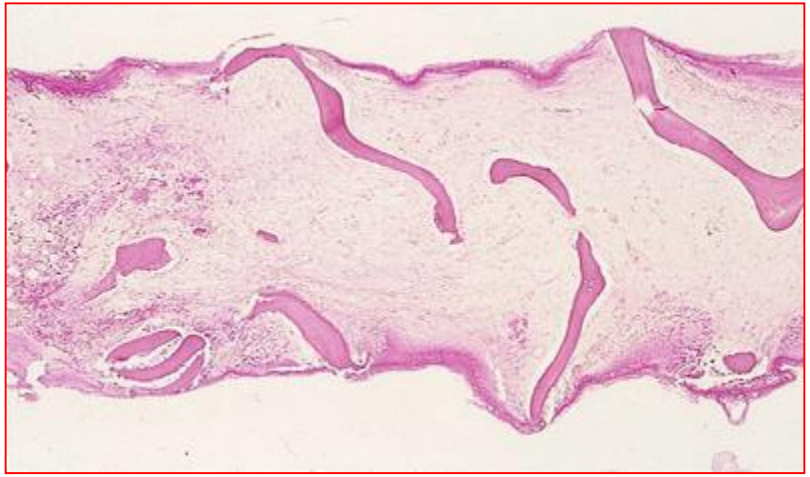
First line treatments for idiopathic aplastic anemia

Standard treatments and lines of research: immunosuppressive drugs



Antonio M. Risitano, M.D., Ph.D.
Head of Hematology and
Bone Marrow Transplantation Unit
AORN San Giuseppe Moscati Avellino

Disclosures

Name of Company	Type of affiliation (example: grant; personal fees, non-financial support; intellectual Property - patents & copyrights; royalties)
Alexion	Research support; Member of an advisory Board; Lecture fees
Alnylam	Research support
Novartis	Research support; Member of an advisory Board; Lecture fees
Pfizer	Lecture fees
Achillion	Member of an advisory Board;
Apellis	Member of an advisory Board; Lecture fees
Biocryst	Member of an advisory Board;
RA pharma	Research support
Amyndas	Consultant
Samsung	Member of an advisory Board;
Roche	Member of an advisory Board;
Jazz	Lecture fees

Aplastic anemia:

Pathophysiology

Pathophysiology of aplastic anemia

Hematopoietic stem cell

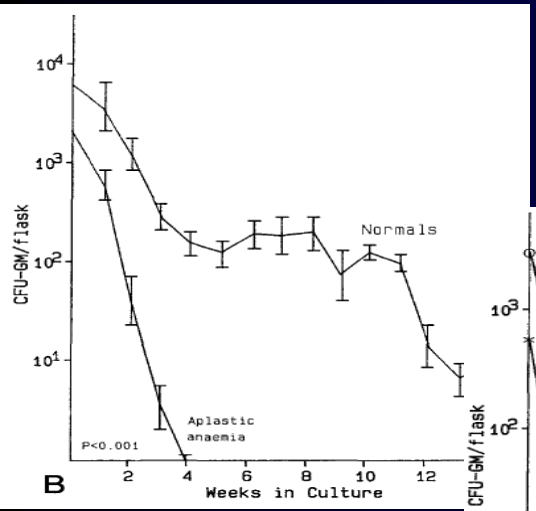
Hematopoietic stem cells in AA

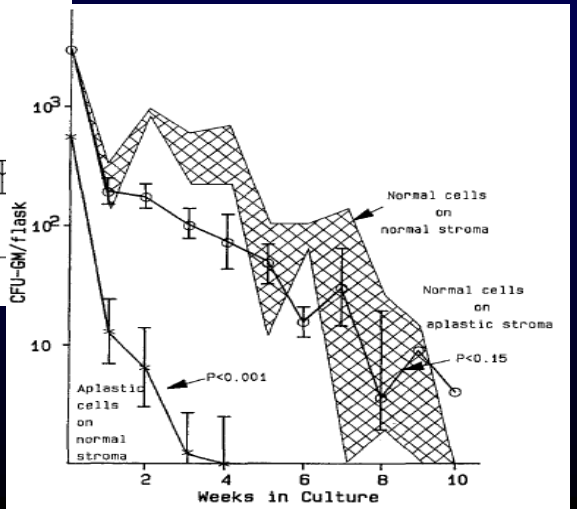
Hematopoietic progenitor cultures

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

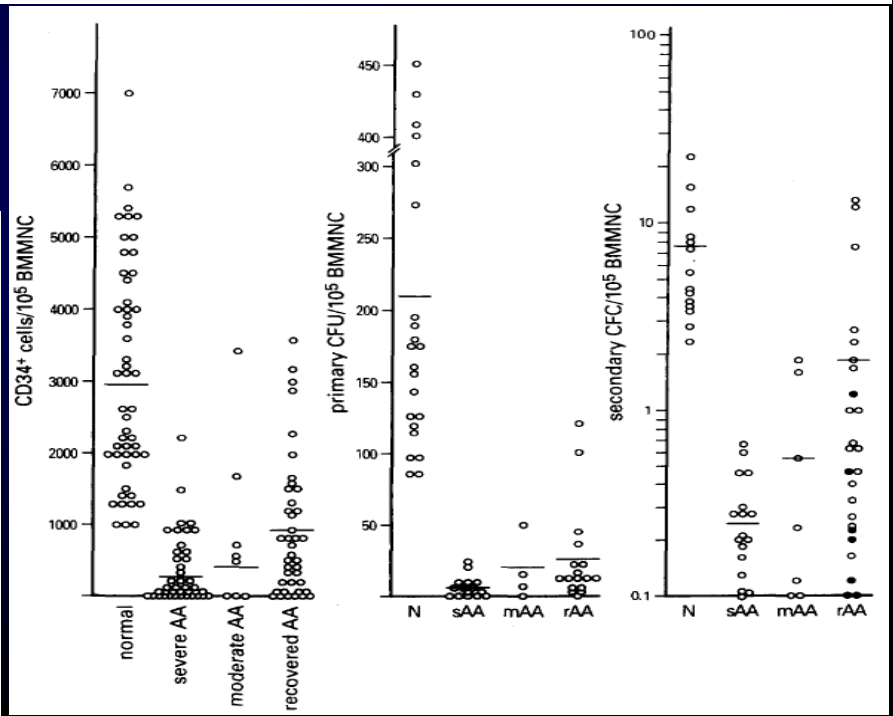




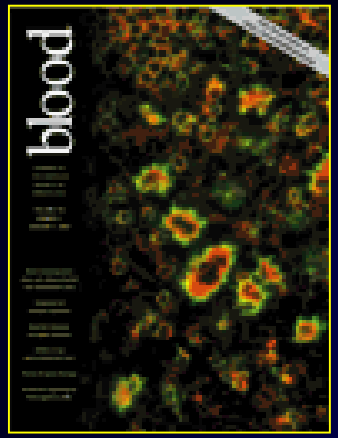
blood 1996 88: 1983-1991

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

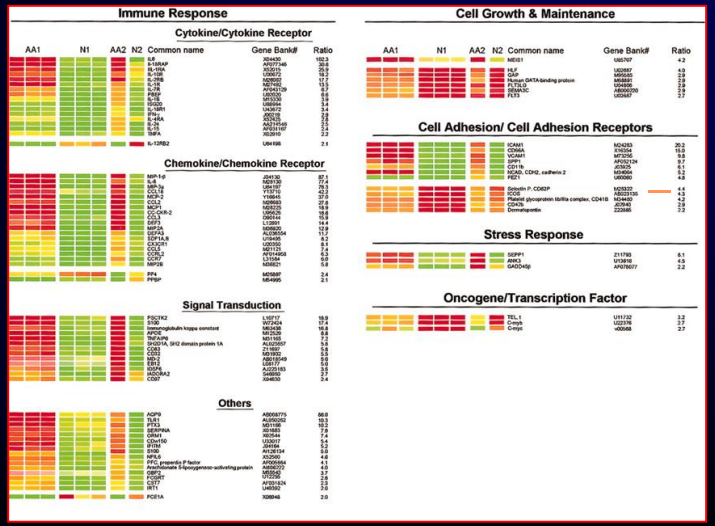


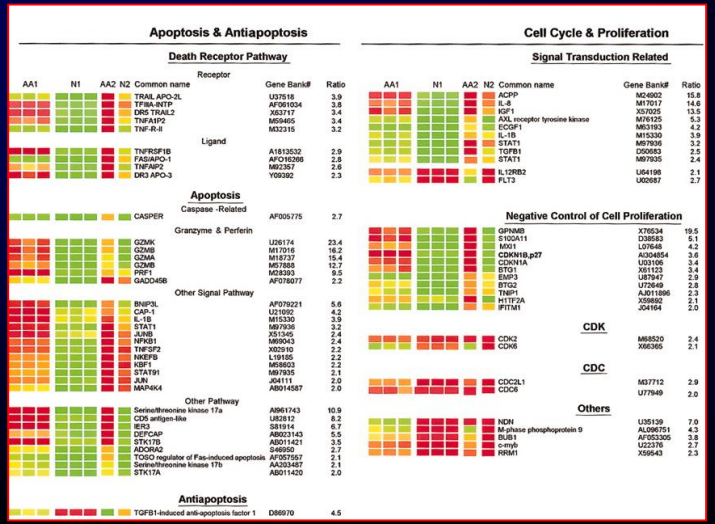
RED CELLS

BLOOD, 1 JANUARY 2004 • VOLUME 103, NUMBER 1

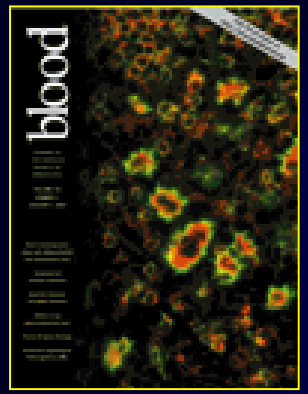
Gene expression profiling in CD34 cells to identify differences between aplastic anemia patients and healthy volunteers

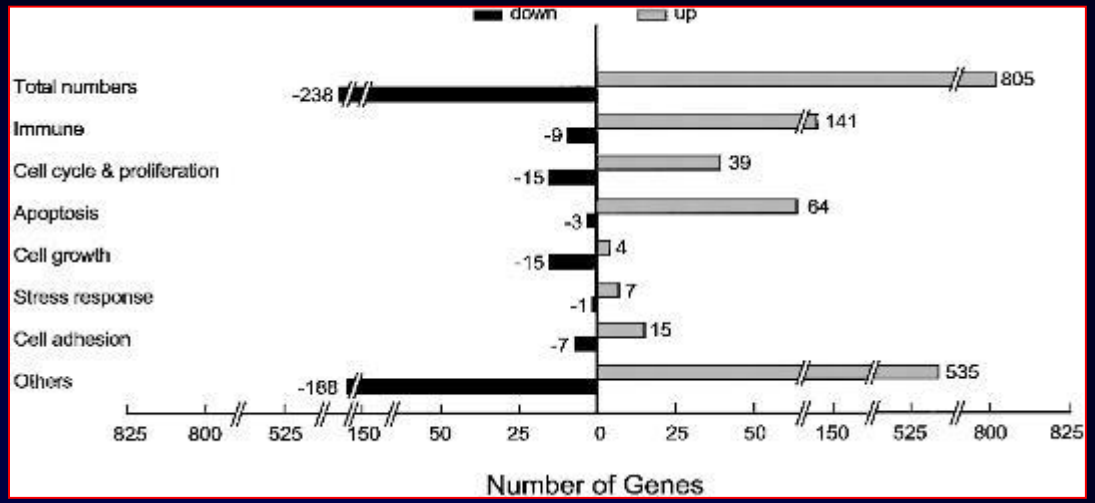
Weihua Zeng, Gubir Cher, Sachiko Kajigaya, Olga Nunez, Alexandra Charrow, Eric M. Billings, and Neal S. Young





Differential expression of specific gene classes among normal and AA CD34+





Over-expressed

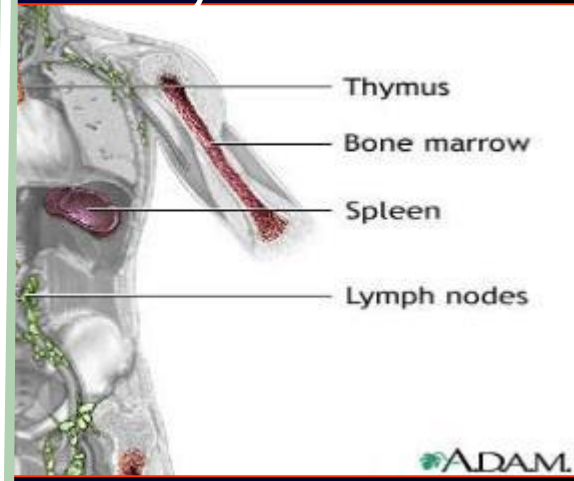
- Apoptosis
- Stress response
- Cytokine/chemokine transduction
- Defense/immune response genes
- Cell cycle/proliferation inhibitors

"…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

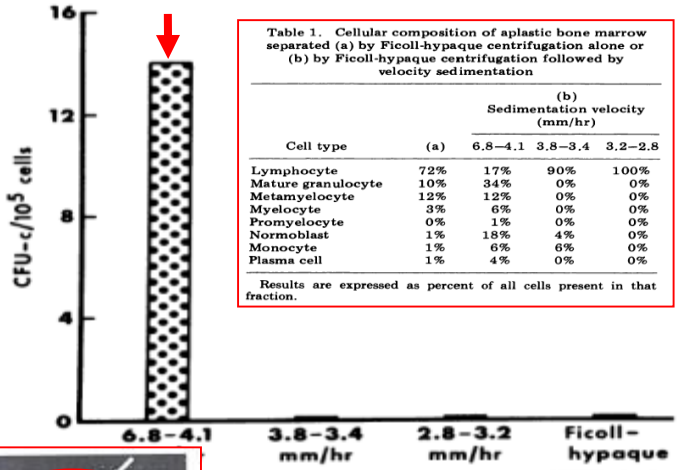
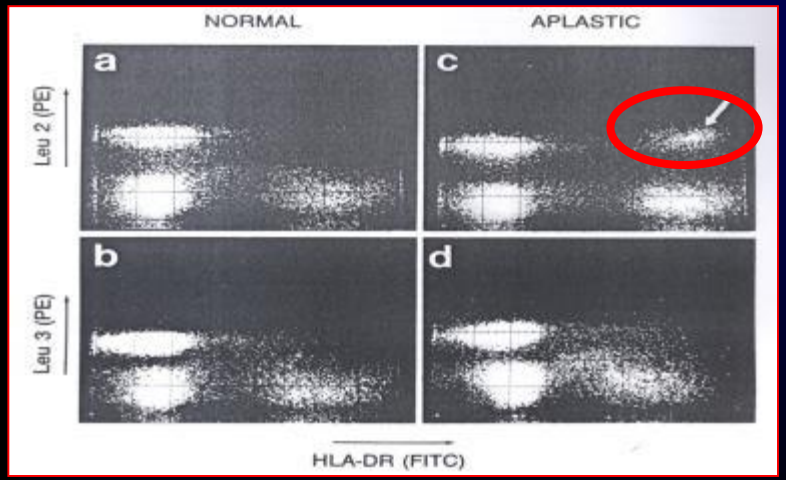


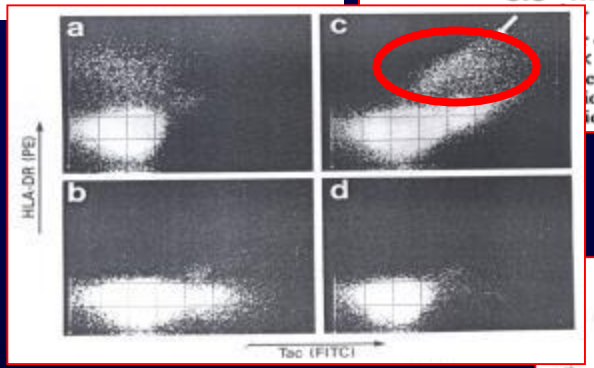
Table 1. Cellular composition of aplastic bone marrow separated (a) by Ficoll-hypaque centrifugation alone or (b) by Ficoll-hypaque centrifugation followed by velocity sedimentation

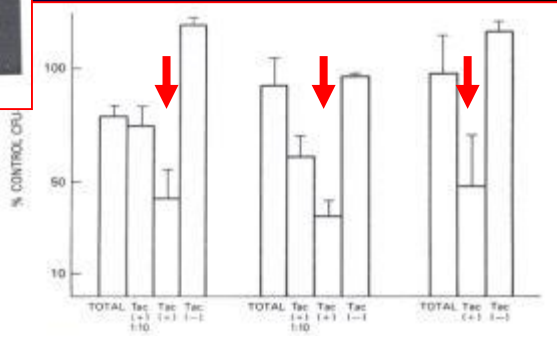
Cell type	(a)	(b) Sedimentation velocity (mm/hr) 6.8–4.1	3.8–3.4	3.2–2.8
Lymphocyte	72%	17%	90%	100%
Mature granulocyte	10%	34%	0%	0%
Metamyelocyte	12%	12%	0%	0%
Myelocyte	3%	6%	0%	0%
Promyelocyte	0%	1%	0%	0%
Normoblast	1%	18%	4%	0%
Monocyte	1%	6%	6%	0%
Plasma cell	1%	4%	0%	0%

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

...of granulocyte-monocyte colonies (CFU-c) after ...10⁵ 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.







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

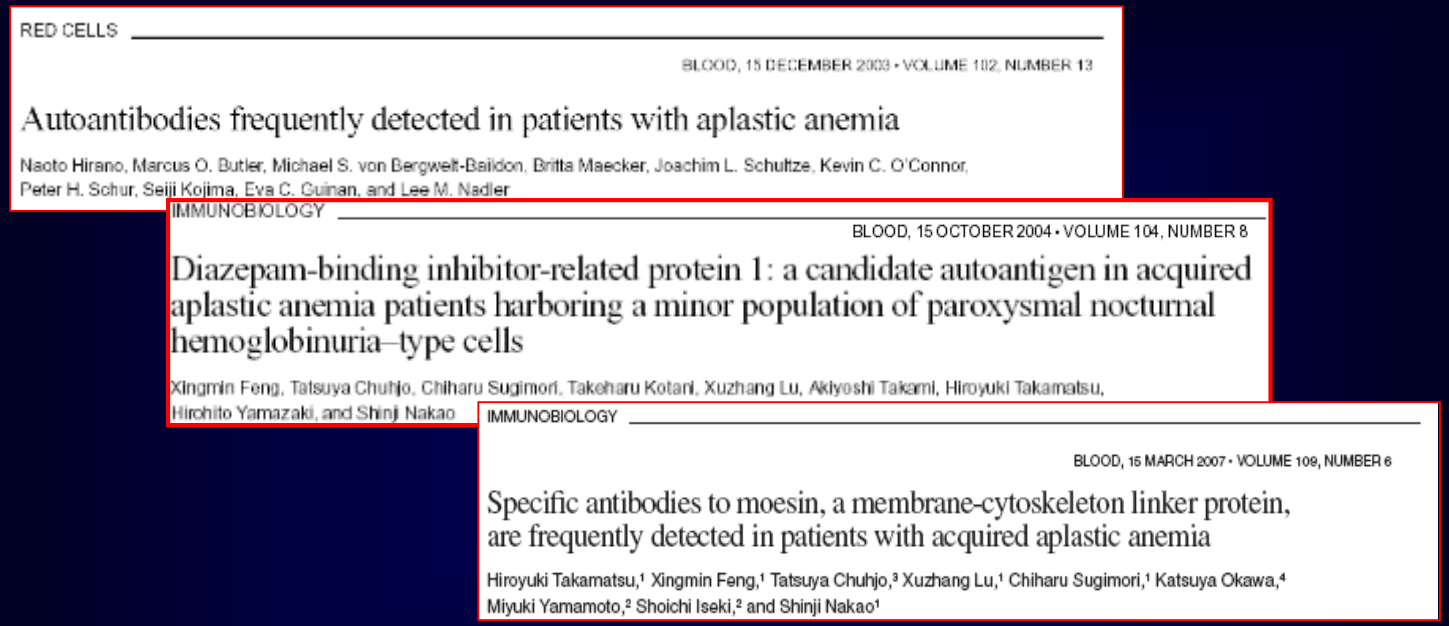
Oligoclonal and polyclonal CD4 and CD8 lymphocytes in aplastic anemia and paroxysmal nocturnal hemoglobinuria measured by Vβ CDR3 spectratyping and flow cytometry

BLOOD, 1 JULY 2002 • VOLUME 100, NUMBER 1

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

Candidate auto-antigens in aplastic anemia

Evidence of auto-antibodies in AA patients



RED CELLS

BLOOD, 15 DECEMBER 2003 • VOLUME 102, NUMBER 13

Autoantibodies frequently detected in patients with aplastic anemia

Naoto Hirano, Marcus O. Butler, Michael S. von Bergwelt-Baildon, Britta Maecker, Joachim L. Schultze, Kevin C. O'Connor, Peter H. Schur, Seiji Kojima, Eva C. Guinan, and Lee M. Nadler

IMMUNOBIOLOGY

BLOOD, 15 OCTOBER 2004 • VOLUME 104, NUMBER 8

Diazepam-binding inhibitor-related protein 1: a candidate autoantigen in acquired aplastic anemia patients harboring a minor population of paroxysmal nocturnal hemoglobinuria–type cells

Xingmin Feng, Tatsuya Chuhjo, Chiharu Sugimori, Takeharu Kotani, Xuzhang Lu, Akiyoshi Takami, Hiroyuki Takamatsu, Hirohito Yamazaki, and Shinji Nakao

IMMUNOBIOLOGY

BLOOD, 15 MARCH 2007 • VOLUME 109, NUMBER 6

Specific antibodies to moesin, a membrane-cytoskeleton linker protein, are frequently detected in patients with acquired aplastic anemia

Hiroyuki Takamatsu,[1] Xingmin Feng,[1] Tatsuya Chuhjo,[3] Xuzhang Lu,[1] Chiharu Sugimori,[1] Katsuya Okawa,[4] Miyuki Yamamoto,[2] Shoichi Iseki,[2] and Shinji Nakao[1]

- ✓**A pathogenic antibody-mediated autoimmune response?**
- ✓**Non-pathogenic antibodies as markers of the underlying immune derangement?**
- ✓**An Ag-specific B-cell response interplaying with a T-cell response?**
  - ***These putative auto-Ag may trigger (as whole proteins or derived epitopes) a cytotoxic T-cell response in vitro (but Ag-specific T-cells were never demonstrated in vivo in AA patients)***

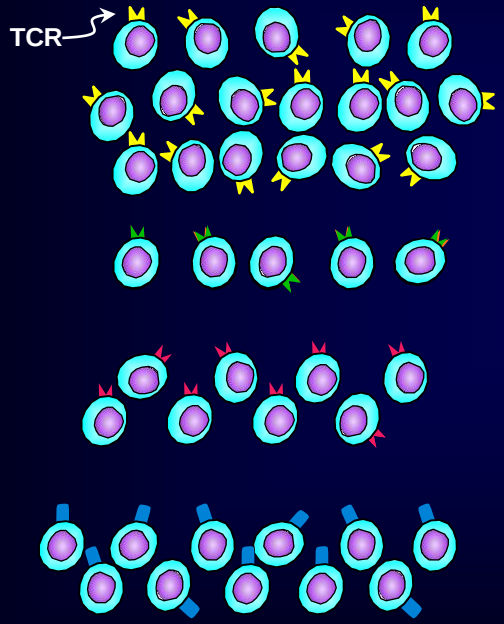
Immune pathophysiology of aplastic anemia

– Escape from self-tolerance maintenance mechanisms

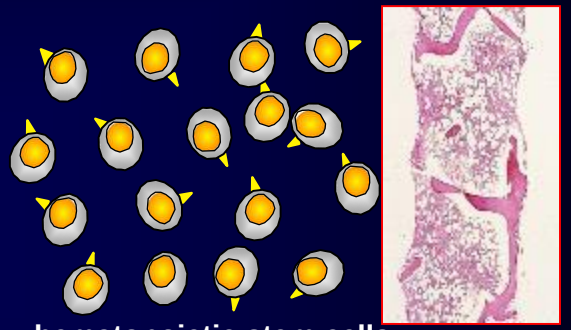
T-cell pool

– Newly expressed Ags (exogenous, mutated, cross-reacting, tissue specific)

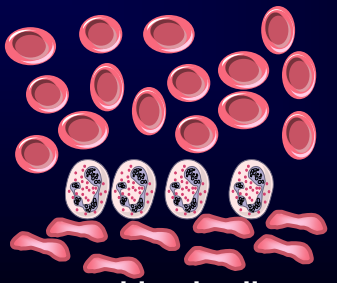
TCR



oligoclonal T-cell response

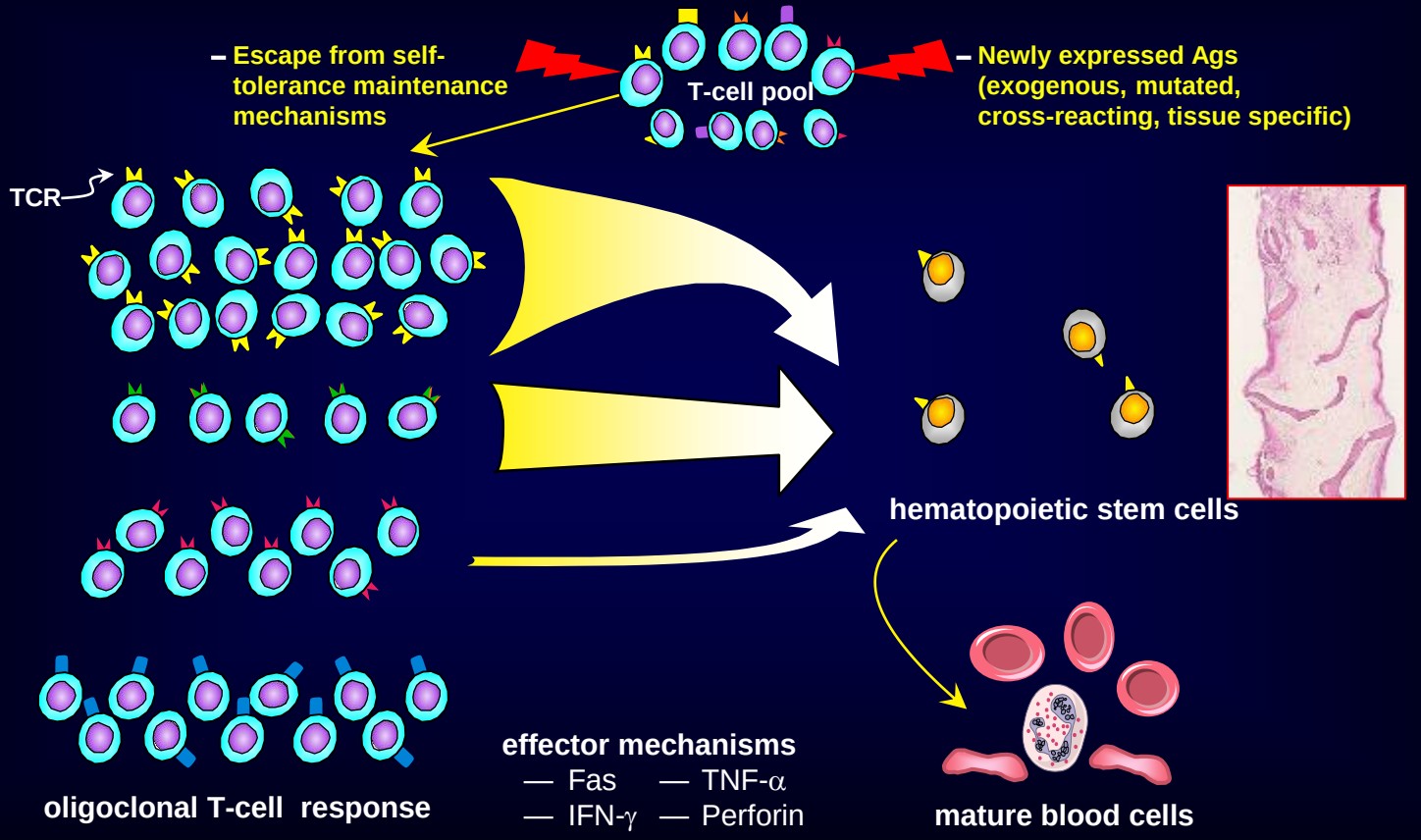


hematopoietic stem cells



mature blood cells

Immune pathophysiology of aplastic anemia

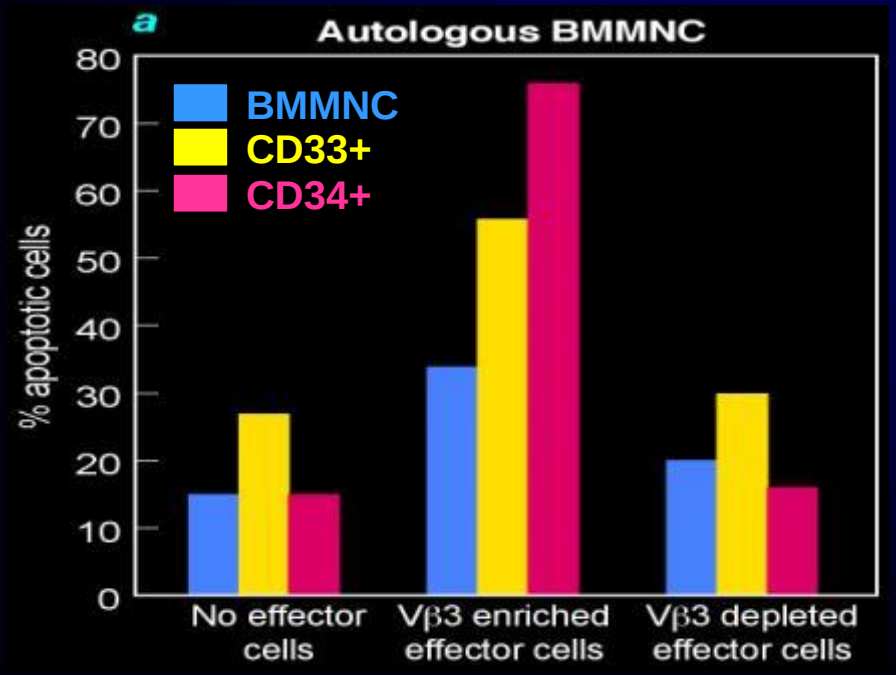


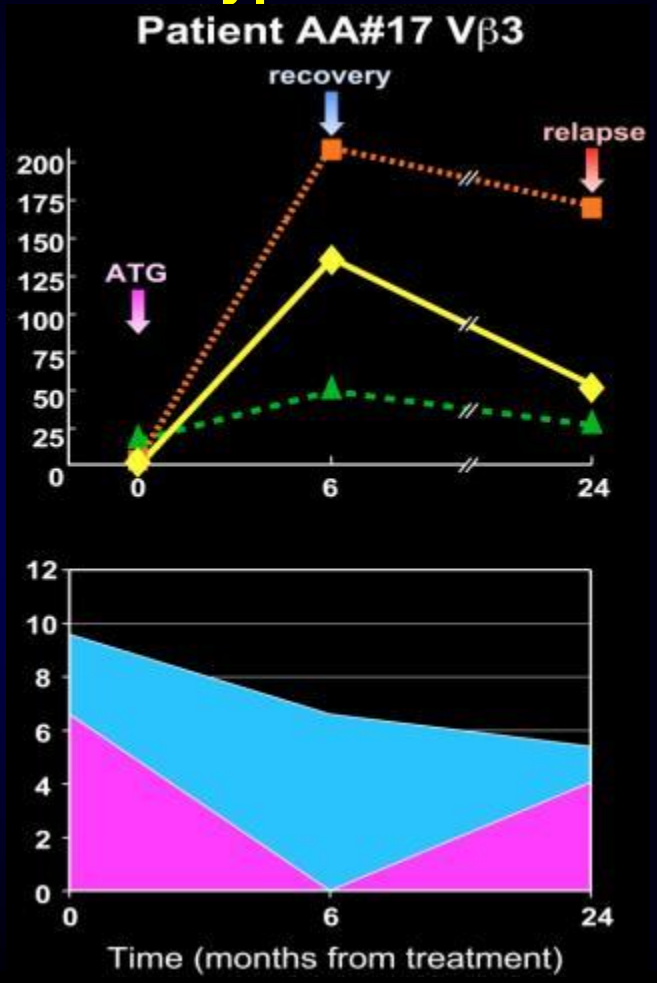
Molecular Tracking of Pathogenic Clonotypic T-cells

