Diagnosis of Bone Marrow Failure in adults: inherited versus acquired

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EBMT

European Reference Network
for rare or low prevalence complex diseases
Network Hematological Diseases (ERN EuroBloodNet)

Conflicts of interest

1. **Employment or Leadership Position**
   University Hospital RWTH Aachen
2. **Advisory Role or Expert Testimony**
   Pfizer
3. **Stock Ownership**
   No conflicts to disclose
4. **Patent, Copyright, Licensing**
   Novartis
5. **Honoraria**
   Novartis, Janssen and Pfizer
6. **Financing of Scientific Research**
   Novartis and Pfizer
7. **Other Financial Relationships**
   No conflicts to disclose
8. **Immaterial Conflicts of Interest**
   RepeatDx

Practical issues before starting

- ✓ **30-35min presentation (30 slides max) + 15 min Q&A session**
- ✓ **Microphones will be muted by host to avoid back noise**
- ✓ **Please, stop your video to improve internet conexion**
- ✓ **Send your questions during the presentation through the chat, they will be gathered and answered after the presentations.**

Case report #1

- 03/13 21-year old male Patient with petechial bleeding *admitted to external community hospital*
  Lab: Platelets: 5/nl, Hb: 13,3 g/dl, WBC: 4,4/nl (granulocytes: 2.0/nl)

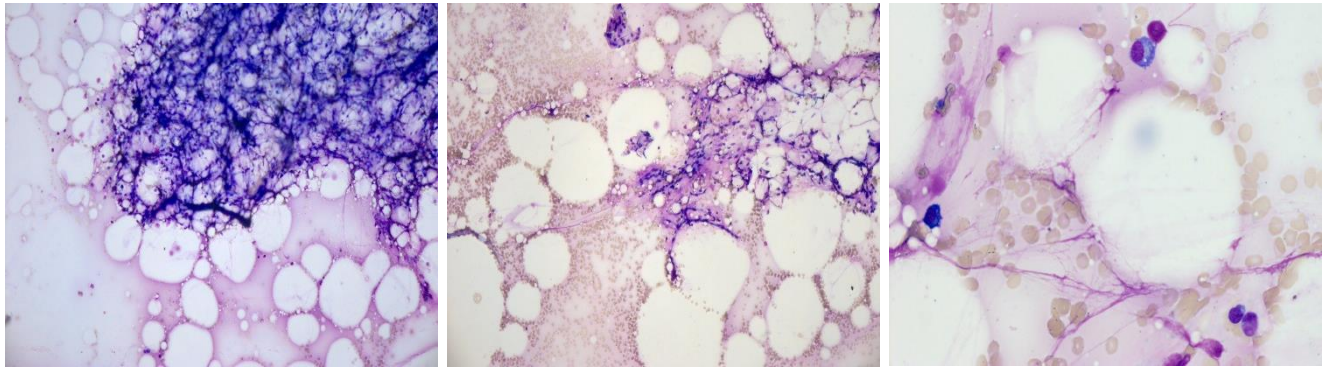
  PMH: viral infect with fever up to 38.5°C 2 weeks ago; no previous surgery, no medication

  Dx: V.a. ITP ⇨ PDN 2mg/kg KG, after 1 week: transient increase of platelets to 30/nl
- 04/13 Follow up: Lab: **Platelets: 8/nl,** Hb 10,4 g/dl, WBC: 3,7/nl, (granulocytes: 1.2/nl),

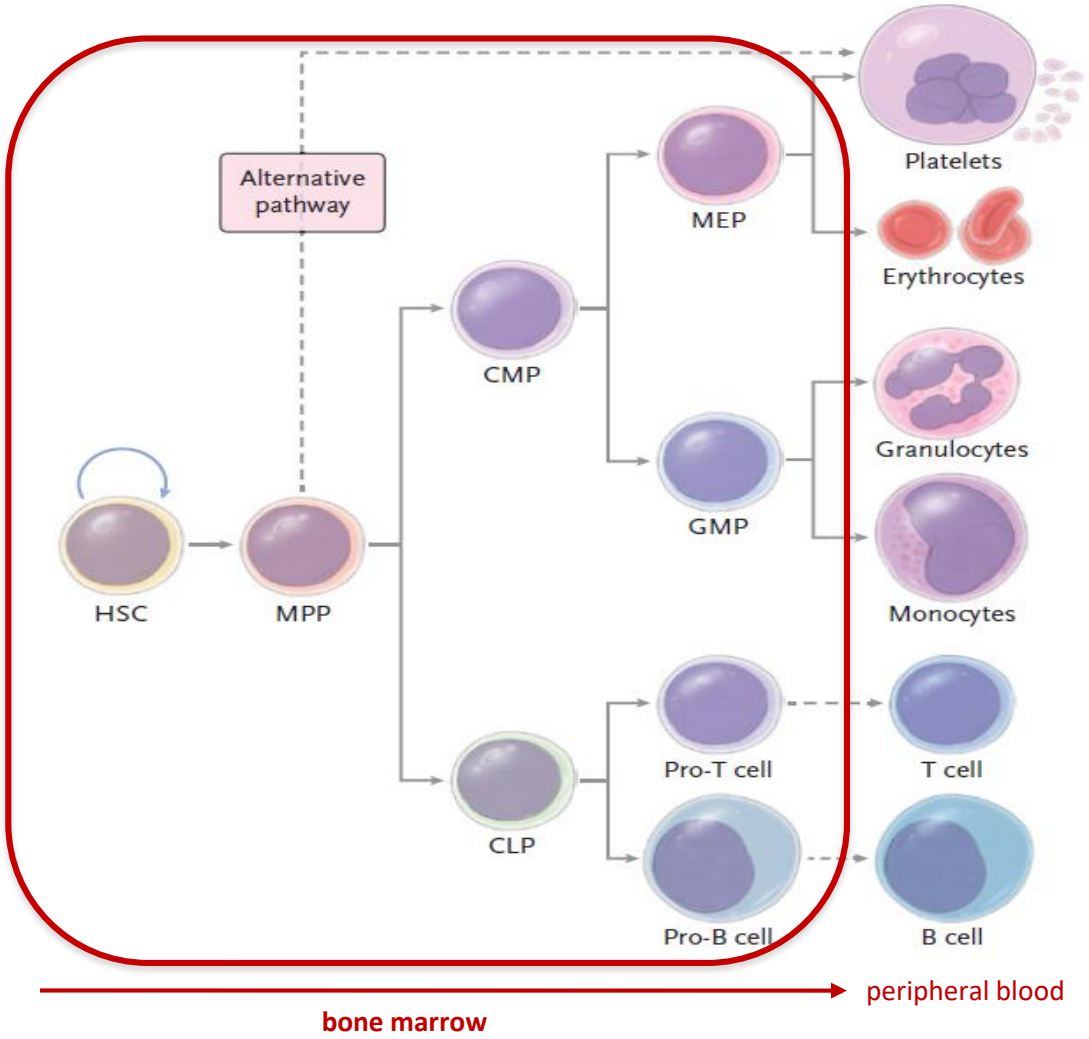
  Reticulocytes: 11/nl (NW 26-78)

  BM-Cytology: Aplastic megakaryopoesis, significantly hypoplastic erythro-/granulopoesis.
  BM-Histology: Aplastic bone marrow with (relative) interstitial T-Lymphocytosis

  Dx.: severe Aplastic Anemia



The hematopoietic system



Hematopoiesis:

- highly dynamic process of coordinated proliferation vs. differentiation decisions,
- strictly regulated by intrinsic and extrinsic factors

- daily blood cell production:
app. 1 x 10^{12} blood cells,

lifetime prodcution:
>1 x 10^{15} blood cells

- Key regulator: **hematopoietic stem cells (HSC)** in the bone marrow

- Blood cell half lives:
  - Granulocytes: 1-4 days
  - Platelets: 9-10 days
  - Erythrocytes 120 days

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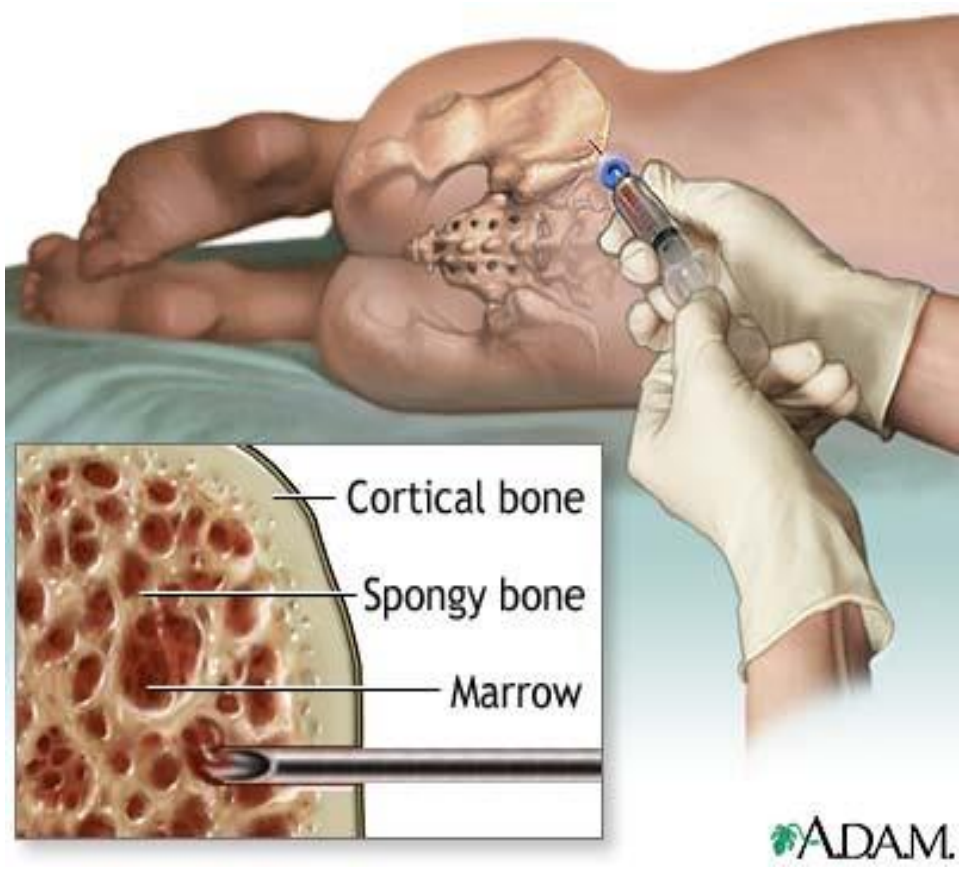
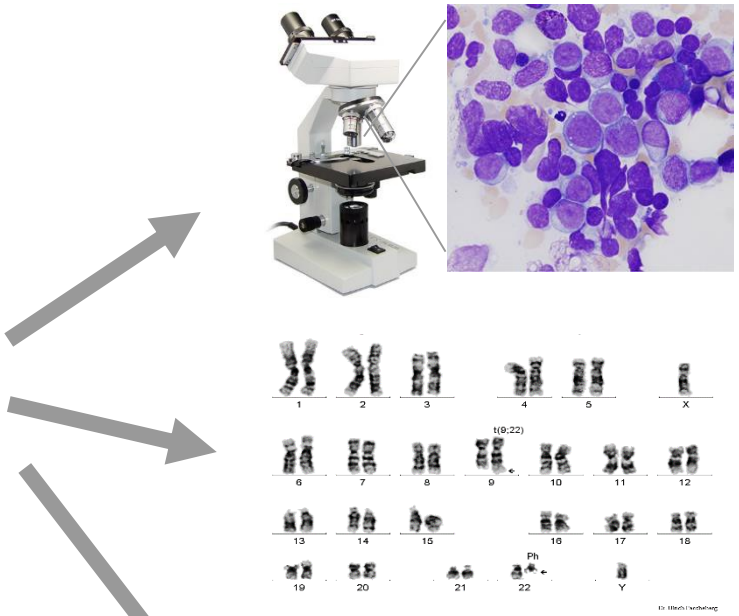
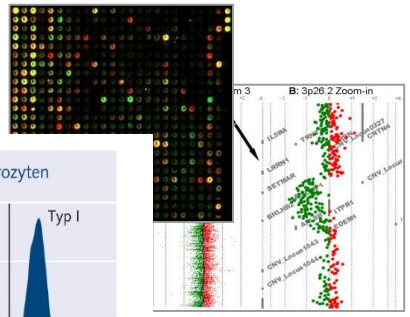
N Engl J Med 2019;380:2237-45.
DOI: 10.1056/NEJMra1804280

