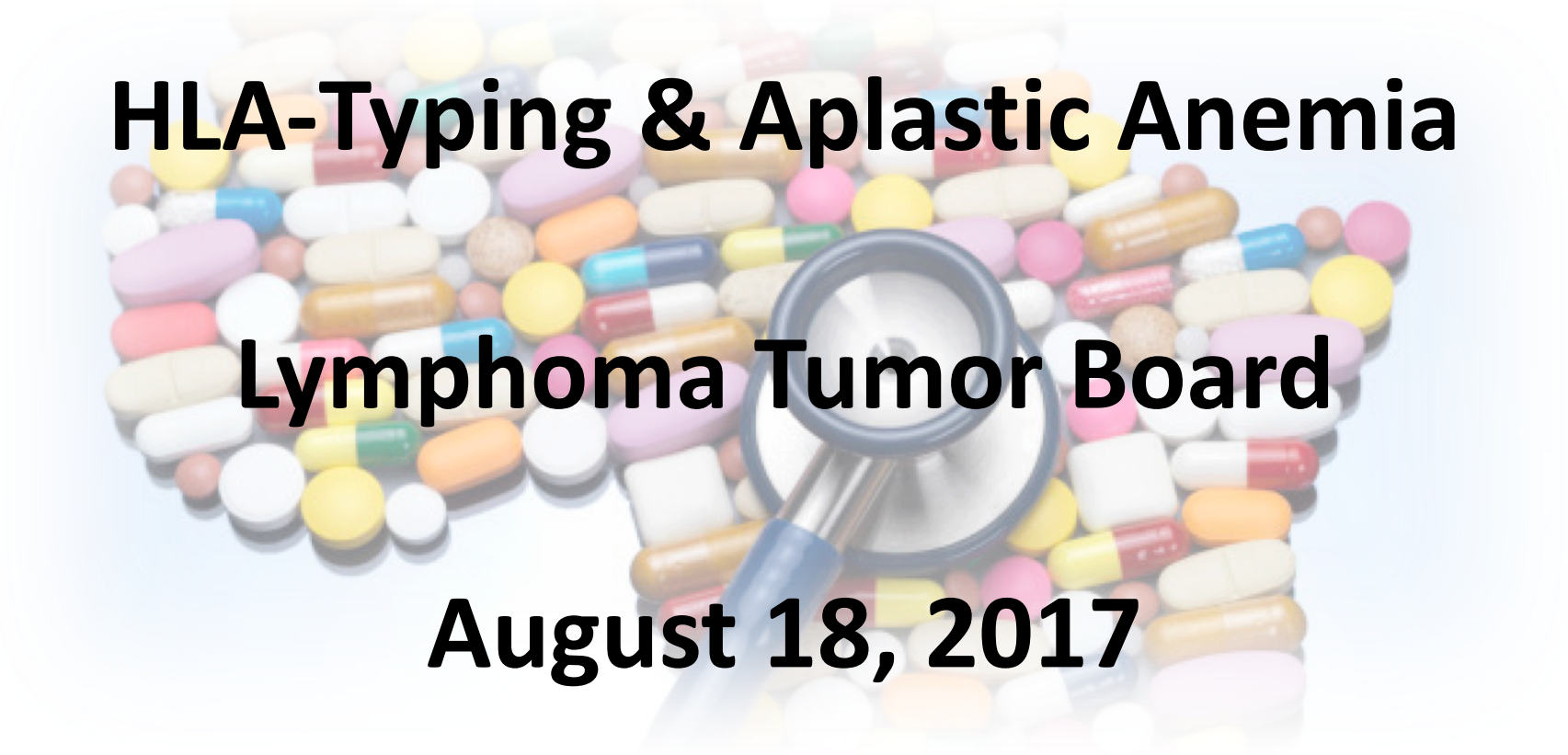


HLA-Typing & Aplastic Anemia

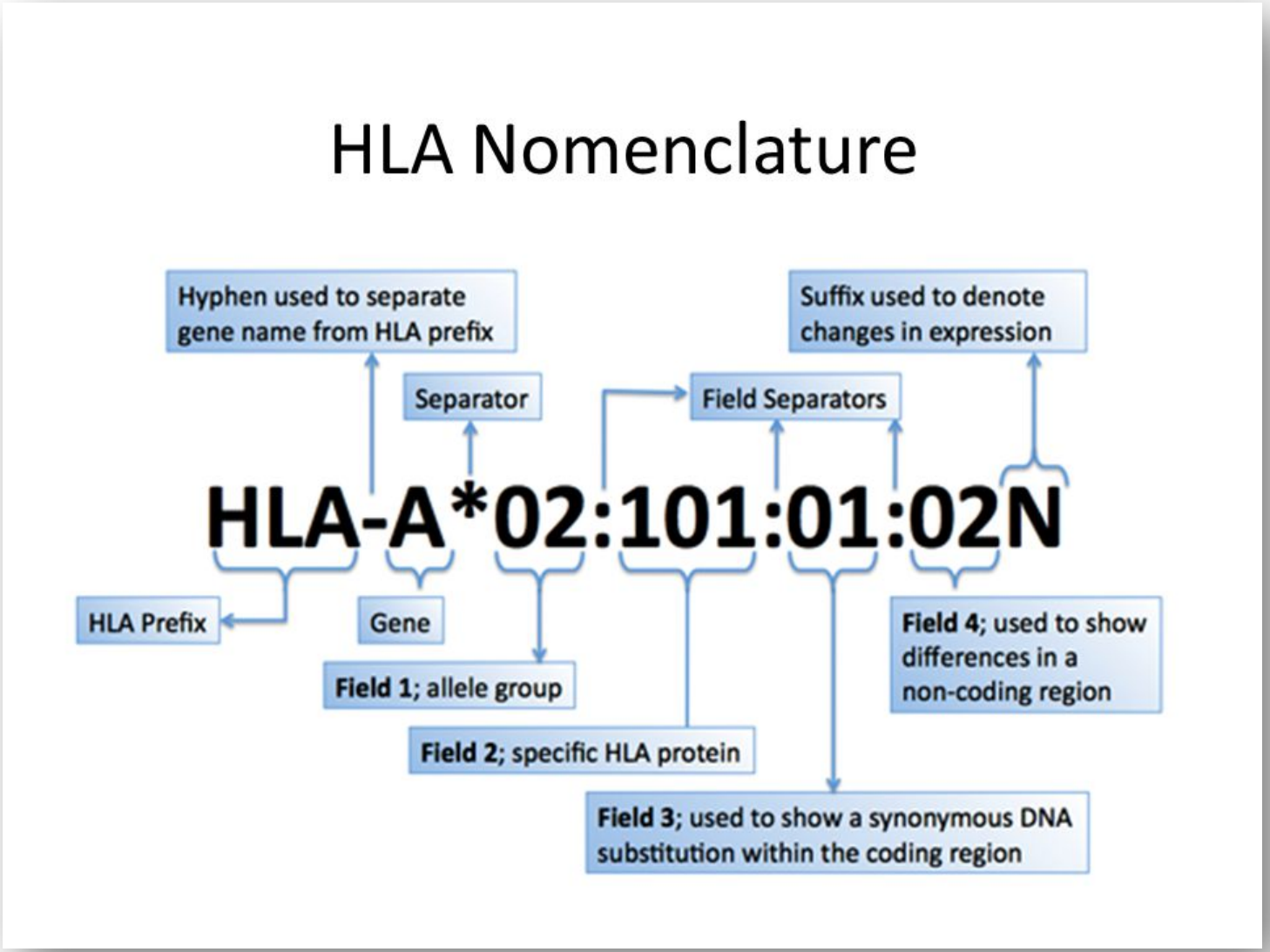
Lymphoma Tumor Board

August 18, 2017

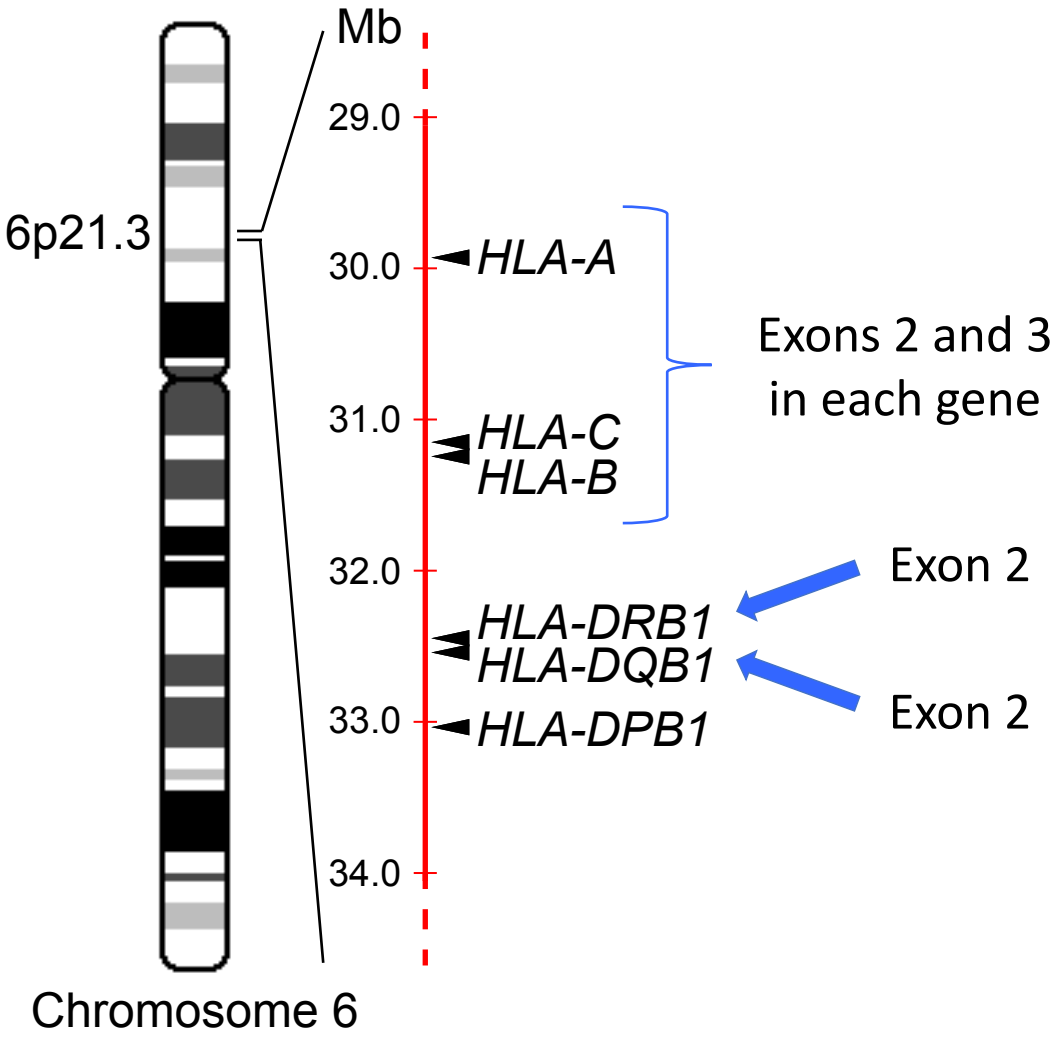
HLA TYPING METHODS

1950's	discovery of HLA system
1960's	serological typing
1980's	first HLA genes cloned, sequenced
1990's	DNA/PCR based HLA typing
1999	sequence entire MHC (HGP)
2000	database of all HLA alleles
2000's	SBT, Luminex SSO
~2012	Next-generation sequencing

First step is to perform HLA Typing



What is involved in HLA typing, anyway?



