Aplastic Anemia:
Current Thinking on the Disease, Diagnosis, and Non-Transplant Treatment

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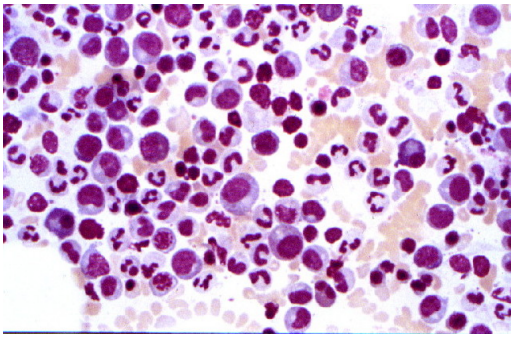
Aplastic Anemia
Diagnosis

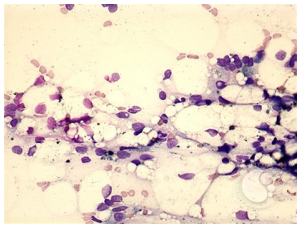
- Pancytopenia
  - Neutropenia
  - Anemia
  - Thrombocytopenia
- Empty or hypocellular bone marrow for age
- Normal karyotype
- Other bone marrow disorders excluded

Normal bone marrow aspirate (50x)

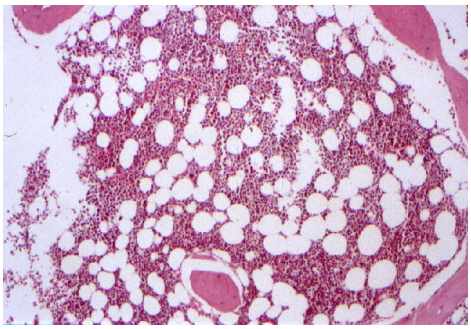


Bone Marrow Aspirate

Aplastic Anemia

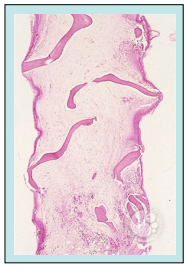


Normal bone marrow biopsy (10x)



Bone Marrow Biopsy

Aplastic Anemia



Aplastic Anemia
Severity

Blood Cells	*Moderate*	*Severe*	*Very Severe*
Neutrophils ($1.8\text{-}6.8 \times 10^9$/L)	0.5-1.2	0.2-0.5	<0.2
Reticulocytes ($21\text{-}80 \times 10^9$/L)	50-60	<50	<50
Platelets ($140\text{-}450 \times 10^9$/L)	20-70	<20	<20

Aplastic Anemia
USA Epidemiology

- 2 per million
- 2 peaks
  - Children and young adults
  - Adults >60 years

Aplastic Anemia
Etiology

Primary (idiopathic)

↓

autoimmune

Secondary
- Acquired
  - Hepatitis
  - Other viruses
  - Chemotherapy
  - Ionizing radiation
  - Medications
- Inherited

Aplastic Anemia
Secondary — Acquired

- Hepatitis
  - B,C, undiscovered
- Other viruses
  - Epstein Barr virus
  - Parvovirus B19
  - HIV
- Viruses in the setting of chronic hemolysis
  - Sickle cell anemia
  - Thalassemia
  - Hereditary spherocytosis
  - Autoimmune
- Chemotherapy
- Ionizing Radiation
- Medications
  - Gold
  - Chloramphenicol
  - Anti-epileptics
  - Anti-arrythmics
- Pregnancy
- PNH
- Thymoma
- Graft-vs-Host Disease

Aplastic Anemia
Secondary — Inherited

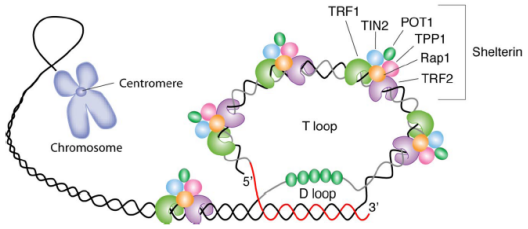
- Fanconi's anemia
  - *Abnormal DNA repair*
- Dyskeratosis congenita
  - *Shortened chromosomal telomeres*
- Shwachman-Diamond syndrome
- Reticular dysgenesis
- Amegakaryocytic thrombocytopenia
- Familial myelodysplasia
- Down's syndrome
- Familial (unnamed)