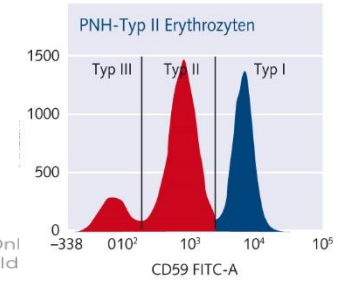
The bone marrow as „diagnostic window“ into hematopoiesis



mineralium.com; Nature.com; journalmed.de; ; genechips.de

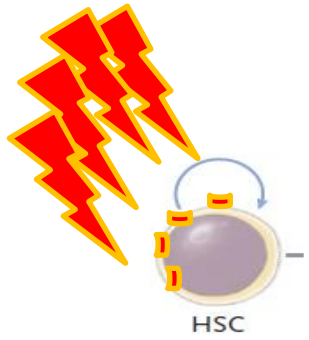


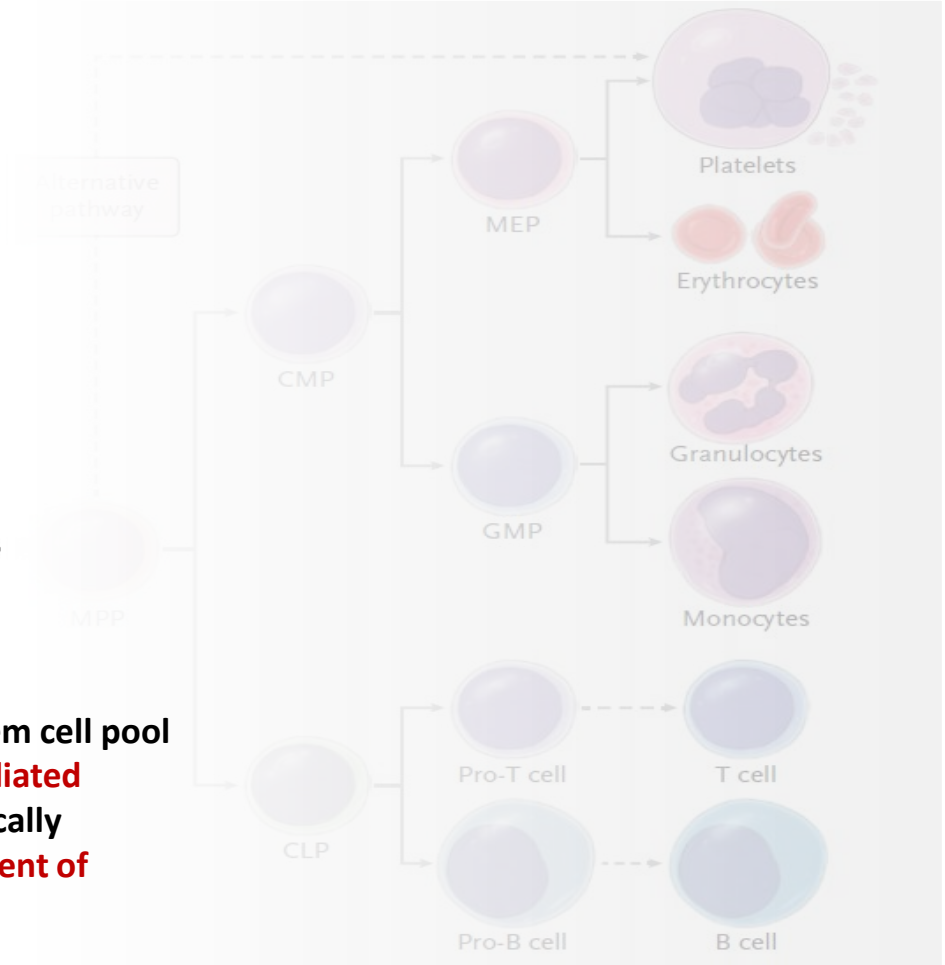




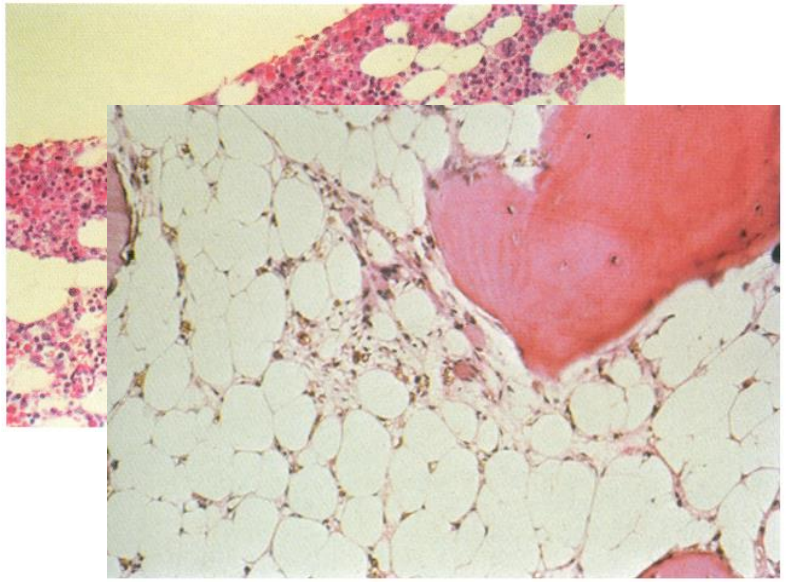
- Morphology
- *Cytogentics*
- *Genetics*
- *Phenotype*

Aplastic Syndromes (BMFS)
Reduction of the stem cell pool





Exhaustion of the stem cell pool by autoimmune-mediated distruction or genetically determined impairment of replicative potential



consequences:

- clinical phenotype defined by lack of functional blood cells:
- red cells -> **anemia**
- WBC -> **Infections**
- Platelets -> **Bleeding**

bone marrow → peripheral blood

N Engl J Med 2019;380:2237-45.
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Hallmarks of aplastic Anemia (AA)[1,2]

Reduction of hematopoietic precursors in the bone marrow (BM hypoplasia)	Pancytopenia (Reduction of red cells, white cells and platelets)	Exclusion of other hematopoietic systems disorders and malignacies

- For diagnosis of AA, 2 of the following criterai will have to be met[3]:

Hb < 10 g/l (Retikulocytes <60/nl)	Platelets < 50/nl	Neutrophils < 1.5/nl

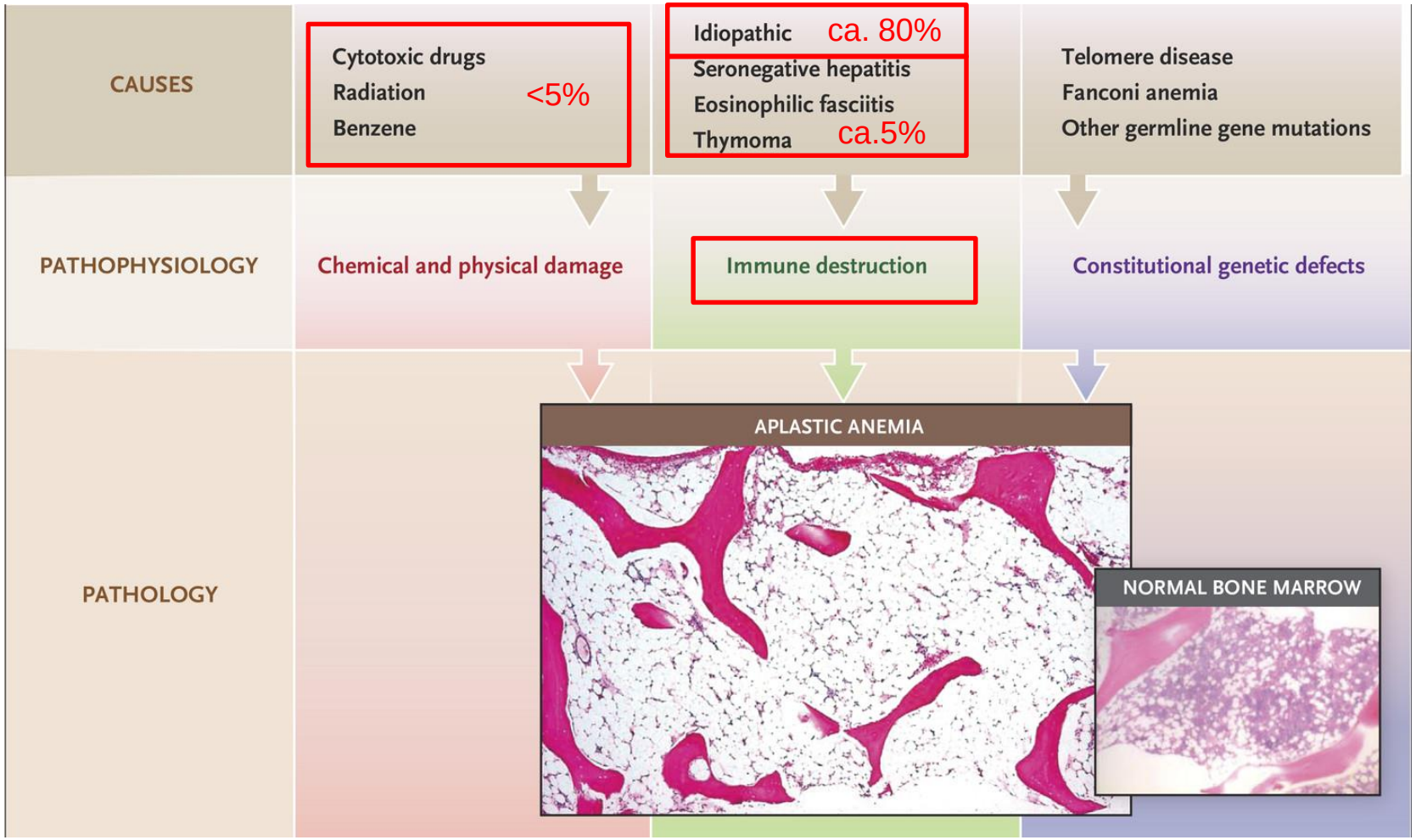
- Estimated Incidence: app. 2 per 1 Million inhabitants per year in North America and Europe[1]
- Two-peak incidence: 10-25 years and >60 years: male = female
- Clinical symptoms of pancytopenia
- AA has significant implications on lifestyle and hrQoL of affected patients[4]
- Treatment delay associated with substantial morbidity and mortality[1]
- Long-term complications: development of secondary MDS, AML or PNH
- Concomittant with PNH as AA/PNH overlap syndrome

1. Scheinberg P. Aplastic anemia: therapeutic updates in immunosuppression and transplantation. *Hematology Am Soc Hematol Educ Program*. 2012;2012:292-300.
2. Desmond R, et al. Eltrombopag restores trilineage hematopoiesis in refractory severe aplastic anemia that can be sustained on discontinuation of drug. *Blood*. 2014;123(12):1818-1825.
3. Kilick SB, et al. Guidelines for the diagnosis and management of aplastic anaemia. *Br J Haematol*. 2018;172(2):187-207.
4. Frickhofen N, et al. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. *Blood*. 2003;101(4):1236-1242.

Learning objectives of the webinar

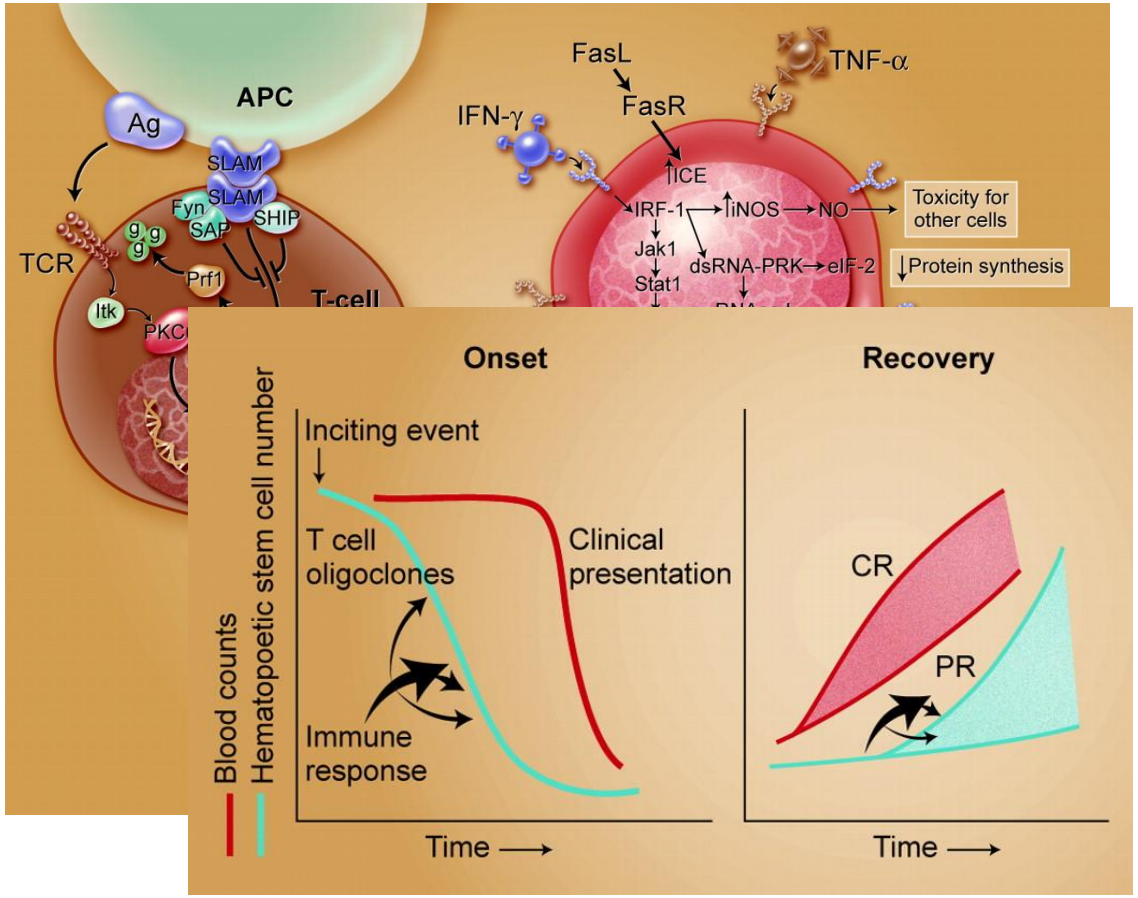
1. **Diagnostic algorithm of aplastic anemia and related bone marrow failure syndrom**es
2. Differential diagnosis (including limitations) of inherited vs. acquired BMFS in adults
3. Introduction into Telomere biology disorders (TBD)
4. Consequences of the correct identifiction of late-onset (hereditary) TBD in adults

Causes of Aplastic Anemia



NS Young. N Engl J Med 2018;379:1643-1656.