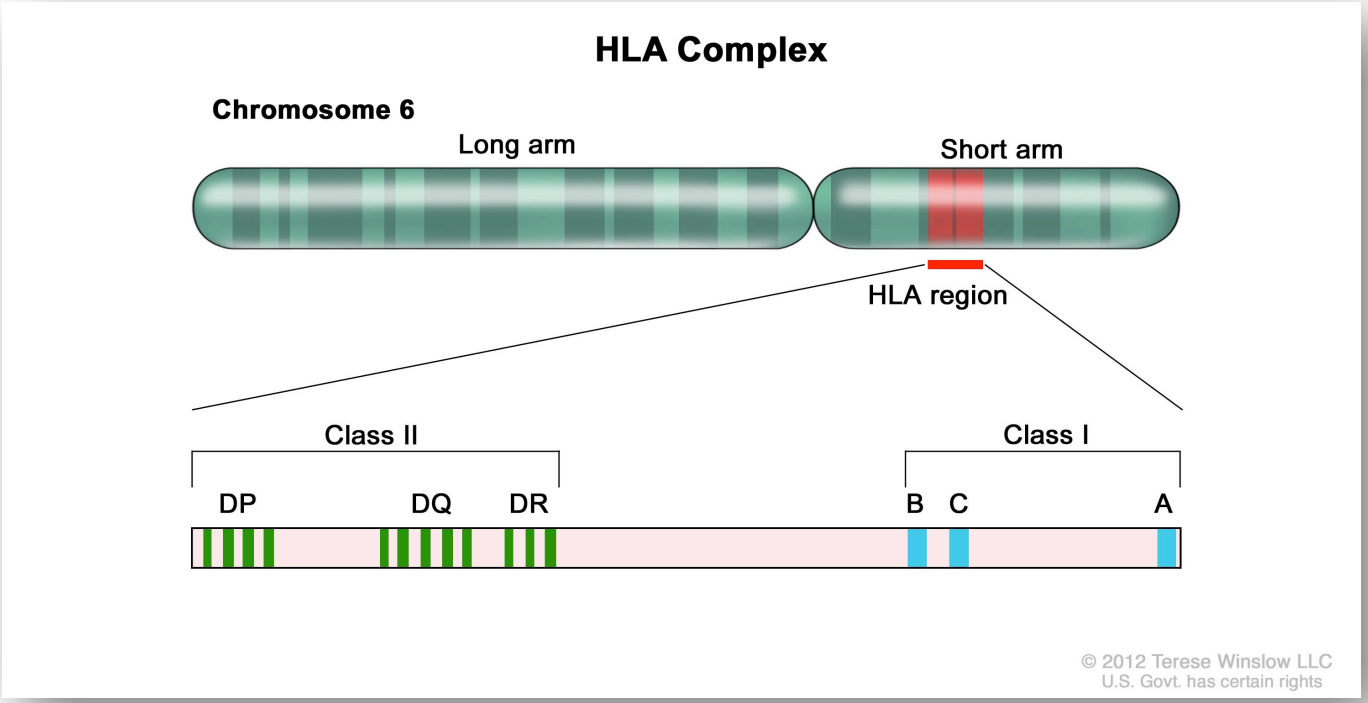
8 exons total

~275 nt each

TOTAL: 2,200 bp

Haploid genome: 3.3 x 10^9 bp

$< 1 \times 10^{-6}$ of the genome


HLA Complex
Chromosome 6
Long arm
Short arm
HLA region
Class II
Class I
DP
DQ
DR
B
C
A
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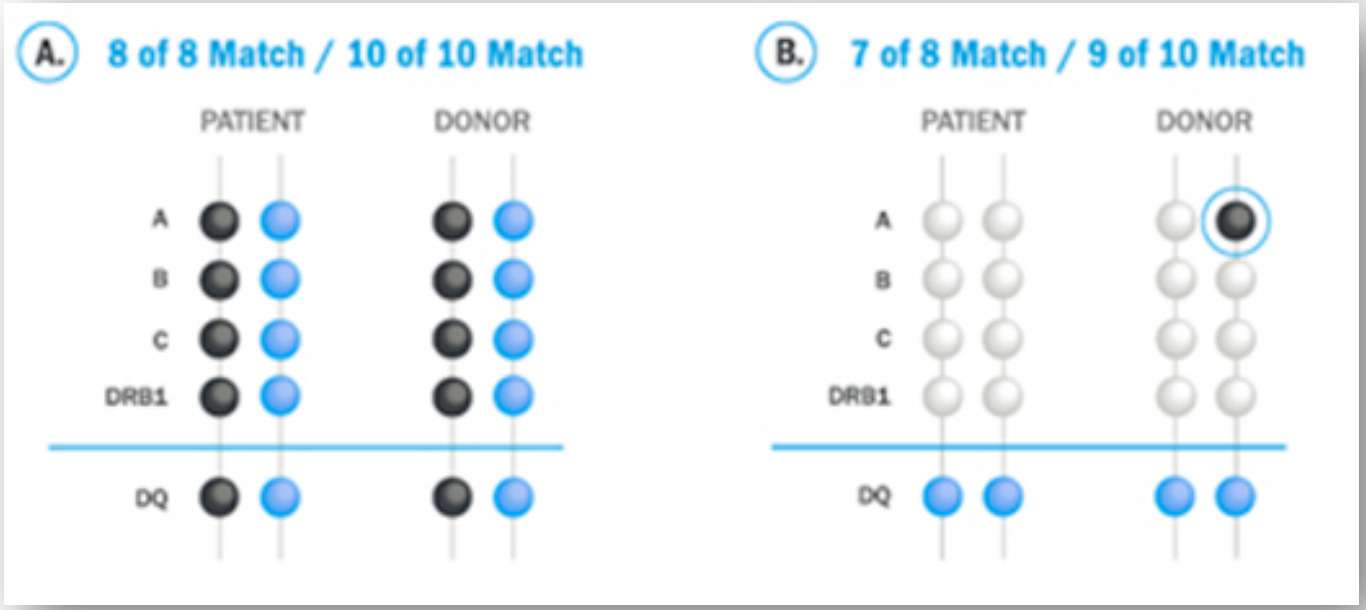
A.
8 of 8 Match / 10 of 10 Match
PATIENT
DONOR
A
B
C
DRB1
DQ
B.
7 of 8 Match / 9 of 10 Match
PATIENT
DONOR
A
B
C
DRB1
DQ

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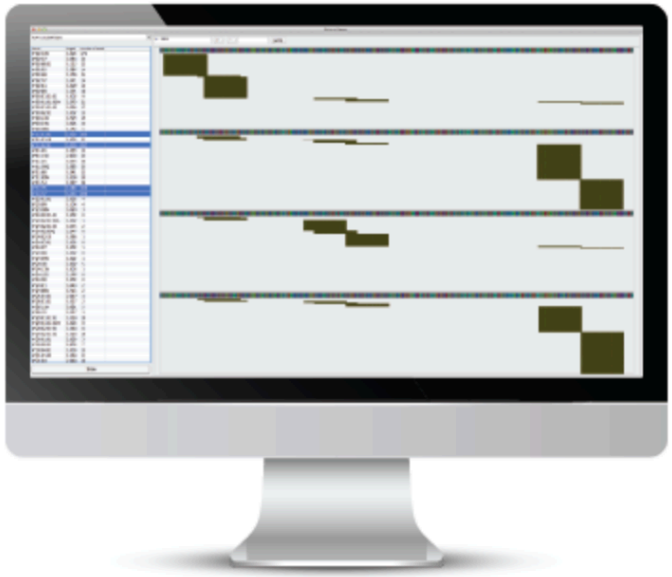
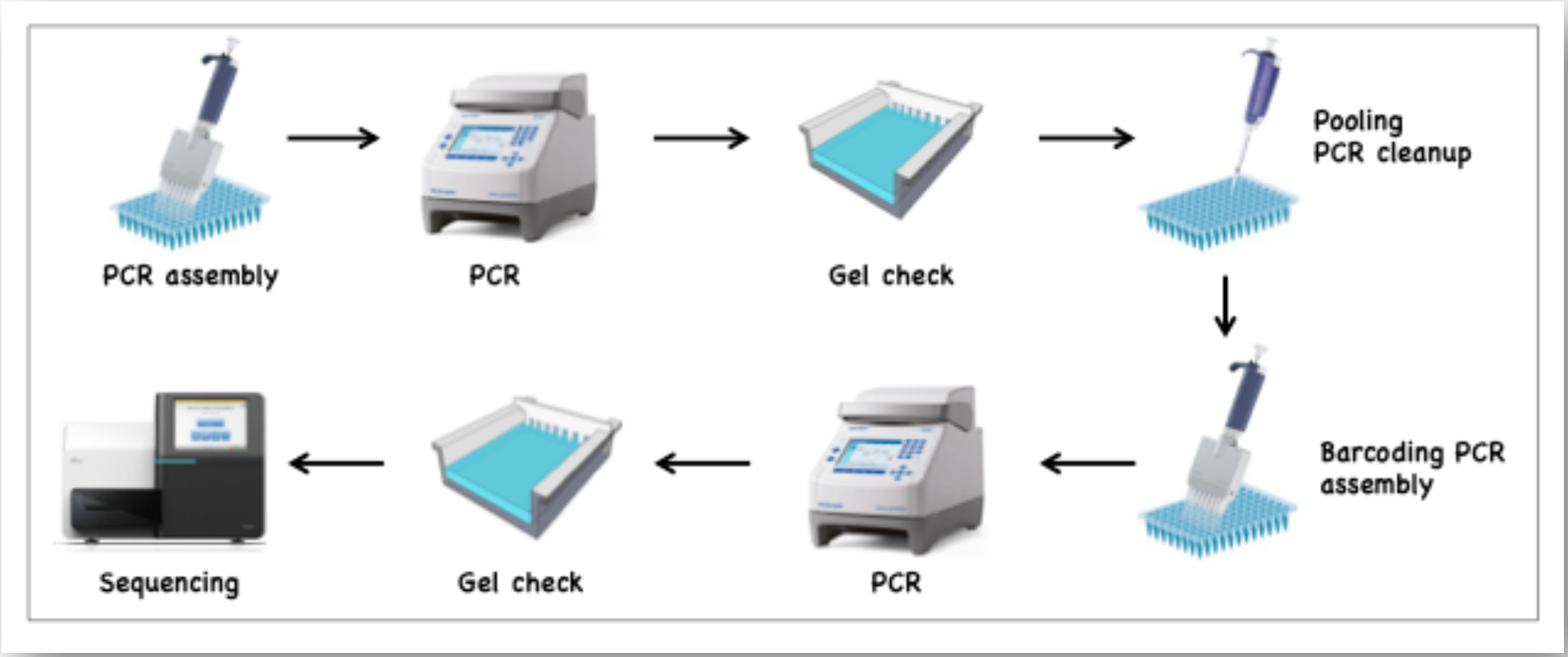