Pathophysiology of Primary Aplastic Anemia

- **Autoimmunity**
  - Cytotoxic T-cells shut down/destroy hematopoietic stem cells (HSC)
  - Mature blood elements are not made
- **Shortened telomeres**
  - Fewer HSC
  - Reduced pool of blood progenitor cells
  - 10% of patients with primary AA have mutations in telomere rebuilding genes

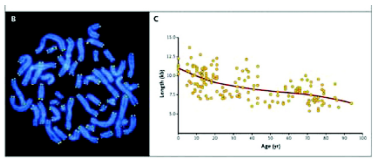
Hematology Am Soc Hematol Educ Prog 2006: 72
Mech Ageing Dev 2008; 129: 35

Telomeres



Blood 2008; 111: 4446

Telomere Attrition Over Time



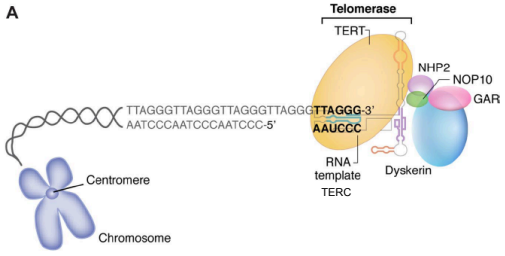
Hematology Am Soc Hematol Educ Program 2010: 30

Telomere Attrition

- 50-100 base pairs of telomeric DNA is lost with each cell division
- Attrition is dampened by rebuilding some lost base pairs after each cell division:
  - TERT and TERC
- Short for age telomeres in stem cells results in:
  - Reduced proliferation
  - Abnomral cellular repair (Fibrosis)
  - Genomic instability
  - Chromosome fusions/translocations/gain or loss (Cancer)

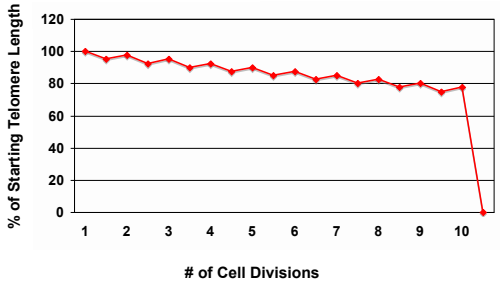
Blood 2008; 111: 4446

Rebuilding Telomeres

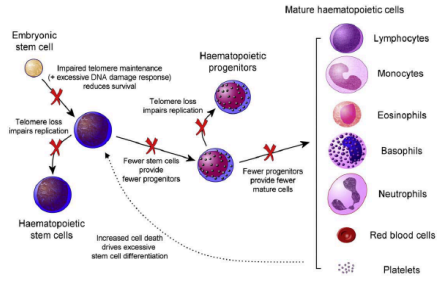


Blood 2008; 111: 4446

Telomere Attrition



Hematopoietic Stem Cells and Telomeres



Biochimica et Biophyica Acta 2009; 1792: 371

Treatment of Primary (Autoimmune) Aplastic Anemia:

Immunosuppression

Treatment of Severe Aplastic Anemia with Immunosuppression

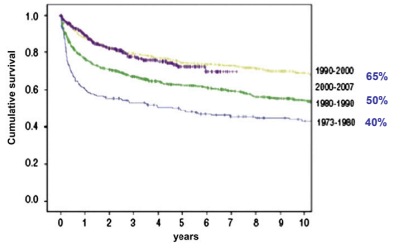
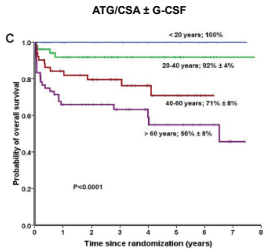


Figure 1. Survival up to 10 years for patients (n = 2400) with SAA treated with ATG reported to the EBMT database having received ATG ± CSA as a first-line treatment.