Lancet 2004; 364: 355–64 Mechanisms of Disease

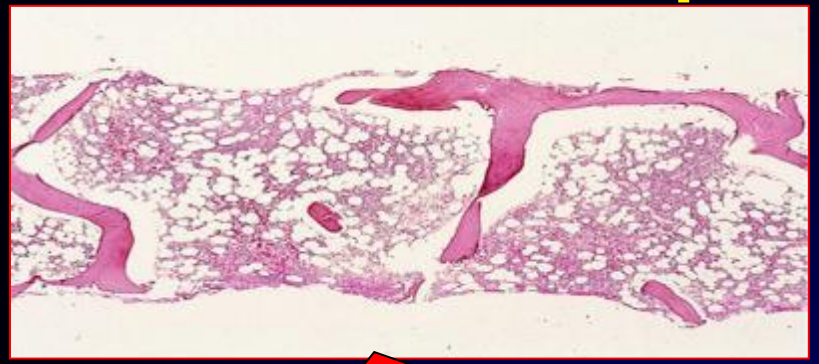
In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR β-CDR3 sequencing

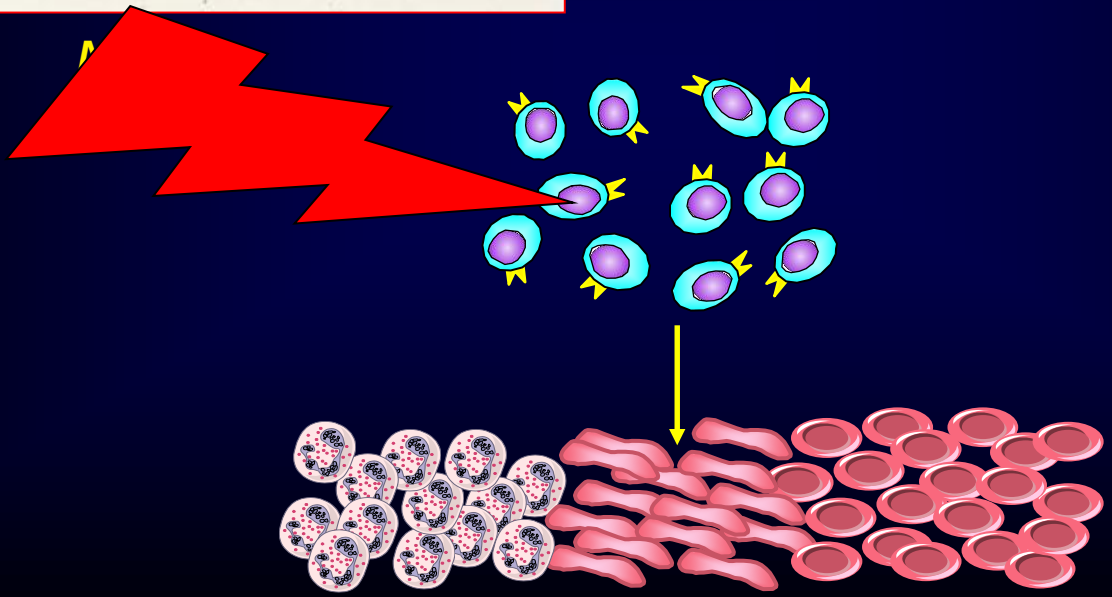
Antonio M Risitano, Jaroslaw P Maciejewski, Spencer Green, Magdalena Plasilova, Weihua Zeng, Neal S Young



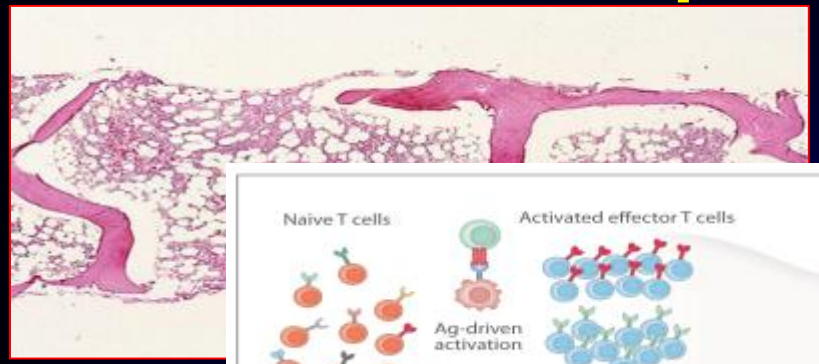


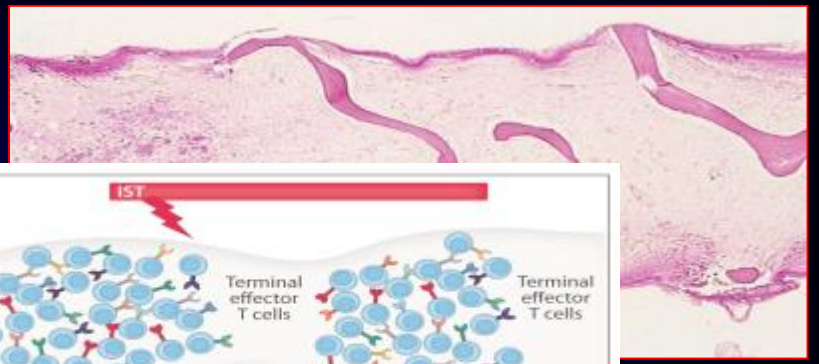
Aplastic anemia

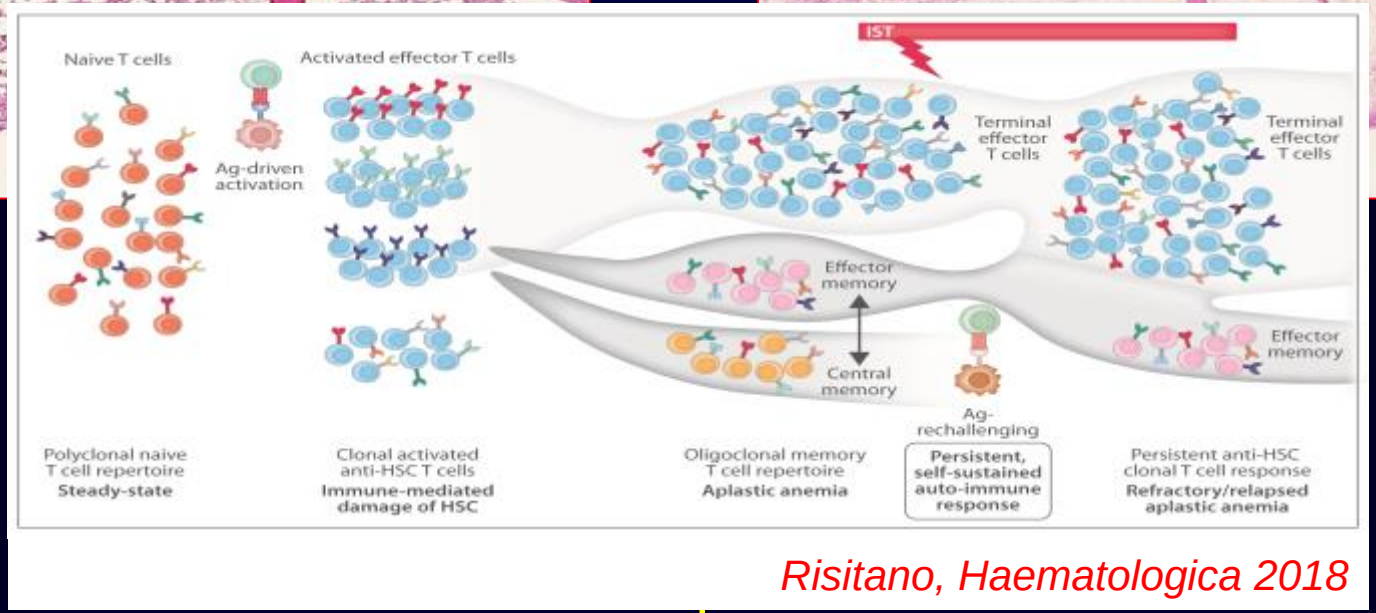




Aplastic anemia







Risitano, Haematologica 2018

Cytopenia

Aplastic anemia:

Diagnosis

Aplastic anemia

Diagnosis

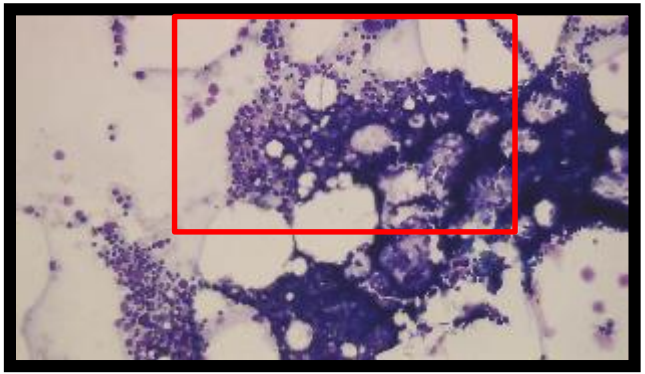
Full blood counts:

- Pancytopenia
- At least 2 cellular lines are decreased

Aplastic anemia

Diagnosis

- **Required:**
  - **bone marrow aspirate**
  - trephine biopsy should be done



- Celullarity should not be based on aspirate
- fragments and trails are **hypocellular**
- variable amounts of residual hemopoietic cells
- prominent **fat spaces**
- megakaryocytes and granulocytic cells are:
  - reduced or absent
  - without dysplasia

Aplastic anemia

Diagnosis

- **Required:**
  - bone marrow aspirate
  - **trephine biopsy should be done**

A trephine is crucial to assess:
overall cellularity
topography of hemopoietic cells
to exclude an abnormal infiltrate
Tangential biopsies: subcortical marrow normally is hypocellular

Bone marrow cellularity is age dependent

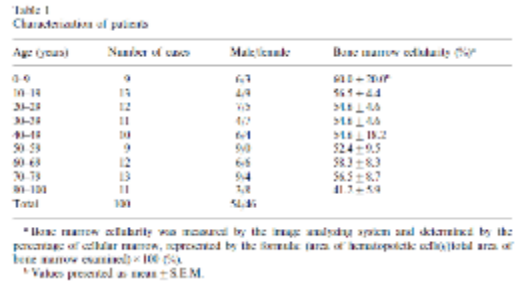


Table 1
Characteristics of patients

Age (years)	Number of cases	Male/female	Bone marrow cellularity (%)*
0–9	9	6/3	60.0 ± 20.0
10–19	15	4/9	56.5 ± 4.4
20–29	12	7/5	54.6 ± 4.6
30–39	11	6/5	54.6 ± 4.6
40–49	10	6/4	54.6 ± 10.2
50–59	9	5/4	52.4 ± 9.5
60–69	12	6/6	58.2 ± 8.3
70–79	13	5/8	56.5 ± 8.7
80–100	11	3/8	41.7 ± 5.9
Total	100	51/49	

* Bone marrow cellularity was measured by the image analysing system and determined by the percentage of cellular marrow, represented by the formula (area of hematopoietic cells)/(total area of bone marrow examined) × 100 (%).
† Values presented as mean ± S.E.M.

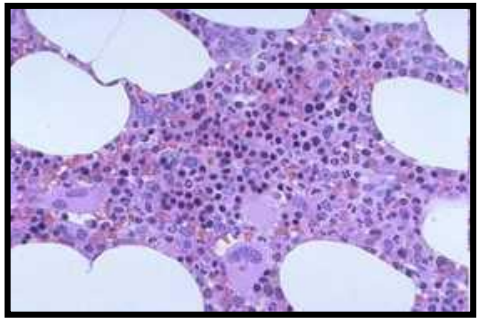
Proliferation stable
Apoptosis: ↑ ageing

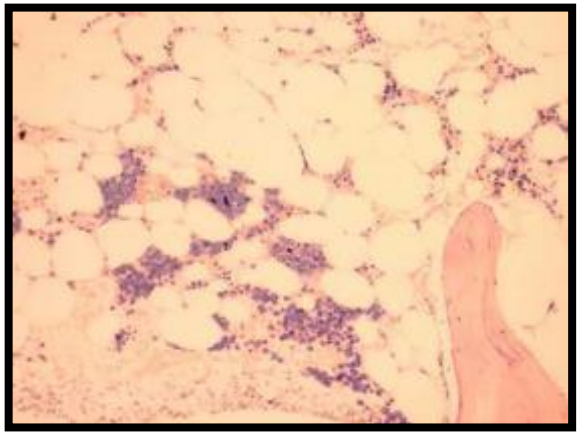
Ogawa et al. Mechanisms of Ageing and Develop 117 (2000) 57-68

Aplastic anemia

Summary

- Pancytopenia
- Persistent, unexplained marrow aplasia
  - Hematopoiesis replaced by fat cells
- No specific marker
  - Diagnosis by exclusion
- Severity need to be defined





Aplastic anemia

Cytogenetics and flow cytometry

- Due to hypocellular bone marrow frequently insufficient metaphases
- FISH for chromosomes 5 and 7 should be considered
- isolated del(13q) favorable long-term outcome
- An abnormal cytogenetic clone does not imply the diagnosis of MDS or AML
- Cytogenetic abnormalities can be present in up to 12% of typical AA patients
- Detection of small PNH clones has implications for defining the disease.
  - About 50% are ‘aplastic’ with small clones and no hemolysis.
- PNH clone size measurements:
  - at presentation
  - serial monitoring should be performed at least yearly

Aplastic anemia

Differential diagnosis

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

Aplastic anemia

Differential diagnosis

- **Fanconi anemia:**

- Positive chromosomal breakage test (MMC or DEB) that still represents the diagnostic gold standard.

- **Screening: telomere length**

- **Dyskeratosis congenita**

- Asymptomatic:
  - Frequent association with TERC, TERT mutation
    - (10% all idiopathic forms)
  - Rarely, with TINF2 gene mutation
- Recognizable phenotype of DC:
  - TINF2, NHP2, NOP10, DKC1 mutation

Aplastic anemia

Severity

Based on **peripheral values** and **bone marrow** findings

Severe AA (SAA)

At least two of the following three criteria have to be fulfilled:

- Reticulocytes <60x10^9/L (using an automated analyzer) or < 20 x 10^9/l (manual count)*

- Platelets < 20x10^9/L

- Neutrophil count <0.5 x10^9/L

Very severe AA (vSAA)

Same criteria of SAA have to be fulfilled; but the neutrophil count has to be < 0.2 x10^9/l

Non- severe AA

Patients not fulfilling the criteria for SAA and vSAA.

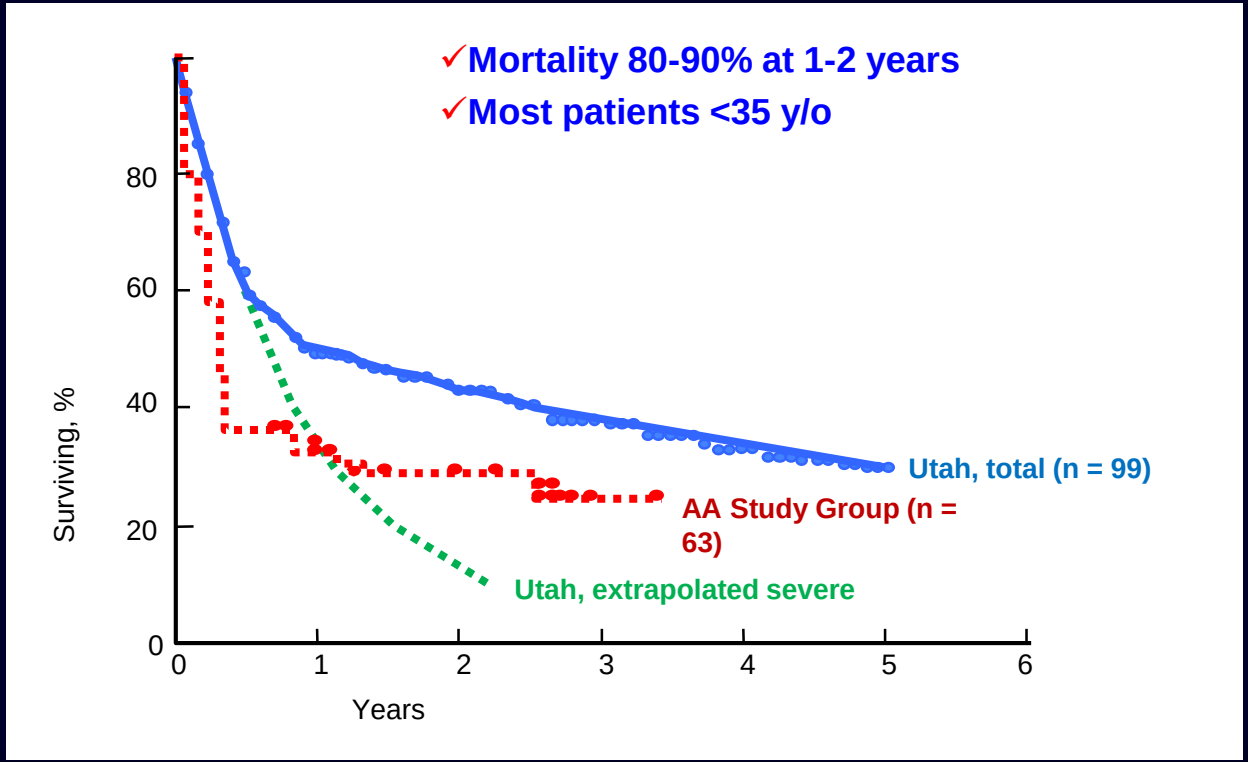
* The different values are because automated count may over-estimate the counting at low level of reticulocyte counts, i.e. it reads 50x10^9/L but in reality they are less

Aplastic anemia:

Disease course and treatment

Aplastic anemia: the natural history

In the '70s almost always a fatal disease



Camitta et al, Blood 1979; 53:504
Williams et al, Sem Hematol 1973; 10:195

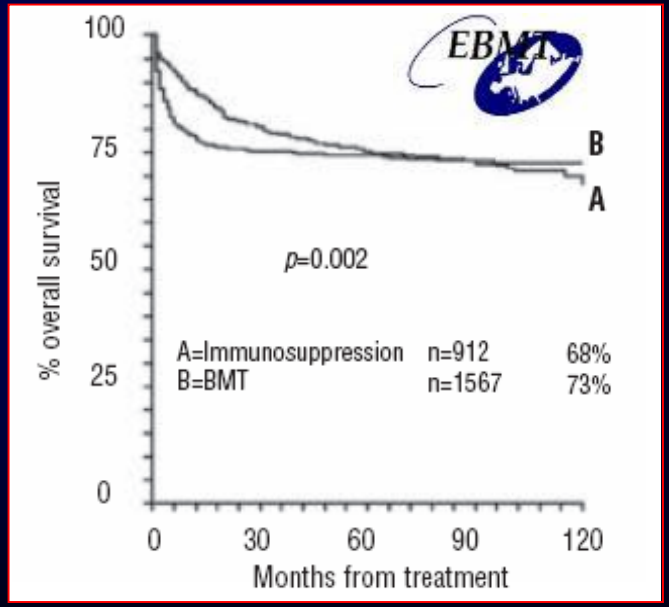
To transplant or not to transplant?

IST HSCT

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

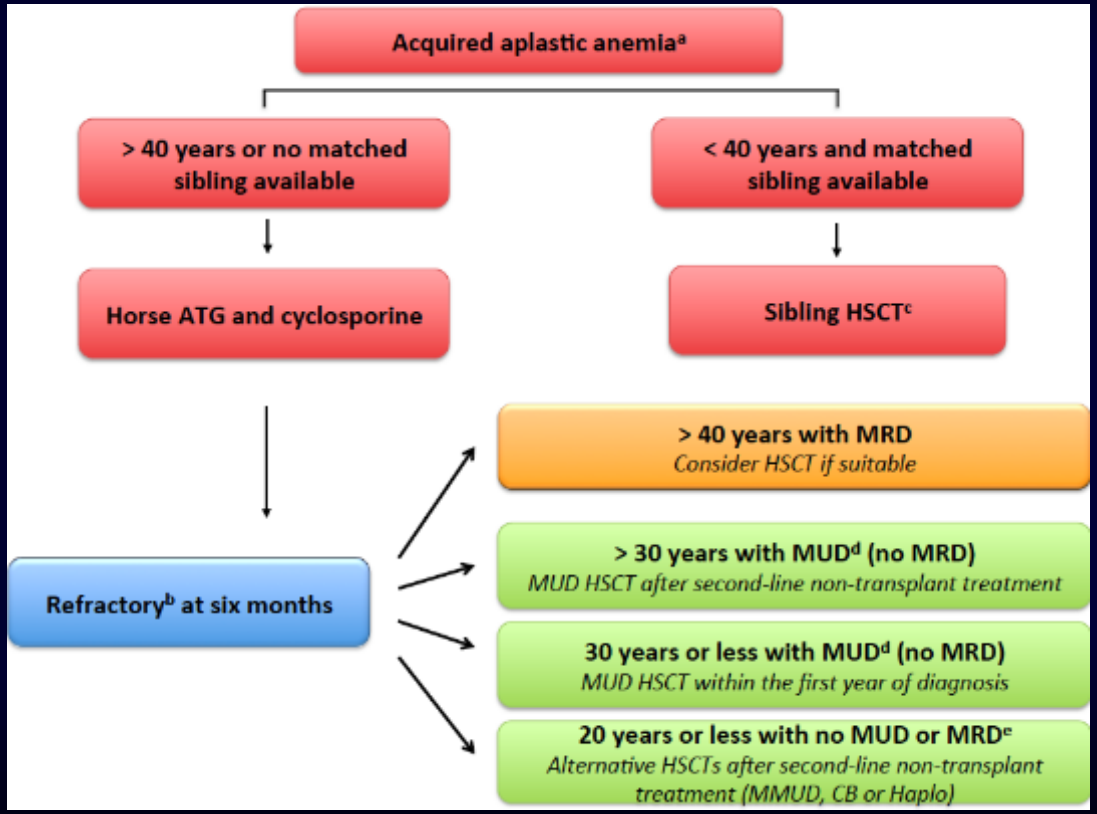
Anna Locasciulli, Rosi Oneto, Andrea Bacigalupo, Gerard Socié, Elisabeth Korthof, Albert Bekassy, Hubert Schrezenmeier, Jakob Passweg, Monika Führer
on the Behalf of the Severe Aplastic Anemia Working Party of the European Blood and Marrow Transplant Group (SAA-WP, BMT).



Locasciulli et al, Haematologica 2007

Treatment algorithm of aplastic anemia

Updated to 2017



Peffault De Latour, ASH Educational

Toward a cure for aplastic anemia

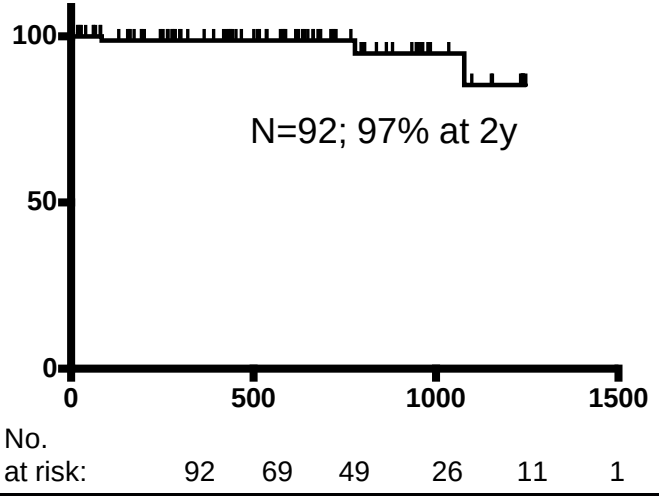
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia

Danielle M. Townsley, M.D., Phillip Scheinberg, M.D., Thomas Winkler, M.D., Ronan Desmond, M.D., Bogdan Dumitriu, M.D., Olga Rios, R.N., Barbara Weinstein, B.S.N., Janet Valdez, P.A., Jennifer Lotter, P.A., Xingmin Feng, Ph.D., Marie Desierto, B.S., Harshraj Leuva, M.B., B.S., Margaret Bevans, Ph.D., Colin Wu, Ph.D., Andre Larochelle, M.D., Ph.D., Katherine R. Calvo, M.D., Cynthia E. Dunbar, M.D., and Neal S. Young, M.D.