Idiopathic Aplastic Anemia Pathophysiology



- Idiopathic AA mostly auto-imune-mediated
- Auto-reactive T-cells deplete HSC pool
- Process mediated by Interferon-y (and TNF-a): leading to induction of apoptosis in HSC
- Immune escape of GPI-negative HSC clones can lead to clinical PNH or AA/PNH syndrome
- GPI-deficiency can serve as a surrogate marker for autoimmune etiology and predictor of response to immunosuppressive treatment (IST)

Young NS et al. Blood 2006

Disease severety of Aplastic Anemia

- Severity of AA can be devided into 3 degrees[1]:

Classification and Criteria		
Moderate AA (mAA)	**Severe AA (sAA)**	**Very severe AA (vsAA)**
Patients with AA, who do not fullfill criteria of sAA or vsAA	BM-cellularity< 25 %, or 25 %-50 % with < 30% residual hematopoietic cells ***and*** 2 out of 3 of the following criteria: • Neutrophil count < 0.5/nl • Platelet count < 20/nl • Reticulcyte count < 20/nl	Criteria of sAA, but with • Neutrophil count < 0,2/nl

1. Marsh JCW, et al. Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol. 2009;147(1):43-70.

Diagnostic Algorithm of suspected Aplastic Anemia

- Detailed Past Medical History: regarding (incl. past) medication, drugs and family history of hematologic and oncologic disorders
- History of radiation exposure, Infections, travelling
- **clinical exam:** Signs of infection, anemia, bleeding, jaundice, hepatic and or splenic enlargment, lymphadenopathy, nail dastrophy, leukoplakia, abnormal skin pigmentation, skeletal or dental malformations, signs of growth retardation, impaired pulmonary function
- Differential blood counts; reticulocyte count (at least 2x)
- Bone marrow **cytology** (incl. iron-staining), bone marrow **histology**
- Bone marrow **cytogenetics**
- **Lab**: Ferritin, Vitamin B12, Folic acid, LDH, Bilirubine (direct and indirect); Quick, PTT, Fibrinogen, CRP, AST/ALT, AP, Creatinin, uric acid, blood glucose, immunoglobulins, protein electropheresis, Anti-nuclear antibodies, Anti-DS antibodies
- **Immunophenotyping** (GPI-Deficiency)
- **Serology**: EBV, CMV, Hepatitis-A,-B,-C, HIV, Parvovirus B19
- Lung x-ray, abdominal ultrasound
- HLA-Typing
- Screening for hereditary forms: "**chromosomal breakage test**", **Telomere screening** (+potential NGS)

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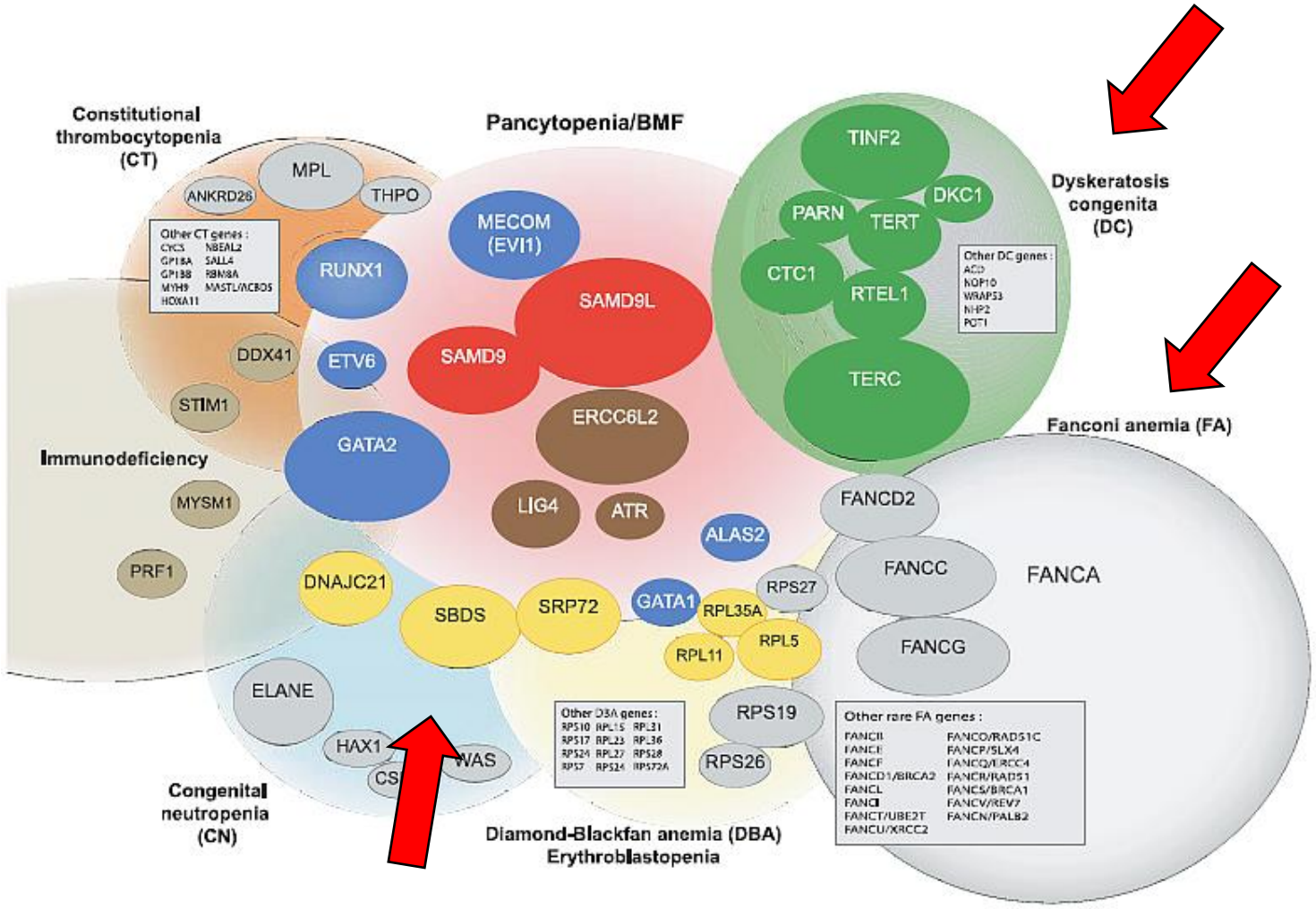
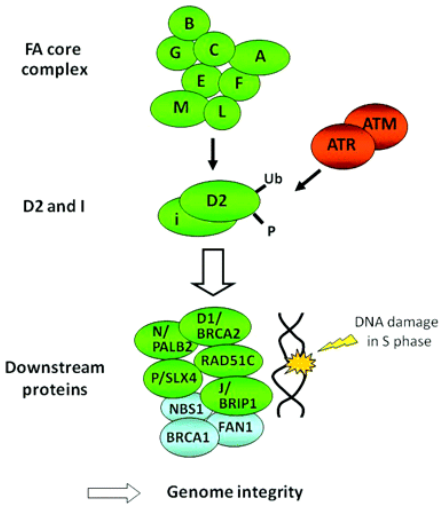
The genetic landscape of inherited bone marrow failure syndromes

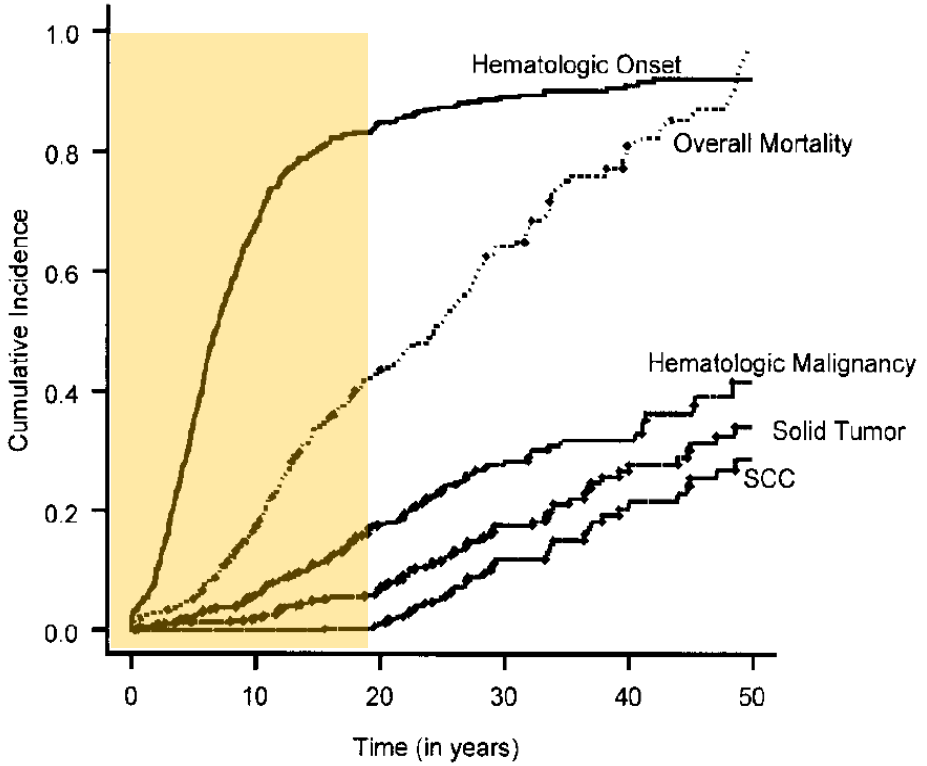


- **In adults**, three major groups of inherited disorders are related to BMF as the main symptom:
  - TBD,
  - FA and
  - SBDS

Fanconi Anemia – in adults

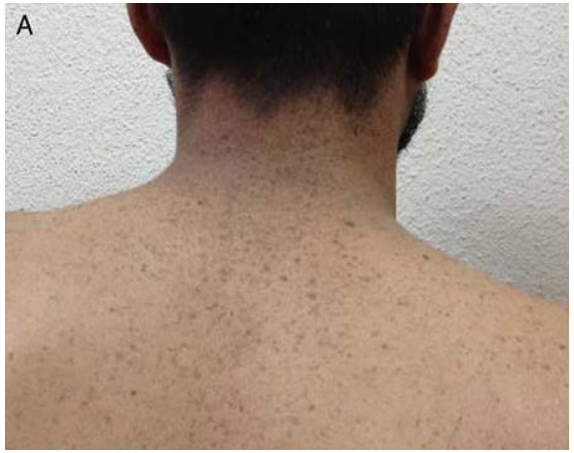
- **Autosomal recessive** inheritance
- Pathophysiology: **DNA damage repair**, especially crosslink repair including nucleotide excision repair and homologous recombination
- approx. **9% diagnosed in the age >18 years**
  - oldest patient diagnosed with 55 years!
- Two main manifestations in adults:
  - BMF
  - AML/MDS
    - Incidence of **FA-related AML: 0.2%**
- No exact evidence about the frequency in AA.
- BMF mediated by FA indistinguishable from acquired AA
  - -> **CBT recommend up to the age of 35 years** (when FA is suspected)