Workflow for HLA typing by next-generation sequencing



Sample ID	Locus	Allele 1	Allele 2	Comments	Allele 1 Ambiguities
Warren-KENGONZI-HARRET-S1	A	A*33:03:01	A*36:01		
Warren-KENGONZI-HARRET-S1	B	B*47:03	B*53:01:01		
Warren-KENGONZI-HARRET-S1	C	C*04:01:01	C*06:02:01		
Warren-KENGONZI-HARRET-S1	DPA1	DPA1*01:03:01	DPA1*01:03:01		
Warren-KENGONZI-HARRET-S1	DPB1	DPB1*02:01:02	DPB1*04:01:01		DPB1*02:01:19
Warren-KENGONZI-HARRET-S1	DQA1	DQA1*01:02:01-new	DQA1*01:02:01	DQA1*01:02:01-new with ex4 variations (p663 T>C;p688 A>G)	
Warren-KENGONZI-HARRET-S1	DQB1	DQB1*05:01:01	DQB1*06:02:01		
Warren-KENGONZI-HARRET-S1	DRB1	DRB1*11:01:02	DRB1*11:01:02		
Warren-KENGONZI-HARRET-S1	DRB345	DRB3*02:02:01	DRB3*02:02:01		
Warren-KENGONZI-HARRET-S2	A	A*33:03:01	A*36:01		
Warren-KENGONZI-HARRET-S2	B	B*47:03	B*53:01:01		
Warren-KENGONZI-HARRET-S2	C	C*04:01:01	C*06:02:01		
Warren-KENGONZI-HARRET-S2	DPA1	DPA1*01:03:01	DPA1*01:03:01		
Warren-KENGONZI-HARRET-S2	DPB1	DPB1*02:01:02	DPB1*04:01:01		DPB1*02:01:19
Warren-KENGONZI-HARRET-S2	DQA1	DQA1*01:02:01-new	DQA1*01:02:01	DQA1*01:02:01-new with ex4 variations (p663 T>C;p688 A>G)	
Warren-KENGONZI-HARRET-S2	DQB1	DQB1*05:01:01	DQB1*06:02:01		
Warren-KENGONZI-HARRET-S2	DRB1	DRB1*11:01:02	DRB1*11:01:02		
Warren-KENGONZI-HARRET-S2	DRB345	DRB3*02:02:01	DRB3*02:02:01		

Sample ID	Locus	Allele 1	Allele 2	Comments	Allele 1 Ambiguities
Warren-NYAKATO-MARIAM-S1	A	A*33:03:01	A*36:01		
Warren-NYAKATO-MARIAM-S1	B	B*47:03	B*53:01:01		
Warren-NYAKATO-MARIAM-S1	C	C*04:01:01	C*06:02:01		
Warren-NYAKATO-MARIAM-S1	DPA1	DPA1*01:03:01	DPA1*01:03:01		
Warren-NYAKATO-MARIAM-S1	DPB1	DPB1*02:01:02	DPB1*04:01:01		
Warren-NYAKATO-MARIAM-S1	DQA1	DQA1*01:02:01-new	DQA1*01:02:01	DQA1*01:02:01-new with ex4 variations (p663 T>C;p688 A>G)	
Warren-NYAKATO-MARIAM-S1	DQB1	DQB1*05:01:01	DQB1*06:02:01		
Warren-NYAKATO-MARIAM-S1	DRB1	DRB1*11:01:02	DRB1*11:01:02		
Warren-NYAKATO-MARIAM-S1	DRB345	DRB3*02:02:01	DRB3*02:02:01		
Warren-NYAKATO-MARIAM-S2	A	A*33:03:01	A*36:01		
Warren-NYAKATO-MARIAM-S2	B	B*47:03	B*53:01:01		
Warren-NYAKATO-MARIAM-S2	C	C*04:01:01	C*06:02:01		
Warren-NYAKATO-MARIAM-S2	DPA1	DPA1*01:03:01	DPA1*01:03:01		
Warren-NYAKATO-MARIAM-S2	DPB1	DPB1*02:01:02	DPB1*04:01:01		DPB1*02:01:19
Warren-NYAKATO-MARIAM-S2	DQA1	DQA1*01:02:01-new	DQA1*01:02:01	DQA1*01:02:01-new with ex4 variations (p663 T>C;p688 A>G)	
Warren-NYAKATO-MARIAM-S2	DQB1	DQB1*05:01:01	DQB1*06:02:01		
Warren-NYAKATO-MARIAM-S2	DRB1	DRB1*11:01:02	DRB1*11:01:02		
Warren-NYAKATO-MARIAM-S2	DRB345	DRB3*02:02:01	DRB3*02:02:01		