OS - Not censored for HSCT

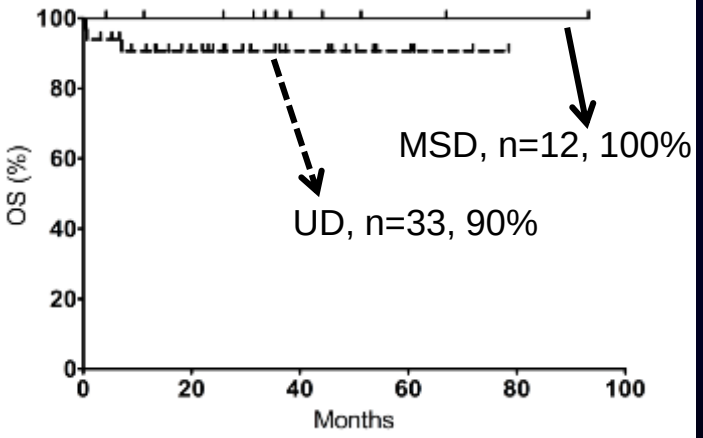


Biology of Blood and Marrow Transplantation
journal homepage: www.bbmt.org
ASBMT

Mixed T Cell Chimerism After Allogeneic Hematopoietic Stem Cell Transplantation for Severe Aplastic Anemia Using an Alemtuzumab-Containing Regimen Is Shaped by Persistence of Recipient CD8 T Cells

Francesco Grimaldi, Victoria Potter, Pilar Perez-Abellan, John P. Velchamy, Muhammad Atif, Rosemary Grain, Monica Sen, Steven Best, Nicholas Lea, Carmel Rice, Antonio Pagliuca, Ghulam J. Mufti, Judith C. W. Marsh, Linda D. Barber

OS – MSD vs UD



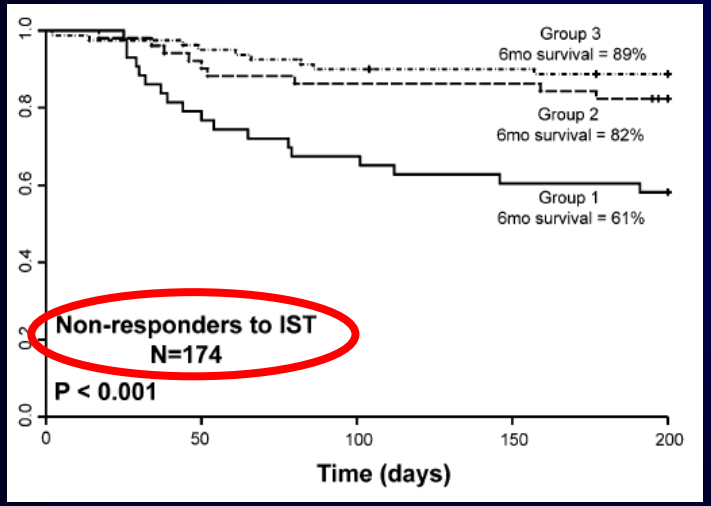
Supportive care

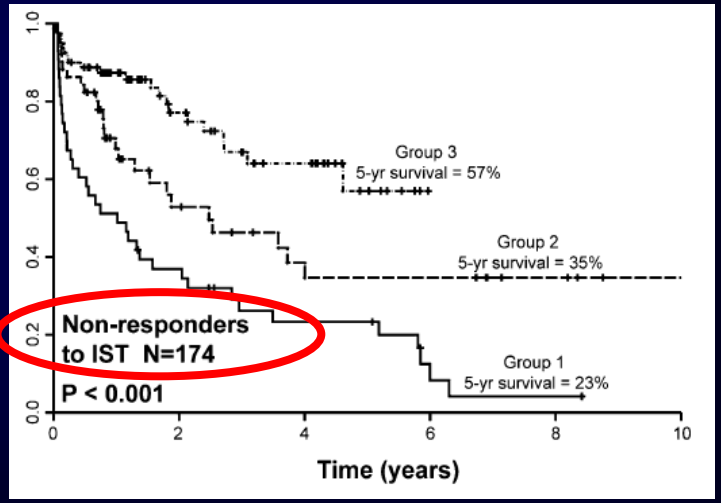
The improvement in anti-infectious management

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

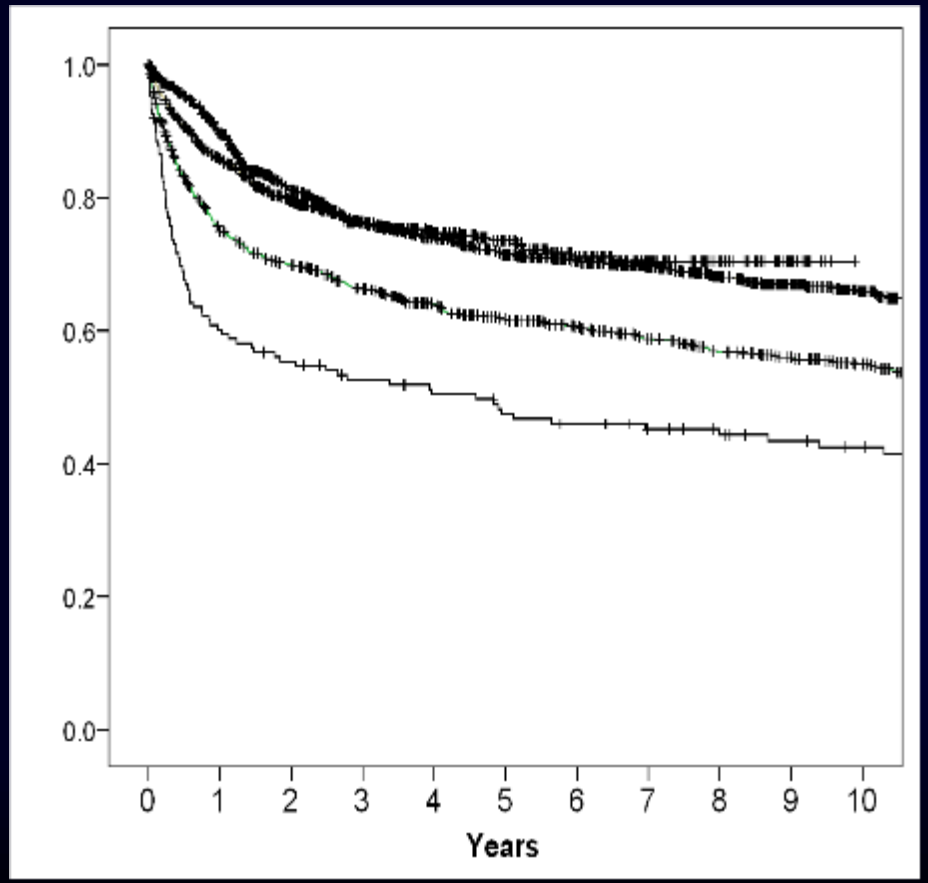




The most relevant breakthrough in AA treatment was the anti-infectious supportive care: keeping AA patients alive until they recover (IST or SCT)

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

Improving IST for AA: chronicle of failures… and unpredictable success

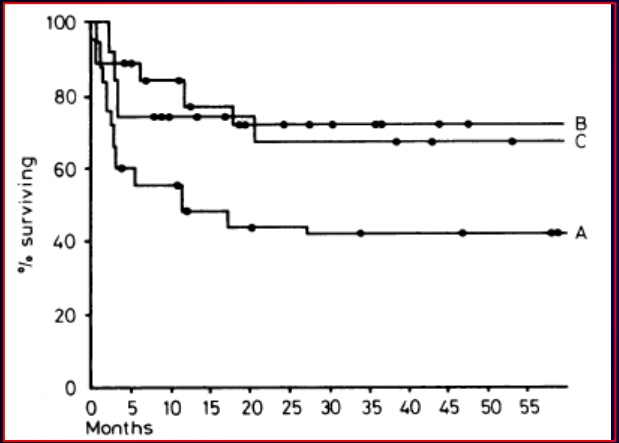
IMMUNOSUPPRESSION AS A TREATMENT FOR SAA

The European pioneers

BRITISH MEDICAL JOURNAL VOLUME 282 14 MARCH 1981

Treatment of severe aplastic anaemia with antilymphocyte globulin or bone-marrow transplantation

BRUNO SPECK, ALOIS GRATWOHL, CATHERINE NISSEN, URS LEIBUNDGUT, DONATELLA RUGGERO, BRUNO OSTERWALDER, HANS PETER BURRI, PIERRE CORNU, MICHEL JEANNET



Group		Criteria	Treatment
A	18	HLA-A, B, Dr identical sibling donor, mixed leucocyte culture not reactive	Cyclophosphamide 50 mg/kg × 4 + bone-marrow transplantation
B	20	HLA-haploidentical, ABO-identical, cross-match negative family donor	Antilymphocyte globulin 40 mg/kg × 4 + bone-marrow infusion Norethandrolone 0·5-1 mg/kg/day by mouth
C	12	No donor	Antilymphocyte globulin 40 mg/kg × 4 Norethandrolone 0·5-1 mg/kg/day by mouth

ALG may be effecive as treament of SAA even without stem cell support, with results at least equivalent to stem cell transplantation

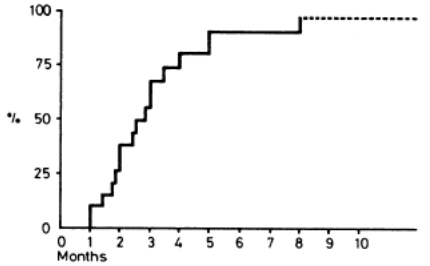


FIG 2—Time required in groups B and C (n = 13 and n = 6 respectively) until patients self-sustaining and transfusion independent.

- ***Gluckman et al, Br J Haematol 1982***
  n=170 ATG vs ATG + haplo-SCT vs HD-MP OS 62,7% no diff among arms

Background

- Standard IST for patients with SAA/vSAA who are not eligible for HSCT is **horse antithymocyte globulin (hATG) plus ciclosporin** (CsA) since 20 years

Add androgens to ATG[1] → No increase in response rate

Add or replace ATG with high-dose corticosteroids[2] → No increase in response rate; high toxicity

1. Champlin RE, et al. Blood. 1985;66:184-8. 2. Marmont AM, et al. Prog Clin Biol Res. 1984;148:271-87. 3. Tisdale JF, et al. Lancet. 2000;356:1554-9. 4. Tisdale JF, et al. Blood. 2002;100:4668-70. 5. Scheinberg P, et al. Blood. 2014;124:2820-3. 6. Scheinberg P, et al. Br J Haematol. 2006;133:606-11. 7. Scheinberg P, et al. Haematologica. 2009;94:348-54. 8. Locasciulli A, et al. Haematologica. 2004;89:1054-61.

G-CSF, granulocyte-colony stimulating factor.

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

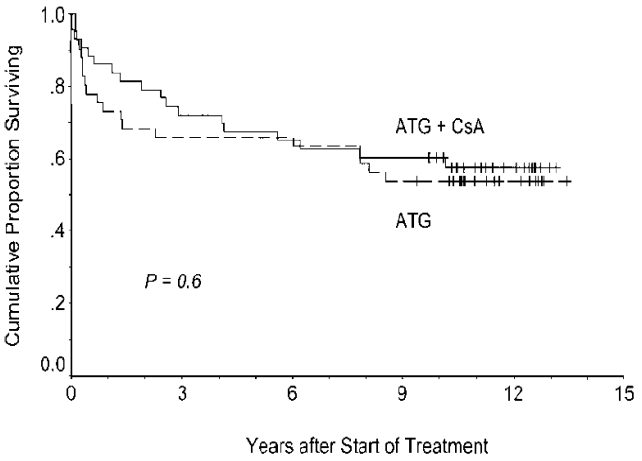
N Frickhofen, JP Kaltwasser, H Schrezenmeier, A Raghavachar, HG Vogt, F Herrmann, M Freund, P Meusers, A Salama, and H Heimpel

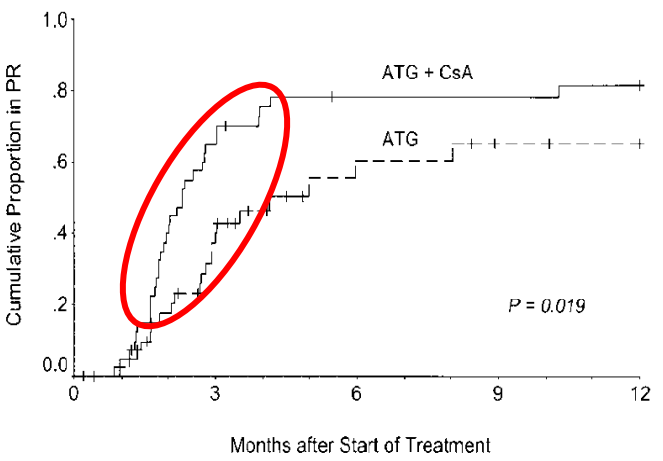
1991

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

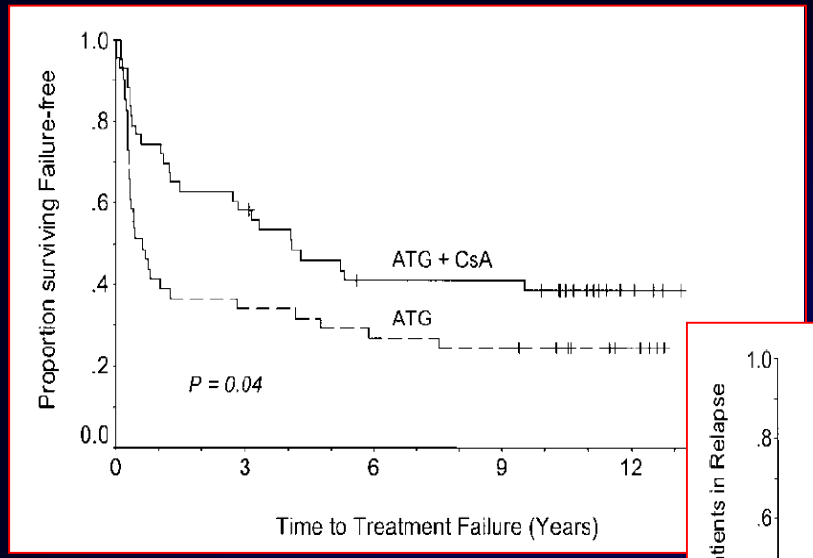




✓ **CyA speed hematological response without affecting survival**

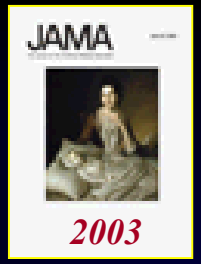
IMPROVING ATG-BASED IMMUNOSUPPRESSION

The benefit of combining ATG and cyclosporine A



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

Frickhofen et al, Blood 2003



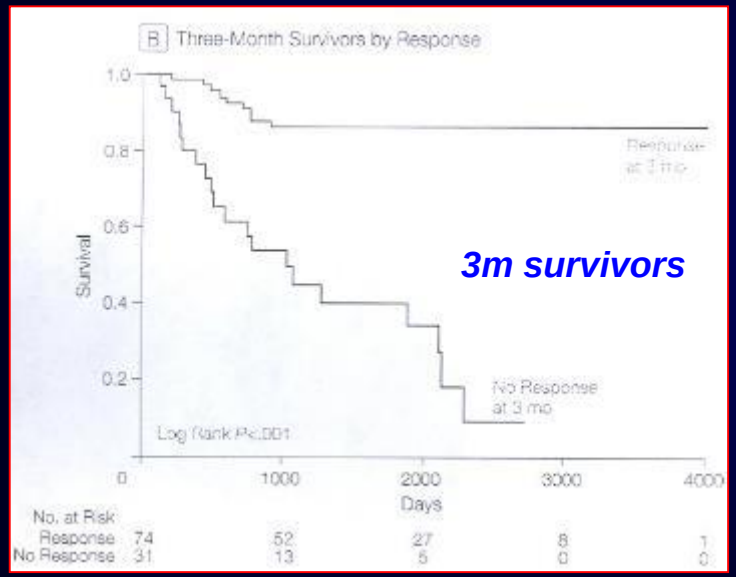
Antithymocyte Globulin and Cyclosporine for Severe Aplastic Anemia

Association Between Hematologic Response and Long-term Outcome

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

n=112

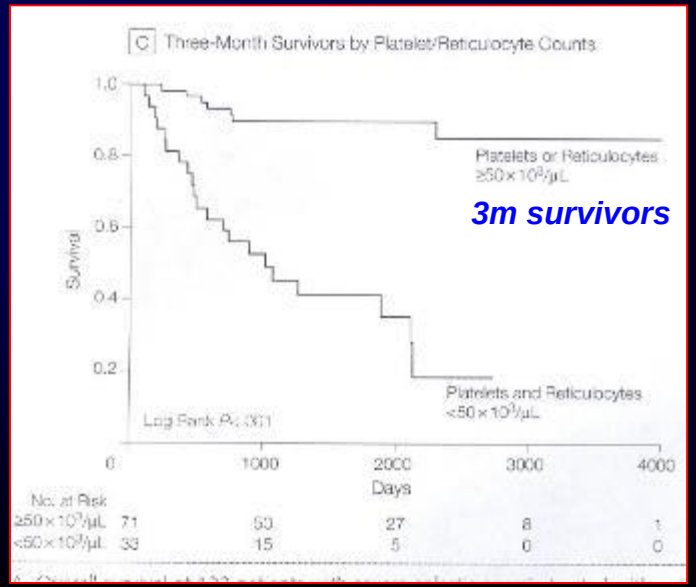
hATG x 4 (40mg/kg) + CsA x 6 m



3m survivors

OS 55% @7y;

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



3m survivors

Hematological response is the main predictor for outcome

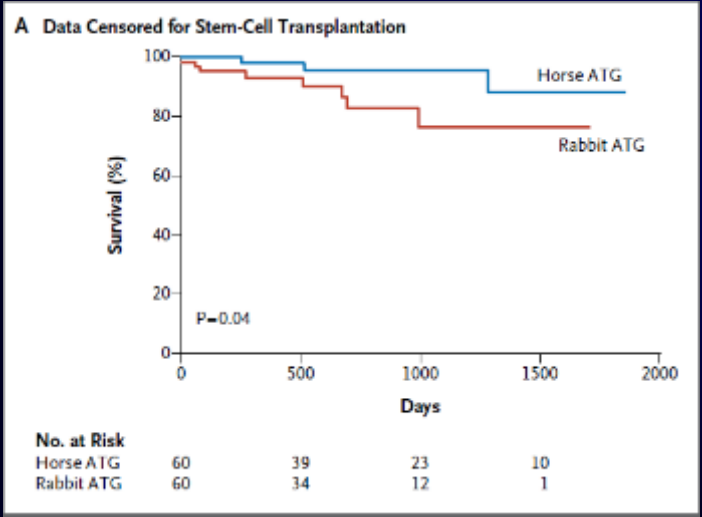
NEJM

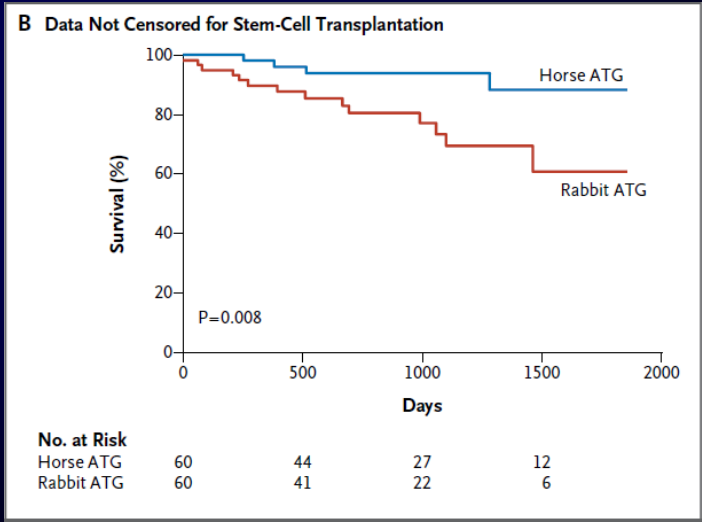
NEJM 2011

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)*





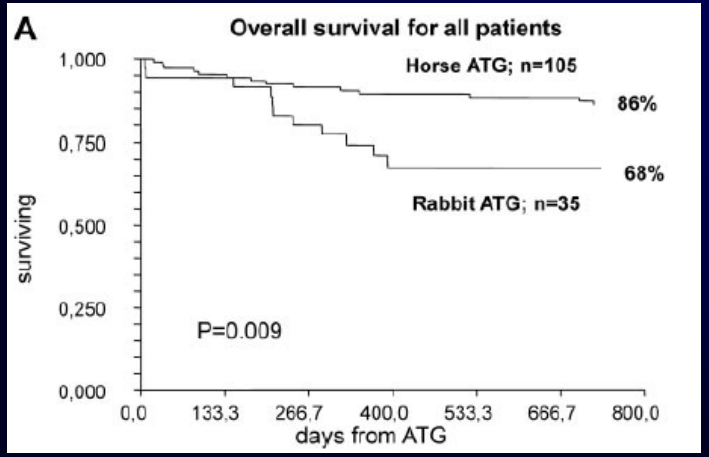
rATG is inferior to hATG in first line treatment of SAA, as indicated by hematological response and survival

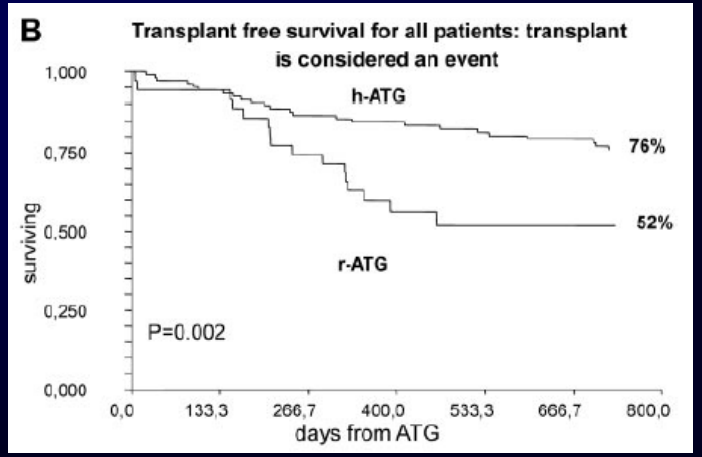
Prospective study of rabbit antithymocyte globulin and cyclosporine for aplastic anemia from the EBMT Severe Aplastic Anaemia Working Party

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

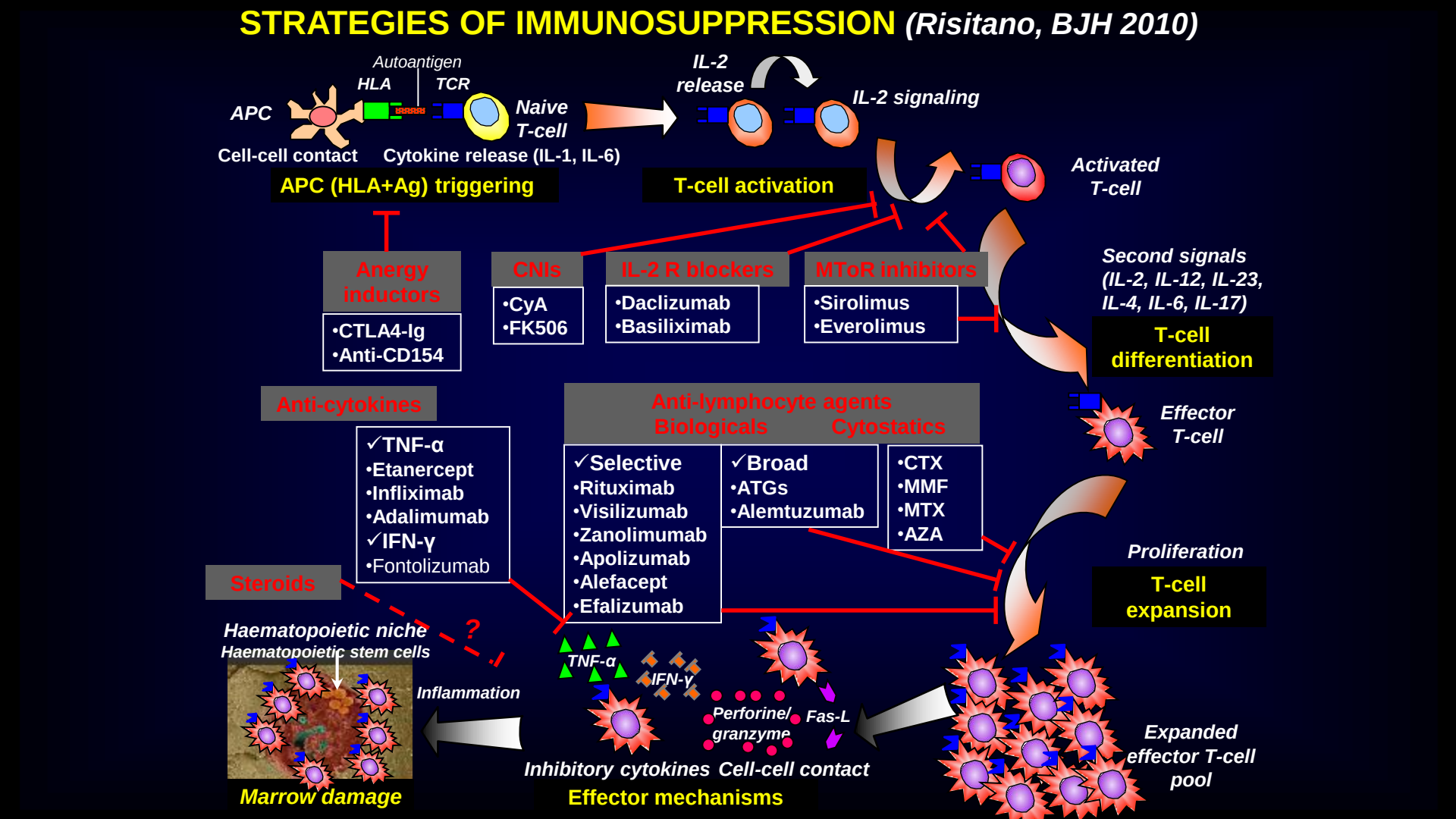
- ✓***Phase II pilot study rATG + CyA (n=35)***
- ✓***Retrospective matched comparison (pair-matched) with hATG + CyA (n=105)***
- ✓***Pilot rATG + CyA study: OR 40% @ 6m (CR 3%, PR 37%)***





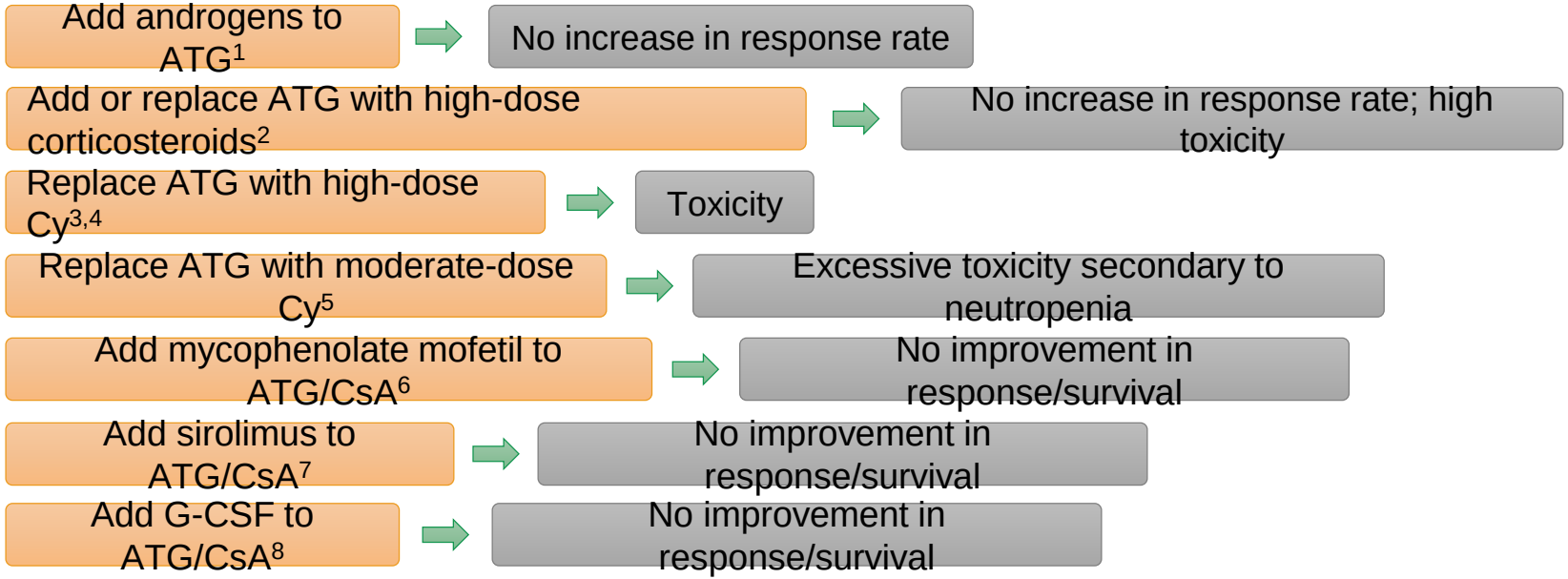
rATG is inferior to hATG in first line treatment of SAA, as indicated by hematological response and survival

STRATEGIES OF IMMUNOSUPPRESSION *(Risitano, BJH 2010)*



Background

- Standard IST for patients with SAA/vSAA who are not eligible for HSCT is **horse antithymocyte globulin (hATG) plus ciclosporin** (CsA) since 20 years



1. Champlin RE, et al. Blood. 1985;66:184-8. 2. Marmont AM, et al. Prog Clin Biol Res. 1984;148:271-87. 3. Tisdale JF, et al. Lancet. 2000;356:1554-9. 4. Tisdale JF, et al. Blood. 2002;100:4668-70. 5. Scheinberg P, et al. Blood. 2014;124:2820-3. 6. Scheinberg P, et al. Br J Haematol. 2006;133:606-11. 7. Scheinberg P, et al. Haematologica. 2009;94:348-54. 8. Locasciulli A, et al. Haematologica. 2004;89:1054-61.