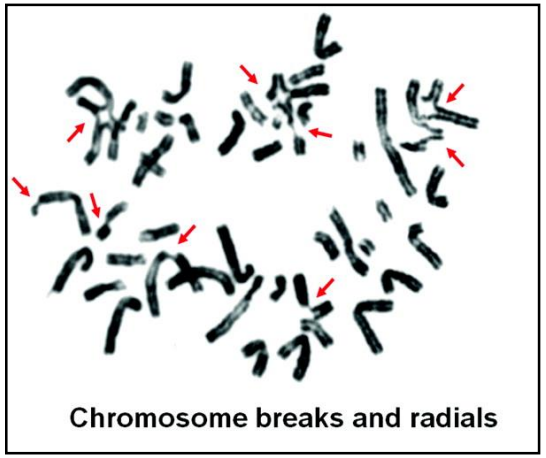




Soulier J, Am Soc Hematol Edu Pro, 2011
Kuttler DI et al. Blood 2003

Fanconi Anemia – Clinical Presentation





- Microsomia (40%): Short stature
- Skin (40%): Generalized hyperpigmentation; cafe au lait spots, hypopigmented areas
- Upper Limbs, unilateral or bilateral (35%):Thumbs (35%): Absent or hypoplastic, bifid, duplicated, rudimentary
- Radii (7%): Absent or hypoplastic (only with abnormal thumbs)
- Skeletal: Head (20%): Microcephaly, hydrocephaly,
- Females (2%): Hypogenitalia, bicornuate uterus, malposition,
- Ears (10%): Deaf (conductive), abnormal shape
- Cardiopulmonary (6%): Congenital heart disease, patent ductus arteriosus, atrial &ventricular septal defect, situsinversus, truncus arteriosus
- Gastrointestinal (5%): Atresia (esophagus, duodenum, jejunum)
- Central Nervous System (3%): Small pituitary, pituitary stalk interruption syndrome, absent corpus callosum, cerebellar hypoplasia, hydrocephalus, dilated ventricle

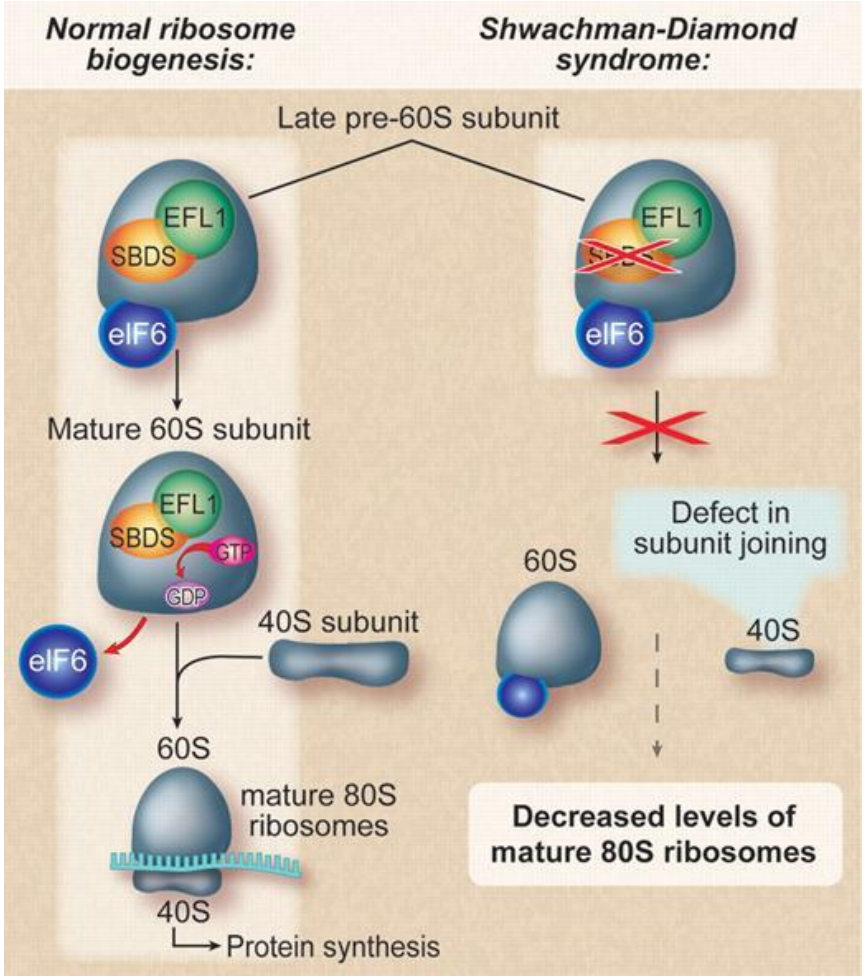
Not typical in adults

- **In adults only few and frequently moderate symptoms**

Shimamura A, Blood reviews 2010, Aslan D J of Pediatric Hematol 2017.

Shwachman-Diamond Syndrome – Diagnosis and Clincial Presentation in adults

- Autosomal recessive inheritance, estimated 1:100.000
- Pathophysiology: **Impaired ribosomal** composition of the 60S and 40S subunits -> ribosomal stress -> HSC depletion
- Diagnostic:
  - Genetic analysis for mutations in the **SBDS gene** (>90% of all cases): c.183_184delinsCT or c.258+2T>C
- Clinical chemistry: low levels of tryptase, elastase in the stool, **pancytopenia with prominent neutropenia**
- **Clinical presentation**: short stature (in children: 50% in the lower third of the percentile curves), pancreatic insufficiency
- **50% of all SDS registry patients without clinical phenotype**
- Few data about the incidence in patients with AA:
  - In pediatric patients: incidence approx. 1%
  - In adult patients: heterozygous SBDS mutations in 5%:
    - no response to immunosuppressive therapy ?!?

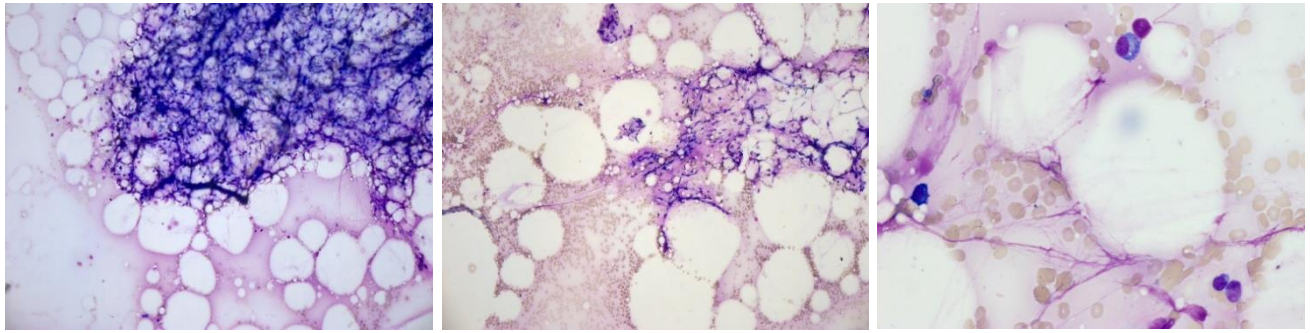


Myers KC, J Pediatric 2014, Narla A Blood 2011

Case report #1 (follow up)

- 05/13 Referred to University Hospital RWTH Aachen: additional diagnostic results
  - Lab: Platelets: 13/nl, Hb: 8,2 g/dl, WBC: 0.1/nl, Reticulocytes 7/nl
  - family and past medical history normal
  - telomere length and chromosomal breakage test: normal
  - karyotype: 46, xy
  - small PNH clone (2%)
  - **exclusion of hereditary reasons of BMF**

  Dx.: acquired very severe Aplastic Anemia: -> Treatment ?
- 6/13 Allogeneic stem transplantation from a matched familiy donor
- 10/21 Patient alive and well



Case report #2

- 06/2014: **49 year old female patient** with no past medical history

  Detection of asymptomatic, **isolated thrombocytopenia** at routine checkup:

  Platelets 23/nl, no signs of bleeding
- 07/2014: Dx of ITP; treatment with steroids, no improvement
- 02/2015: Evidence of bleeding; Treatment with Immunoglobulins, no improvement of blood counts.

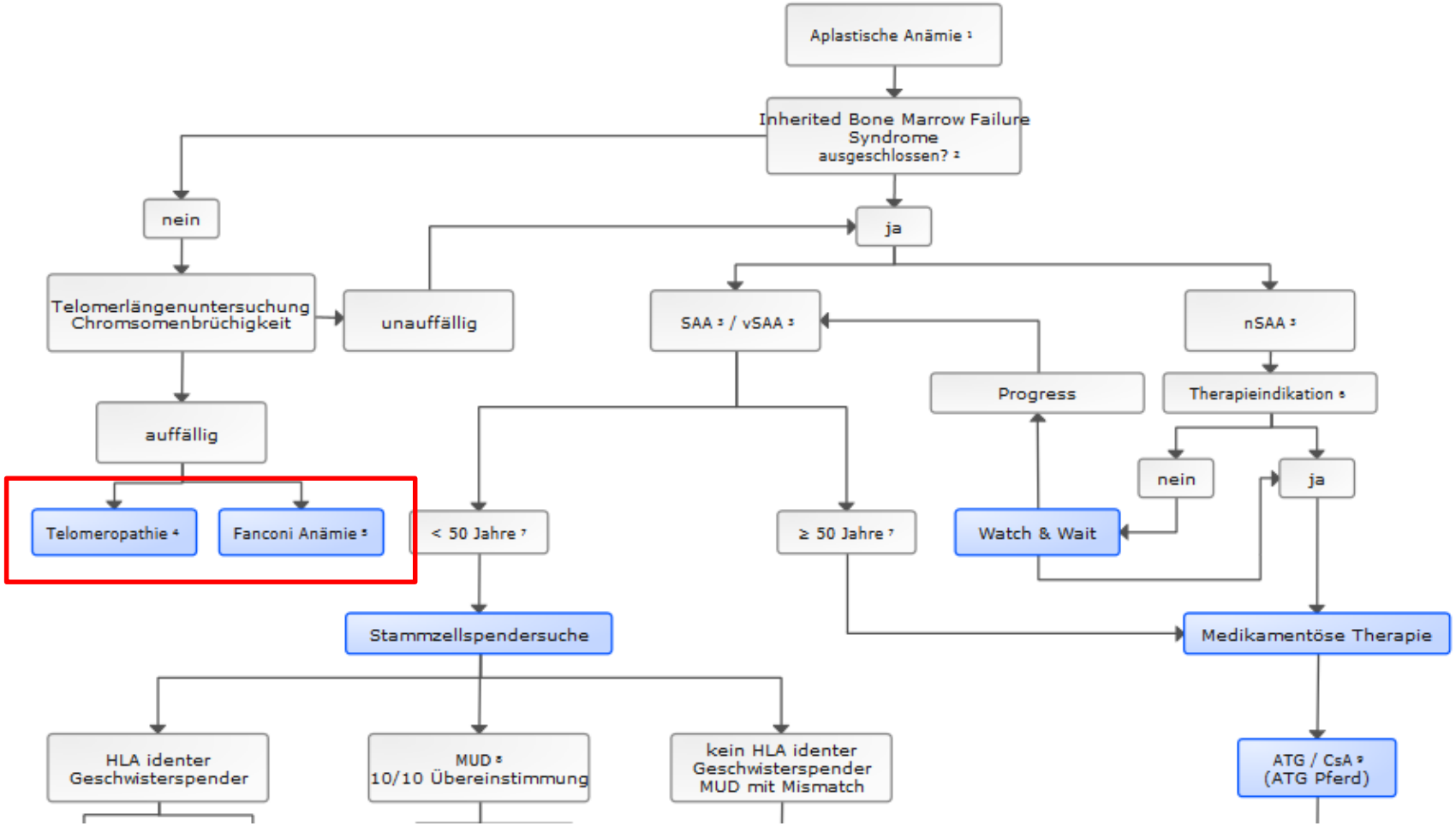
  BM cytology: **hypocellular BM**

  cytogenetics: 46, XX.

  Molecular genetics: ASXL1, ETV6, EZH2, RUNX1, TP53: all negative
- 10/2015 gradual **worsening of anemia and neutropenia** over time
- 05/2016**:** **Diagnosis of mAA** (**platelets: 17/nl**, Hb: 11,0 g/dl, neutrophils: 4,01 /nl, reticulocytes 69/nl, **BM: cellularity <10%**), weekly **transfusion dependent** for platelets
- 05/2016: **horse-ATG + CSA**: transient improvement of platelets to 30/nl
- 02/2017: again transfusion-dependent thrombocytopenia (<10/nl) and anemia 8.9 g/dl