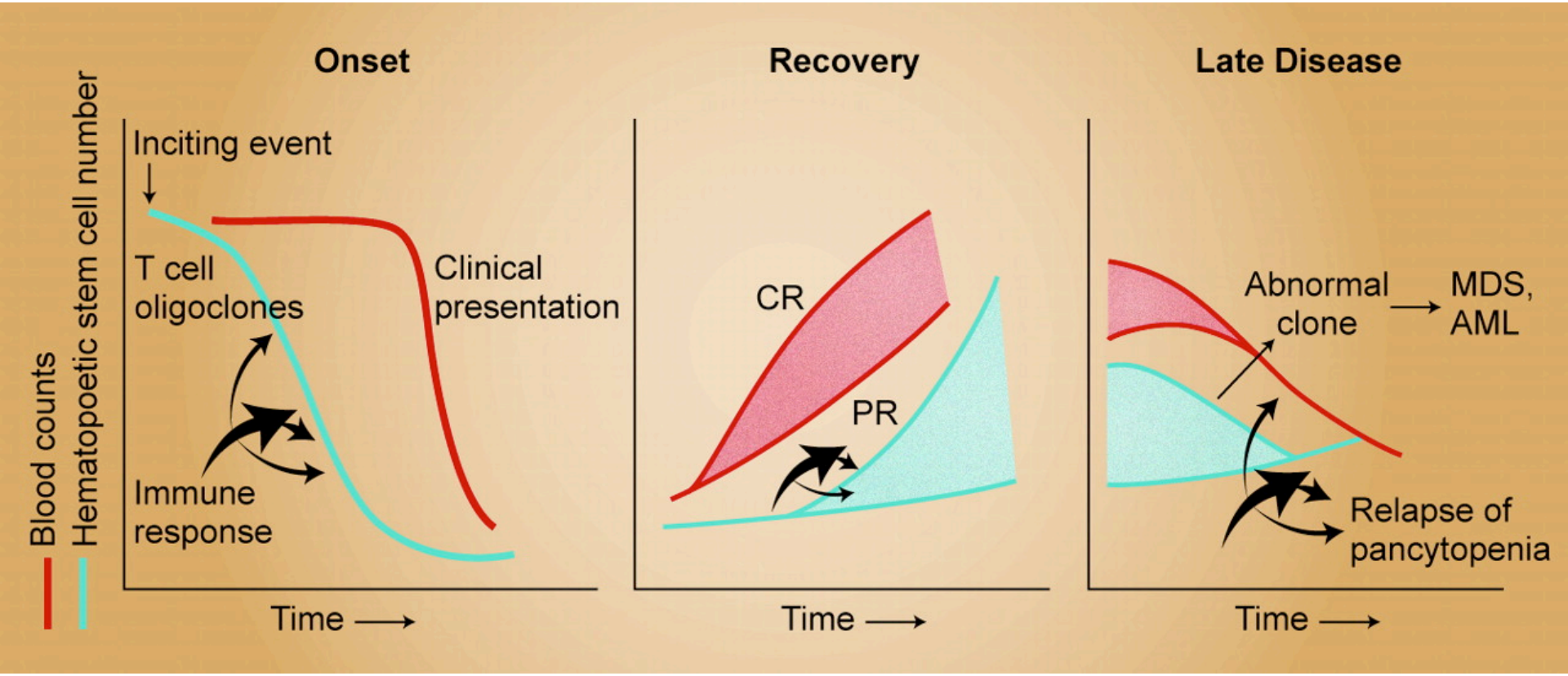
Sample ID	Locus	Allele 1	Allele 2	Comments	Allele 1 Ambiguities
Warren-NYANGONA-ELIZABETH-S1	A	A*33:03:01	A*36:01		
Warren-NYANGONA-ELIZABETH-S1	B	B*47:03	B*53:01:01		
Warren-NYANGONA-ELIZABETH-S1	C	C*04:01:01	C*06:02:01		
Warren-NYANGONA-ELIZABETH-S1	DPA1	DPA1*01:03:01	DPA1*01:03:01		
Warren-NYANGONA-ELIZABETH-S1	DPB1	DPB1*02:01:02	DPB1*04:01:01		DPB1*02:01:19
Warren-NYANGONA-ELIZABETH-S1	DQA1	DQA1*01:02:01-new	DQA1*01:02:01	DQA1*01:02:01-new with ex4 variations (p663 T>C;p688 A>G)	
Warren-NYANGONA-ELIZABETH-S1	DQB1	DQB1*05:01:01	DQB1*06:02:01		
Warren-NYANGONA-ELIZABETH-S1	DRB1	DRB1*11:01:02	DRB1*11:01:02		
Warren-NYANGONA-ELIZABETH-S1	DRB345	DRB3*02:02:01	DRB3*02:02:01		
Warren-NYANGONA-ELIZABETH-S2	A	A*33:03:01	A*36:01		
Warren-NYANGONA-ELIZABETH-S2	B	B*47:03	B*53:01:01		
Warren-NYANGONA-ELIZABETH-S2	C	C*04:01:01	C*06:02:01		
Warren-NYANGONA-ELIZABETH-S2	DPA1	DPA1*01:03:01	DPA1*01:03:01		
Warren-NYANGONA-ELIZABETH-S2	DPB1	DPB1*02:01:02	DPB1*04:01:01		DPB1*02:01:19
Warren-NYANGONA-ELIZABETH-S2	DQA1	DQA1*01:02:01-new	DQA1*01:02:01	DQA1*01:02:01-new with ex4 variations (p663 T>C;p688 A>G)	
Warren-NYANGONA-ELIZABETH-S2	DQB1	DQB1*05:01:01	DQB1*06:02:01		
Warren-NYANGONA-ELIZABETH-S2	DRB1	DRB1*11:01:02	DRB1*11:01:02		
Warren-NYANGONA-ELIZABETH-S2	DRB345	DRB3*02:02:01	DRB3*02:02:01		

Hematopoiesis: some numbers

- Each day a typical adult produces:
  - 2×10^{11} red blood cells
  - 1×10^{11} white blood cells
  - 1×10^{11} platelets
- Over a lifetime: ~$4\text{-}8 \times 10^{15}$ blood cells
- Maintenance of basal hematopoiesis requires each human HSC to divide ~52 times
- Between the HSC and terminally differentiated circulating blood cells, there are between 17 and 19.5 effective cell divisions, with a net amplification of between ~170,000 and ~720,000