G-CSF, granulocyte-colony stimulating factor.

Aplastic Anemia: Management of Adult Patients

Jaroslaw P. Maciejewski and Antonio M. Risitano

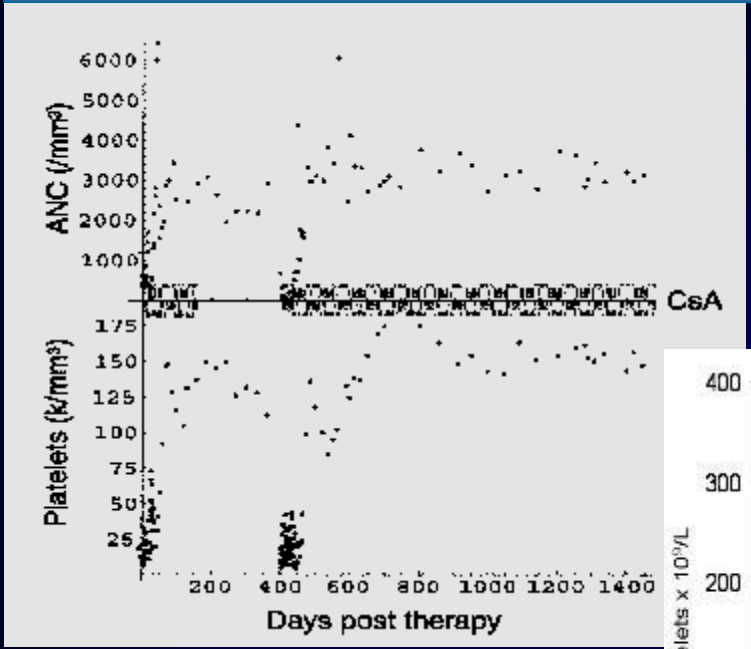
REASONS FOR TREATMENT FAILURE

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

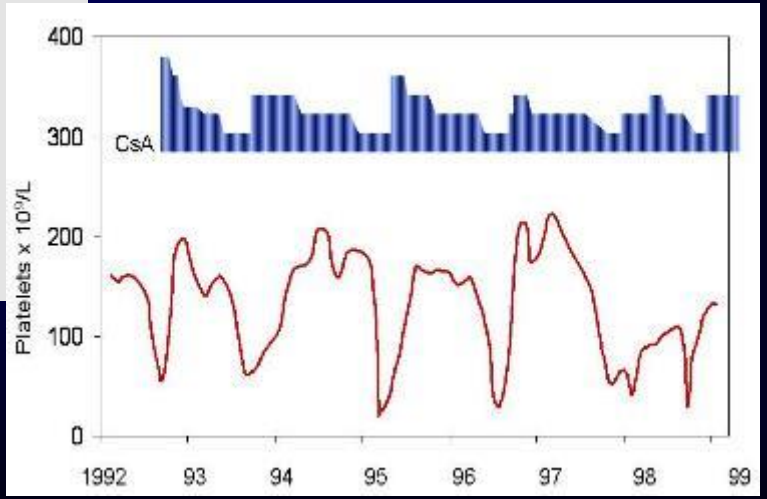
Improve immunosuppressive therapies

RELAPSES AFTER IST

The role of maintenance CyA therapy



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

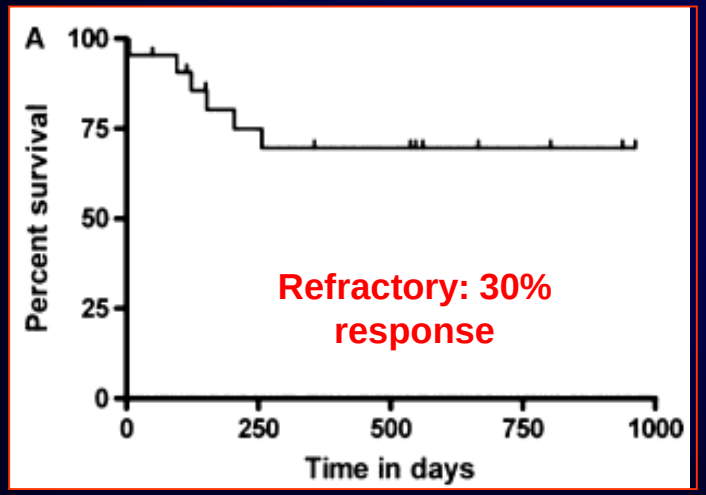


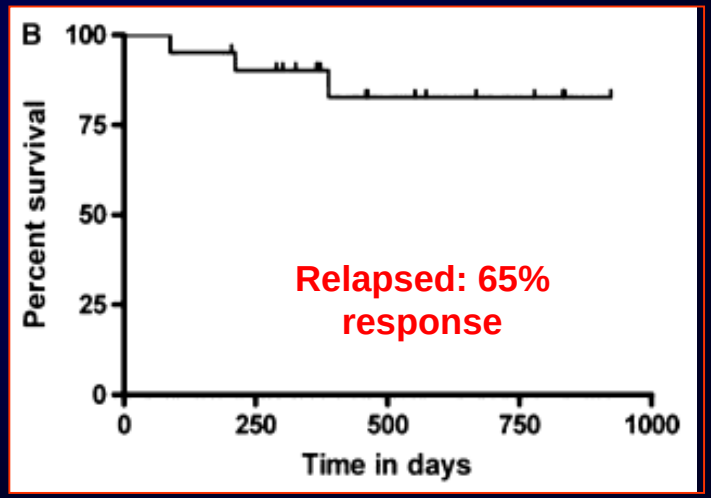
Frickhofen N. Blood. 2003 (101). 1236-1242

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

bjh research paper

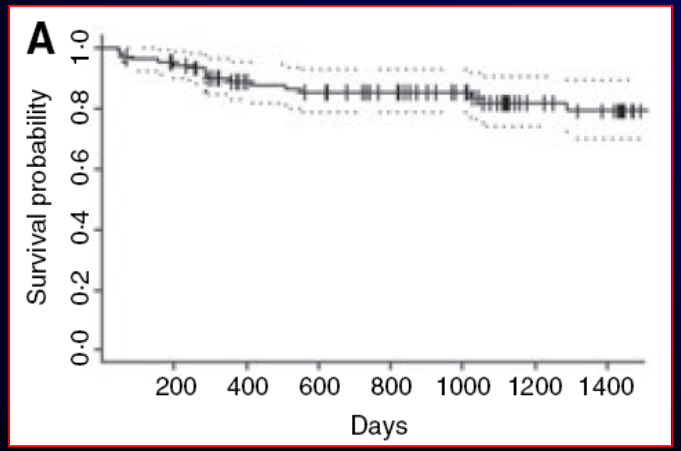
Treatment of severe aplastic anaemia with combined immunosuppression: anti-thymocyte globulin, ciclosporin and mycophenolate mofetil

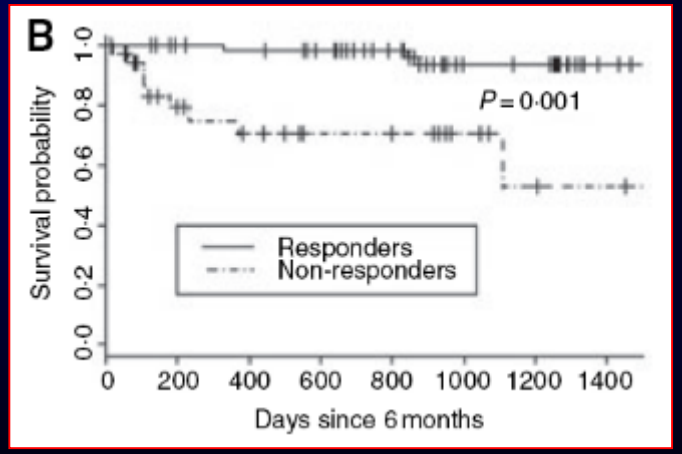
 British Journal of Haematology, **133**, 606–611

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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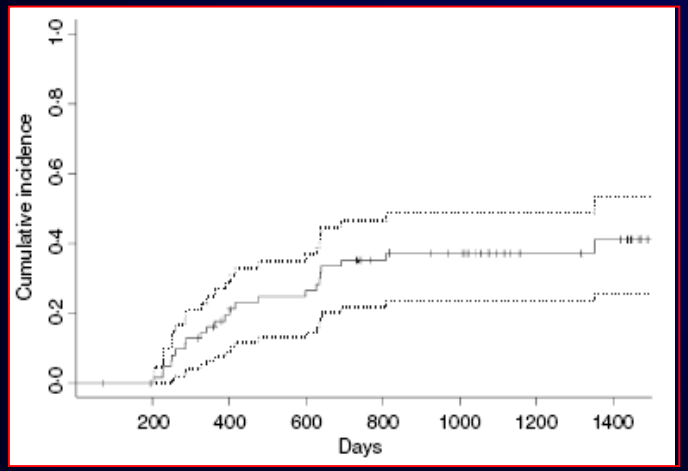
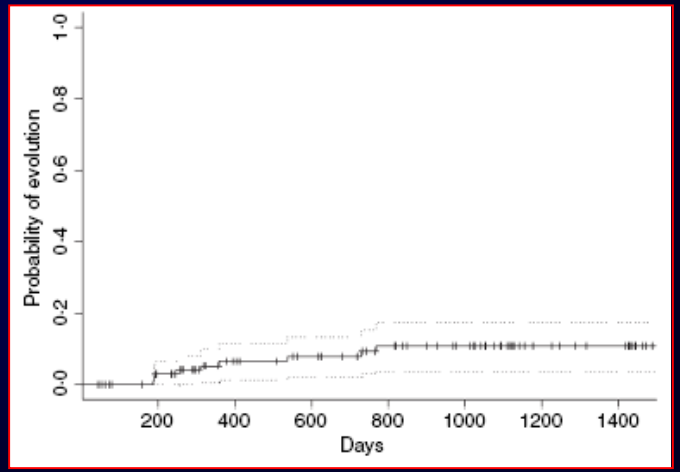




Relapse

Clonal evolution

Sirolimus (Rapamune®)

Original Article

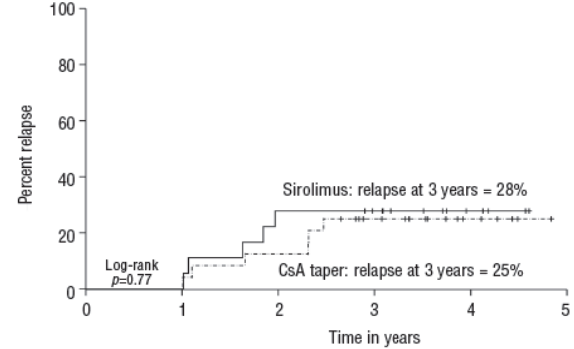
Treatment of severe aplastic anemia with a combination of horse antithymocyte globulin and cyclosporine, with or without sirolimus: a prospective randomized study

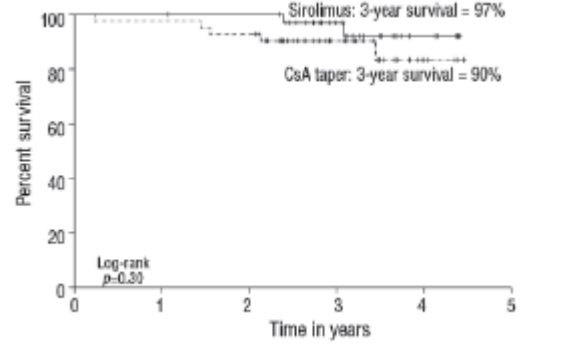
Phillip Scheinberg,[1] Colin O. Wu,[2] Olga Nunez,[1] Priscila Scheinberg,[1] Carol Boss,[1] Elaine M. Sloand,[1] and Neal S. Young[1]

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%)





CYCLOPHOSPHAMIDE FOR TREATMENT OF SAA

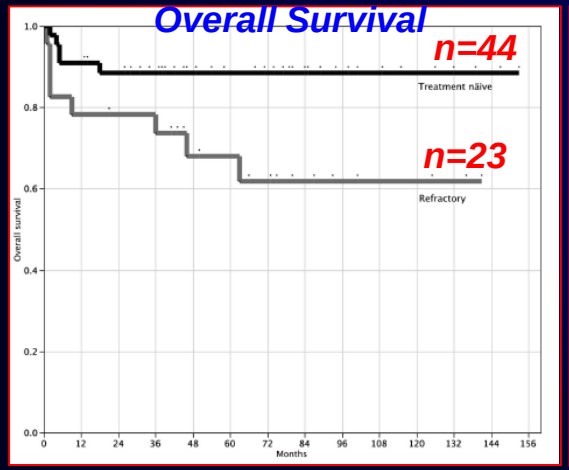
The Johns Hopkins experience

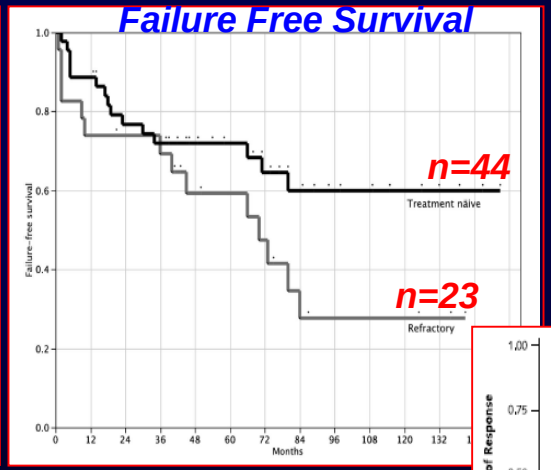
High-dose cyclophosphamide for severe aplastic anemia: long-term follow-up

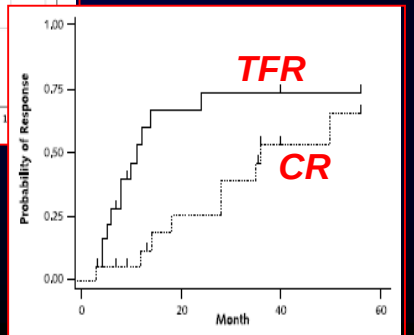
Robert A. Brodsky,[1,2] Allen R. Chen,[2] Donna Dorr,[1] Ephraim J. Fuchs,[2] Carol Ann Huff,[2] Leo Luznik,[2] B. Douglas Smith,[2] William H. Matsui,[2] Steven N. Goodman,[2] Richard F. Ambinder,[2] and Richard J. Jones[2]

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

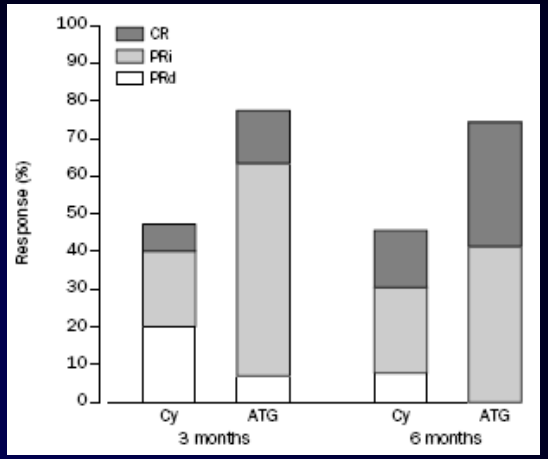
High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial

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)

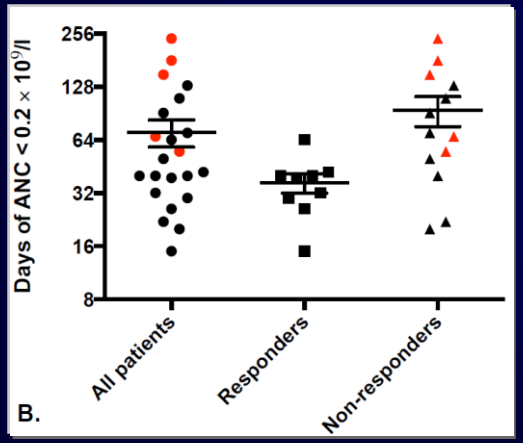
Long-term analysis (median 38m):

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

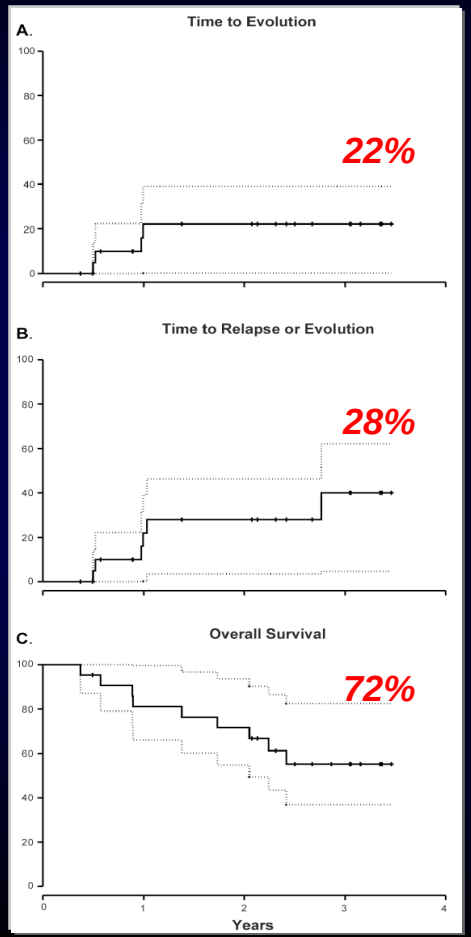
Moderate-dose cyclophospamide plus CsA for AA

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]

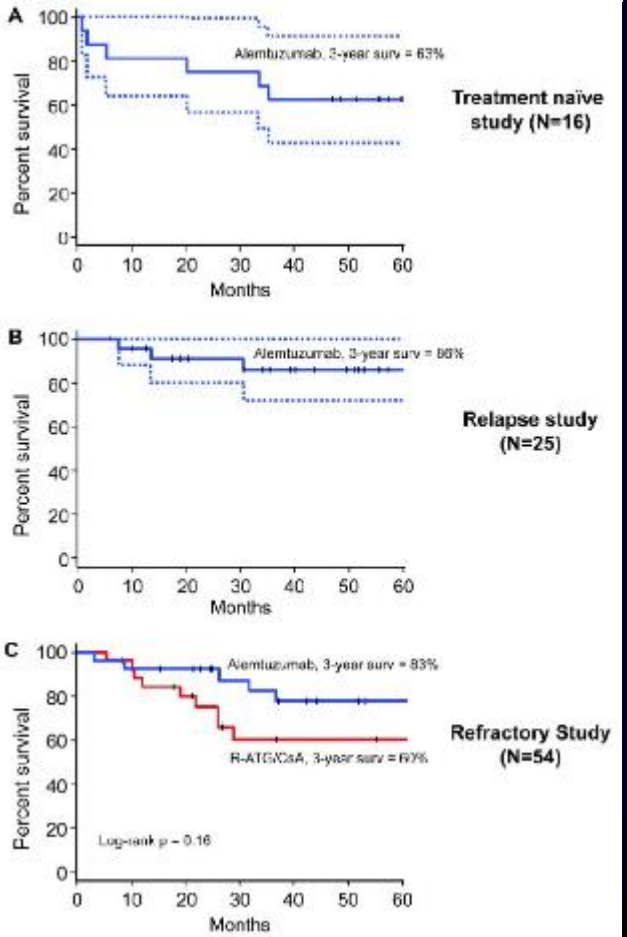
Blood 2012

	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**

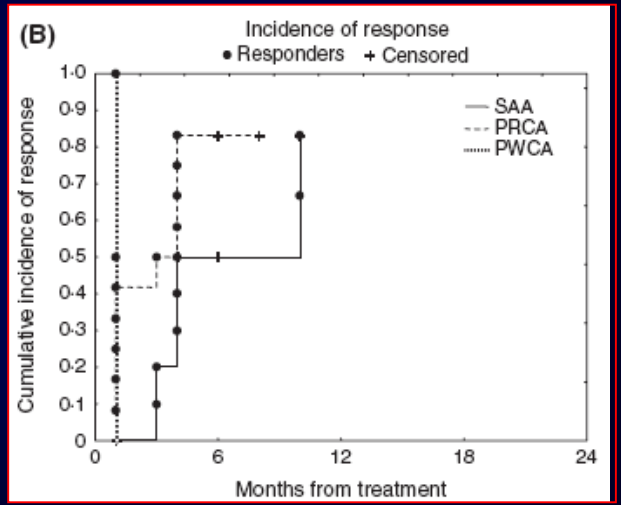
Alemtuzumab is safe and effective as immunosuppressive treatment for aplastic anaemia and single-lineage marrow failure: a pilot study and a survey from the EBMT WPSAA

✓*Phase II prospective study 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

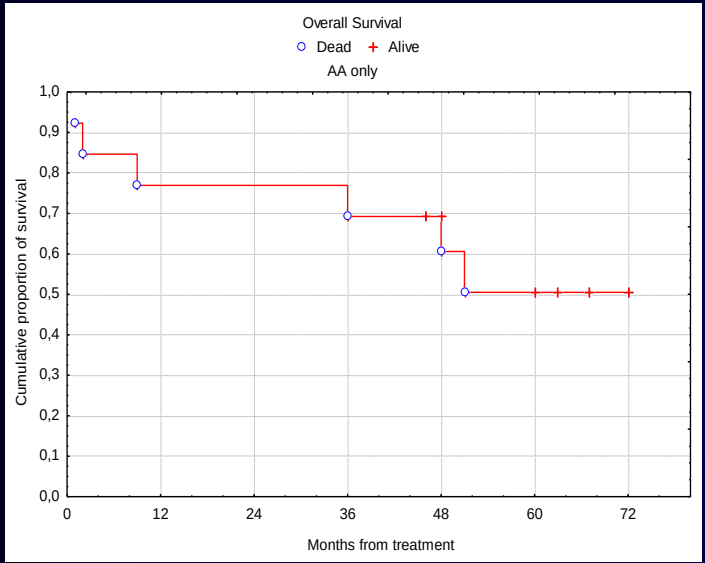
✓Frequent relapses (maintenance IS or retreatment needed)

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

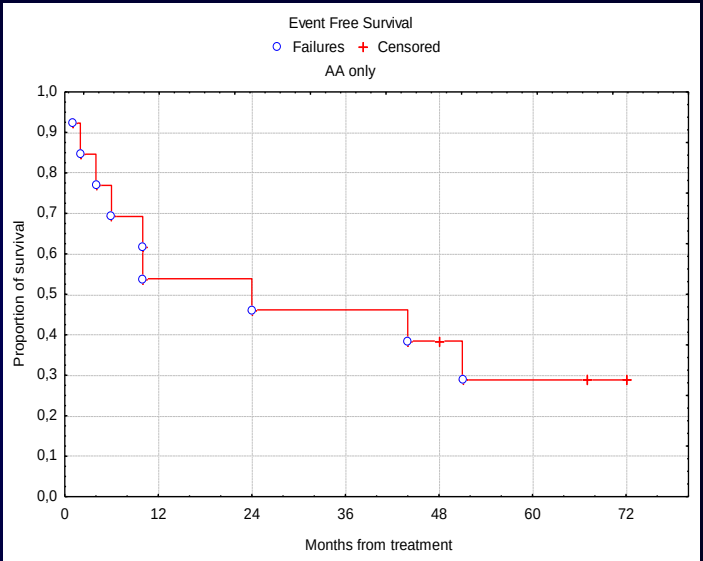
Alemtuzumab for marrow failure syndromes

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

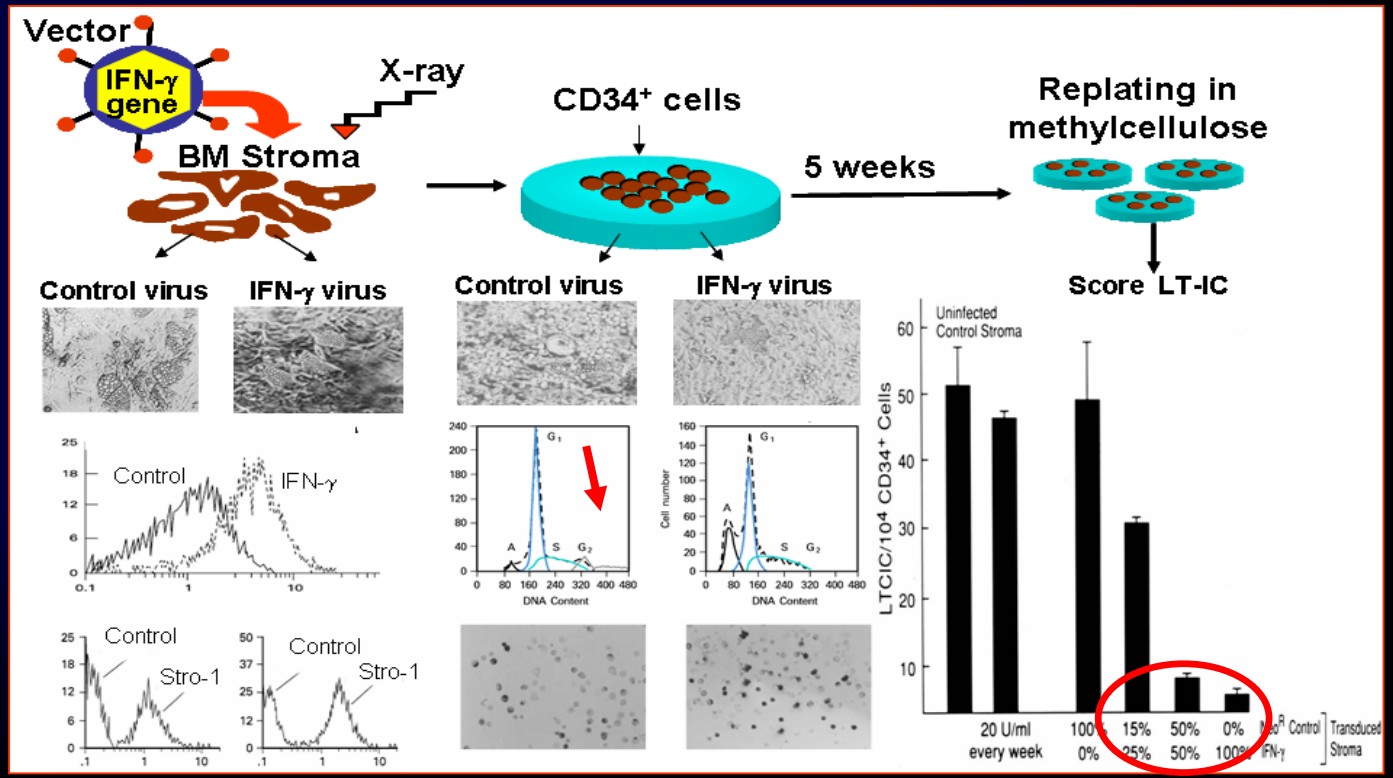
Background

- Eltrombopag as investigational treatment for severe aplastic anemia

Blood, Vol 87, No 10 (May 15), 1996: pp 4149-4157

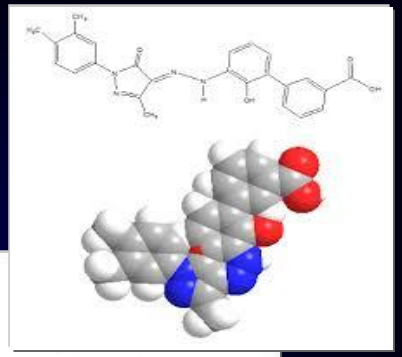
Interferon-γ Constitutively Expressed in the Stromal Microenvironment of Human Marrow Cultures Mediates Potent Hematopoietic Inhibition

