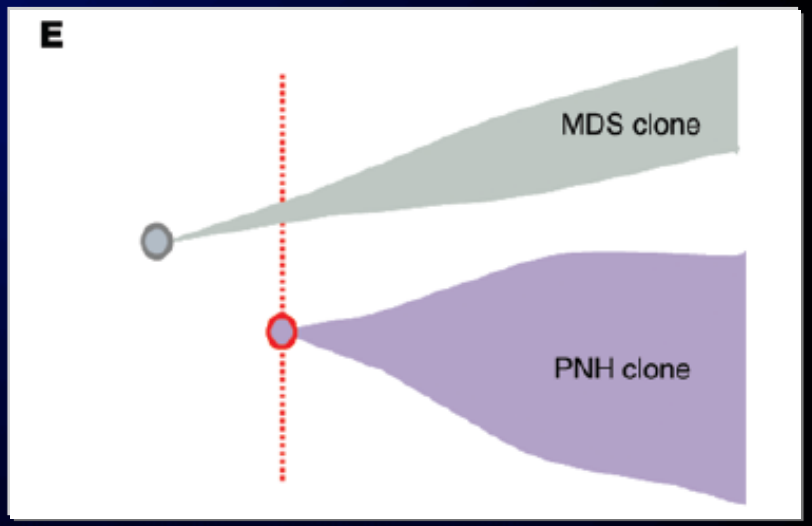
Figure 2. A Model for the Pathogenesis of PNH

Deep sequencing reveals stepwise mutation acquisition in paroxysmal nocturnal hemoglobinuria

Wenyi Shen,[1,2] Michael J. Clemente,[1] Naoko Hosono,[1] Kenichi Yoshida,[3] Bartlomiej Przychodzen,[1] Tetsuichi Yoshizato,[3] Yuichi Shiraishi,[4] Satoru Miyano,[4,5] Seishi Ogawa,[3] Jaroslaw P. Maciejewski,[1] and Hideki Makishima[1]







Is PNH a cancer???

HEMATOPOIESIS AND PNH

Long-term support from a single HSC

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2002 99: 2748-2751
doi:10.1182/blood.V99.8.2748

Long-term support of hematopoiesis by a single stem cell clone in patients with paroxysmal nocturnal hemoglobinuria

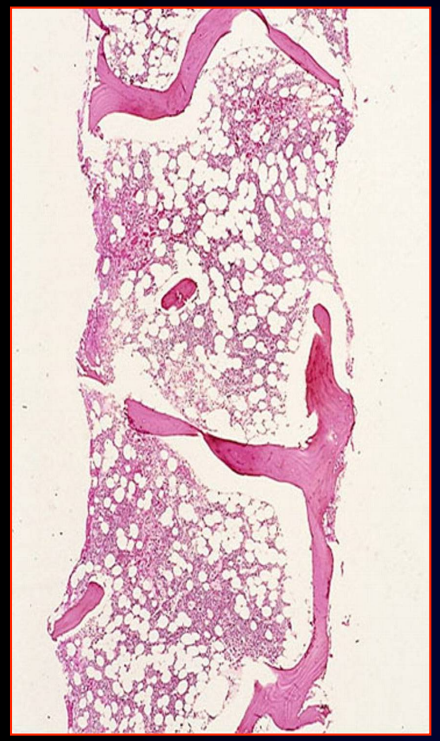
Jun-ichi Nishimura, Toshiyuki Hirota, Yuzuru Kanakura, Takashi Machii, Takashi Kageyama, Shoichi Doi, Hiroshi Wada, Toru Masaoka, Yoshio Kanayama, Hiroshi Fujii, Nobumasa Inoue, Maki Kuwayama, Norimitsu Inoue, Kazuhito Ohishi, and Taroh Kinoshita

Table 2. Summary of CD59 expressions and somatic mutations of PIG-A in 9 patients with PNH at the previous analysis and the current analysis