Rates of production can increase 10-fold

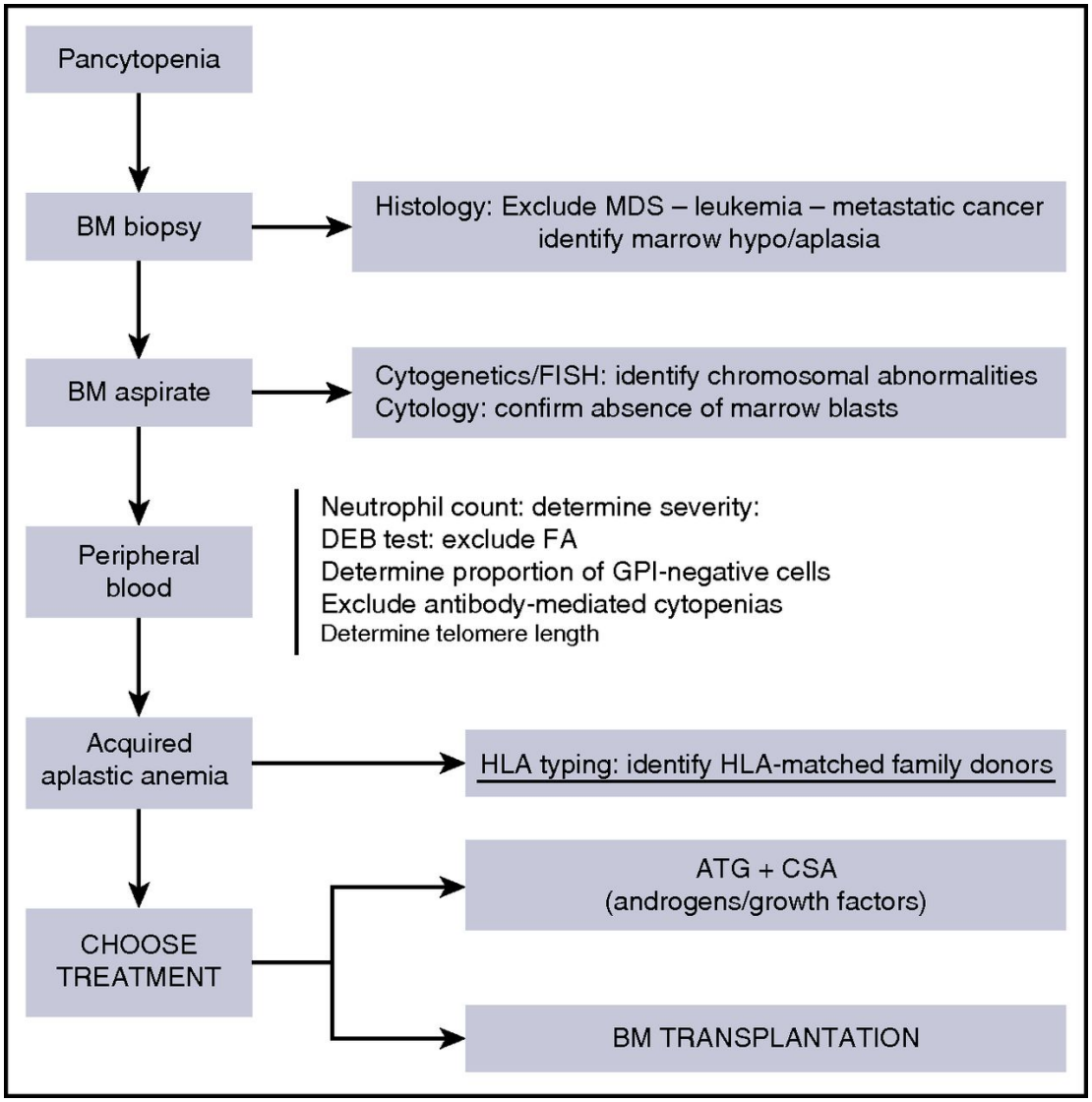
Pathophysiology of acquired aplastic anemia



Pathogenesis and diagnosis of severe aplastic anemia

- Acquired SAA results from immune-mediated destruction of hematopoietic cells
- Late clonal disorders arise in 10-20% of patients after immunosuppressive therapy (IST)
- Do some patients with “SAA” actually have a premalignant disease, and is IST just postponing the inevitable?
- Diagnosis is based on the exclusion of other disorders that can cause pancytopenia and on the Camitta criteria (next slide)

Diagnostic procedures in patients with pancytopenia



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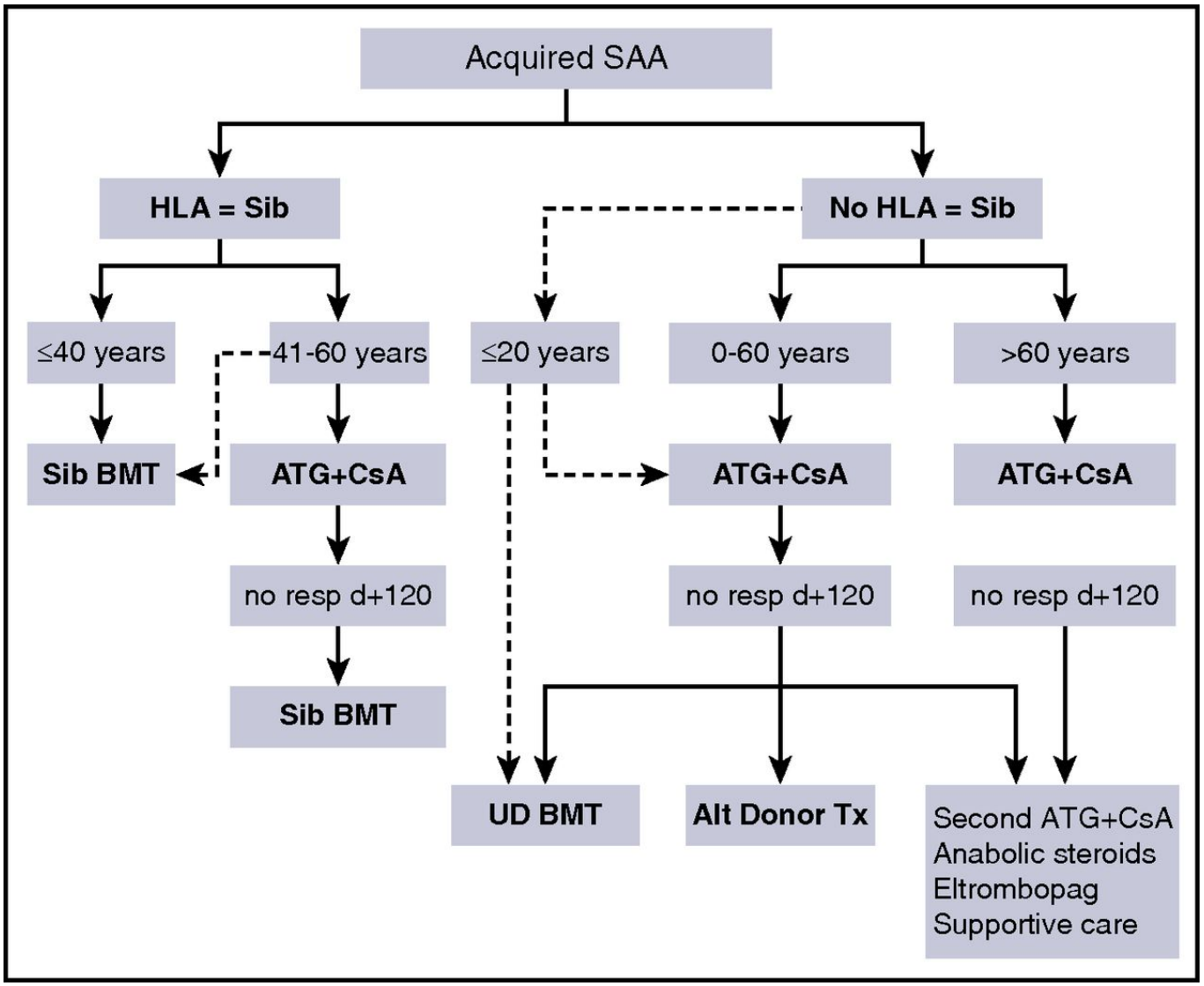
Treatment strategy in patients with acquired aplastic anemia



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Treatment of SAA (1)

- Moderate cases (lack of blood count criteria for SAA) - observation is appropriate when transfusion is not required
- Antibiotics when fever or documented infection occurs in the presence of severe neutropenia (ANC < 500/µL)
- Immunosuppressive therapies are widely used due to lack of transplantation
- ATG-based regimen in combination with cyclosporine
- 60% of patients are responders at 3 or 6 months after initiation of horse ATG

