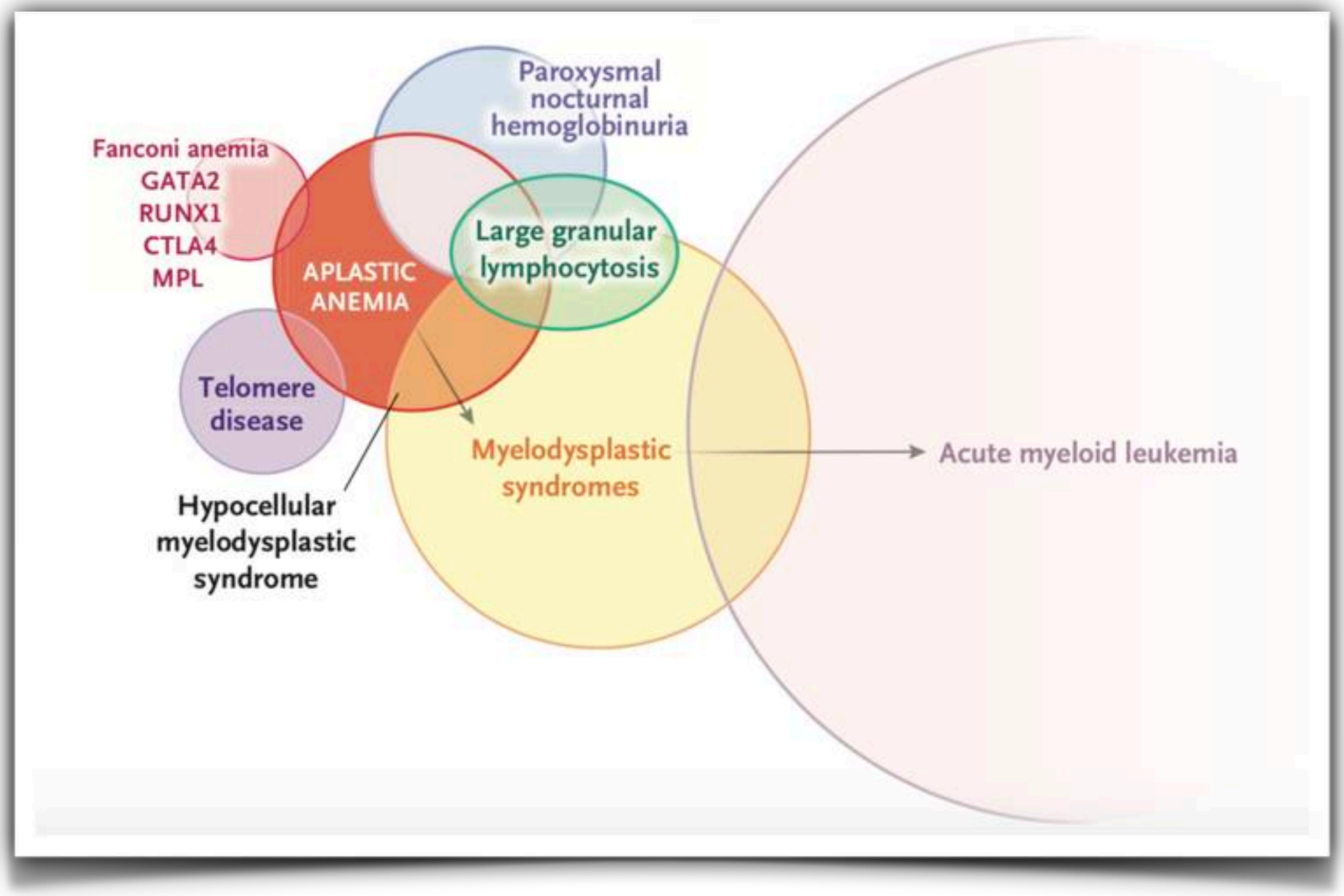
Age associated B cells in acquired aplastic anemia

15th Online Discussion Club (ODC) by the Young-EuNet-INNOCHRON
February 9th, 2023, 15:00- 16:30 CET

Elena E Solomou,MD
Associate Professor Internal Medicine-Hematology
University of Patras Medical School
Greece