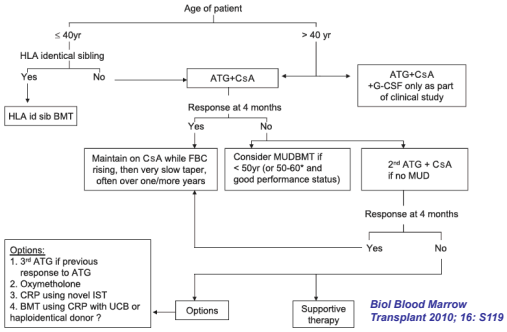
Haematologica 2009; 94: 310

Age and Survival After Immunosuppression



Blood 2011; 117: 4434

Idiopathic Aplastic Anemia Treatment Algorithm



Biol Blood Marrow Transplant 2010; 16: S119

Immunosuppressive Therapy
Primary Aplastic Anemia

- Response definitions
  - Complete
    - Normal blood counts
      - Neutrophils 1.5 x 10^9/L
      - Hemoglobin ≥13 g/dL (men), ≥ 12 g/dL (women)
      - Platelets ≥150 x 10^9/L
    - No use of growth factors or transfusions
  - Partial
    - Transfusion and growth factor independence
    - Blood counts less than normal
  - Overall
    - Complete + Partial

Immunosuppression: Treatment Strategy

- ATG (antithymocyte globulin)
  - 5 daily doses
- CSA (cyclosporine)
  - Twice daily
  - Blood levels: 500-800 (1st month), then 200-500 ng/mL
  - Through 6 months, then attempt taper
- Corticosteroids
  - 1 mg/kg/day
  - First 14 days, then taper

NEJM 1991; 324: 1297

Table 1. Different brands of antithymocyte globulin

ATG Brand	Cells used for Immunization	Animal Species	Recommended Dose	Comment
ATGAM	Human Thymocytes	Horse	40 mg/kg x 4	Standard treatment in the US
Lymphoglobuline	Human Thymocytes	Horse	15 mg/kg x 5	No longer available
Thymoglobuline	Human Thymocytes	Rabbit	3.75 mg/kg x 5	Few studies published, only ATG available in Europe
ATG-Fresenius	Jurkat T-ALL cell line	Rabbit	5 mg/kg x 5	Inferior response data in studies with a limited number of patients

T-ALL indicates T-cell acute lymphoblastic leukemia.

Hematology 2010; 36-42

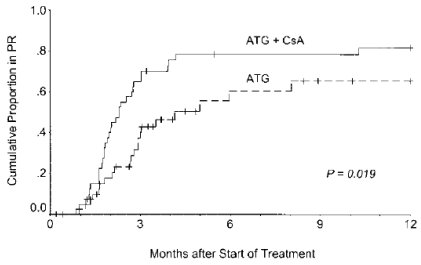
hATG vs hATG + CSA

	Response at 4 mo (all patients)	*Response at 4 mo (severe)*	*Time to response (days)*	*FFS at 11 years*	*Relapse at 11 years*
ATG + CSA	70%	65%	60	39%	45%
ATG	41%	31%	82	24%	30%
P value	0.02	0.01	0.02	0.04	0.4

*ATG = horse ATG (lymphoglobuline)

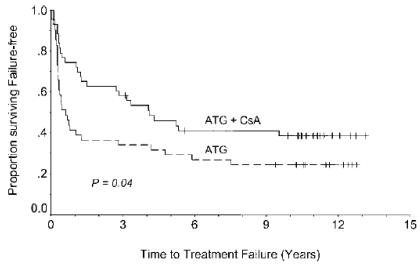
Blood 2003; 101: 1236

Kinetics of Response Time to Immunosuppresssion



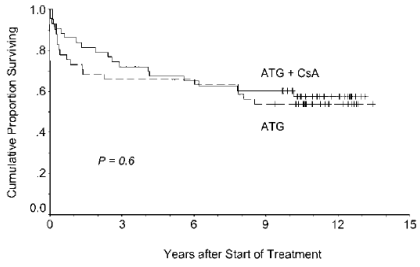
Blood 2003; 101: 1236

Failure Free Survival after Immunosuppression



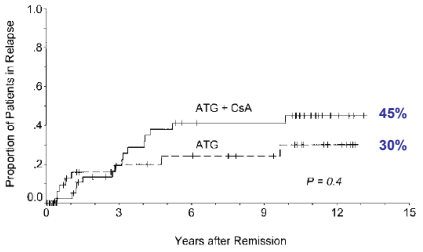
Blood 2003; 101: 1236

Survival After Immunosuppression



Blood 2003; 101: 1236

Aplastic Anemia Relapse After Immunosuppression



Blood 2003; 101: 1236

Relapse of Aplastic Anemia After Immunosuppression

- No one relapsed while on CSA
- Time from stopping CSA to relapse
  - 27 months (2-29)
- 2nd response to ATG/CSA better if
  - relapsing from a Complete Response