  Referred to University Hospital RWTH Aachen

German Onkopedia guidelines Aplastic Anemia



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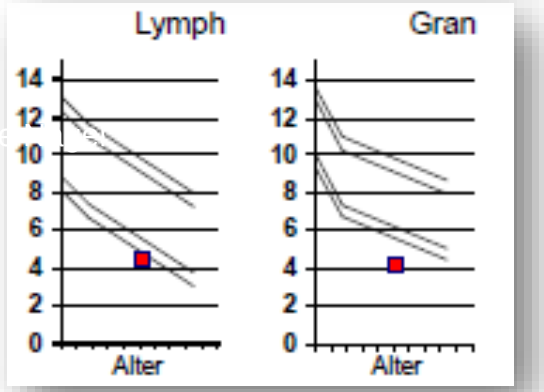
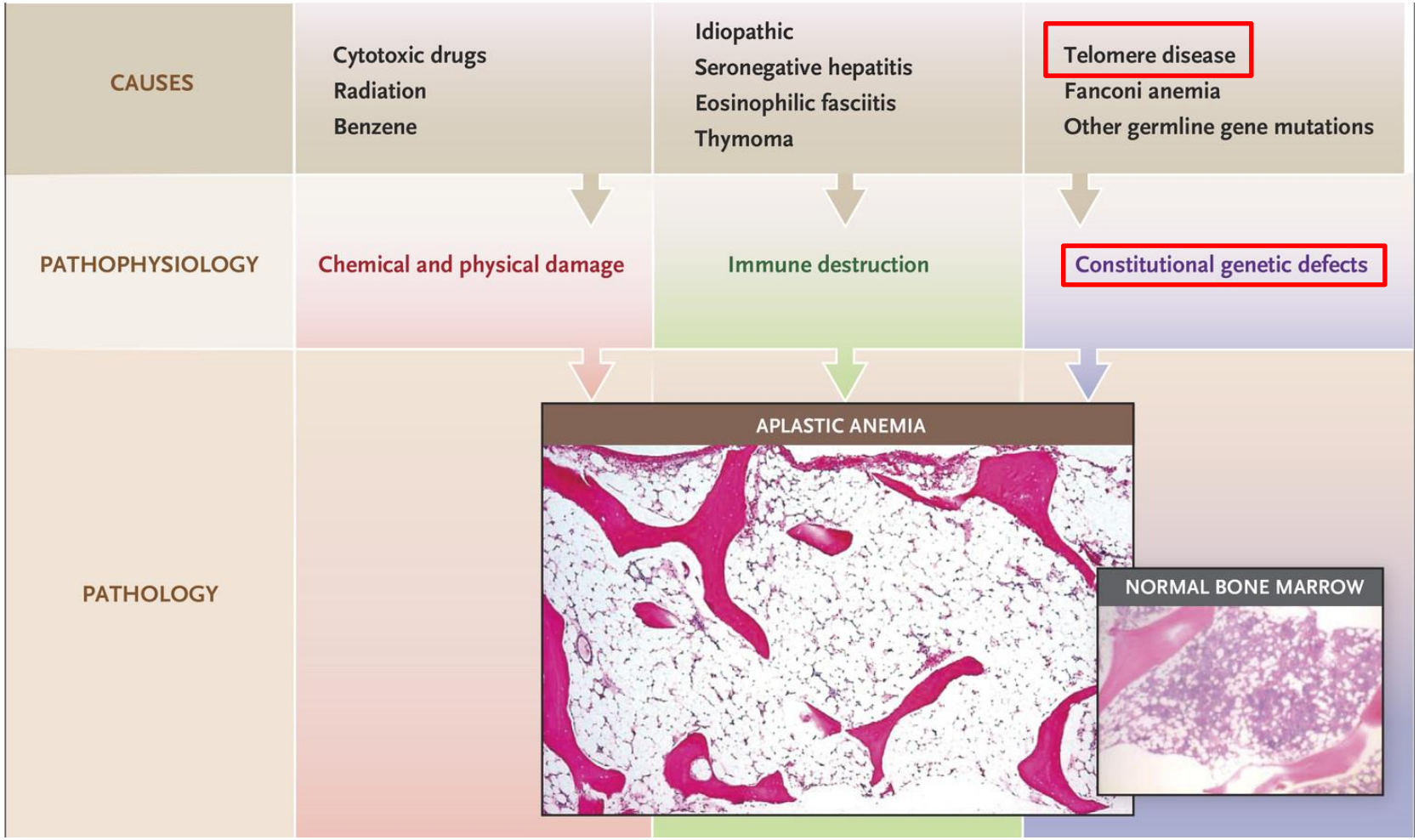
  Referred to University Hospital RWTH Aachen
- 03/2017**:** **Diagnosis of Telomere biology disorder (TBD, here: cryptic DKC)** based on short telomeres and detection of **TERC 73 G>A Mutation**, no clinical signs of DKC
- 05/2017 Treatment initiation with **Danazol**: Increase of platelets to 69/nl and Hb to 12,5 g/dl (within 4 months)



Causes of Aplastic Anemia



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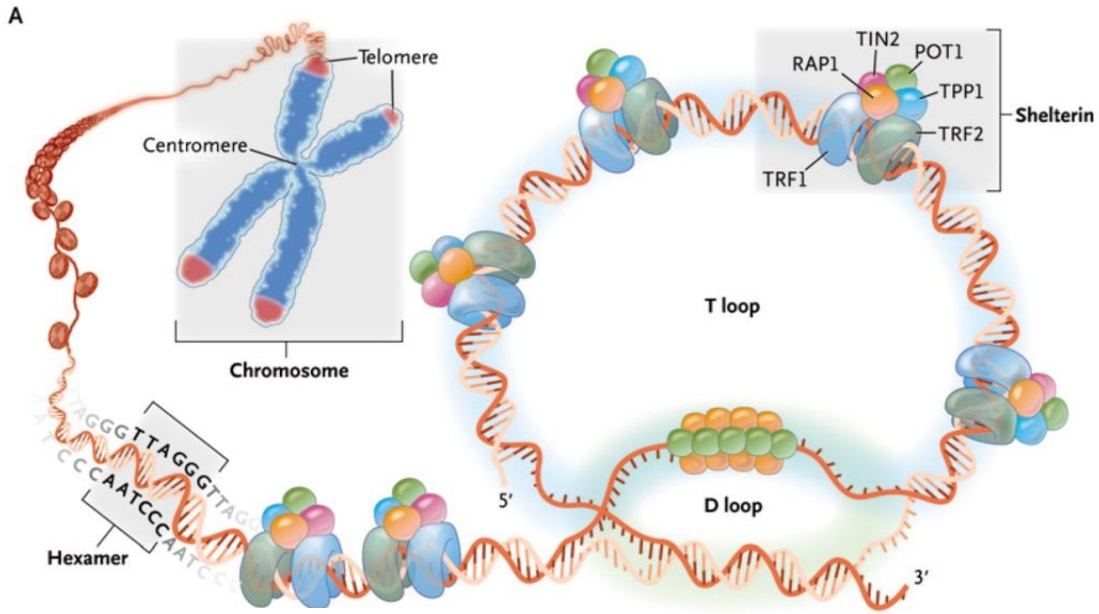
Introduction into Telomere biology



Calado, Young (2009) ***N. Engl. J. Med***

The Nobel Prize in Physiology or Medicine 2009

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"

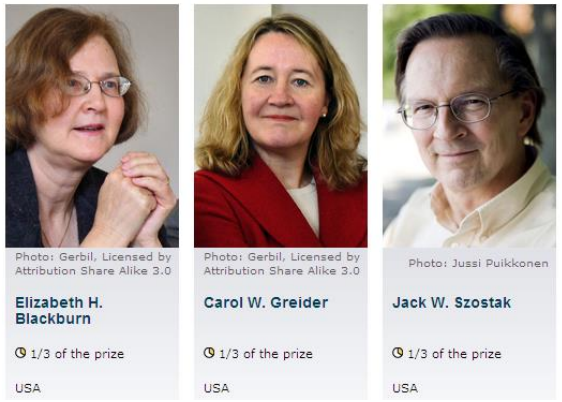
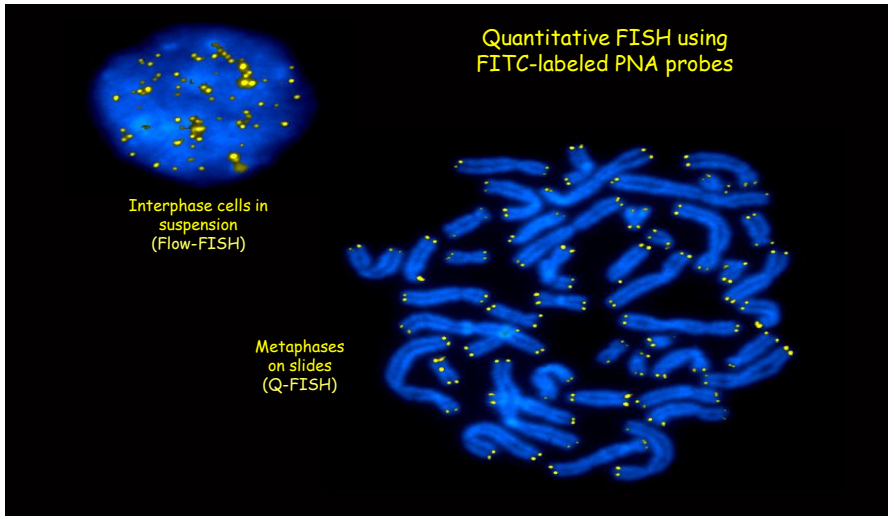
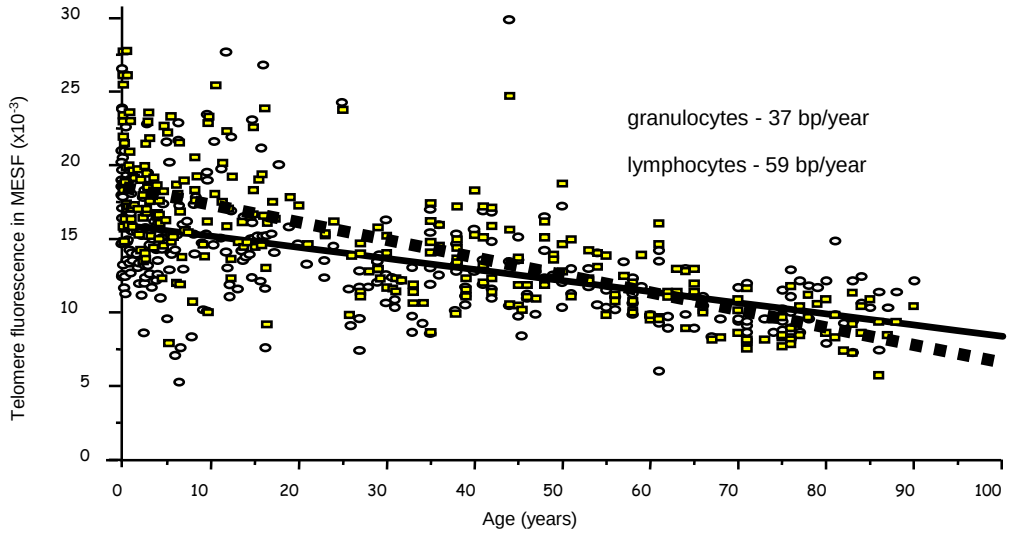


Photo: Gerbil, Licensed by Attribution Share Alike 3.0	Photo: Gerbil, Licensed by Attribution Share Alike 3.0	Photo: Jussi Puikkonen
Elizabeth H. Blackburn	**Carol W. Greider**	**Jack W. Szostak**
1/3 of the prize	1/3 of the prize	1/3 of the prize
USA	USA	USA