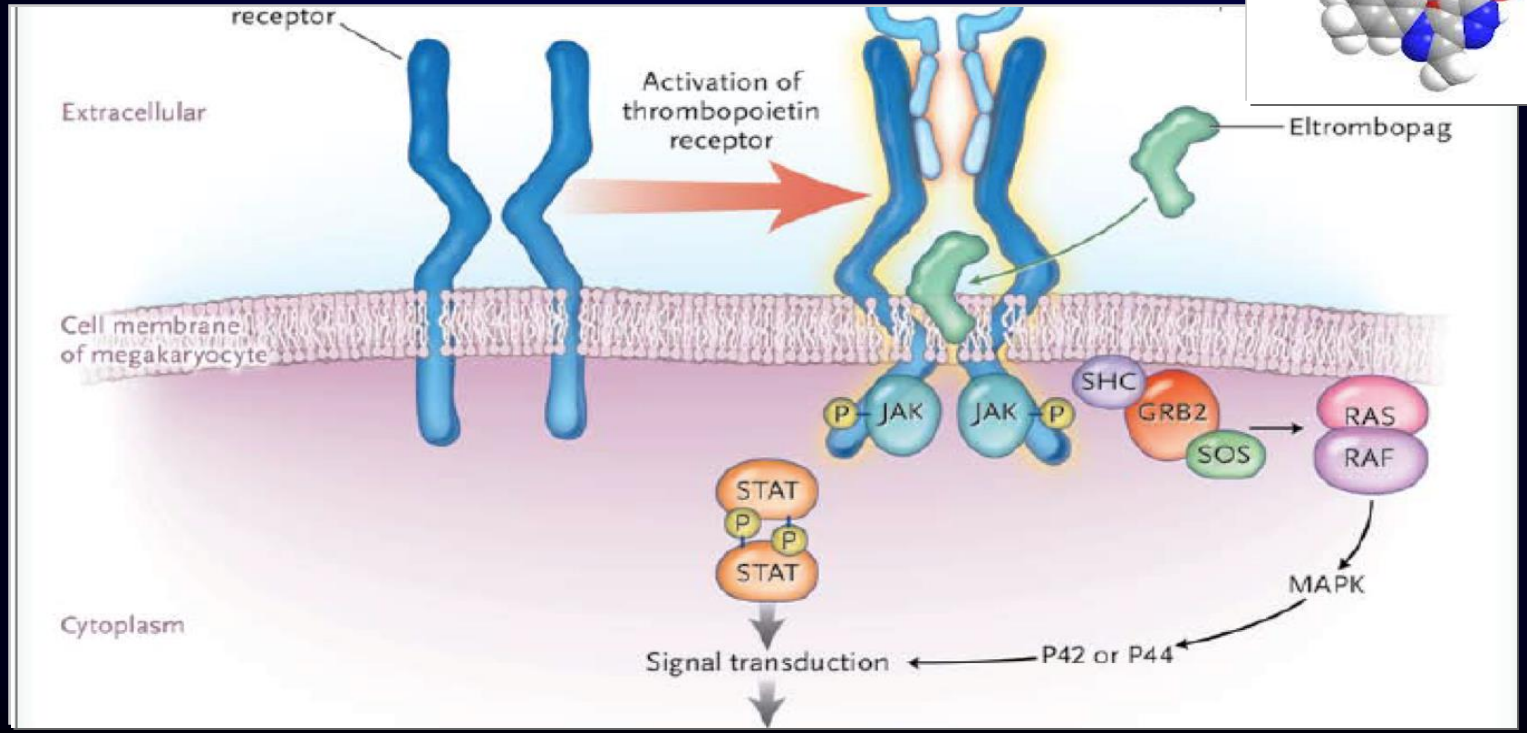
By Carmine Selleri, Jaroslaw P. Maciejewski, Tadatsugu Sato, and Neal S. Young



ELTROMBOPAG

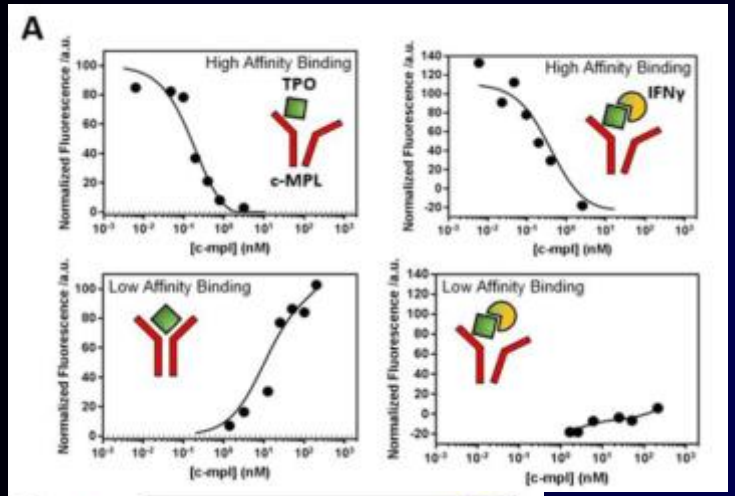
A Tpo-mimetic agent

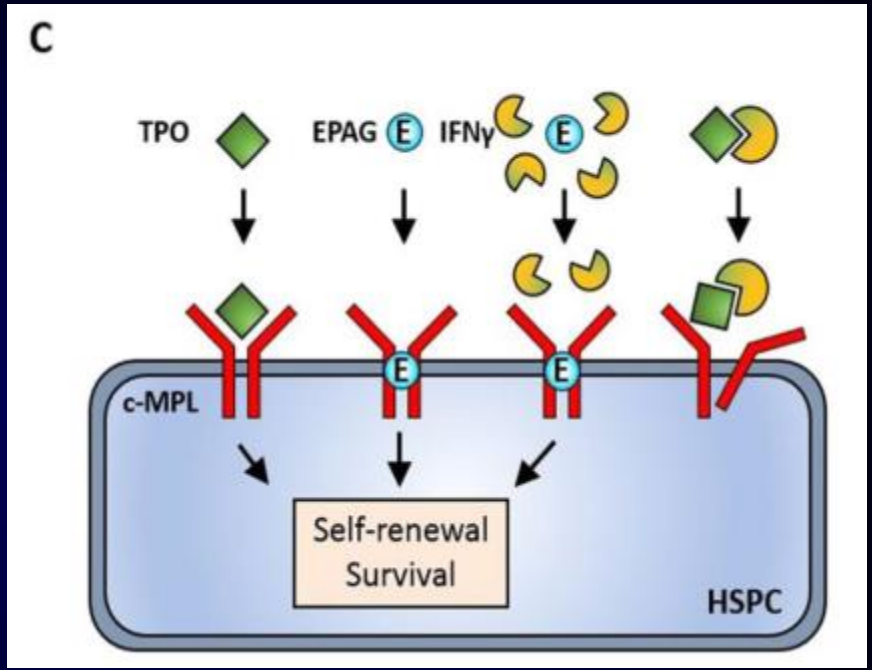




Interferon-γ and hematopoietic stem cells

A novel mechanism of inhibition



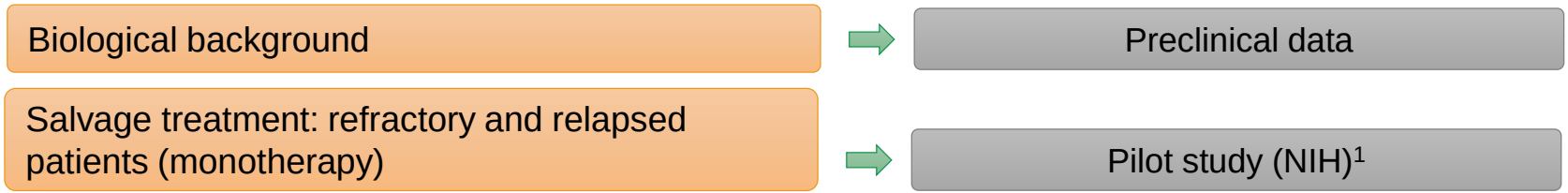


Eltrombopag may overcome the inhibitory effect of hemopoiesis exerted by IFN-γ via the c-MPL pathway

Alvarado et al, ASH 2017

Background

- Eltrombopag as investigational treatment for severe aplastic anemia



1. Olmes MJ, et al. N Engl J Med. 2012 Jul 5;367(1):11-9. 2. Townsley D, et al. New Engl J Med. 2017;376(16)1540-1550.

ELTROMBOPAG IN REFRACTORY SAA

The status of art

NEJM

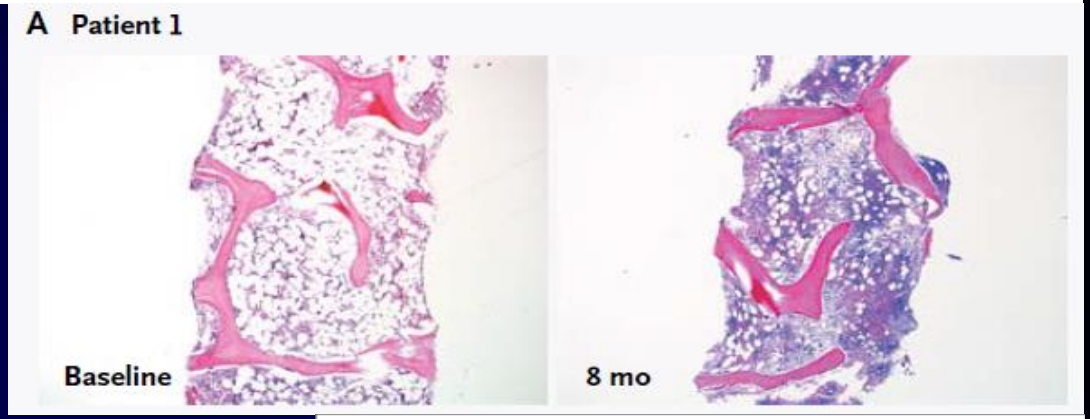
Eltrombopag and Improved Hematopoiesis in Refractory Aplastic Anemia

Phase II study

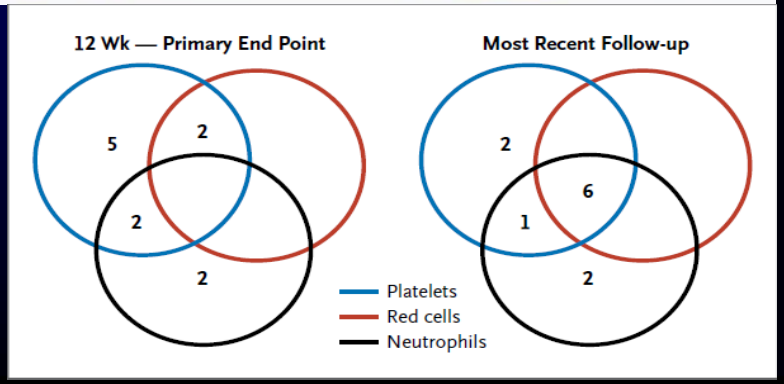
n=25

Refractory SAA

Eltrombopag 50-150 mg, orally, for 12 weeks



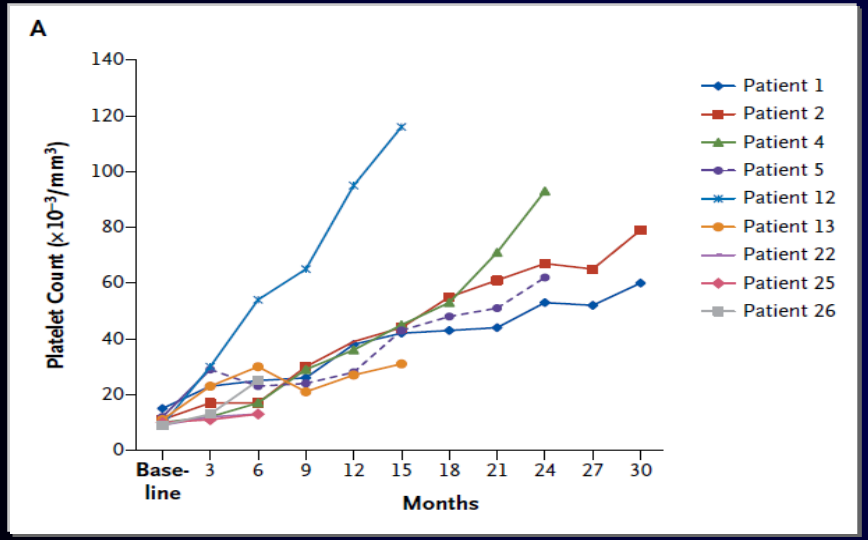
- ✓44% hematological response (at least 1 lineage)
  - ✓ Plt response 36%
  - ✓ Hb response 24%
  - ✓ ANC response 36%
- ✓Increased marrow cellularity (resp.)
- ✓Minimal toxicity (liver?), no fibrosis

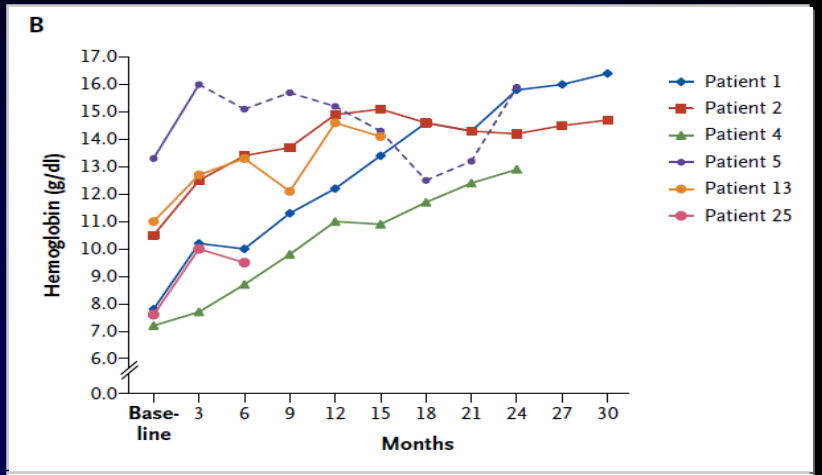


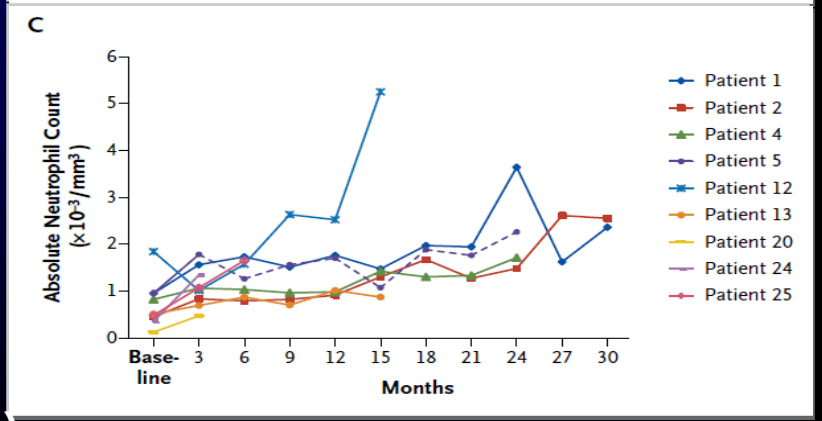
ELTROMBOPAG IN REFRACTORY SAA

The status of art

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

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]

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.

AMERICAN SOCIETY OF HEMATOLOGY

BLOOD, 20 MARCH 2014 • VOLUME 123, NUMBER 12

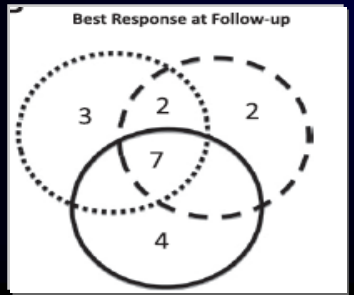
✓Additional 18 patients (n=43), OR 17/43 (40%)

✓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]*

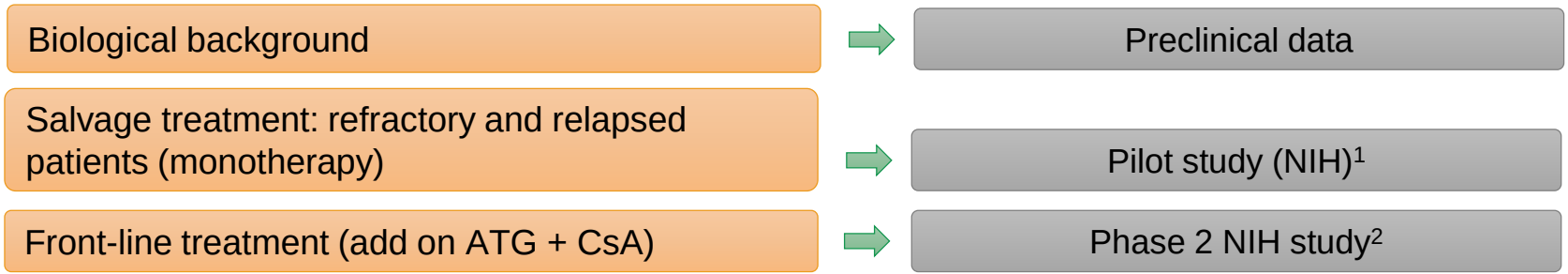


CGH (SNP-based)

Age (y)	Response	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]	46XYdel13q[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

Background

- Eltrombopag as investigational treatment for severe aplastic anemia



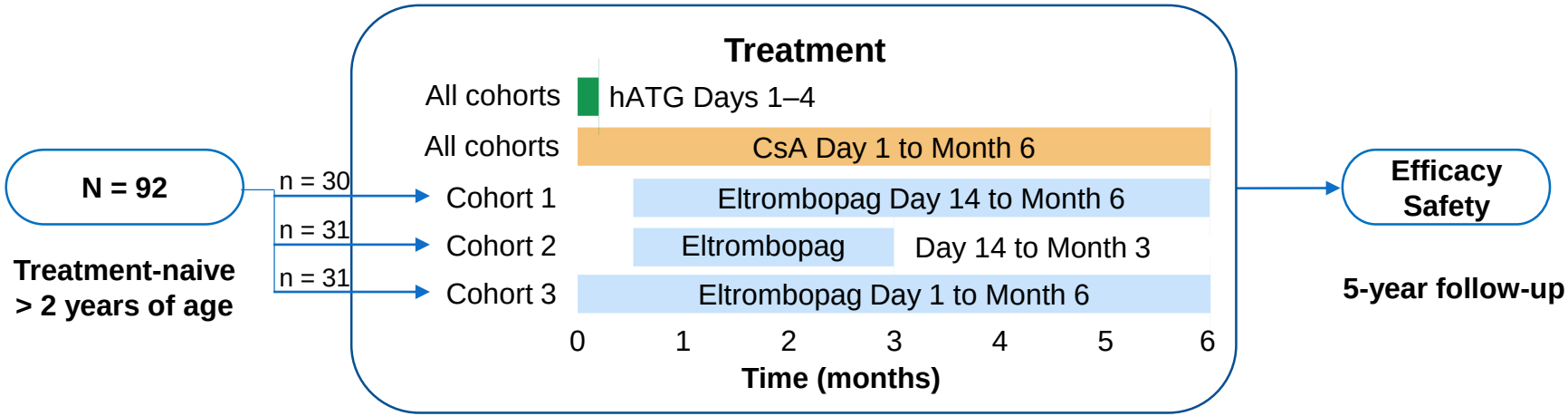
1. Olmes MJ, et al. N Engl J Med. 2012 Jul 5;367(1):11-9. 2. Townsley D, et al. New Engl J Med. 2017;376(16)1540-1550.

Background


ORIGINAL ARTICLE

Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia

- A phase 2, open-label, interventional, single-arm, sequential cohort study of **eltrombopag in combination with immunosuppression in the first-line** treatment of patients with SAA



Townsley DM, et al. N Engl J Med. 2017;376:1540-50.

Background

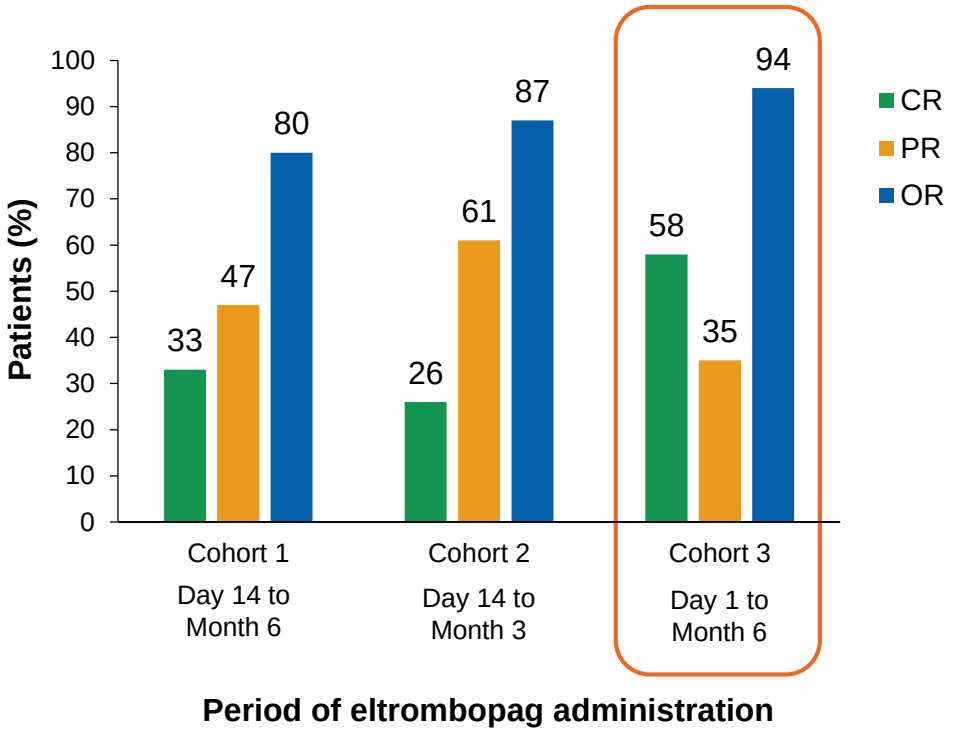
At 6 months

CR

- Platelet count 100 × 10^9/L
- Neutrophil count ≥ 1 × 10^9/L
- Hemoglobin level 10 g/dL

PR

- Blood counts not meeting criteria for SAA or CR



Townsley DM, et al. N Engl J Med. 2017;376:1540-50.

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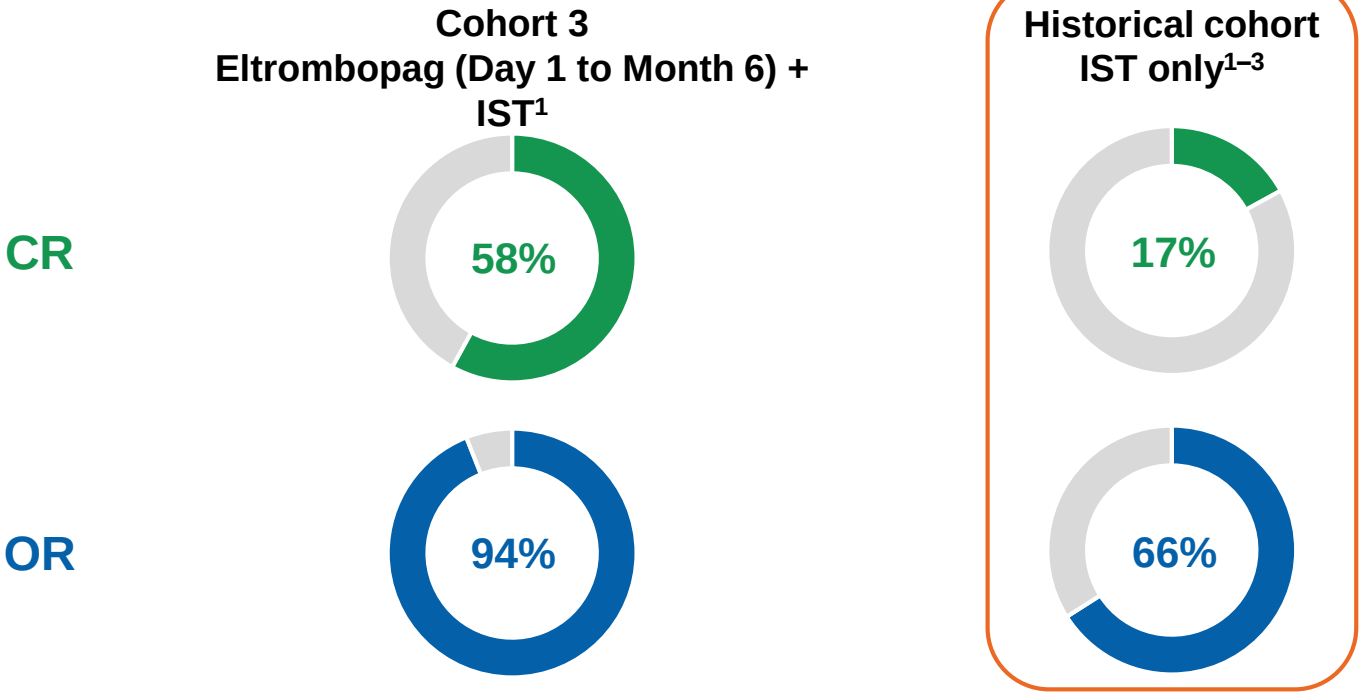
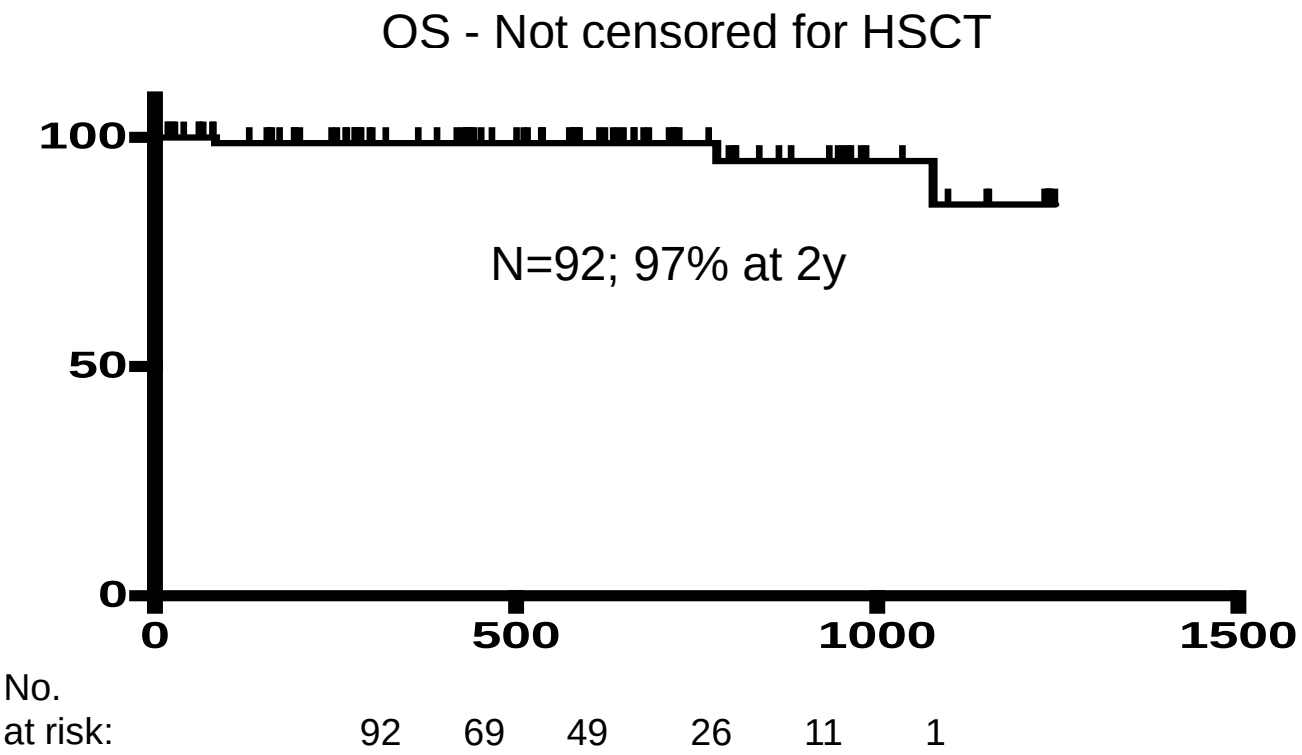


1. Townsley DM, et al. N Engl J Med. 2017;376:1540-50. 2. Scheinberg P, et al. Haematologica. 2009;94:348-54. 3. Scheinberg P, et al. N Engl J Med. 2011;365:430-8.

Background



Townsley DM, et al. N Engl J Med. 2017;376:1540-50.