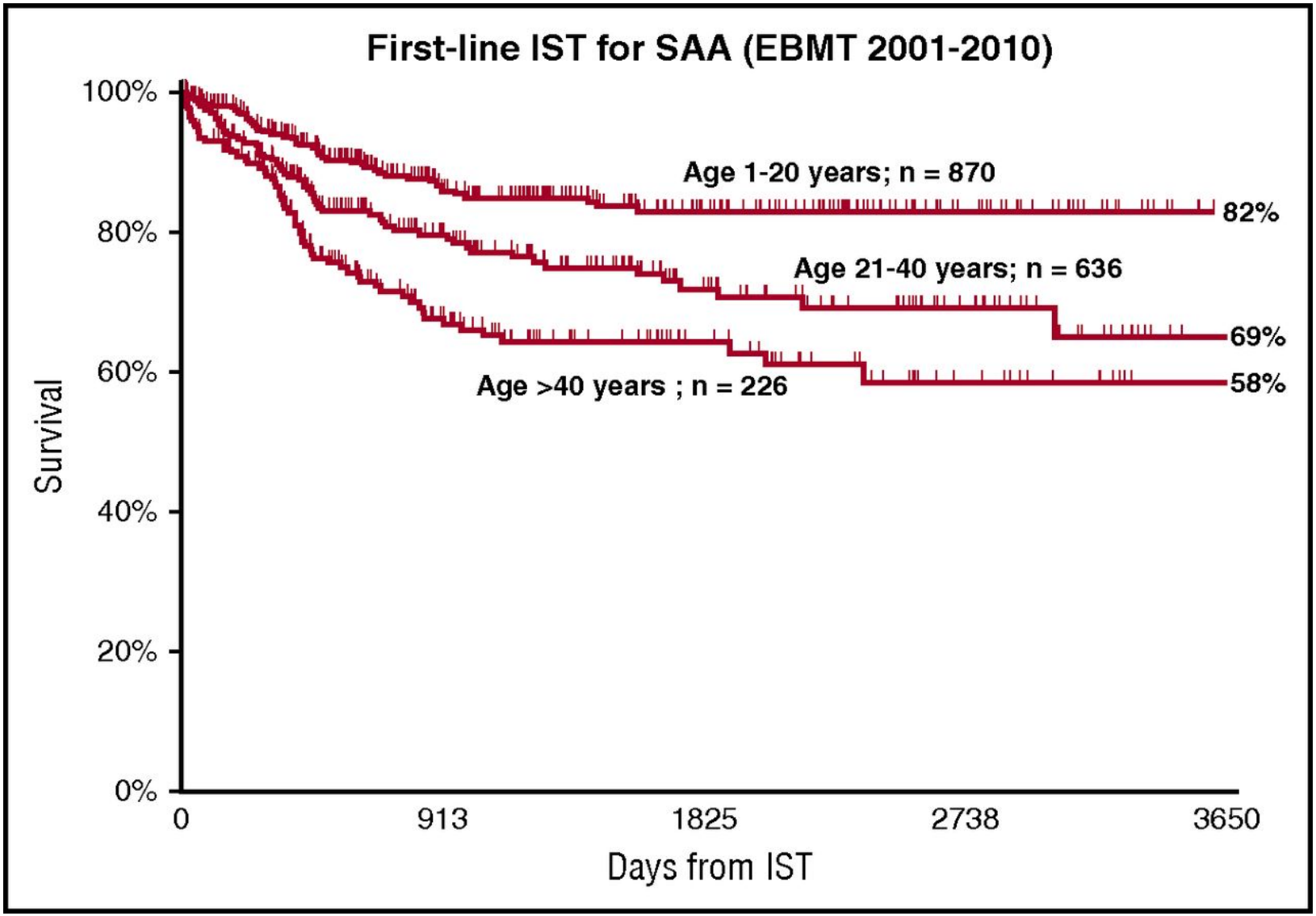
Treatment of SAA (2)

- Perform ATG skin test if available for hypersensitivity to horse serum and desensitize those by intradermal injection
- Platelets should be maintained at more than 20,000/μL during ATG administration
- Patients need to be free of infection before initiating ATG
- ATG administered at a dose of 40 mg/kg over 4 hours, daily for 4 days
- Prednisone 1 mg/kg is started on day 1 and continued for 2 weeks as prophylaxis for serum sickness
- Acetaminophen and diphenhydramine are conventional premedications for treatment with ATG

Treatment of SSA (3)

- Responders have better survival prospects than do nonresponders
- Long-term prognosis is predicted by the robustness of the early blood count response
  - Defined as either platelets or reticulocytes > 50 X 10^9/L [50,000/µL] 3 months after treatment.
- Corticosteroids are of unproven benefit, and inferior in efficacy, to conventional immunosuppression regimens
- Should not transfuse platelets prophylactically in SAA patients who have a platelet count more than 10,000/µL and who are not bleeding

The age effect in patients receiving first-line IST



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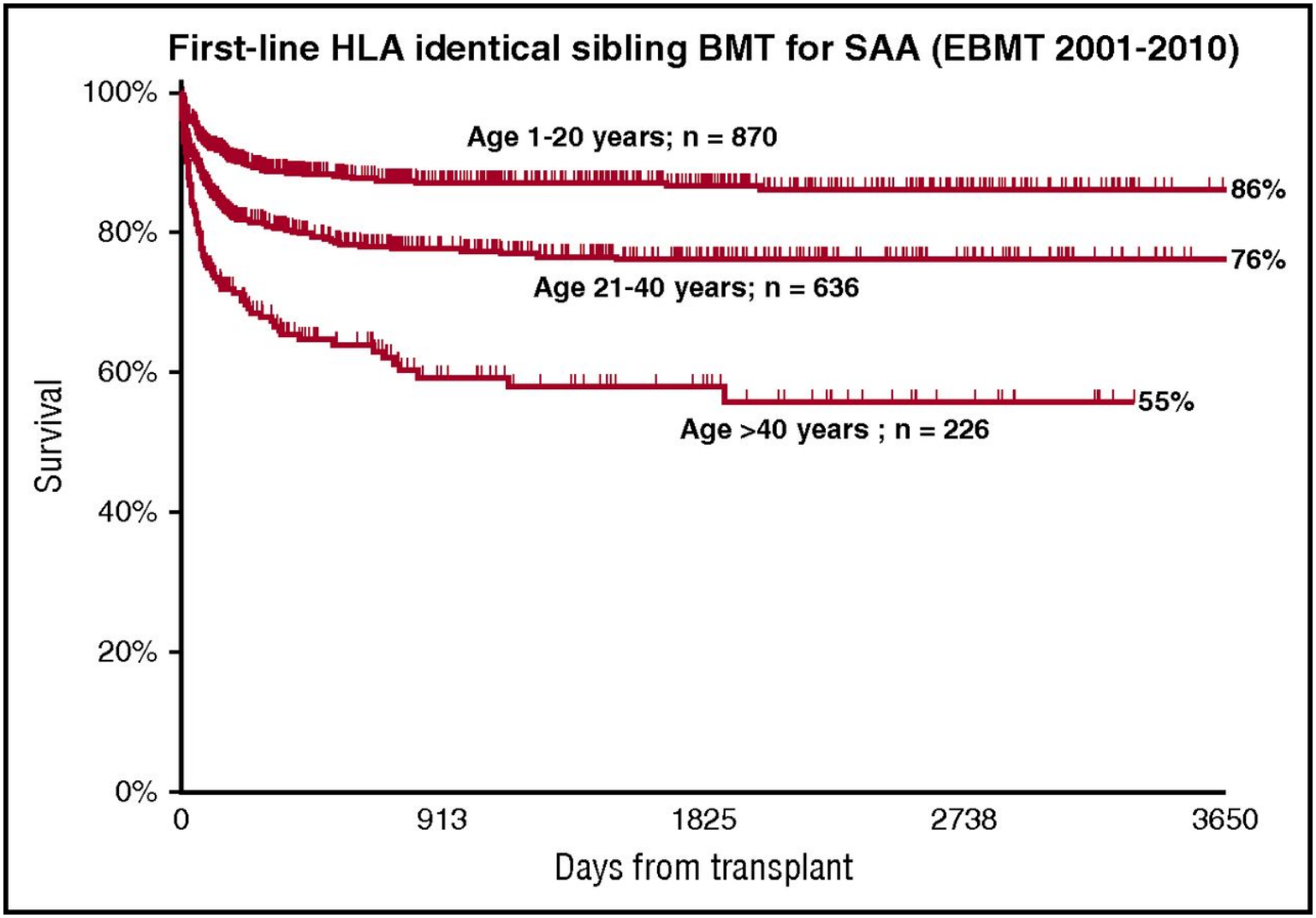
A strong age effect in patients with aplastic anemia, after transplantation from an HLA-identical sibling