Brümmendorf and Lansdorp (1999) unpublished



Rufer, Brümmendorf (1999) ***J. Exp. Med.***

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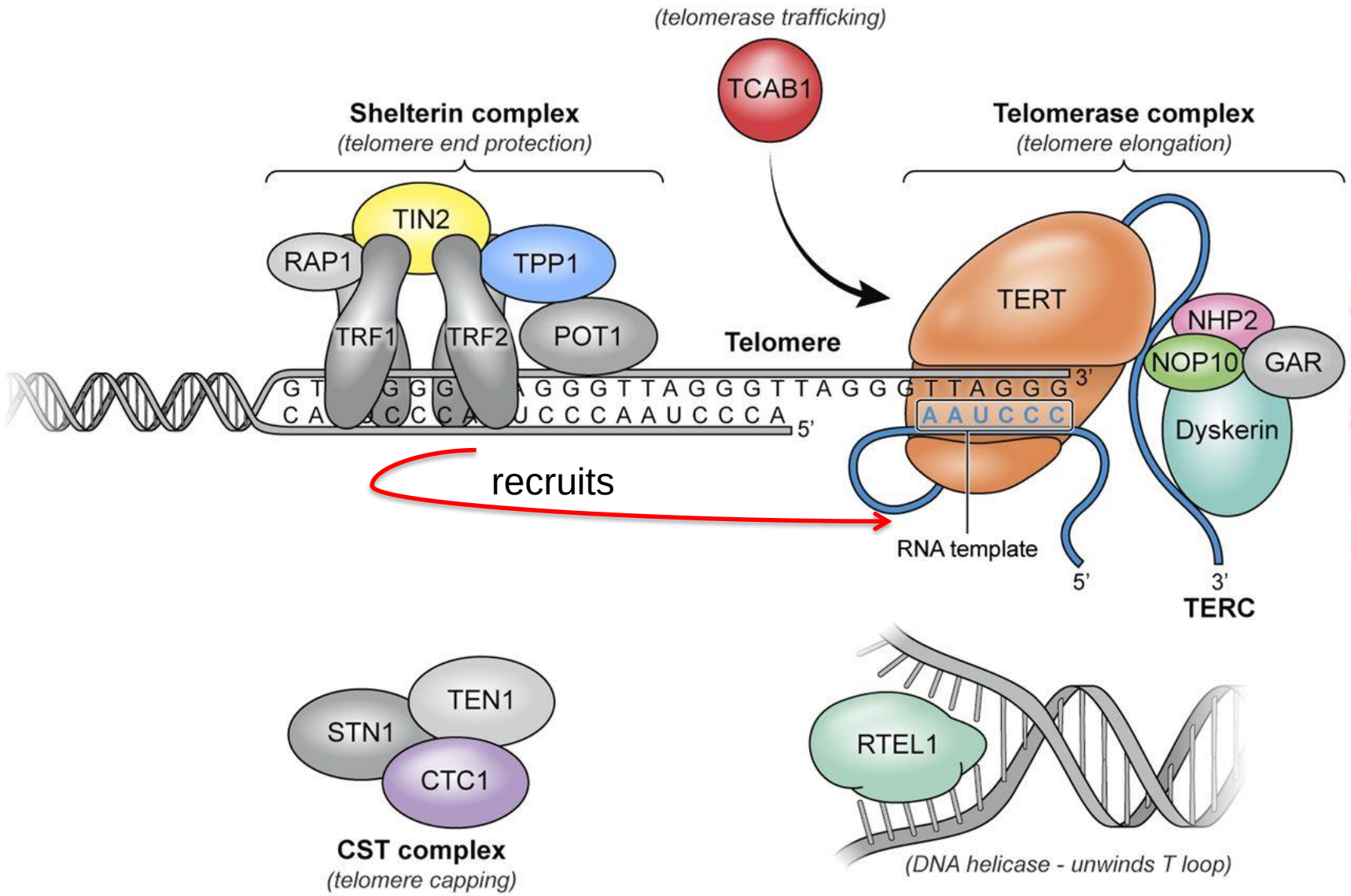
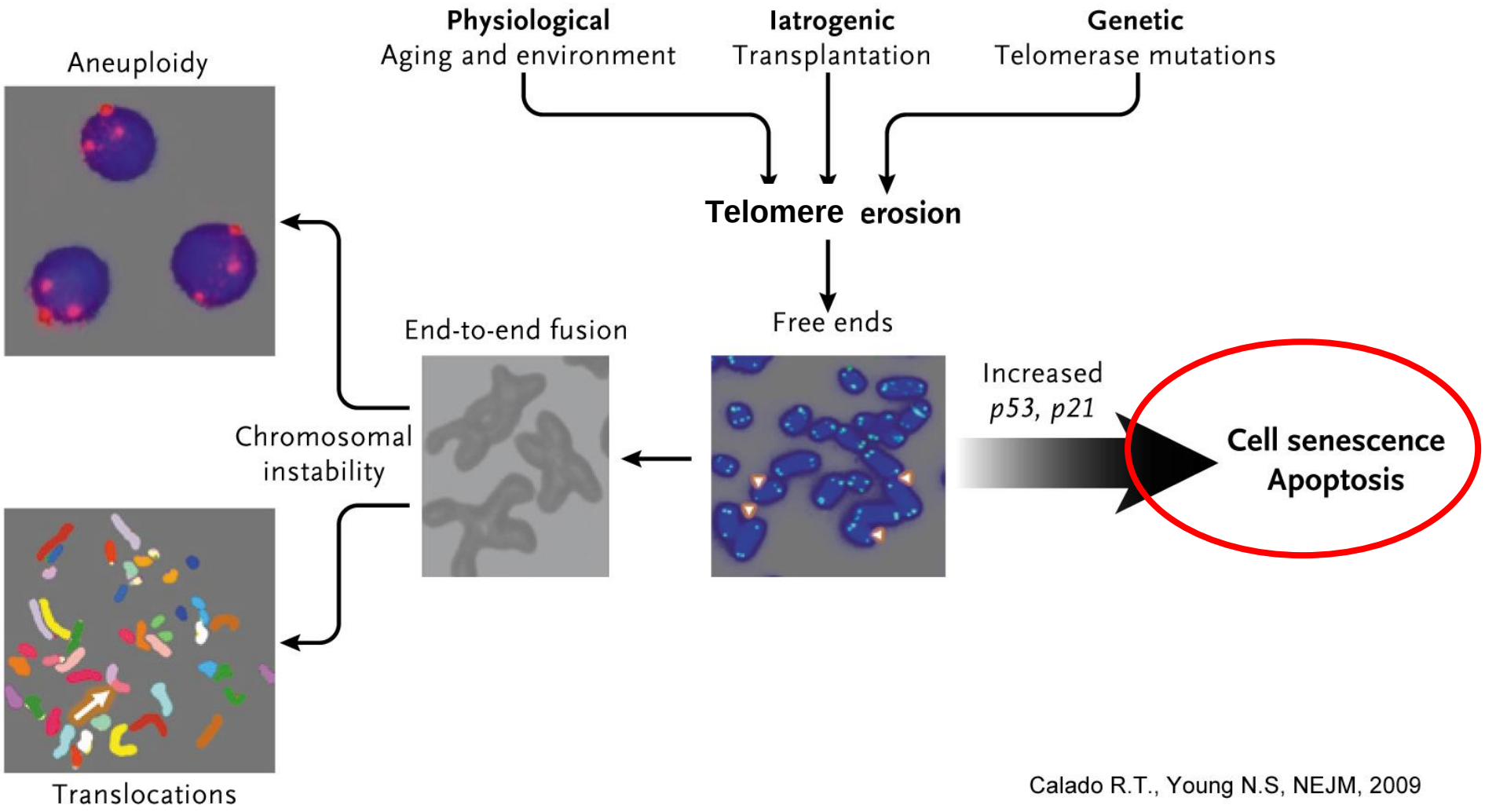
Telomere/Telomerase: components



Danielle M. Townsley et al. Blood 2014;124:2775-2783

Causes and Consequences of impaired telomere maintenance

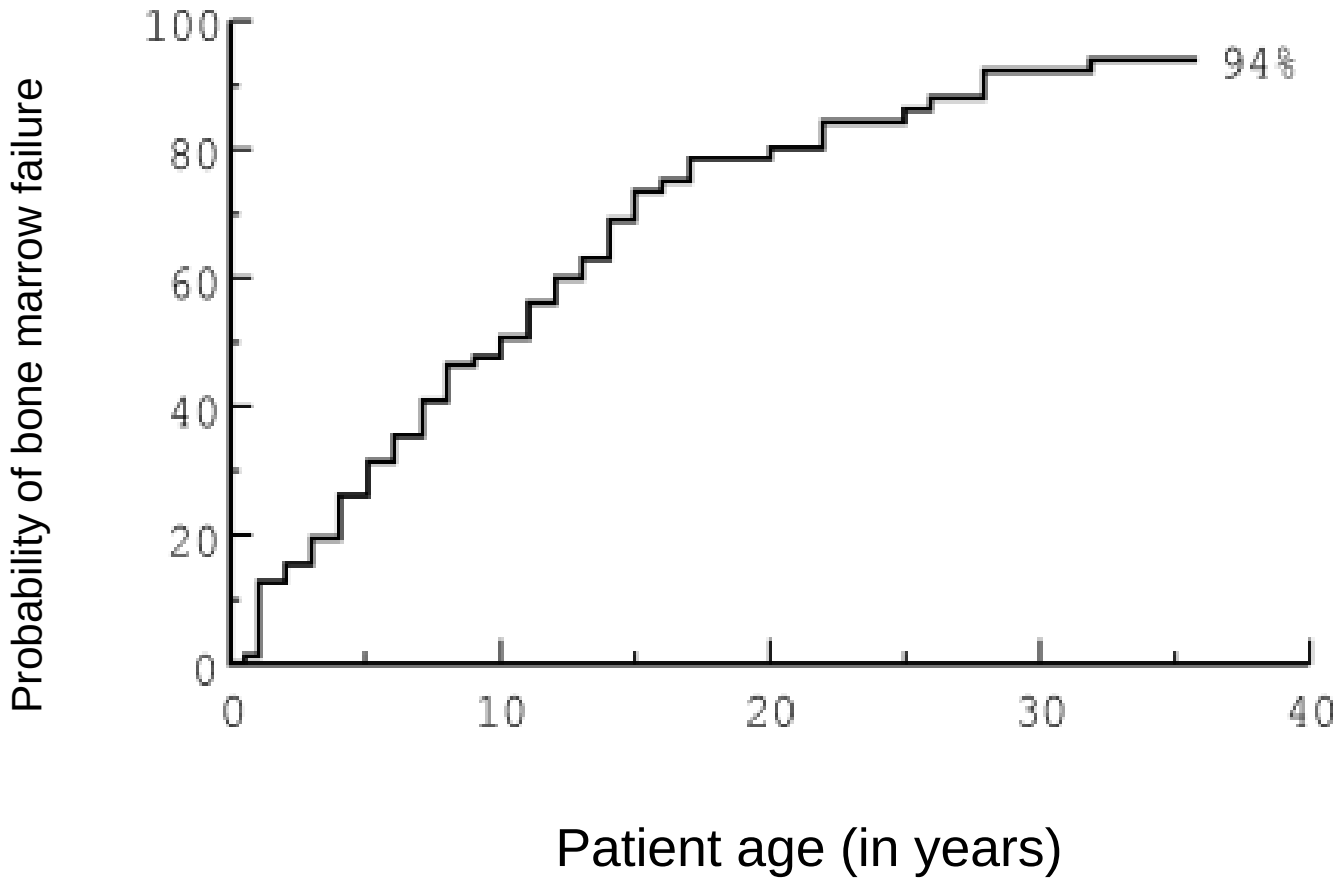


Calado R.T., Young N.S, NEJM, 2009

Learning objectives of the webinar

1. Diagnostic algorithm of aplastic anemia and related bone marrow failure syndromes
2. Differential diagnosis (including limitations) of inherited vs. acquired BMFS in adults
3. Introduction into Telomere biology disorders (TBD)
4. **Consequences of the correct identifiction of late-onset (herediatry) TBD in adults**

Probability of bone marrow failure in patients with Dyskeratosis congenita



from: Dokal I., *Br. J. Hematol. 110:768 (2000)*

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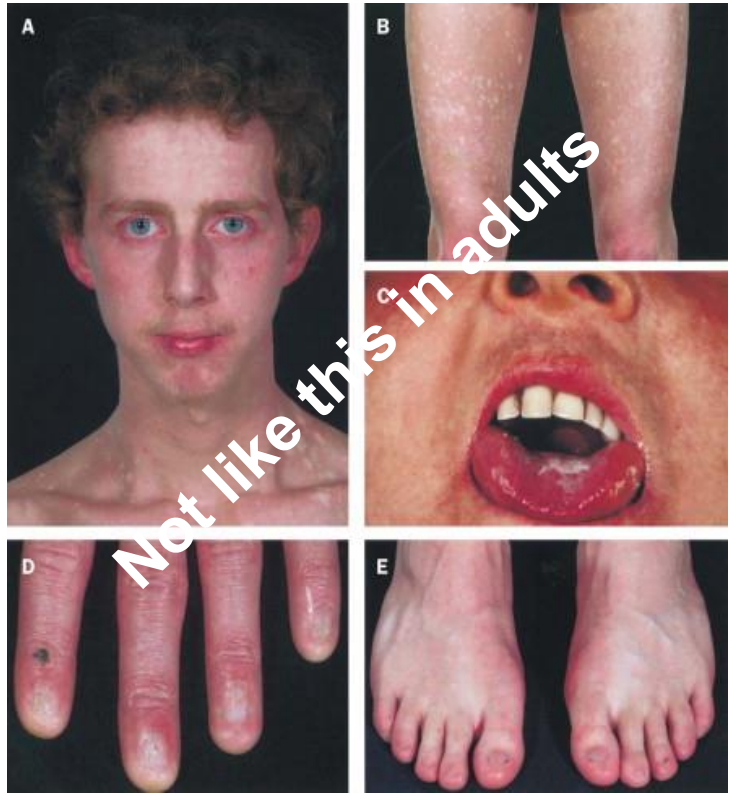
Telomere biology disorders – Clincial presentation

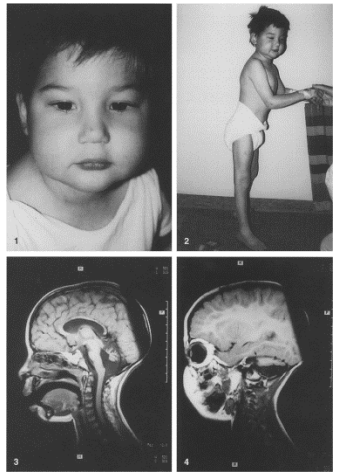


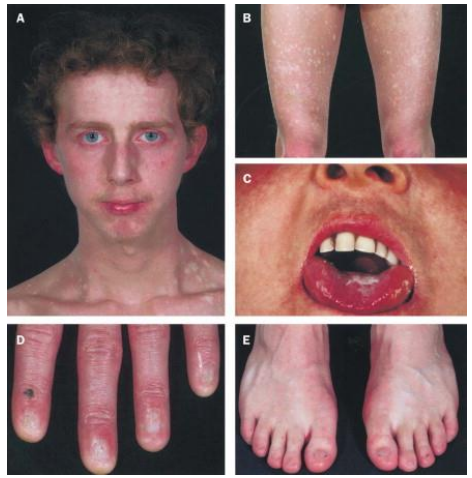
Clinical feature/abnormality	% of patients*
Major/common features	
Abnormal skin pigmentation	89
Nail dystrophy	88
BM failure	85.5
Leucoplakia	78
Other recognized somatic features	
Epiphora	30.5
Learning difficulties/developmental delay/ mental retardation	25.4
Pulmonary disease	20.3
Short stature	19.5
Extensive dental caries/loss	16.9
Esophageal stricture	16.9
Premature hair loss/greying/sparse eyelashes	16.1
Hyperhiderosis	15.3
Malignancy	9.8
Intrauterine growth retardation	7.6
Liver disease/peptic ulceration/enteropathy	7.3
Ataxia/cerebellar hypoplasia	6.8
Hypogonadism/undescended testes	5.9
Microcephaly	5.9
Urethral stricture/phimosis	5.1
Osteoporosis/aseptic necrosis/scoliosis	5.1
Deafness	0.8