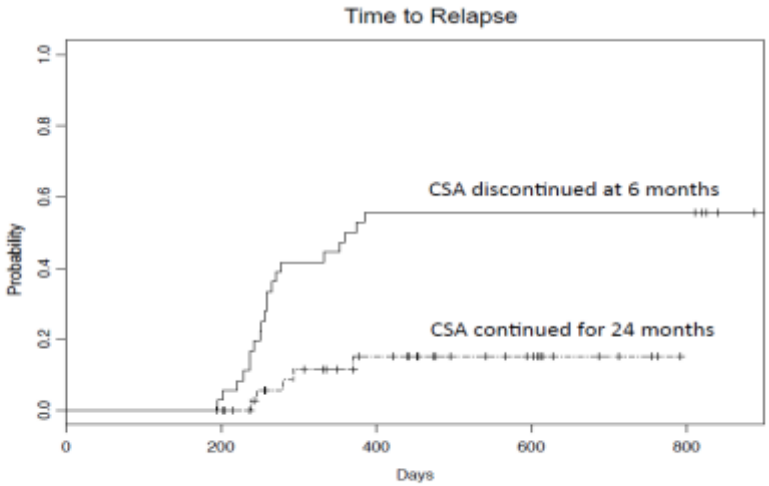
Background

THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

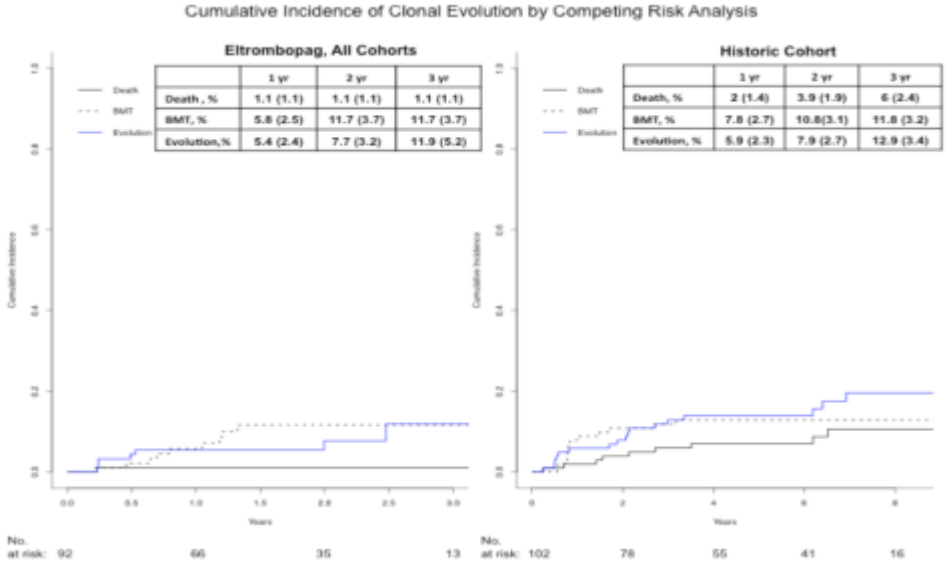
Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia

Danielle M. Townsley, M.D., Philip Scheinberg, M.D., Thomas Winkler, M.D., Ronan Desmond, M.D., Bogdan Dumitriu, M.D., Olga Rios, R.N., Barbara Weinstein, B.S.N., Janet Valdez, P.A., Jennifer Lotter, P.A., Xingmin Feng, Ph.D., Marie Desierto, B.S., Harshraj Leuva, M.B., B.S., Margaret Bevans, Ph.D., Colin Wu, Ph.D., Andre Larochelle, M.D., Ph.D., Katherine R. Calvo, M.D., Cynthia E. Dunbar, M.D., and Neal S. Young, M.D.



No. at risk:

CSA discontinued	92	35	17	16	16
CSA continued	92	43	24	11	1



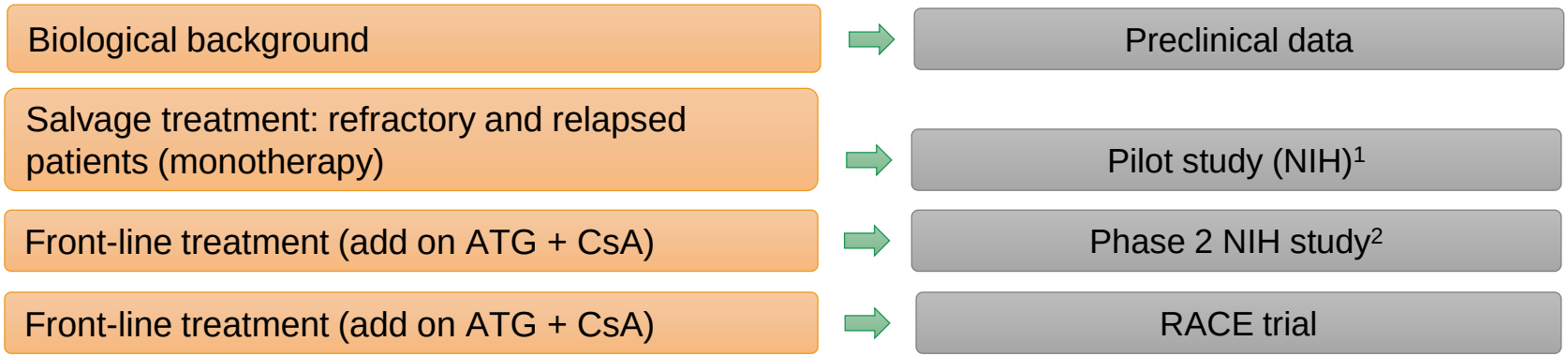
Eltrombopag, All Cohorts	1 yr	2 yr	3 yr
Death , %	1.1 (1.1)	1.1 (1.1)	1.1 (1.1)
BMT, %	5.8 (2.5)	11.7 (3.7)	11.7 (3.7)
Evolution,%	5.4 (2.4)	7.7 (3.2)	11.9 (5.2)

Historic Cohort	1 yr	2 yr	3 yr
Death, %	2 (1.4)	3.9 (1.9)	6 (2.4)
BMT, %	7.8 (2.7)	10.8(3.1)	11.8 (3.2)
Evolution, %	5.9 (2.3)	7.9 (2.7)	12.9 (3.4)

Townsley DM, et al. N Engl J Med. 2017;376:1540-50.

Background

- Eltrombopag as investigational treatment for severe aplastic anemia



1. Olmes MJ, et al. N Engl J Med. 2012 Jul 5;367(1):11-9. 2. Townsley D, et al. New Engl J Med. 2017;376(16)1540-1550.

EBMT European Society for Blood and Marrow Transplantation

A prospective

Randomized multicenter study comparing horse

Antithymocyte globuline (hATG) +

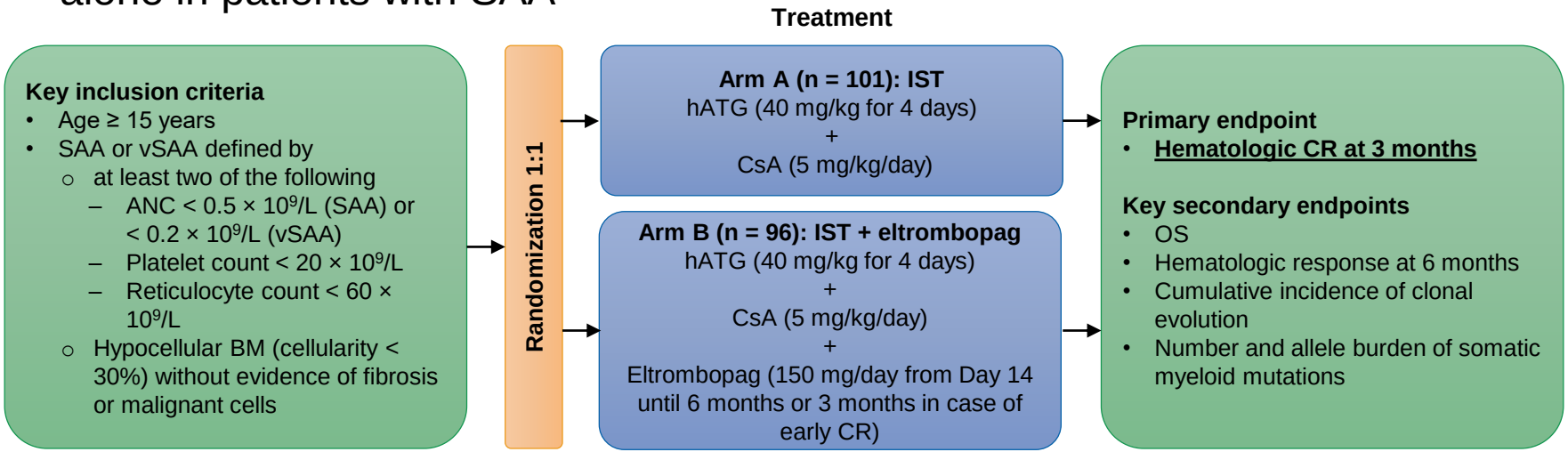
Cyclosporine A (CsA)

with or without

Eltrombopag as front-line therapy for SAA patients

RACE design

- The **RACE trial** is an investigator-driven, open-label, phase 3, randomized trial comparing the combination of hATG, CsA, and eltrombopag with IST alone in patients with SAA



Central laboratory King's college, London

Stratification based on disease severity age and center

ANC, absolute neuthrophil count; vSAA, very severe aplastic anemia.

RACE definitions & primary endpoint

- **RACE criteria for response**
  - **CR:** Hb >100 g/L, neutrophils >1.0x109/L and platelets >100x109/L
  - **PR:** no longer meets SAA criteria, Transfusion independence, Hb >8gr/dL, neutrophils >0.5x109/L and platelets >20x109/L (different from NIH)
  - **NR**: not meeting criteria for response
- **Clonal evolution**
  - Acute leukemia, myelodysplastic syndrome and/or new karyotypic abnormality
- **Primary endpoint**
  - To detect an increase in CR from 7% in arm A to 21% in arm B at 3m (at least 96 patients per arm)

RACE trial

- **Inclusion period**: July 2015 - April 2019
- **Patients**: 205 treatment naïve patients enrolled in 6 countries and 24 sites
- **Median Follow-up:** 18 months

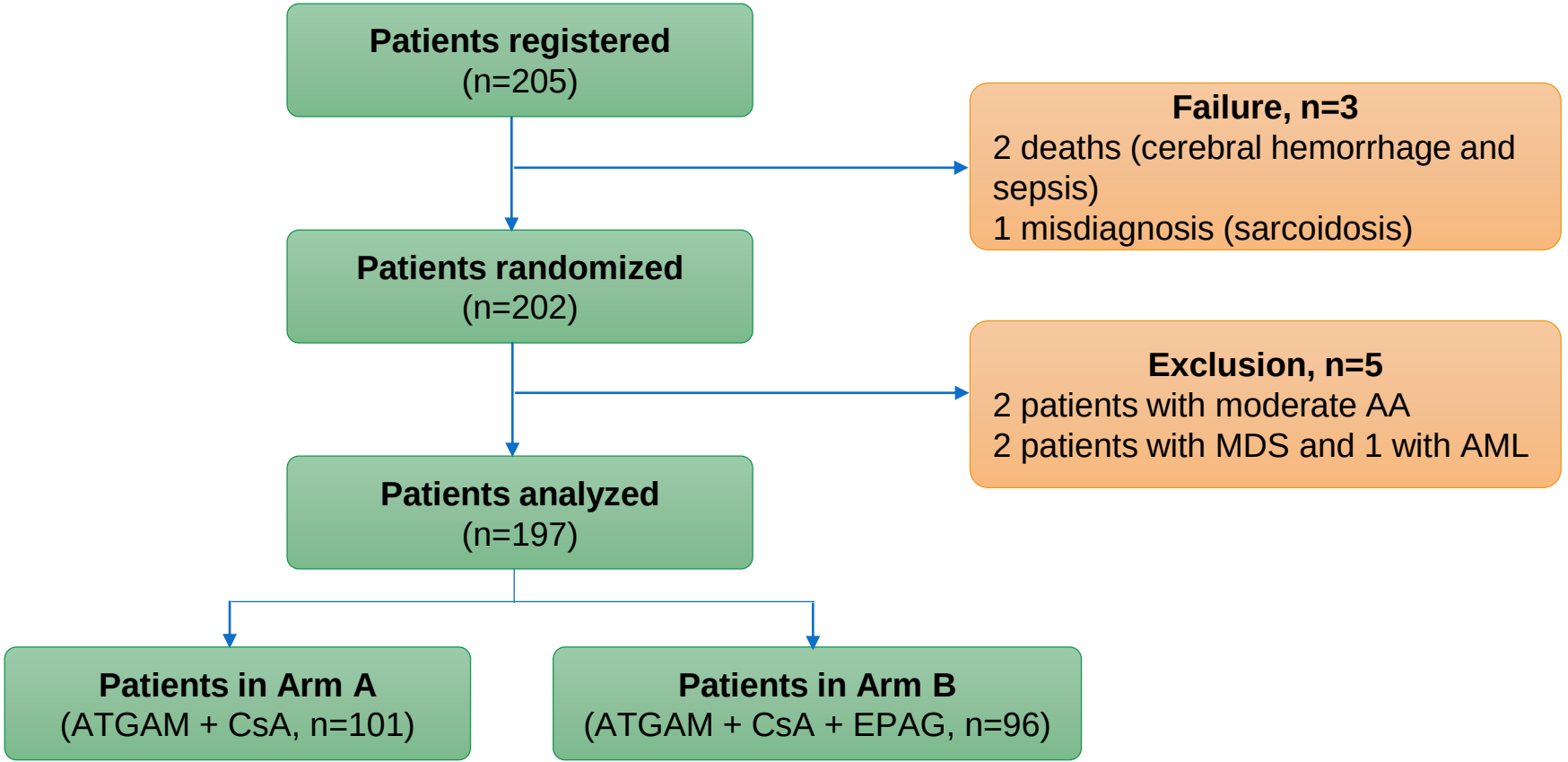
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 6, 2022 VOL. 386 NO. 1

Eltrombopag Added to Immunosuppression in Severe Aplastic Anemia

R. Peffault de Latour, A. Kulasekararaj, S. Iacobelli, S.R. Terwel, R. Cook, M. Griffin, C.J.M. Halkes, C. Recher, F. Barraco, E. Forcade, J.-C. Vallejo, B. Drexler, J.-B. Mear, A.E. Smith, E. Angelucci, R.A.P. Raymakers, M.R. de Groot, E. Daguindau, E. Nur, W. Barcellini, N.H. Russell, L. Terriou, A.-P. Iori, U. La Rocca, A. Sureda, I. Sánchez-Ortega, B. Xicoy, I. Jarque, J. Cavenagh, F. Sicre de Fontbrune, S. Marotta, T. Munir, J.M.L. Tjon, S. Tavitian, A. Praire, L. Clement, F. Rabian, L. Marano, A. Hill, E. Palmisani, P. Muus, F. Cacace, C. Frieri, M.-T. van Lint, J.R. Passweg, J.C.W. Marsh, G. Socié, G.J. Mufti, C. Dufour, and A.M. Risitano, for the Severe Aplastic Anemia Working Party of the European Society for Blood and Marrow Transplantation*

RACE flow chart



Baseline characteristics

	Arm A	Arm B	Total
No. of patients	101 (51.3%)	96 (48.7%)	197 (100%)
Age (median, min-max)	52 (15-81)	55 (16-77)	53 (15-81)
Age categories (n, %)			
<18 y	7 (6.9%)	2 (2.1%)	9 (4.6%)
18-<40	29 (28.7%)	27 (28.1%)	56 (28.4%)
40-<65	43 (42.6%)	43 (44.8%)	86 (43.7%)
>65	22 (21.8)	24 (25.0%)	46 (23.4%)
Sex (n, %)			
Male	52 (51.5%)	56 (58.3%)	108 (54.8%)
Female	49 (48.5%)	40 (41.7%)	89 (45.2%)
Severity of AA (n, %)			
SAA	67 (66.3%)	62 (64.6%)	129 (65.5%)
vSAA	34 (33.7%)	34 (35.4%)	68 (34.5%)
PNH granulocytes >1.0% (n, %)	44 (44.9%)	33 (35.5%)	77 (40.3%)

RACE treatment protocol

Treatment	*Dose (units)*	*Route*	*Treatment Period*
ATGAM (Pfizer)	*40 mg/kg/day*	*i.v., 12-18 h infusion*	*Day 1, 2, 3 and 4*
Cyclosporine A	*5 mg/kg/day*	*Orally*	*Day 1-365 (adjusted on blood levels)*
Eltrombopag	*150 mg every 24 h (50 mg tablets x3)*	*Orally*	*Day 14-90 (or 14-180)*

8.1.2 Horse ATG (ATGAM)

Patients will receive horse-ATG (ATGAM) for 4 consecutive days (days 1-4), at the dose of 40 mg/kg, as a i.v. injection lasting 12-18 hours. As prevention of ATGAM-related side effects, including serum sickness, corticosteroids will be administered at the dose of 1 mg/kg/day (either intravenously or orally) for at least 7 days (see below) and then tapered and stopped within 2-3 weeks post treatment. A pre-medication with paracetamol (e.g. 1000 mg) and/or anti-histaminic medications (e.g. clorpheniramine 10 mg) are allowed as well.

RACE treatment protocol

Anaphylaxis and allergic reactions
Anaphylaxis is uncommon, but may occur at any time during therapy with ATGAM.More frequently, allergic reaction include skin rash, fever and chills. Prophylactic administration of corticosteroids and/or anti-histamine may decrease the frequency of this reaction.

Respiratory distress
May indicate an anaphylactoid reaction. Discontinue infusion of ATGAM. If distress persists, administer an antihistamine, epinephrine, corticosteroids, or some combination of the three.

Pain in chest, flank, or back
May indicate anaphylaxis or hemolysis. Treatment is that indicated above for those conditions.

Hypotension
May indicate anaphylaxis. Stop infusion of ATGAM and stabilize blood pressure with pressors if necessary.

Chills and fever
Occur frequently in patients receiving ATGAM. ATGAM may release endogenous leukocyte pyrogens. Prophylactic and/or therapeutic administration of antihistamines, antipyretics, or corticosteroids generally controls this reaction.

RACE treatment protocol (suggested monitoring)

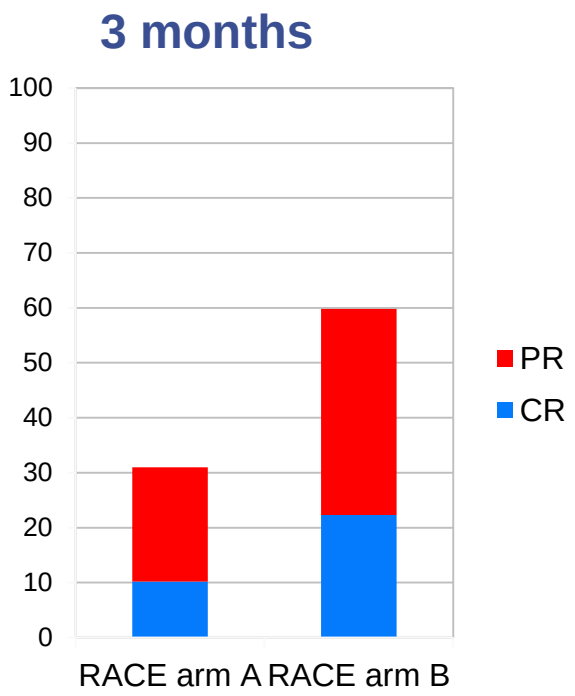
Time	Pre	d0-1	w1,2,3,4	m1.5,2,2.5	m3	m4,5	m6	m7,8	m9	m10,11	m12	m15	m18	M21	m24
Medical history	X														
Diagnosis	X														
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Signs and symptoms	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Transfusion record	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bone Marrow	X*						X*				X				X*
Trephine biopsy	X						X				X				X
Immunophenotype	X*						X*				X				X*
DEB test	X^														
Biochemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Telomeres	X*						X*								X*

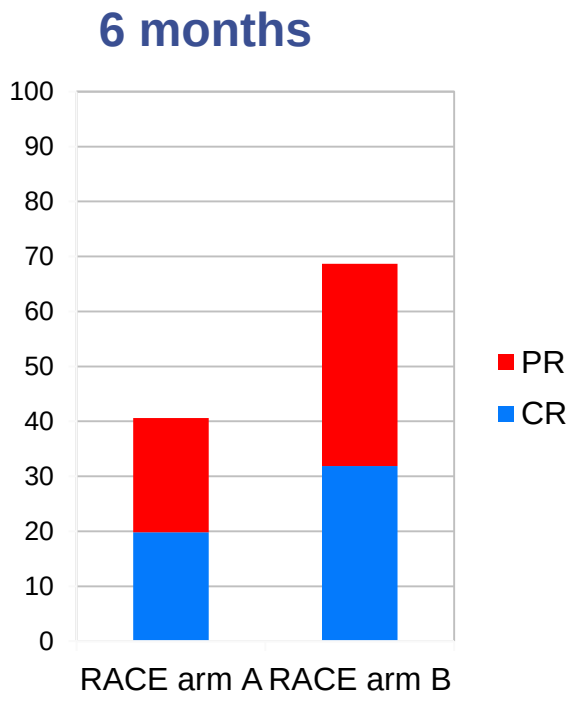
Hematological response

- The **RACE study** was powered to detect an increase in CR from 7% in arm A to 21% in arm B at 3 months (primary endpoint).

- **3 months*:**
  - CR: Arm A 9.9% & Arm B 21.9% (OR 3.2, p=0.012)
  - OR: Arm A 30.7% & Arm B 59.4% (OR 2.99, p<0.001)

- **6 months*:**
  - CR: Arm A: 19.8% & Arm B 31.6% (OR 2.13, p=0.031)
  - OR: Arm A: 40.6% & Arm B 68.4% (OR 3.63, p<0.001)



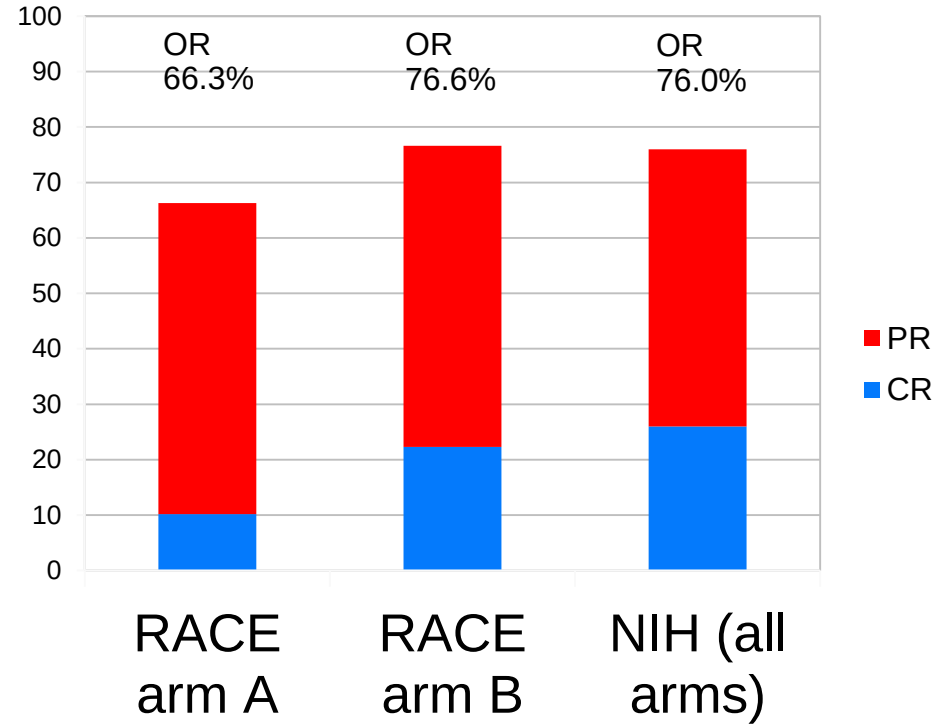


**Prior transplantation, clonal evolution or death were considered as no response at 3 and 6m*

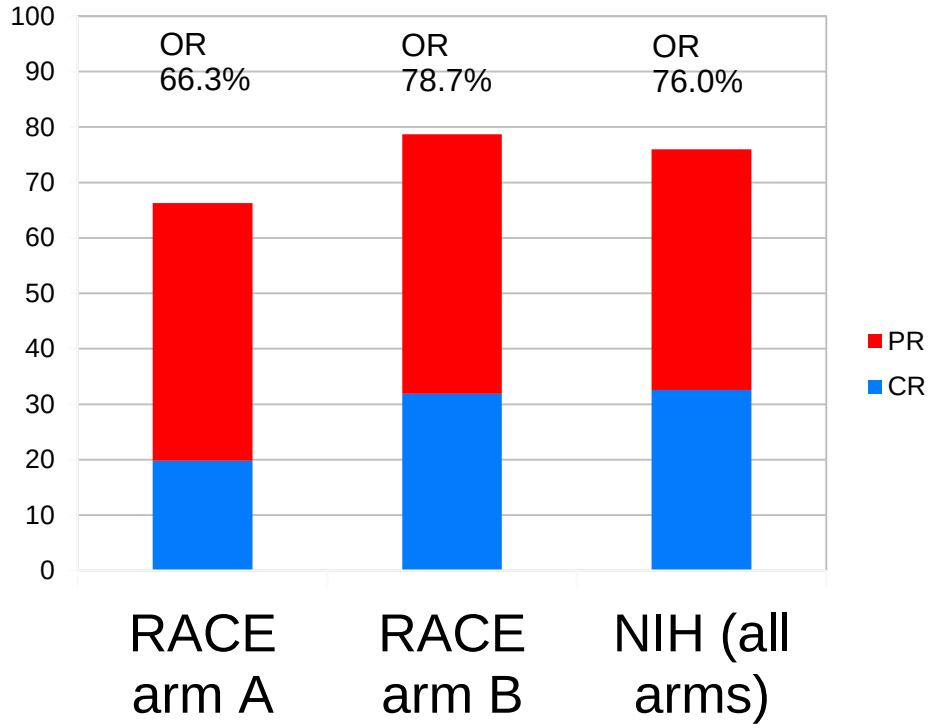
Hematological response

NIH criteria

3 months

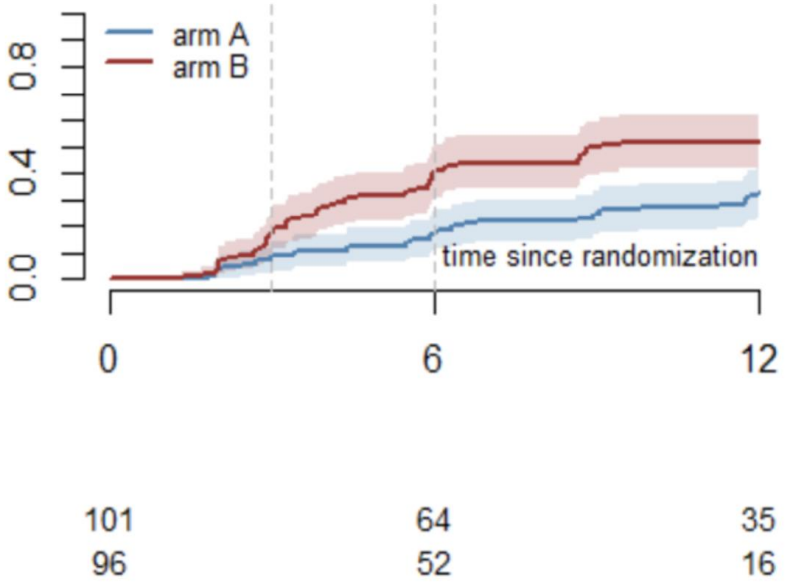


6 months

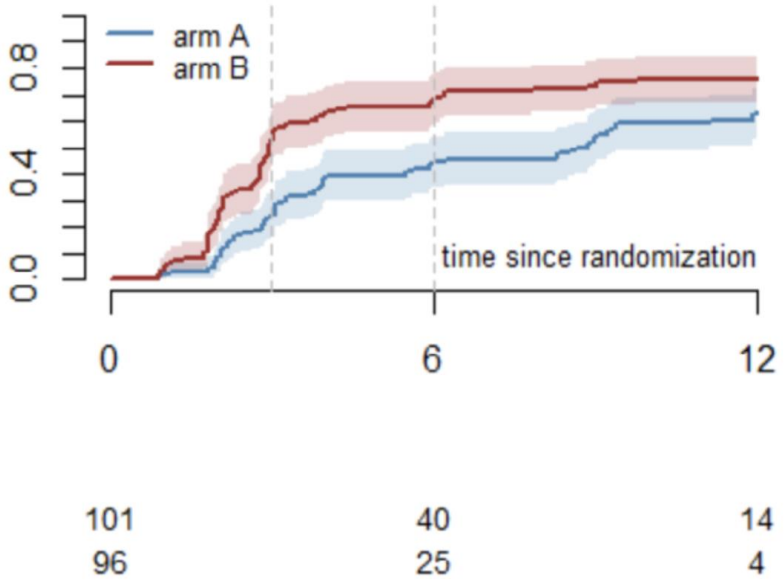


Hematological response

Time to complete response:
9.1 months (arm B) and not reached (arm A) (p=0.007)