		Previous analysis			Current analysis			
		PB PMN		BM Colonies/bursts	PB PMN		BM MNC	Duration
Patient	Mutation	CD59⁻ (%)	mRNA (AS)	DNA (AS)	CD59⁻ (%)	DNA (AS)	DNA (AS)	(y)
J4	298C to T	50	11/22	ND	98	3/10	ND	7
	273C to A		0/10	ND		2/10	ND	
J5	1309C del	65	10/20	ND	97	9/9	ND	7
J3	383A to G	97	18/20	ND	93	4/10	ND	7
J11	408T del	83	15/20	ND	82	7/18	ND	7
J13	116C to A	87	18/20	ND	73	7/10	ND	7
J19	987 T ins	78	14/27	13/25	61	5/10	10/10	8
	338 T to C		ND	2/25		0/15	1/16	
	1003 G to T		2/27	4/25		0/10	0/10	
	1028 AA del		0/27	2/25		0/10	0/10	
J12	936A del	94	14/20	ND	33	4/16	ND	6
	322A del		1/12	ND		2/11	ND	
J15	Int 5 3′splice site G to A	99	(Major)	ND	57	4/10	ND	7
	368A ins		(Minor)	ND		1/9	ND	
J16	Int 5 5′splice site T del	98	5/5	ND	43	4/18	ND	10

AS indicates amplified subclones; Duration, the period between the previous analysis and the current analysis; ND, not done.

Bone Marrow Failure and PNH



AA + PNH clone(s)