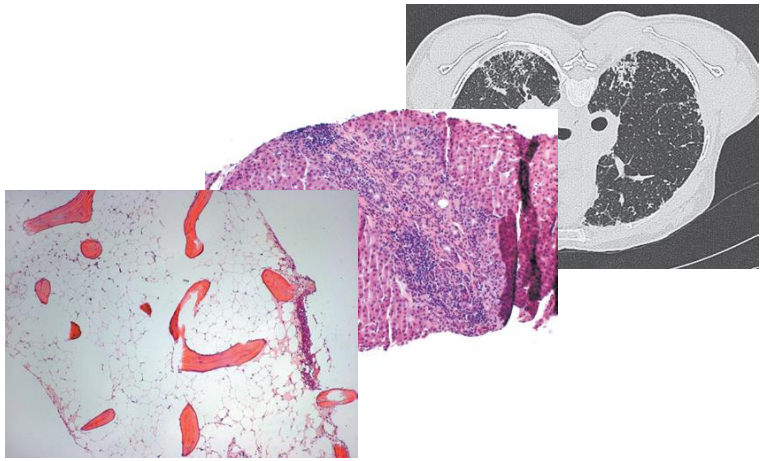
- Cryptic manifestations with **mono/ oligosymptomatic presentation of symptoms e.g. in adults as** sole aplastic anemia
- In case of other clinical features, frequently only subtle presentation

Dokal Lancet 2007, Dokal I ASH edu Book 2011

Definition of adult late-onset TBD (cryptic DKC)







Early childhood

Adolescence

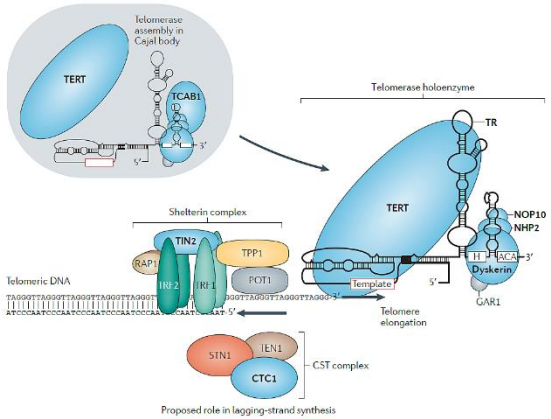
adults

10 years

20 years

30 years

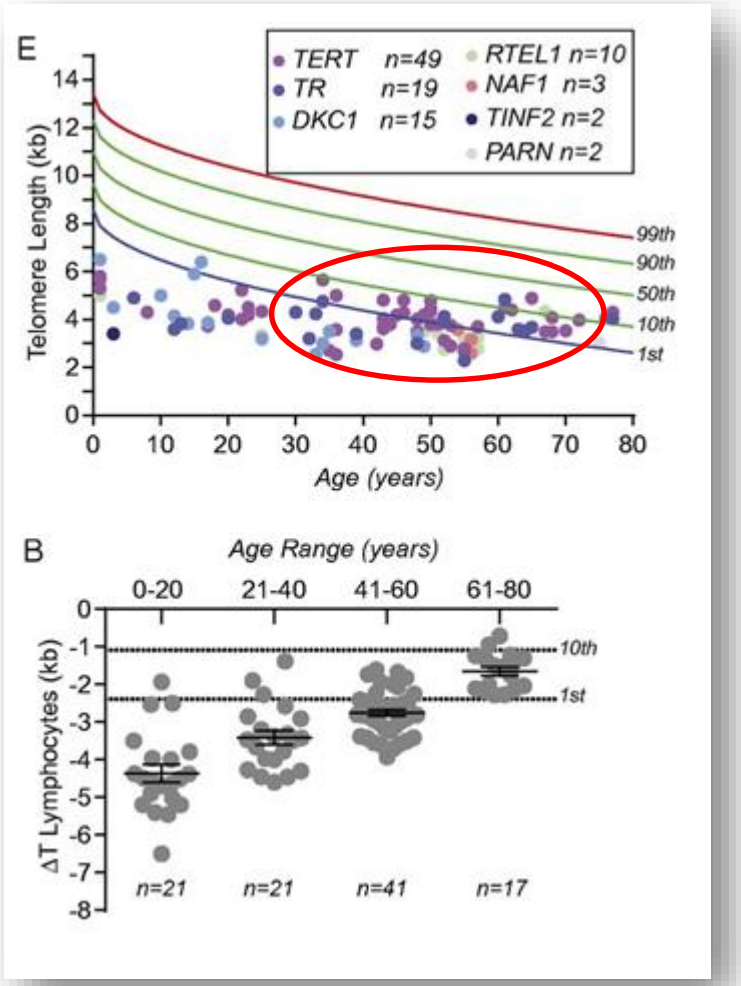
50/60 years



- Same mutation with different phenotypes
- Increased risk for cancer

Fernandaz Garcia, J Blood Med 2014, Dokal Lancet 2001, ASH educational 2011, Armanios Nat Rev Gen 2013

Challenges in the diagnosis of late onset Telomere biology disorders



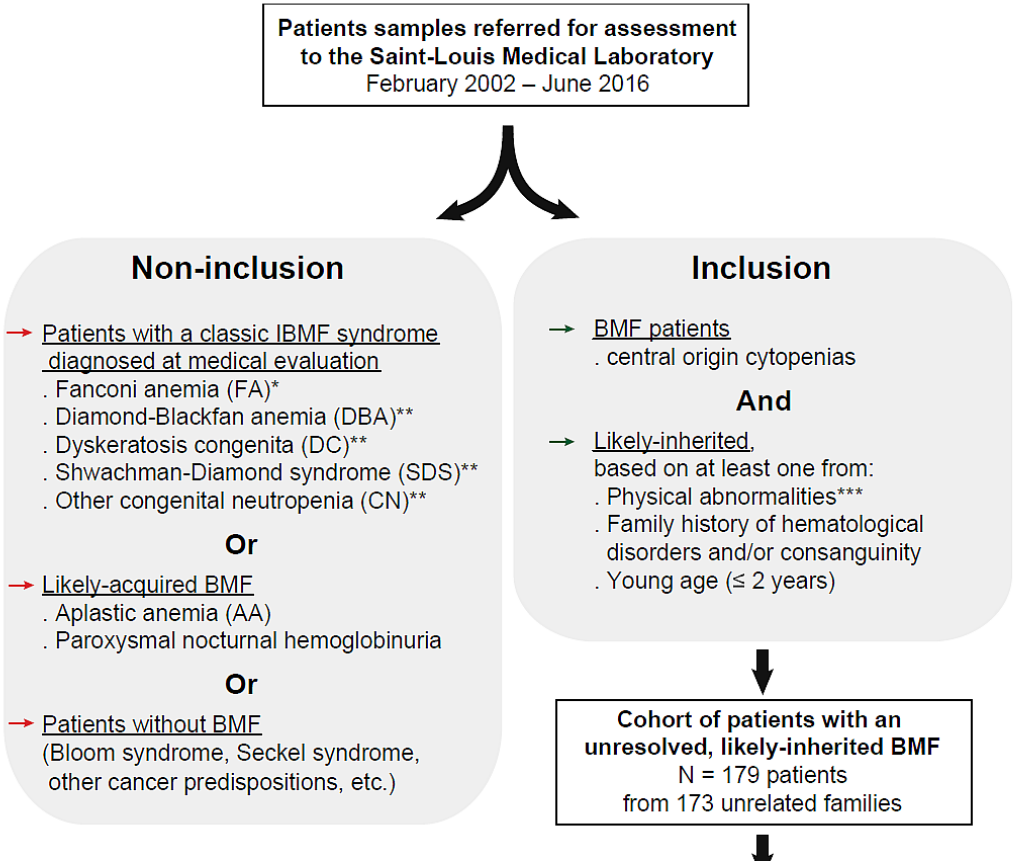
- Patients frequently mono/oligosymptomatic without typical DKC triad
- In patients >40 years telomere length screening is less specific
- Flow-FISH state-of-the- art for diagnosis
- Complicated validation of genetic variants

no consensus criteria – individual decision

Alder PNAS 2018, Ferreira MS Ann NY Ac Sci 2019

What is known about late onset TBDs and bone marrow failure ?

- Retrospective screening in AA patients based on clinical algorithm: Incidence between 1% to 4% for TERT/TERC mutations only ![1]
- Retrospective data identified 15% TBD (TERC, TERT, TINF2 a.o.) pts using physical abnormalities and family history[2]
- Detailed prospective data of adult patients with BMFS (incl. TL screening) in real life are missing



Bluteau Blood 2018 [2]

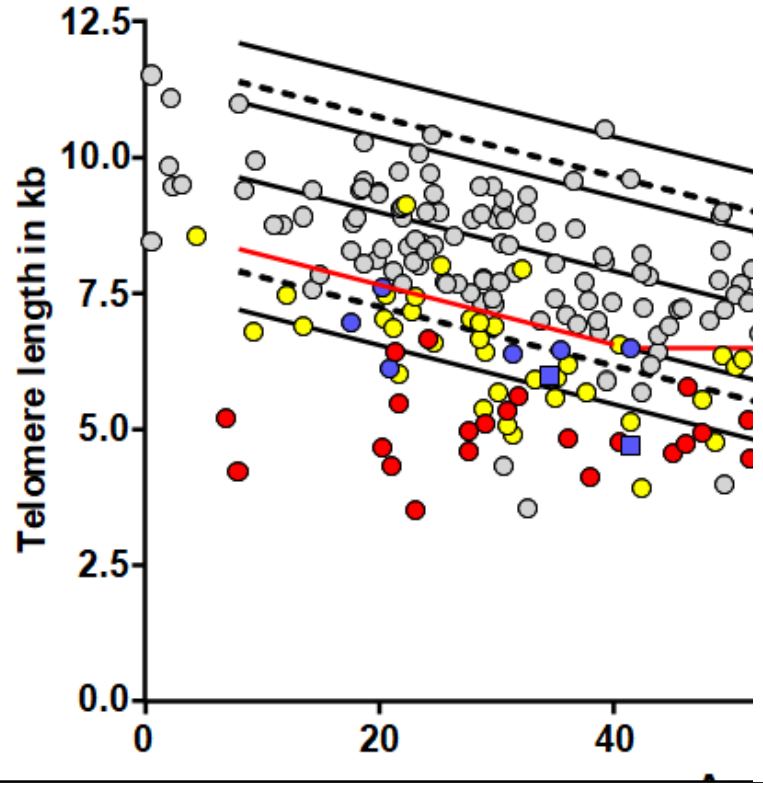
Yamaguchi Blood 2003, NEJM 2005 [1]

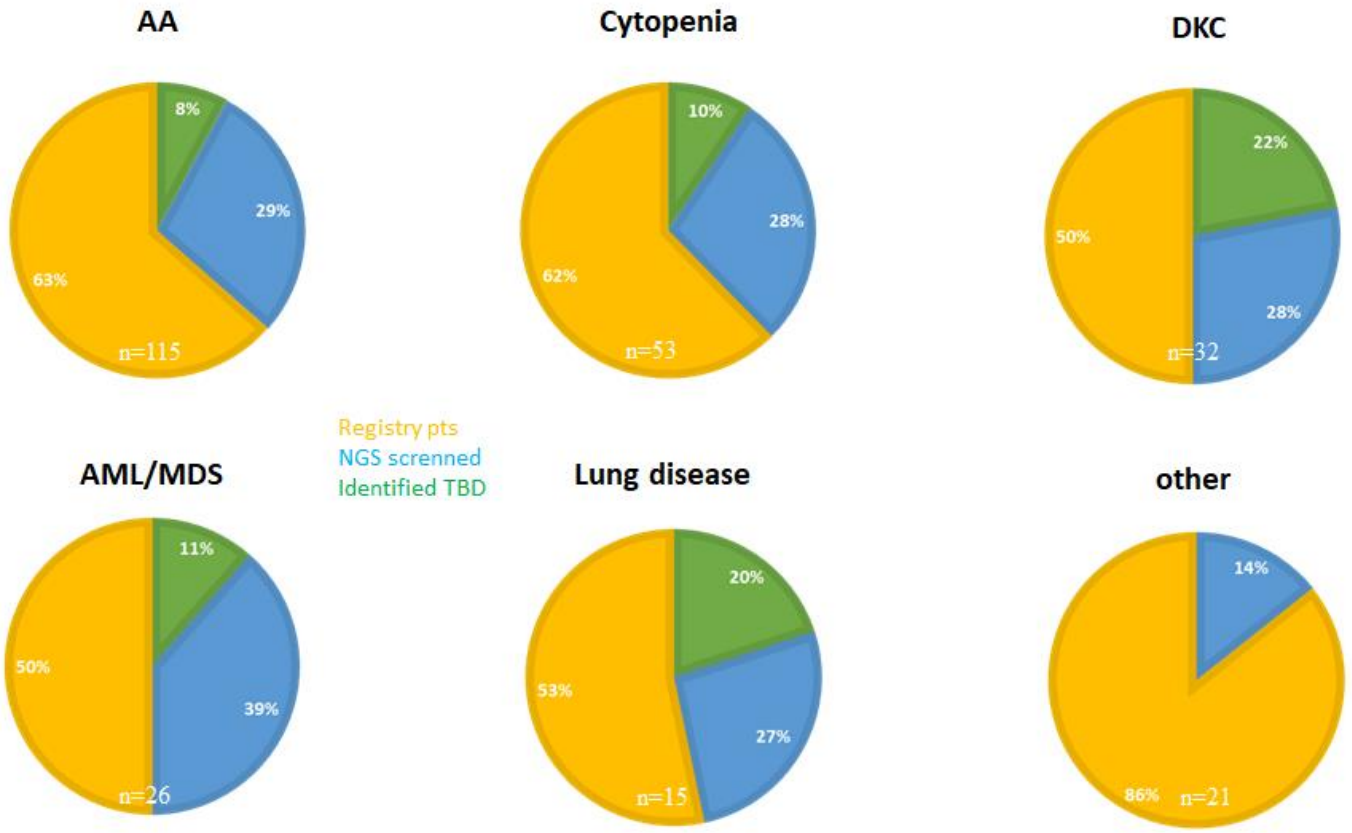
Data of the Aachen TBD-Registry (since 2014)

- **Inclusion criteria:** *"When the **treating physican suspects a TBD**, the patient will be included in the registry"*
- **No exclusion criteria**
- German speaking region ("informed consent" only in german)
- **Screening of TBD included in the German AA guidelines**
- Focus on **adult patients and** real life data
- No costs for the physician or patient !!