Time to first response:
8.8 months (arm A) versus 3 months (arm B) (p=0.005)



Predictors (response)

		(Intercept)	Randomization arm (Arm B versus Arm A)	Age (≥40 versus ≥15 and <40)	Disease severity (vSAA versus SAA)
CR at 3mo	OR	0.29 (0.07,1.24)	2.8 (1.21,6.46)	0.68 (0.29,1.55)	0.22 (0.07,0.67)
	p-value	0.095	0.016	0.354	0.008
OR at 6mo	OR	2.66 (0.84,8.42)	3.52 (1.91,6.5)	0.5 (0.26,0.96)	0.47 (0.25,0.89)
	p-value	0.096	0	0.038	0.021

No correlation found with mutations at baseline, PNH clone, lymphocytes & reticulocytes (Telomere length not tested)

Safety

	Arm A	Arm B	Total
Serious Adverse Events*	135	145	280
Fatal cases	14	8	22
Patients coming off study treatment prematurely requiring second line HSCT	13	11	24
Pregnancy	3	1	4

**Events are classified per SOC (system organ class) according to the CTCAE (Common Terminology Criteria for Adverse Events (US National Cancer Institute of the National Institutes of Health).*

Serious Adverse Events

	Arm A	Arm B	Total
Blood and lymphatic system disorders	17	18	35
Cardiac disorders	6	4	10
Gastrointestinal disorders	2	15	17
General disorders and administration site conditions	10	19	29
Hepatobiliary disorders	4	3	7
Immune system disorders	2	5	7
Infections and infestations	53	43	96
Injury, poisoning and procedural complications	1	2	3
Investigations	2	1	3
Metabolism and nutrition disorders	4	2	6
Musculoskeletal and connective tissue disorders	4	1	5
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	3	2	5
Nervous system disorders	5	5	10
Psychiatric disorders	2	2	4
Renal and urinary disorders	9	11	20
Respiratory, thoracic and mediastinal disorders	8	6	14
Skin and subcutaneous tissue disorders	1	1	2
Surgical and medical procedures	0	1	1
Vascular disorders	2	4	6
Total	**135**	**145**	**280**

Fatal cases

Cause of death	Arm A	Arm B	Total
Hemorrhages	2	0	2
Infections	9	4	13
Salvage treatment	1	0	1
Others:	2	4	6
• Acute Respiratory Distress Syndrome	0	1	1
• Aortic valve disease	0	1	1
• Concomitant lung cancer	1	0	1
• Encephalopathy of unknown origin	1	0	1
• Tamponade	0	1	1
• Thrombosis	0	1	1
Total	***14***	***8***	***22***

Clonal evolution – myeloid malignancy

Arm	Age	AA	Cytogenetics/Karyotypic abnormalities			CE	MDS	somatic mutations +VAF			Response		Relapse
			Baseline	6 months	24 months			Baseline	6 months	24 months	3 mo	6 mo	
A	58	SAA	46XY	46, XY, +Y, -7[4]/46, XY[11]		Yes-6 mo	No	BCOR 0.1%, DNMT3A 1.24%, TET2 0.1%	BCOR 5.01%, DNMT3A 13.24% TET2 13.29%	NST	PR	CE	Yes
B	19	SAA	46,XX[16]	Not done or failed (del13q at 12 &18 months)	Normal	Yes-12 mo	Yes	PIGA 7.74%	PIGA 7.15%	NST	PR	CR	No
B	62	SAA	46,XY [15]	46,XY,-13(q13q34)[2]/46,XY[18]	Unknown (persistent del13q at 12 and 18 months)	Yes-6 mo	No	No mutations	No mutations	NST	NR	NR	No
A	67	SAA	46, XY [20]	45,X,-Y[3]/46,XY[17]	46,XY,del(7)(q22q3?2)[7] /46,XY[18] (No del7q detected 6 and 12 months later)	No	No	No mutations	No mutations	BCOR 1.97%	NR	NR	No

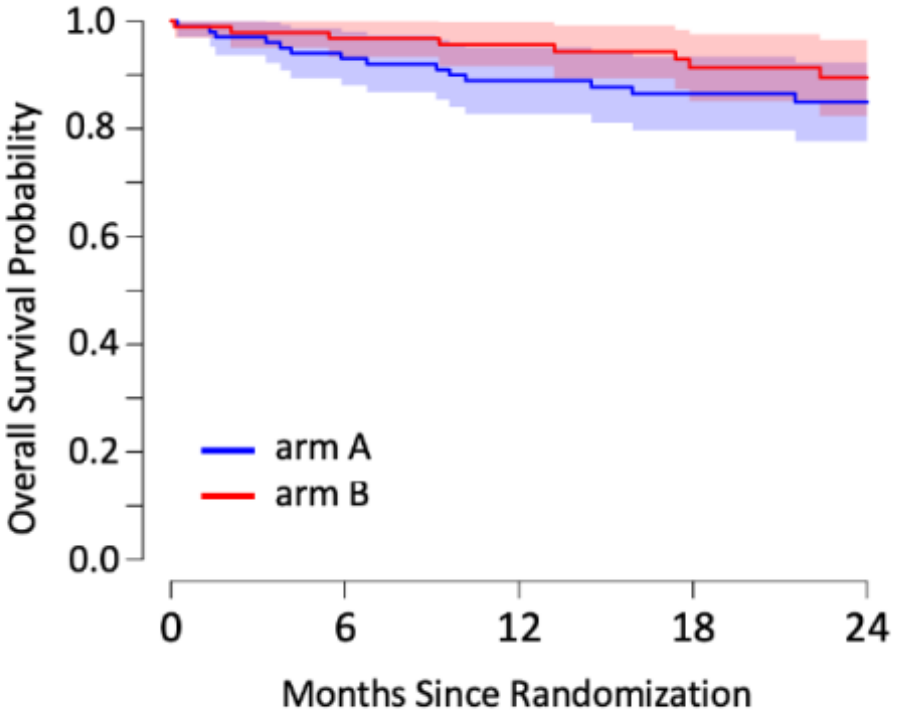
Long-term outcomes

- **HSCT requirement (during study follow-up)**
  - **Arm A:** n=12
  - **Arm B:** n=11
- **Relapse (CI at 18 months)**
  - **Arm A:** 11.3% (95% CI, 2.2% to 20.4%)
  - **Arm B:** 19.1% (95% CI, 9.2% to 28.9%)
- **Ciclosporine independence (at 2 years)**
  - **Arm A:** 18.8%
  - **Arm B:** 27.6%

Overall Survival

Median Follow-up: 24 months

No role of mutational status at baseline, 6 months; neither new mutations between baseline and 6 months



Number of Patients at Risk

arm A	101	93	80	64	26
arm B	96	92	74	58	25

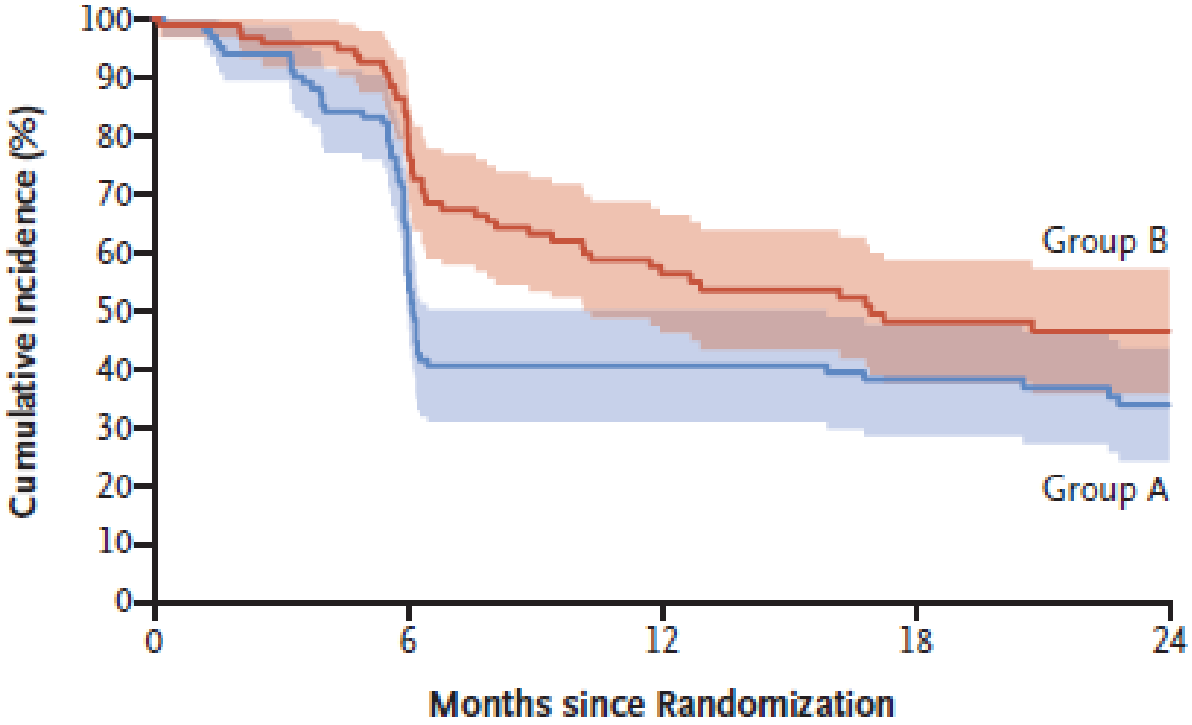
Long-term outcome (EFS)

Arm A: 34%
(95% CI, 24.3% to 43.6%)

and

Arm B: 46.5%
(95% CI, 35.9% to 57.2%)

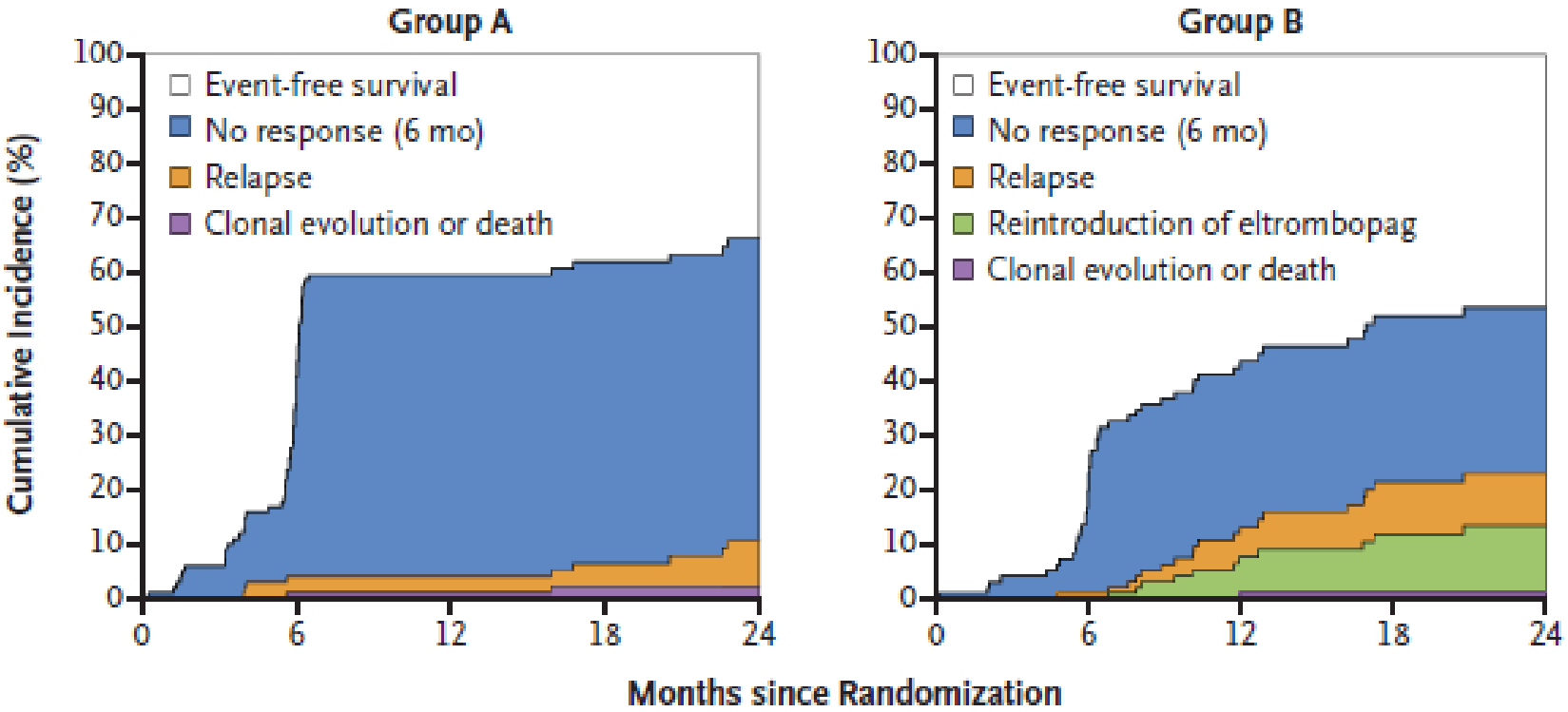
(p=0.002)



No. at Risk					
Group B	96	76	45	31	15
Group A	101	60	38	30	10

Long-term outcome (EFS)

Stacked cumulative incidence curves



Long-term outcome (predictors)

		(Intercept)	Randomization arm (Arm B versus Arm A)	Age (≥40 versus ≥15 and <40)	Disease severity (vSAA versus SAA)
OS	HR		0.57 (0.24,1.37)	3.35 (0.99,11.34)	1.85 (0.8,4.27)
	p-value		0.211	0.052	0.15
EFS	HR		0.42 (0.25,0.72)*	1.99 (1.29,3.06)	1.54 (1.06,2.24)
	p-value		0.002	0.002	0.025
First Response	HR		2.25 (1.53,3.31)*	0.85 (0.6,1.19)	0.42 (0.27,0.65)°
	p-value		0	0.341	0
Relapse	HR		1.32 (0.55,3.21)	3.6 (1.06,12.24)	1.4 (0.56,3.47)
	p-value		0.536	0.04	0.472

Improving IST for AA:
very long term outcome
(cure???)

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!!!

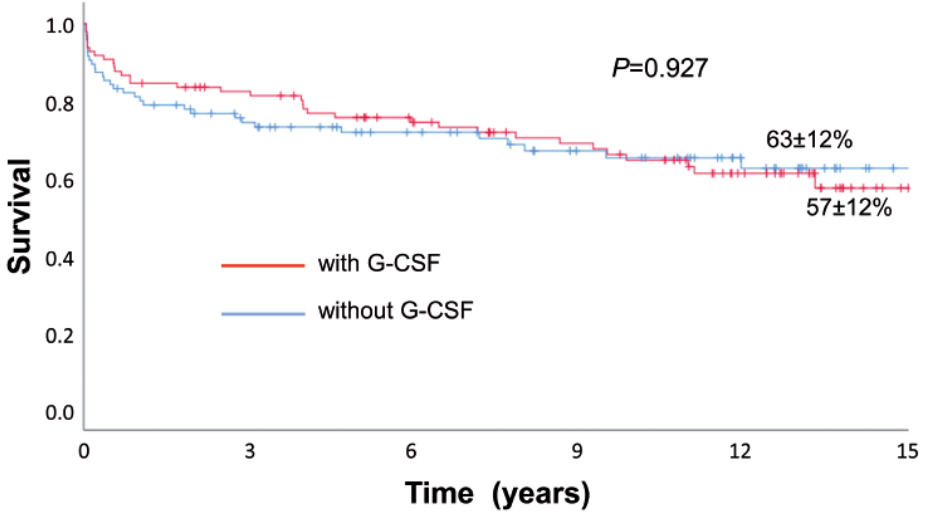
Long-term outcome of a randomized controlled study in patients with newly diagnosed severe aplastic anemia treated with antithymocyte globulin and cyclosporine, with or without granulocyte colony-stimulating factor: a Severe Aplastic Anemia Working Party Trial from the European Group of Blood and Marrow Transplantation

Ferrata Storti Foundation

Haematologica 2020
Volume 105(5):1223-1231

André Tichelli,[1] Régis Peffault de Latour,[2] Jakob Passweg,[1] Cora Knol-Bout,[3] Gérard Socié,[4] Judith Marsh,[5] Hubert Schrezenmeier,[6] Britta Höchsmann,[6] Andrea Bacigalupo,[7] Sujith Samarasinghe,[8] Alicia Rovó,[9] Austin Kulasekararaj,[10] Alexander Röth,[11] Dirk-Jan Eikema,[3] Paul Bosman,[3] Peter Bader,[12] Antonio Risitano[13] and Carlo Dufour[14] on behalf of the SAA Working Party of the EBMT

A



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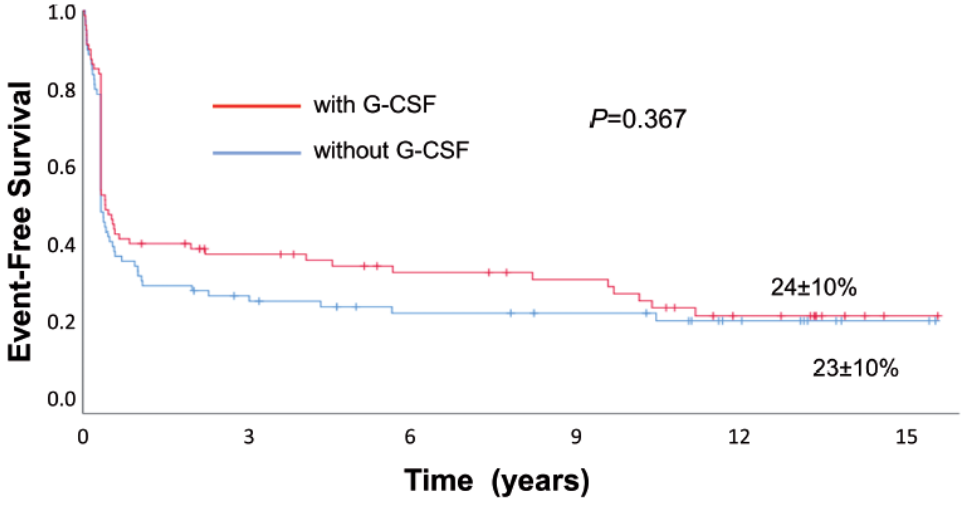

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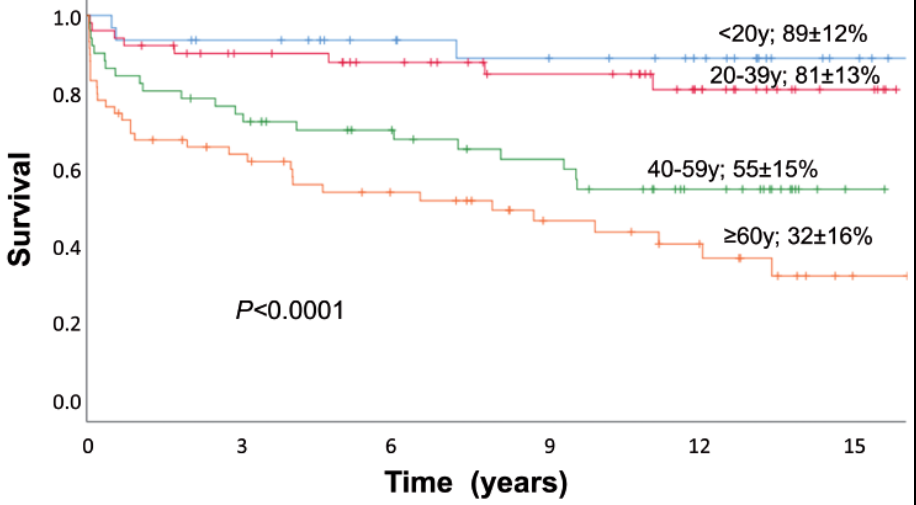

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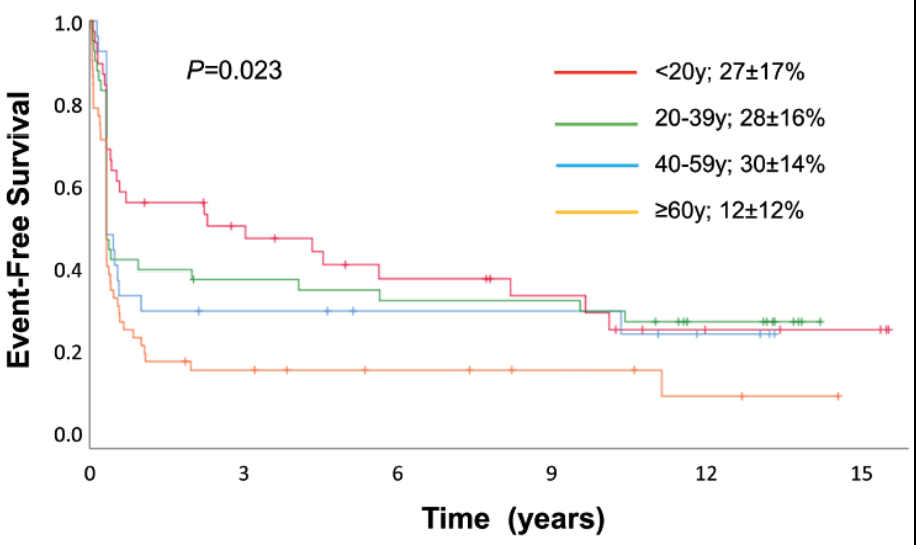
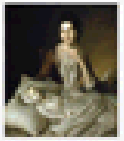


JAMA



2003

Antithymocyte Globulin and Cyclosporine for Severe Aplastic Anemia

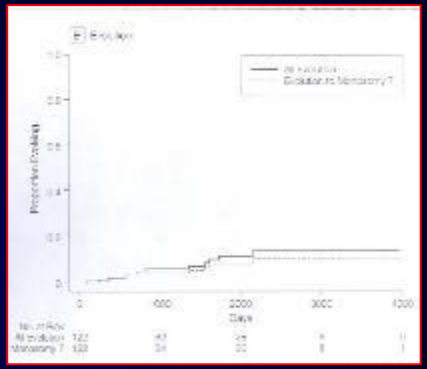
Association Between Hematologic Response and Long-term Outcome

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

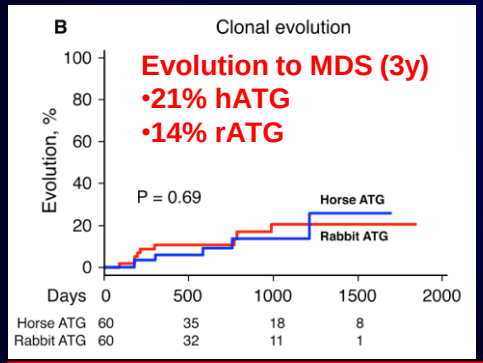
n=112

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

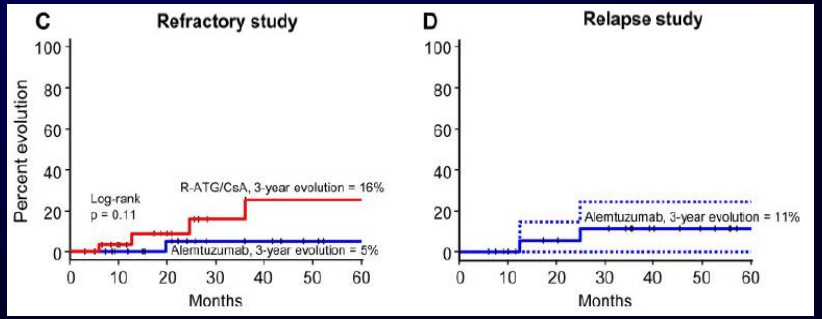
Clonal evolution (3y)
- 11% MDS (especially 7-)
- 10% PNH



NEJM 2011



Blood 2012



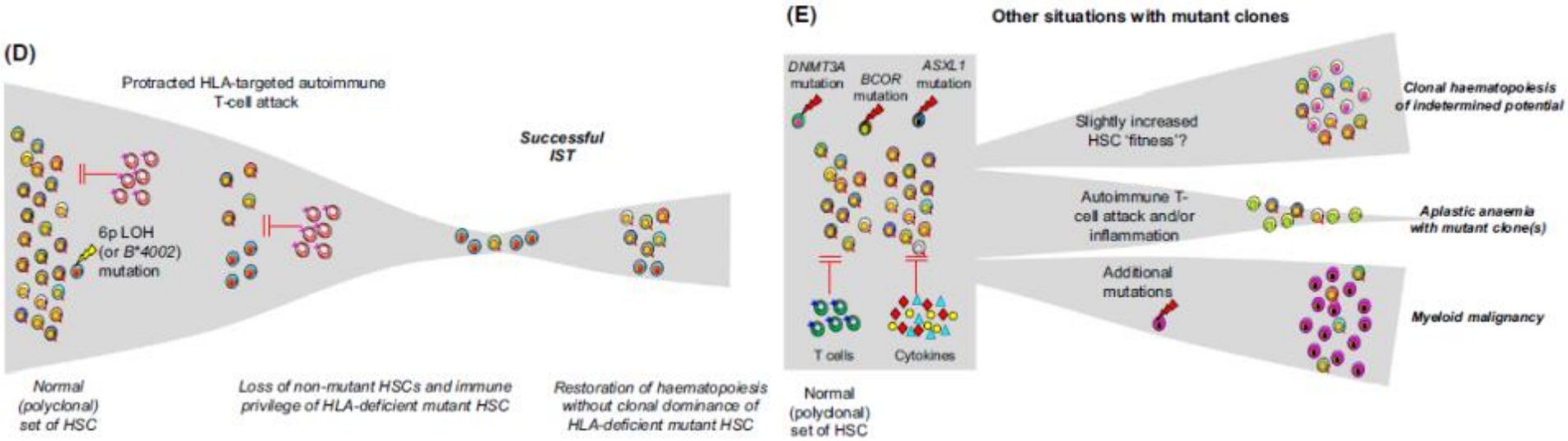
In all recent studies, the incidence of clonal evolution is about 10%, regardless the specific treatment

Somatic mutations in AA (III)

bjh review

Advances in understanding the pathogenesis of acquired aplastic anaemia

Lucio Luzzatto[1] and Antonio M. Risitano[2]