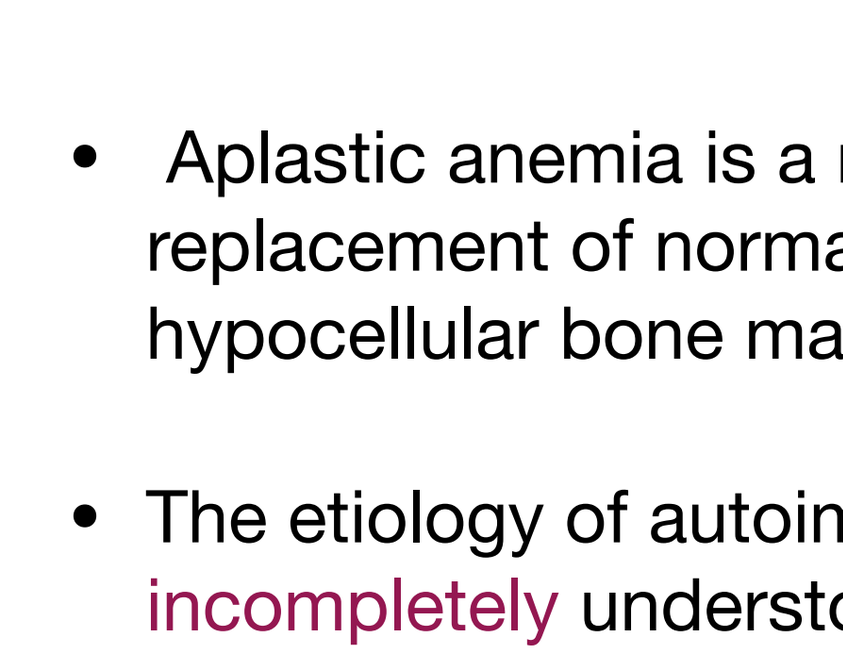
Bone marrow failure syndromes

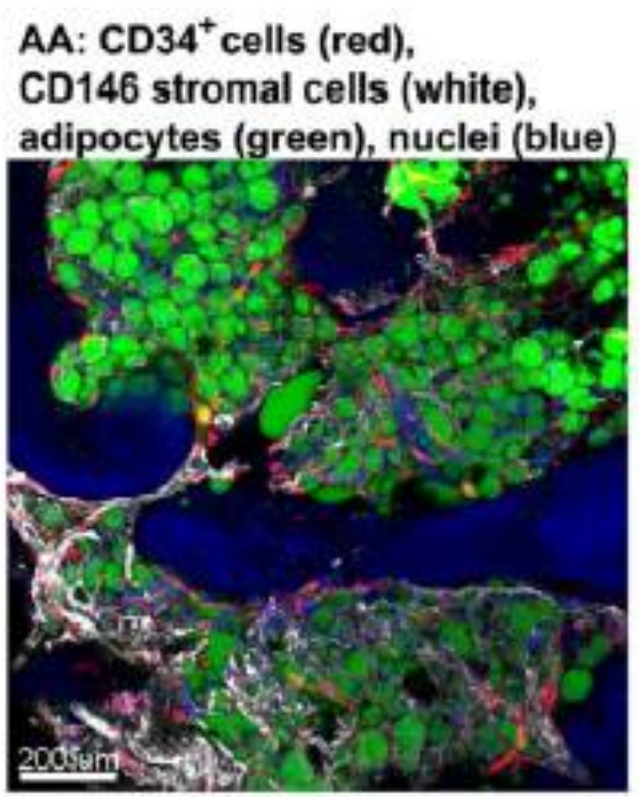


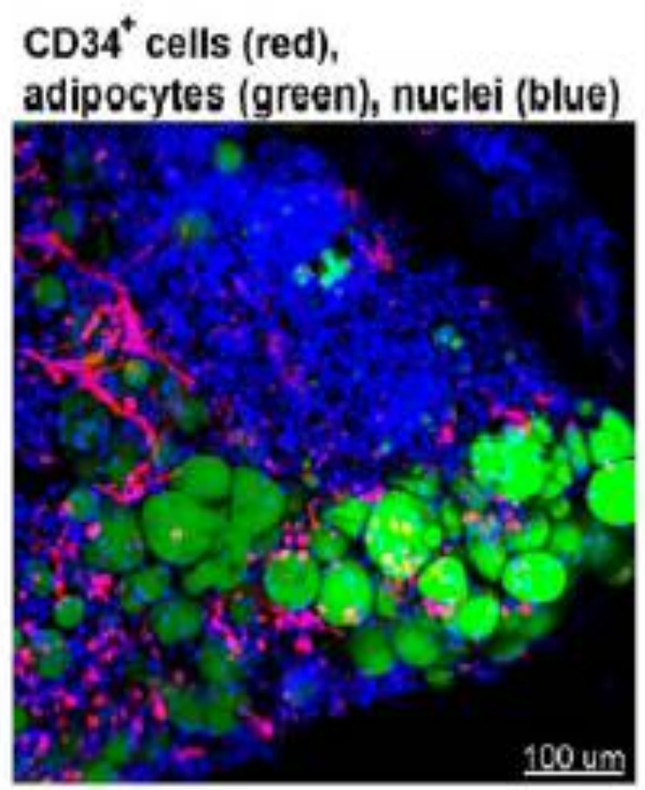
Neal S Young, NEJM October 25th 2018

- Aplastic anemia is a rare autoimmune disease characterized by replacement of normal hematopoietic tissue by fat resulting in hypocellular bone marrow and peripheral blood pancytopenia