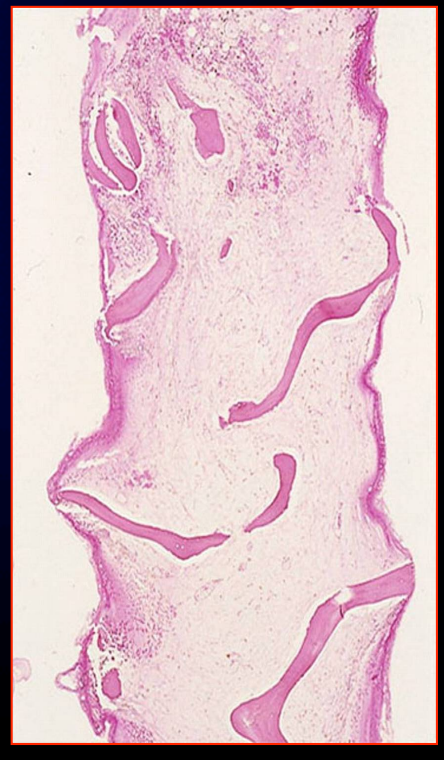
PNH

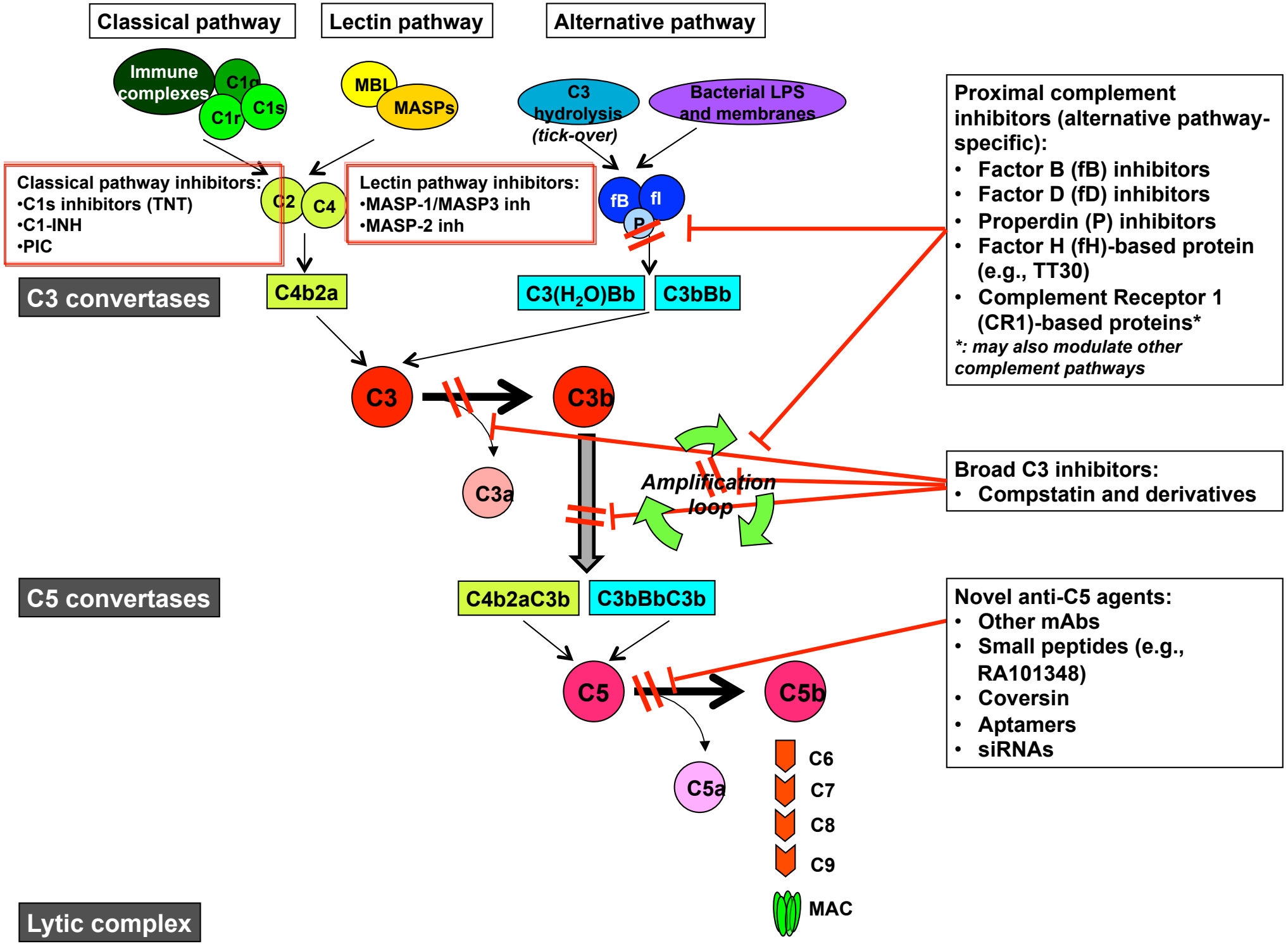
Hemolysis

Cytopenia

Aplastic Anemia

Aplastic anemia




Classical pathway
Lectin pathway
Alternative pathway
Immune complexes
C1q
C1r
C1s
MBL
MASPs
C3 hydrolysis
(tick-over)
Bacterial LPS and membranes
Classical pathway inhibitors:
•C1s inhibitors (TNT)
•C1-INH
•PIC
Lectin pathway inhibitors:
•MASP-1/MASP3 inh
•MASP-2 inh
C2
C4
fB
fI
P
C3 convertases
C4b2a
C3(H2O)Bb
C3bBb
C3
C3b
C3a
Amplification loop
C5 convertases
C4b2aC3b
C3bBbC3b
C5
C5b
C5a
C6
C7
C8
C9
MAC
Lytic complex
Proximal complement inhibitors (alternative pathway-specific):
• Factor B (fB) inhibitors
• Factor D (fD) inhibitors
• Properdin (P) inhibitors
• Factor H (fH)-based protein (e.g., TT30)
• Complement Receptor 1 (CR1)-based proteins*
*: may also modulate other complement pathways
Broad C3 inhibitors:
• Compstatin and derivatives
Novel anti-C5 agents:
• Other mAbs
• Small peptides (e.g., RA101348)
• Coversin
• Aptamers
• siRNAs

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