Blood 2003; 101: 1236

2nd Clonal Disease After Immunosuppression

- Cumulative probability 25 % at 11 years
- PNH
  - 8/84 (6%)
- MDS/AML
  - 4/84 (5%)
- Solid cancer
  - 8/84 (5%)
- Latency
  - MDS/AML - 6.6-9.5 years
  - Solid cancer - 1.2-11.5 years

Blood 2003; 101: 1236

Predicting Response to hATG/CSA

	n	*Response @ 6 months*	*P value*
All patients	316	61%	—
Retic* ≥25 , <25	77/117	80% , 53%	<0.001
Lymphs* ≥1 , <1	139/55	70% , 47%	0.001
Neuts* ≥0.2 , <0.2	128/66	68% , 52%	0.003
Platelets* ≥10 , <10	85/103	65% , 59%	0.22
PNH ≥1% , <1%	61/59	61% , 59%	0.88
Age <18 , 18-60 , >60	58/109 /27	74% , 58% , 53%	0.02

*x10^9/L

Br J Haemtol 2008; 144: 206

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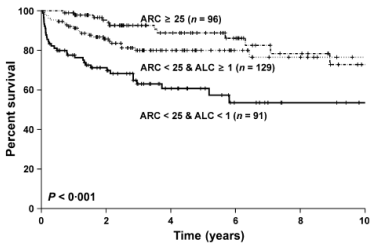
Br J Haemtol 2008; 144: 206

Predicting Response to Immunosuppression

- Multivariate analysis
  - Age (<18 years)
  - Retics (≥25 x10^9/L)
  - Lymphs (≥1 x10^9/L)
- Age and Retics and Lymphs also predict **Survival**

Br J Haemtol 2008; 144: 206

Survival by Reticulocytes and Lymphocytes at Diagnosis



Br J Haemtol 2008; 144: 206

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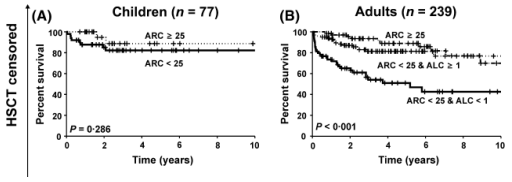


Br J Haemtol 2008; 144: 206

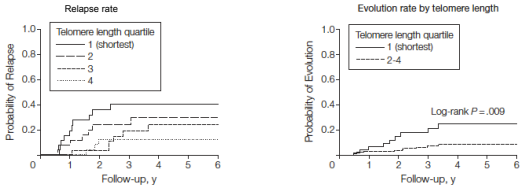
Telomere Length* and Response of SAA to Immunosuppression

Telomere Quartile	*Number*	*Response Rate*
1	46	57% (41-71)
2	46	54% (39-69)
3	45	60% (45-75)
4	46	57% (42-71)

*White blood cells

JAMA 2010; 304: 1358

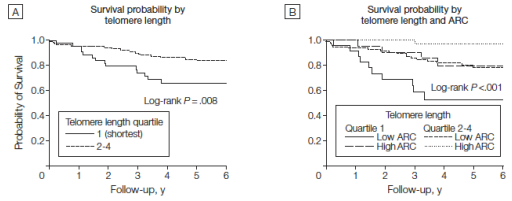
Telomere Length* and Outcomes



*White blood cells

JAMA 2010; 304: 1358

Telomere Length* and Survival



*Blood white cells

JAMA 2010; 304: 1358

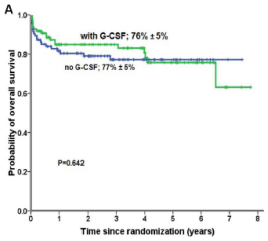
hATG/CSA ± G-CSF

	No G-CSF	*G-CSF**	*P value*
Response	63%	73%	0.54
→ Infections in first 3 months	36%	24%	0.01
→ % days in hospital in first month	87%	82%	0.003
Death at 6 years	24%	22%	0.67
Relapse at 6 years	33%	32%	0.79
2nd Cancer	6%	3%	0.54
PNH at 6 years	22%	16%	0.07