- 85% of the samples from University hospitals from Germany, Austria or Switzerland (n=35)
- 15% from local hospitals or local hematologist/pulmonologist (n=89)

Results of the Aachen TBD Registry TL Screening - the first 272 patients (2014-2017)

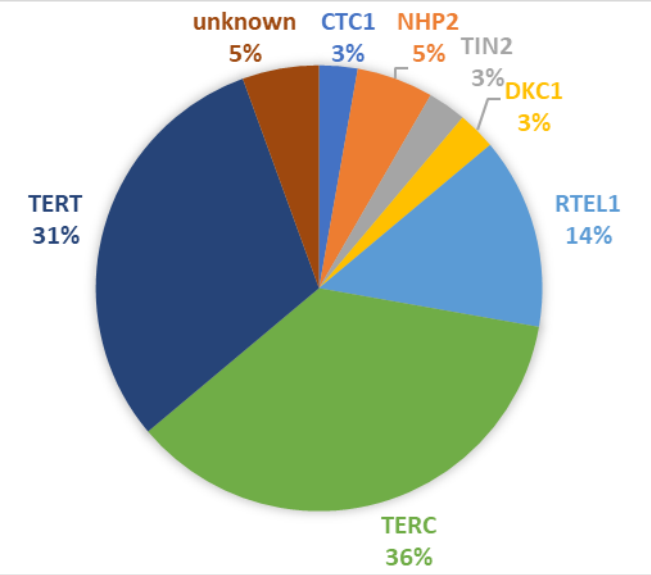




- **Aachen screening algorithm *(Telomere screening only !):***
- *All samples with TL(lymphcoytes) <10%, granulocytes <1% or TL <6.5 kb in pts >40 years are screend with NGS*
- Approx. **10%** confirmed TBD in **screening population**
- 50% of all pts with TL(lymphocyte) < 1% with pathogenic mutation

Clinical features of adult TBD patients: the Aachen cohort (2022)

	N=36
Mean age	36.7 years ± 18.5 SD
Years of follow-up	2.2 years ± 1.3 SD
Age at first manifestation	27.8 years ± 19.1 SD
Death during follow-up	25% (9/36)
Time from first man. to death	11.7 years ± 10.2 SD



	N=36
DKC typical stigmata	44% (75% 1-2)
Other DKC typical manifestations e.g. Epiphora	5%
Family history	72%
Early hair greying	33%
Leukopenia	92%
Anemia	78%
Thrombopenia	75%
Confirmed aplastic/hypocellular BM	70%
Detection of Clonal evolution(MDS gene)	14% (4/28)
Lung disase (mean DLCO)	41% (52)
Liver disease	25% (n=8)
Abnormal liver values (AST/ALT)	75% (n=6)
Enteropathy	5%
Psychiatric disorders	11%
Cardiac diseases	8%
Renal diseases	5%
Neurological dis. (Leukencephalopathy)	5%
Cancer	8%
Osteonecrosis	8%

BMF

breast, sarcoma, HD, NHL

Kirschner et al. Leukemia 2018; Beier and Brümmendorf unpublished data

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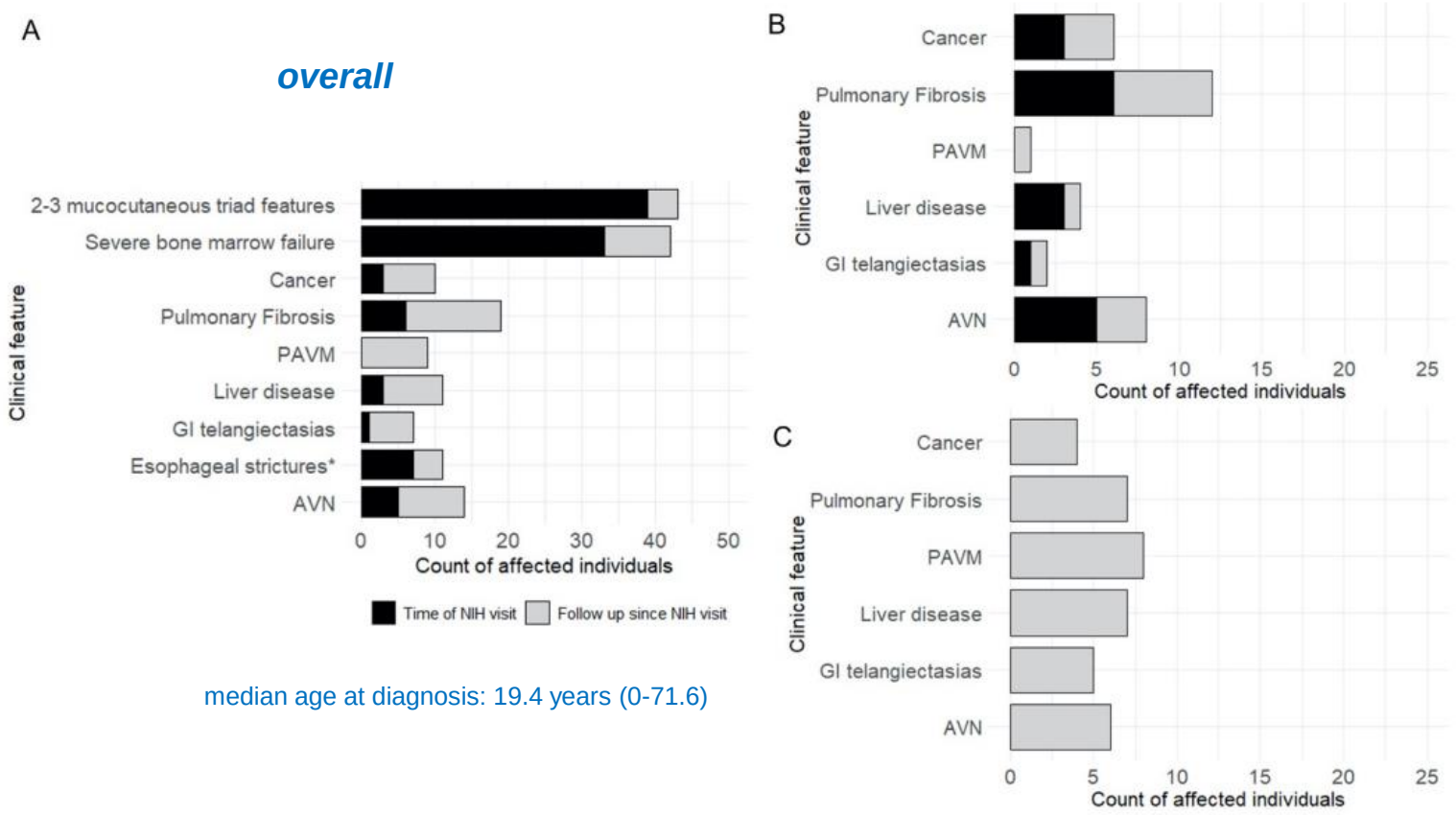
Development of clinical complications in patients with TBD over time (NIH Cohort)



median age at diagnosis: 19.4 years (0-71.6)

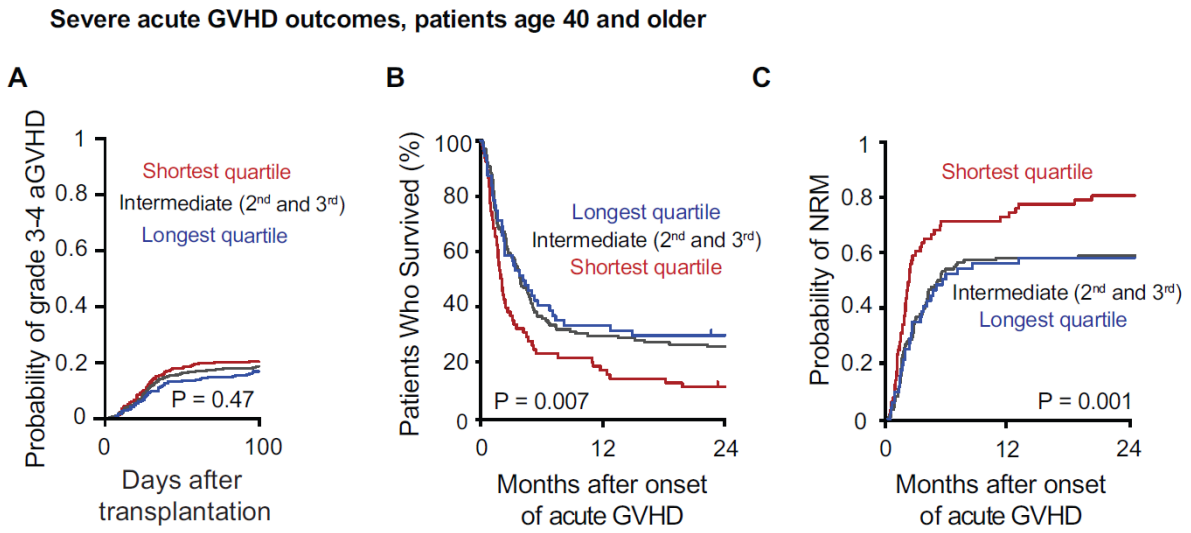
No allogeneic SCT

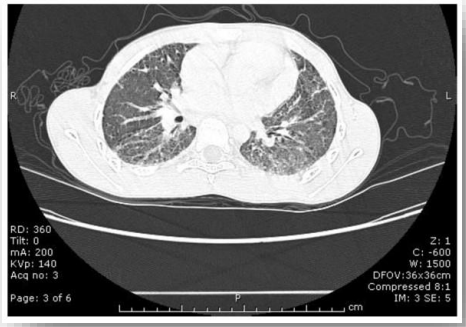
Following allogeneic SCT

Niewisch M.R. et al. Blood 2022

Consequence of diagnosis of underlying TBD in adult BMFS

- Very low probability of persisting response to **ATG**
- High response rates under **androgen treatment**: causal (!) treatment: increasing telomerase activity in defined TBD genotypes, particularly TERC/TERT)
- **Increased risk** of
  - secondary MDS/AML and
  - solid cancer (cancer prevention !)
- Implications for family members (disease anticipation):
  - genetic counselling and cancer prevention
  - role as **potential stem cell donor**
- Implications for allogeneic stem cell transplantation:
  - **High TRM** due to GVHD related complications
  - Differential dx: e.g. chronic lung/liver GVHD vs lung/liver fibrosis
  - in TBD, lung fibrosis main cause of death after allo SCT
  - **Radiation sensitivity**: avoid TBI-containing protocols





Giri BMC Blood Dis, 2011, Myllikmäki Blood 2020

Take home messages

1. Hereditary BMFS (10%) are severely underdiagnosed in adults (think of it !)
2. Clinical presentation regarding affected organ system highly variable
3. Typical DKC trias mostly absent in adults
4. No consensus criteria for diagnosis of adult TBD (cryptic DKC) established
5. Proper diagnosis of TBD has important clinical implications for patients and family members

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Thanks to all co-workers

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Stiftung zur Hilfe bei PNH/AA

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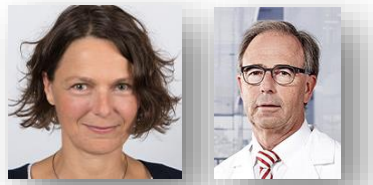

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