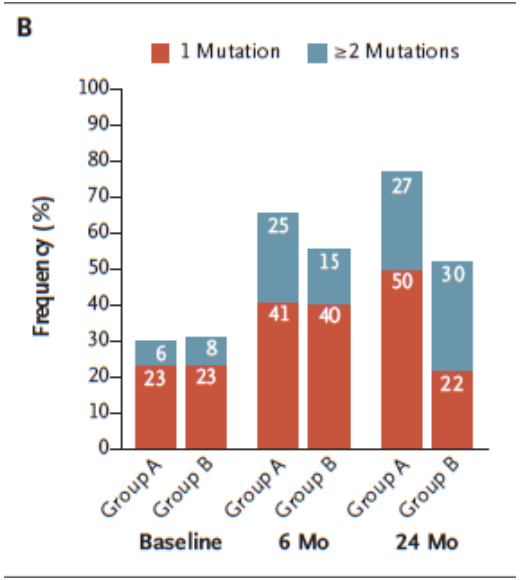
Somatic mutations in AA: RACE

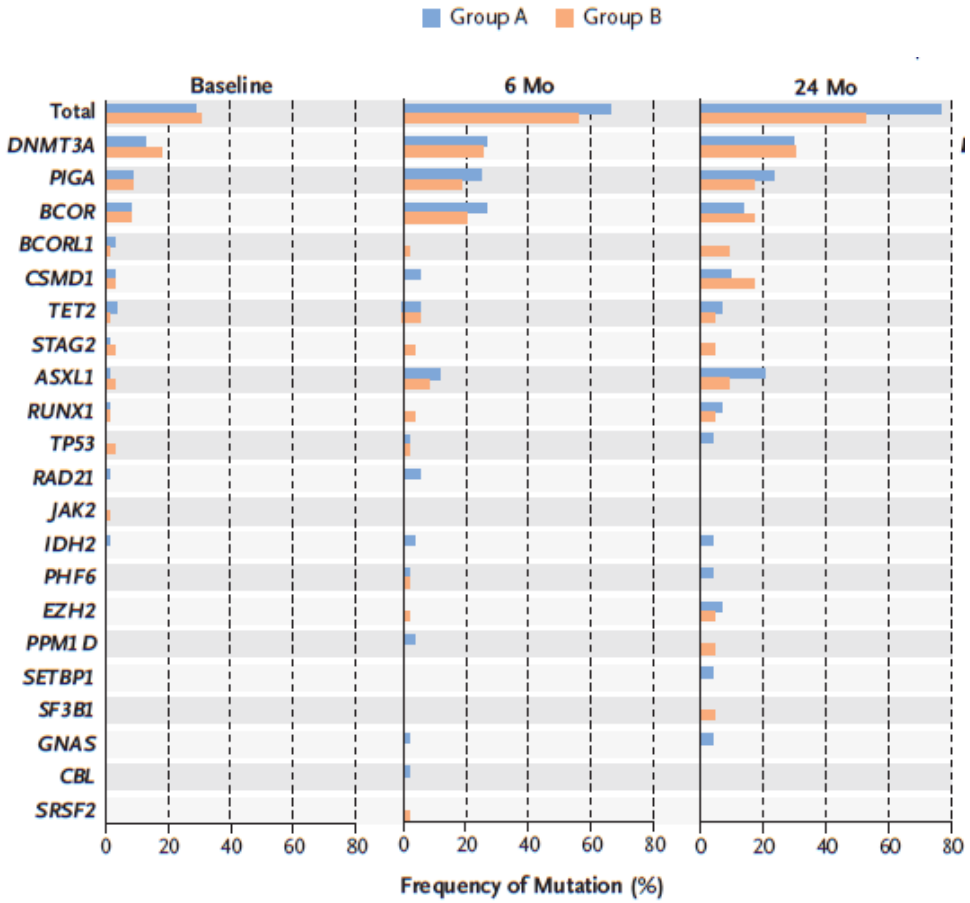
- **Bone marrow samples systematically collected at baseline, 6 months and 24 months**
  - Centralized NGS analysis performed at King's College, using two different gene panels (one standard with 32 genes, and one much larger looking for >250 genes)
  - The 31 gene panel

ASXL1	DNMT3A	BCOR	BCORL1
PIGA	TET2	TP53	U2AF1
RUNX1	ZRSR2	SETBP1	SRSF2
GNAS (Hotspot only)	SF3B1	NRas	KRas
EZH2	JAK2	IDH1	IDH2
MPL	CBL	FLT3	NPM1
STAG2	PHF6	RAD21	PTPN11
CSMD1	ETV6	PPM1D	

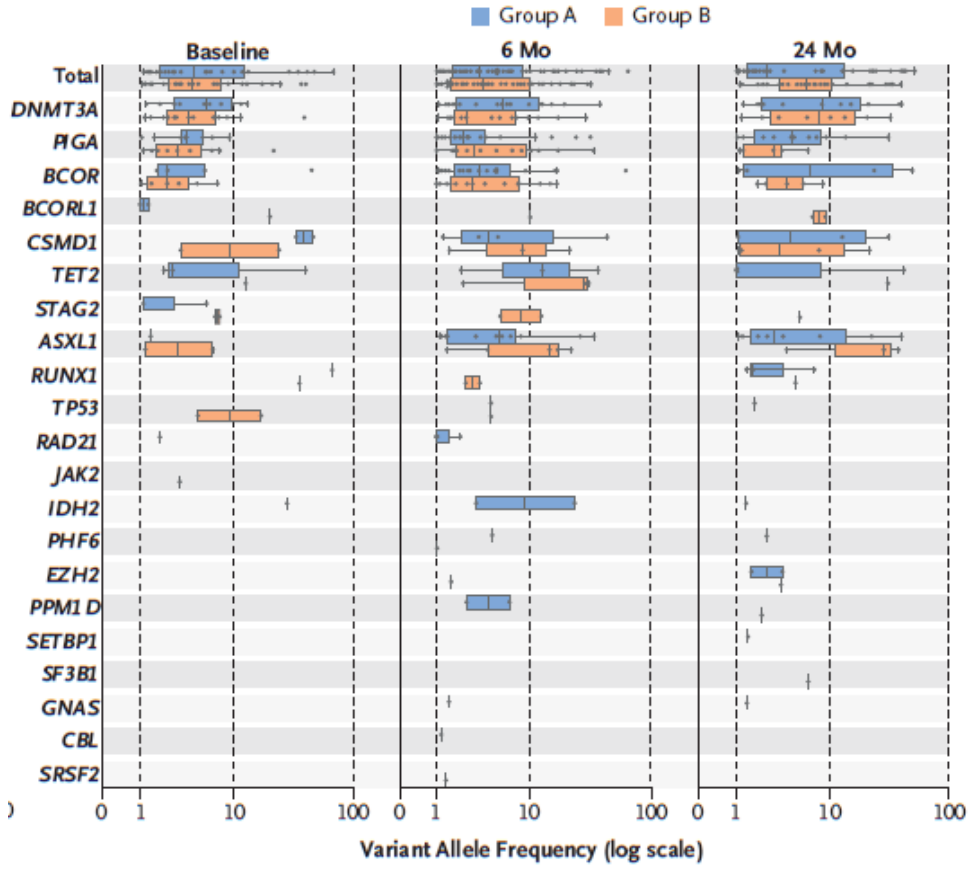
- **Analysis ongoing**
  - Baseline somatic mutations
    - Correlation with hematological response (and clonal evolution)
    - Clonal dominance over time (with impact of treatment arm)
  - 6 and 24 month mutations
    - Impact of treatment arm
    - Correlation with hematological response (and clonal evolution)

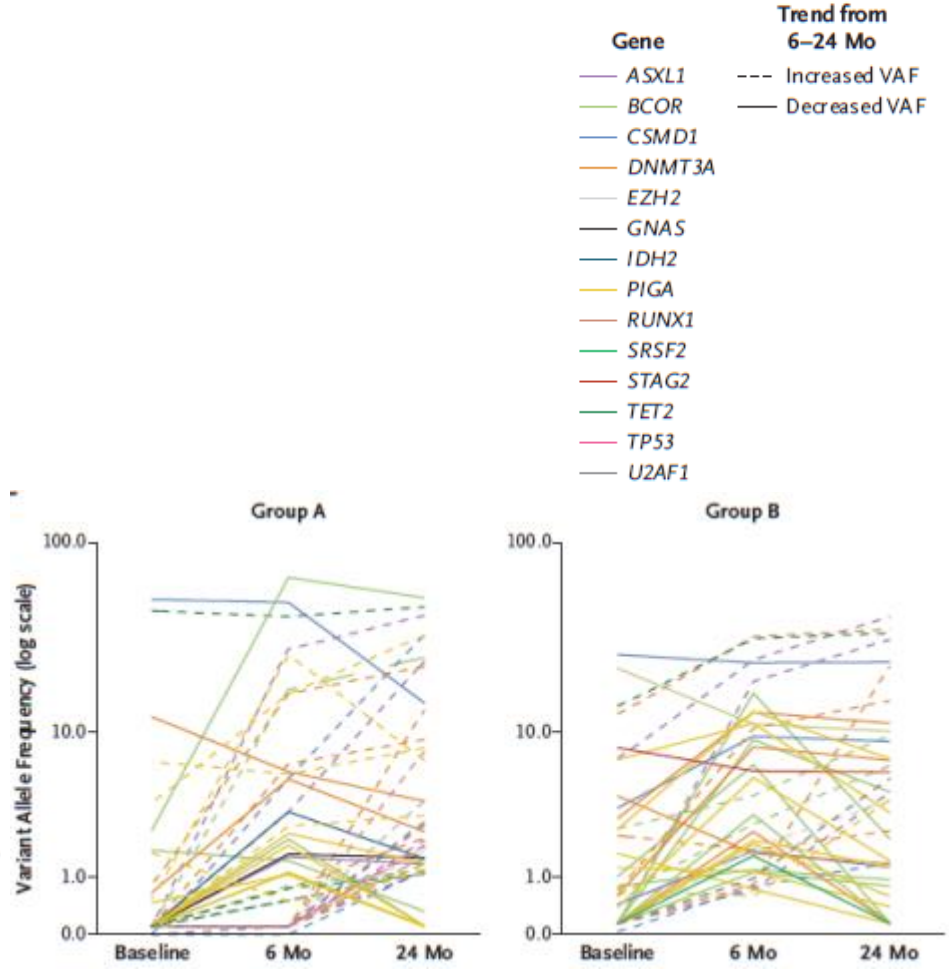
Clonal evolution – somatic mutations (I)





Clonal evolution – somatic mutations (II)





Impact of somatic mutations on response and additional mutations

Mutations at baseline	Overall Response at 6 months, n (%)			
	No	Yes	No	Yes
No	94 (86.2%)	15 (13.8%)	55 (50.9%)	53 (49.1%)
Yes	37 (78.7%)	10 (21.3%)	19 (40.4%)	28 (59.6%)

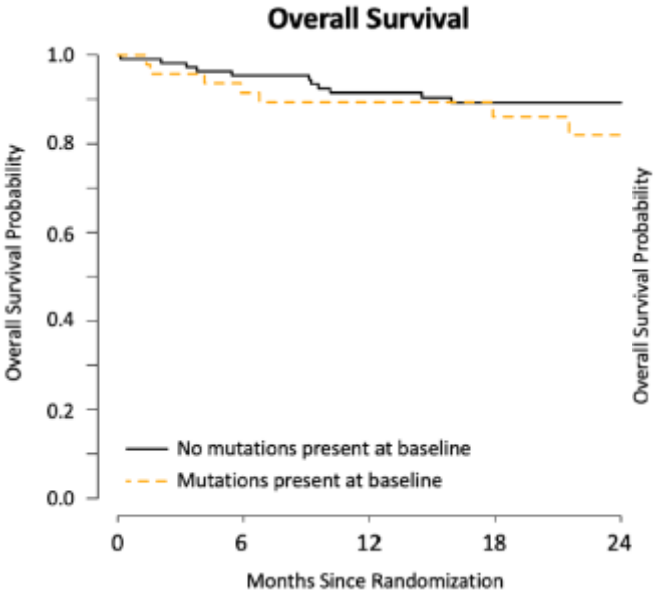
Mutations at 6 months	Overall Response at 6 months, n (%)	
	No	Yes
No	20 (41.7%)	28 (58.3%)
Yes	34 (46.6%)	39 (53.4%)

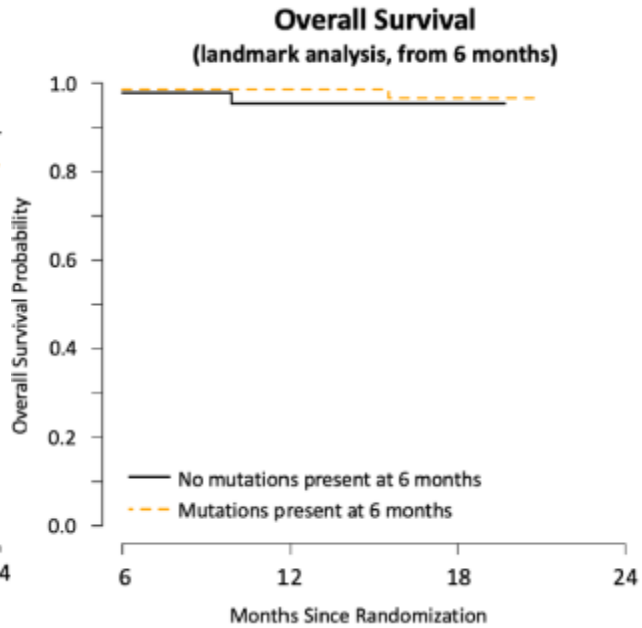
Onset of new/ additional mutations 0-6 months	Overall Response at 6 months, n (%)	
	No	Yes
No	25 (40.3%)	37 (59.7%)
Yes	27 (51.9%)	25 (48.1%)

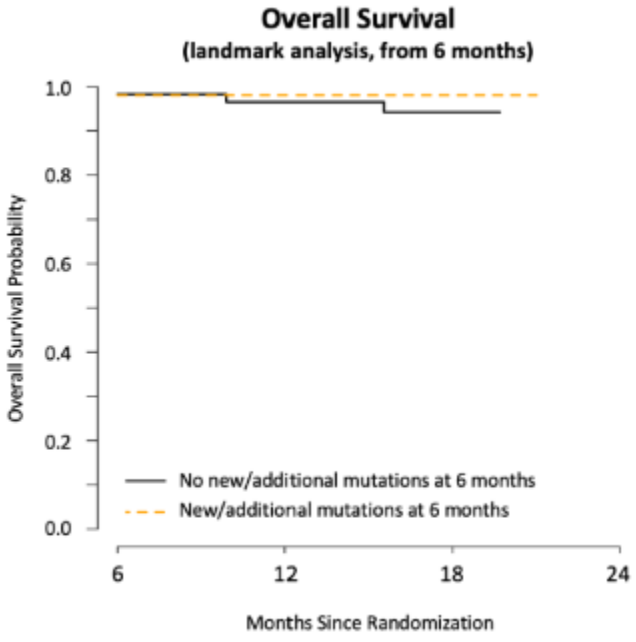
Table S15A: Onset of new/additional mutations at 6 and 24 months

Time Point (months)	Presence of new/ additional mutations	Arm A	Arm B
0-6 (n=114)	No	27 (47.4%)	35 (61.4%)
	Yes	30 (52.6%)	22 (38.6%)
0-24 (n=48)	No	10 (38.5%)	16 (72.7%)
	Yes	16 (61.5%)	6 (27.3%)
6-24 (n=49)	No	18 (66.7%)	18 (81.8%)
	Yes	9 (33.3%)	4 (18.2%)

Impact of somatic mutations on survival







Conclusion - Perspective

- EPAG, when added to standard IST (hATG and CsA), **significantly increases the rate of CR at 3 months** in untreated patients with SAA with **no safety concern** at time of analysis (18 months median follow-up).

- At 24 months, clonal evolution very rare (2-3%) with no difference between arms; but it occurs 10-15 years after the diagnosis of aplastic anemia; the **Long Term Follow-Up study (RACE-2)** is ongoing to answer this question in the future

- **Somatic myeloid mutations assessment (on going):** high sensitivity next generation sequencing analysis was performed at baseline, 6 months and 24 months using a 31 gene target molecular bar coded panel central analysis (central analysis at King's College, London): no increased frequency of somatic mutation in eltrombopag arm, and no impact of somatic mutations on any outcome.

NEJM, 2022

Somatic mutations and clonal evolution

ARTICLE

MYELODYSPLASTIC SYNDROME

Predictors of clonal evolution and myeloid neoplasia following immunosuppressive therapy in severe aplastic anemia

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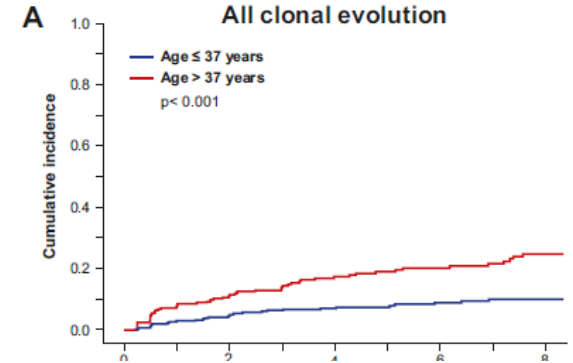
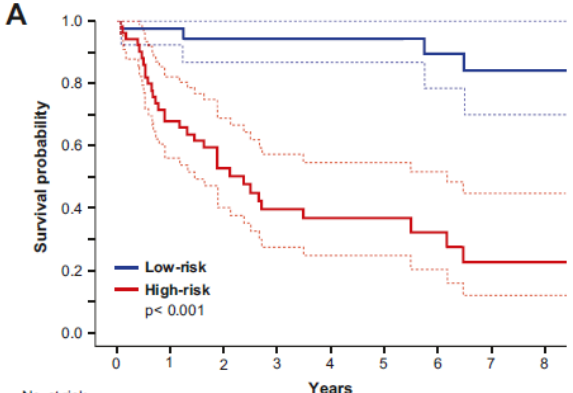
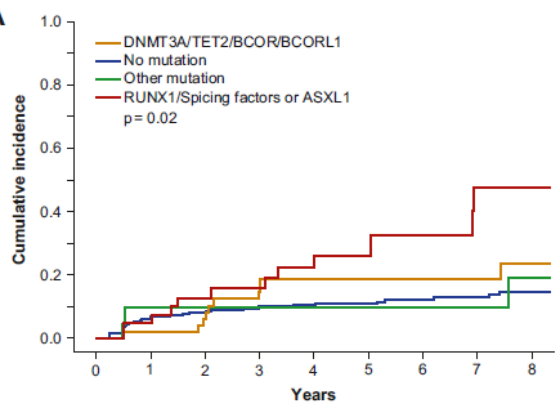
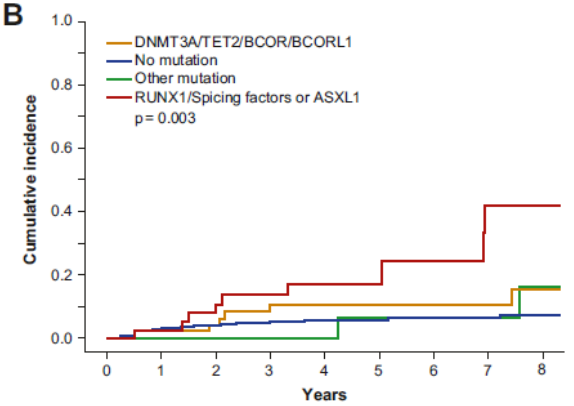
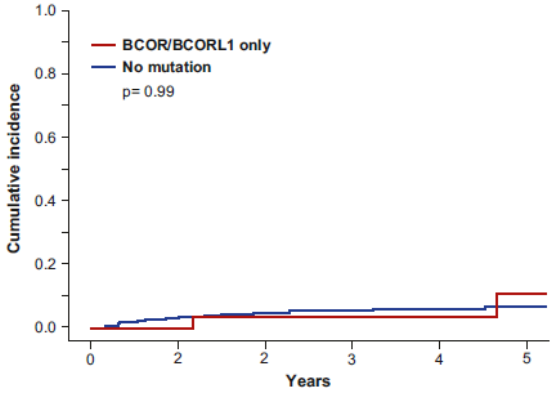
Alina Dulau-Florea[5], Cynthia E. Dunbar[4], Katherine R. Calvo[5], Colin O. Wu[2] and Neal S. Young[1]

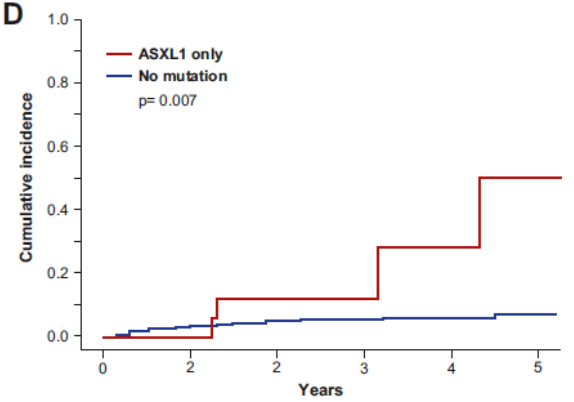










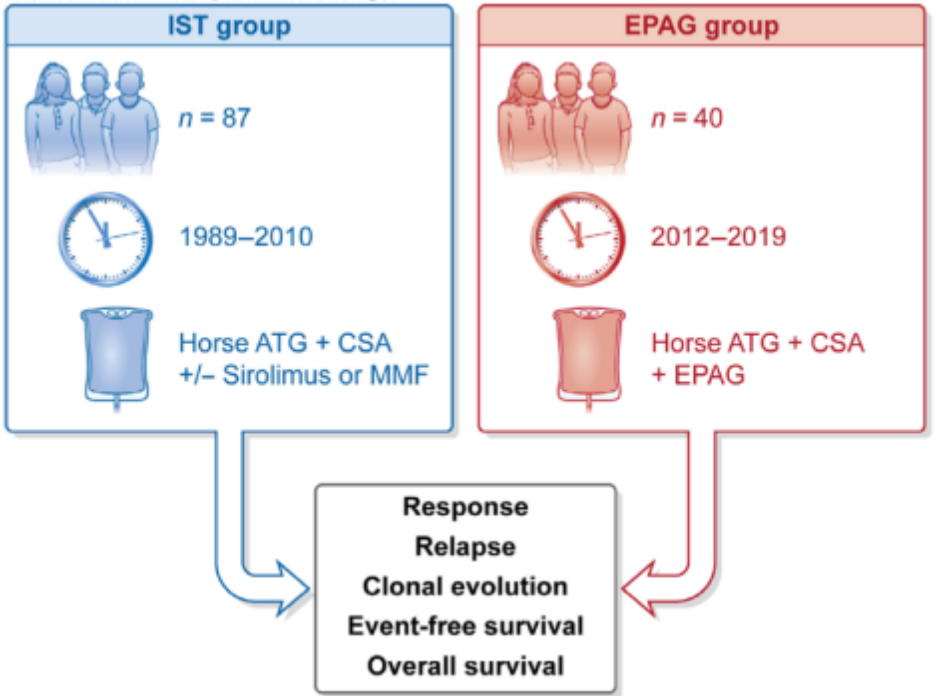


Adults vs pediatric patients

Br J Haematol. 2021 February ; 192(3): 605–614. doi:10.1111/bjh.17232.

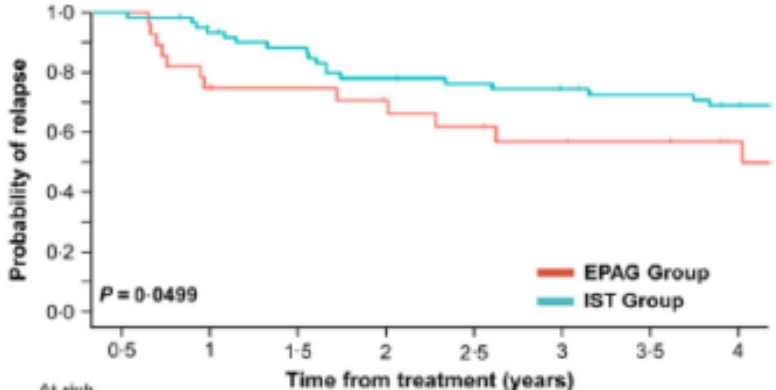
Eltrombopag added to immunosuppression for children with treatment-naïve severe aplastic anaemia

Emma M. Groarke[1], Bhavisha A. Patel[1], Fernanda Gutierrez-Rodrigues[1], Olga Rios[1], Jennifer Lotter[1], Daniela Baldoni[2], Annie St. Pierre[2], Ruba Shalhoub[3], Colin O. Wu[3], Danielle M. Townsley[1], Neal S. Young[1]



Haematological response in paediatric IST group *versus* EPAG group.

	IST group (n = 87)	EPAG group (n = 40)	P
6-month response			
Response, n (%)			
Overall response	63 (72)	28 (70)	0·78
CR	20 (23)	12 (30)	0·42
PR	43 (49)	16 (40)	
NR	19 (22)	4 (10)	
Off study	5 (6)	8 (20)	

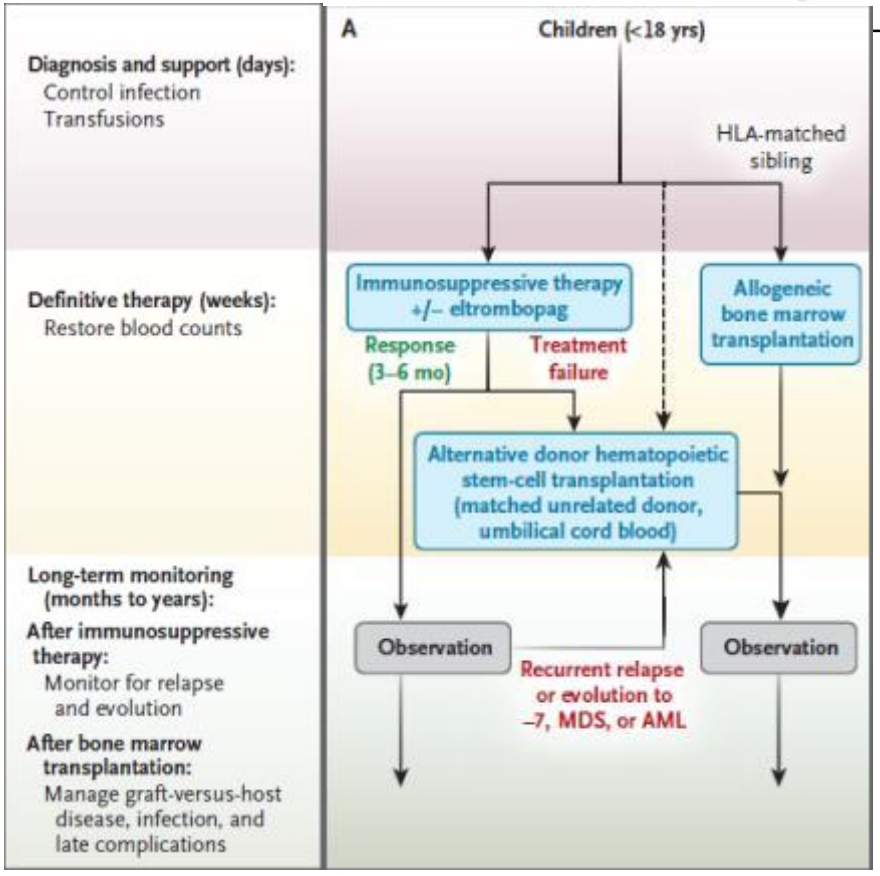


Treatment algorithm of aplastic anemia

The NEW ENGLAND JOURNAL of MEDICINE
REVIEW ARTICLE

Aplastic Anemia

Neal S. Young, M.D.



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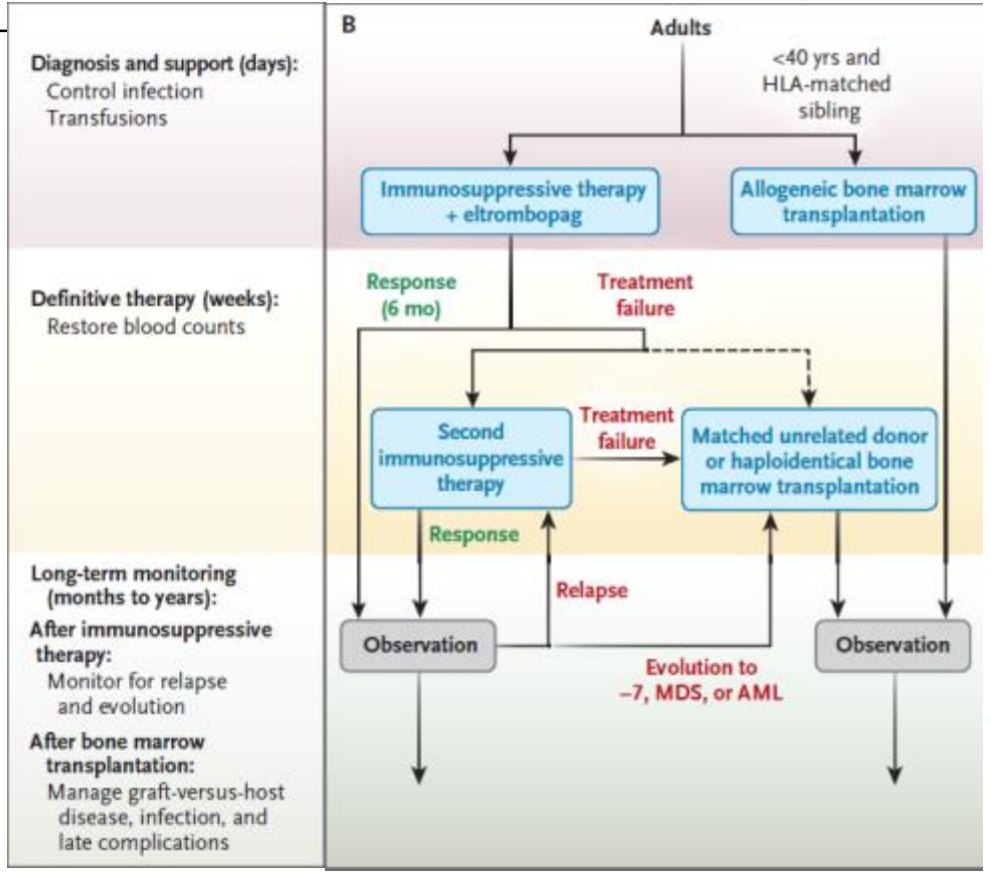

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- all principal investigators and sites
- all patients!

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