**G-CSF, day 8-240*

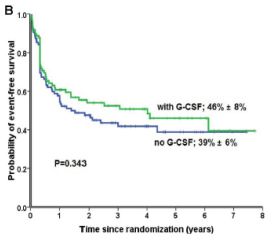
Blood 2011; 117: 4434

Impact of G-CSF on Survival



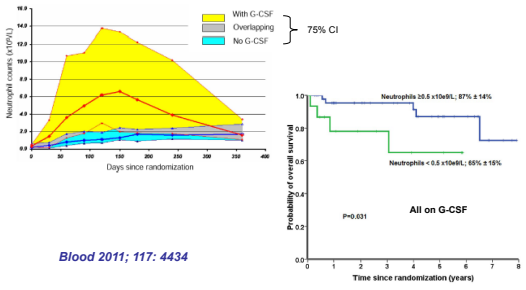
Blood 2011; 117: 4434

Impact of G-CSF on Event - Free Survival



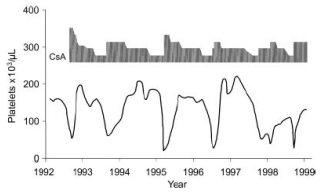
Blood 2011; 117: 4434

Neutrophil Response and Impact on Survival



Blood 2011; 117: 4434

CSA-Dependence of Blood Counts



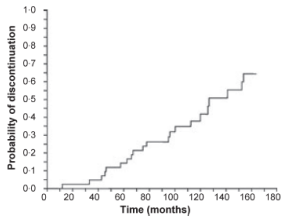
Blood 2003; 101: 1236

Duration of CSA

- 42 children with vSAA/SAA in Italy
- ATG + CSA
- CSA, blood levels 150-300 ng/ml to 6 months
- 71% response rate at 6 months
- CSA tapering
  - 22/33 (67%) come off at 95 months (12-164)
  - Median duration of full dose, 12 months (3-45)
  - Median duration of taper, 19 months (4-64)
  - Probability off CSA at 5 yr (21%), 10 yr (41%)
- 6/33 (18%) CSA-dependent
  - Median time on CSA - 104+ months (89-120+)

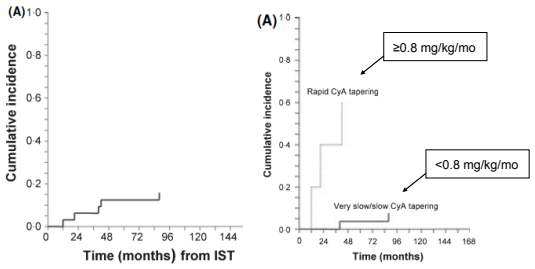
Br J Haematol 2008; 140: 197

Duration of CSA in Children



Br J Haematol 2008; 140: 197

Aplastic Anemia Relapse by CSA Taper Rate



Br J Haematol 2008; 140: 197

hATG/CSA + MMF
(Mycophenolic Acid Mofetil)

- 104 children and adults with vSAA/SAA
- MMF for 18 months
  - 600 mg/m2 twice daily (<12 yr) or 1g twice daily (≥12 yr)
- Response rate 62% at 6 months
- Probability of aplastic anemia relapse 37% at 4 years
  - 15/24 relapses occur during MMF

Br J Haematol 2006; 133: 606

Survival and Relapse, MMF

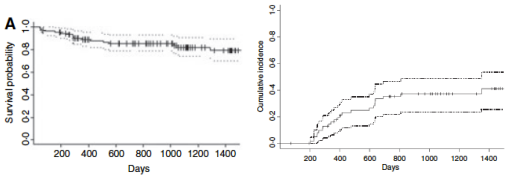


Fig 1. Cumulative incidence of relapses (from day 0 to 1500) among responders (solid line) with 95% confidence intervals (dotted lines).