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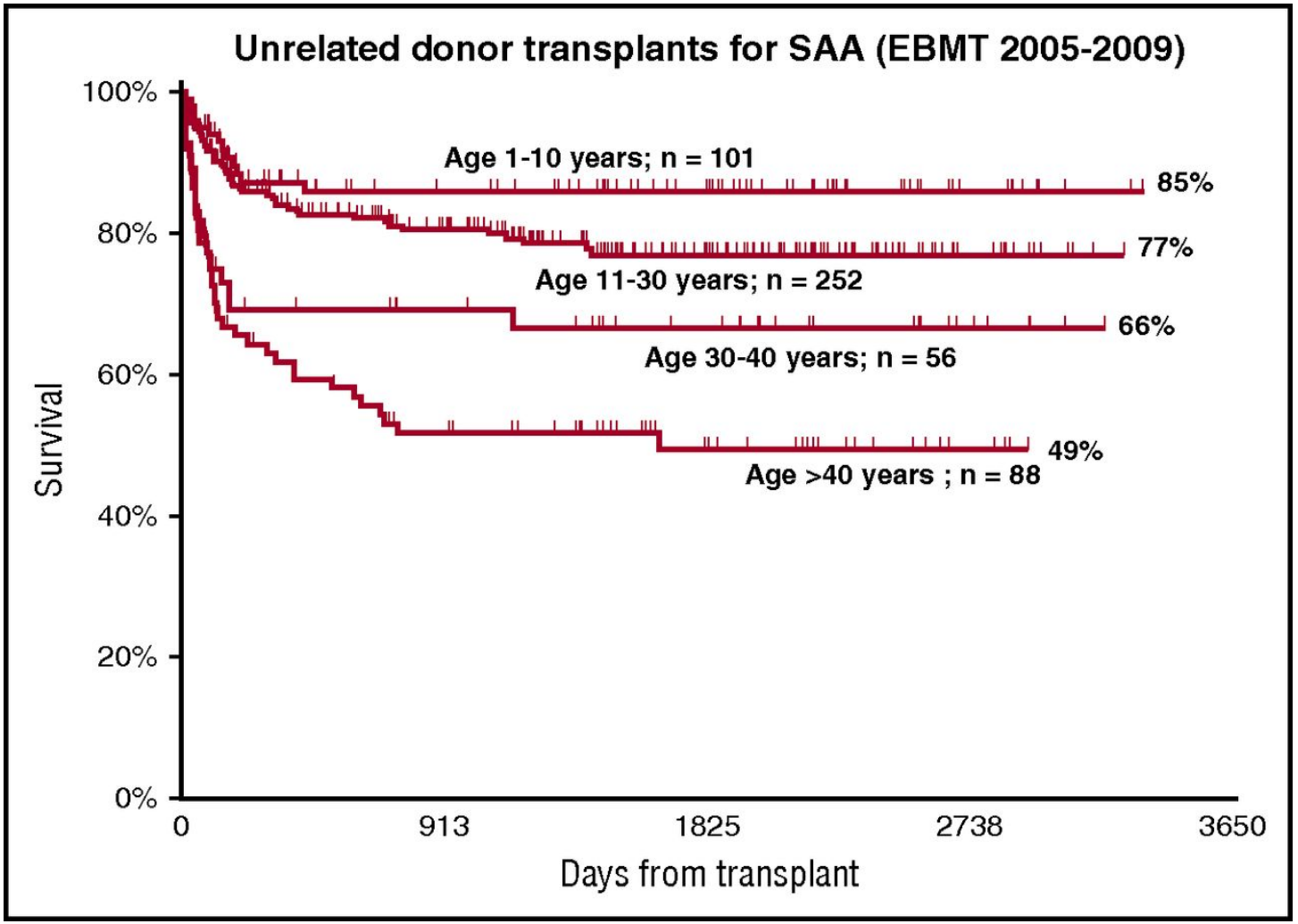
Age effect in URD transplants: best outcome seen for very young patients, for whom first-line URD BMT may be considered



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Relapse and Long Term Follow-up

- Defined as requirement for additional immunosuppression & not necessarily recurrent pancytopenia
- Does not by itself indicate a poor prognosis
- Major reason for relapse - incomplete eradication by ATG of pathogenic clones
- Second course of ATG therapy can be administered to patients with relapsed or refractory disease
- Cyclophosphamide has been used to treat relapsed/refractory SAA, and is associated with a response rate of about 50%
  - Toxicity of high-dose cyclophosphamide: prolonged neutropenia and susceptibility to infection
  - Higher death rates have been reported with use of cyclophosphamide

References

- http://sciscogenetics.com/
- http://slideplayer.com/slide/5679853/
- Young, N. S., Calado, R. T., & Scheinberg, P. (2006). Current concepts in the pathophysiology and treatment of aplastic anemia. Blood, 108(8), 2509-2519. Accessed August 10, 2016. http://dx.doi.org/10.1182/blood-2006-03-010777.
- Scheinberg, P., & Young, N. S. (2012). How I treat acquired aplastic anemia. Blood, 120(6), 1185-1196. Accessed August 10, 2016. http://dx.doi.org/10.1182/blood-2011-12-274019.
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