Br J Haematol 2006; 133: 606

hATG/CSA ± Sirolimus

- N=77 (120 planned), all ages
- hATG/CSA with/without Sirolimus
- 6 month response rate
  - No sirolimus: 62%
  - Yes sirolimus: 51%
- No difference in:
  - Relapse rate
  - Development of clonal disease
  - Survival

Haematologica 2009; 94: 348

Replacing ATG: Cyclophosphamide

- N=67 SAA (44 untreated; 23 refractory)
- Cyclophosphamide 50 mg/Kg ideal weight x 4
- Outcome (untreated)
  - 71% response
  - 58% 10-yr event-free survival
  - 88% 10-yr survival
- Toxicity
  - 5 deaths (11%) - after 5 months (3 fungus)
  - 60 days to neutrophils 0.5 x10^9/L (28-104)
  - 117 days to platelet transfusion-independence (24-640)

Blood 2010; 115: 2136

Survival After Cyclophosphamide

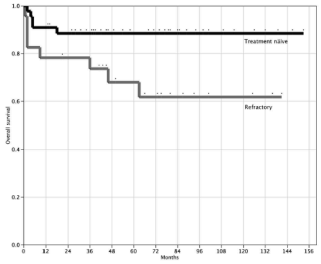


Figure 1. Overall survival after high-dose cyclophosphamide therapy. Overall survival for 44 treatment-naive patients (top line) and 23 patients refractory to prior immunosuppressive therapy (bottom line). *P* = .03 (log-rank test).

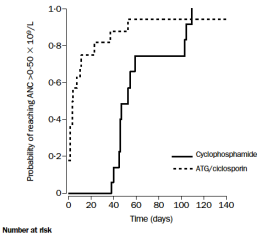
Blood 2010; 115: 2136

hATG/CSA vs Cyclophosphamide/CSA

	ATG	*Cyclophos-phamide*	*P value*
n	16	15	
Response @ 6 mo	75%	46%	0.1
Fungus or Death <3 mo	0 (0%)	6 (40%)	<0.01
Hospital days	12	59	<0.001
IV antibiotics	7%	47%	<0.002
RBC units	11	36	0.02
Platelet transfus.	9	32	0.03
Days G-CSF	3	14	0.001
Days Neuts <0.5	3	53	<0.003

Lancet 2000; 356: 1554

Neutrophil Recovery: hATG vs Cyclophosphamide



Number at risk

Cyclophosphamide	15	15	12	4	4	4	0	0
ATG	16	5	2	1	1	1	1	1

Figure 2: **Kaplan-Meier estimates of proportion of patients reaching an absolute neutrophil count (ANC) of 0·50×10⁹/L**

Lancet 2000; 356: 1554

Replacing hATG with Alemtuzumab

- Alemtuzumab
  - Anti-CD52 antibody
  - 73-103 mg subcutaneous
- Alemtuzumab followed by CSA
- 6 month response: 58% (84% cumulative)
- No clear advantage to hATG

Br J Haematol 2010; 148: 791

Primary Aplastic Anemia Summary -1-

- **Pathophysiology**
  - Overactive T-cells
  - Shortened telomeres
- **Standard Immunosuppression**
  - ATG + CSA
  - 60-70% response rate
  - G-CSF shortens hospitalization and reduces infections
  - No benefit to add MMF or Sirolimus, or using Alemtuzumab
  - Cyclophosphamide is too toxic

Primary Aplastic Anemia Summary -2-

- **CSA**
  - Average duration is 8 years
  - On full dose 1 year
  - 18% are CSA-dependent
  - More relapse and shorter time to relapse with faster tapers (≥0.8 mg/kg/month)
- **Predicting Response and Survival**
  - Young age
  - Higher reticulocyte count
  - Higher lymphocyte count

Primary Aplastic Anemia Summary -3-

- **Shorter Telomeres predict**
  - More relapse
  - Shorter survival
  - More clonal evolution
- **Long-Term problems with Immunosuppression**
  - Relapse
  - PNH
  - MDS/AML
  - 2nd cancers