- The etiology of autoimmune diseases is complex and incompletely understood

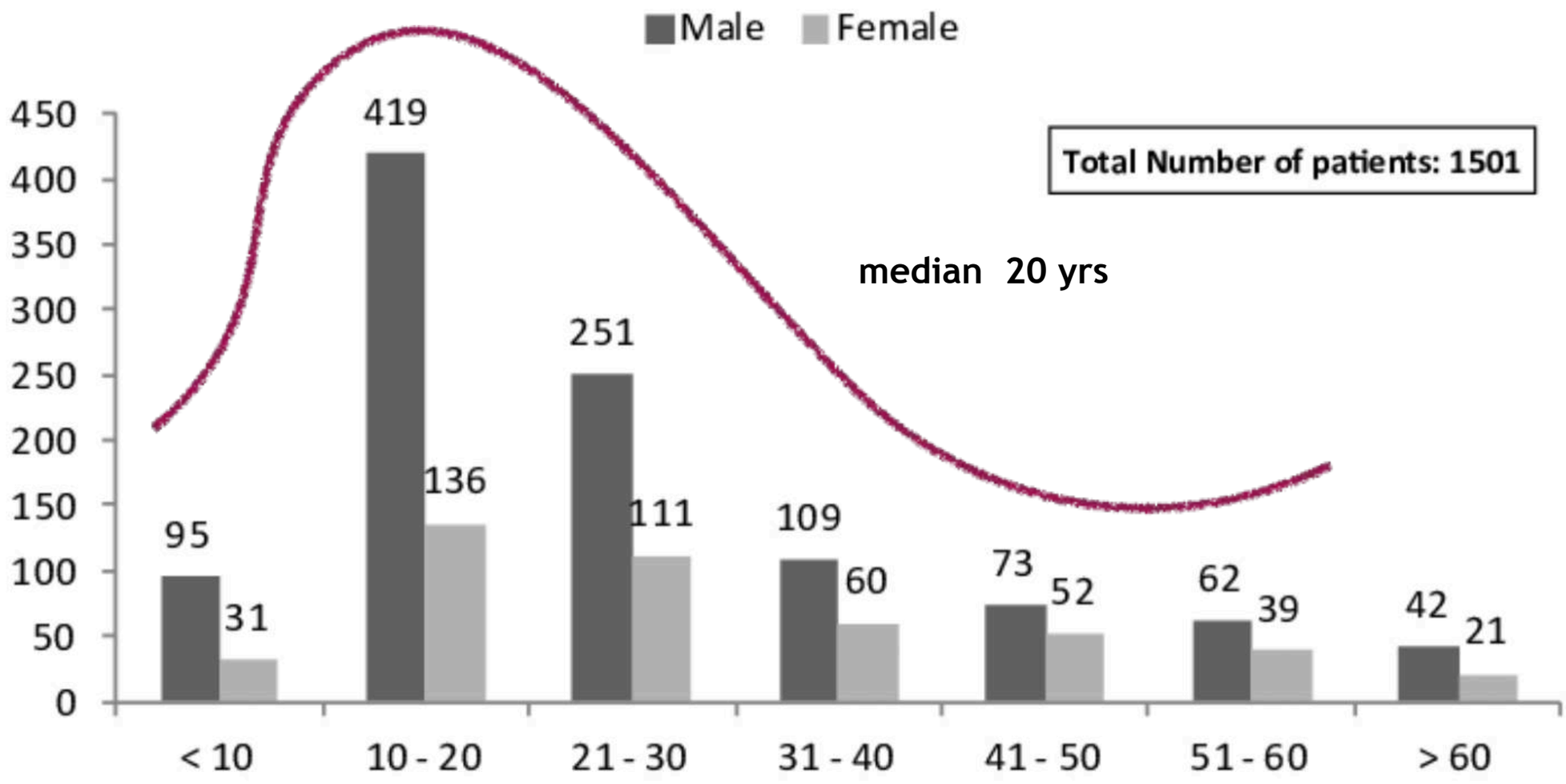






Takaku T et al, Blood 2010; 116: e41-e55

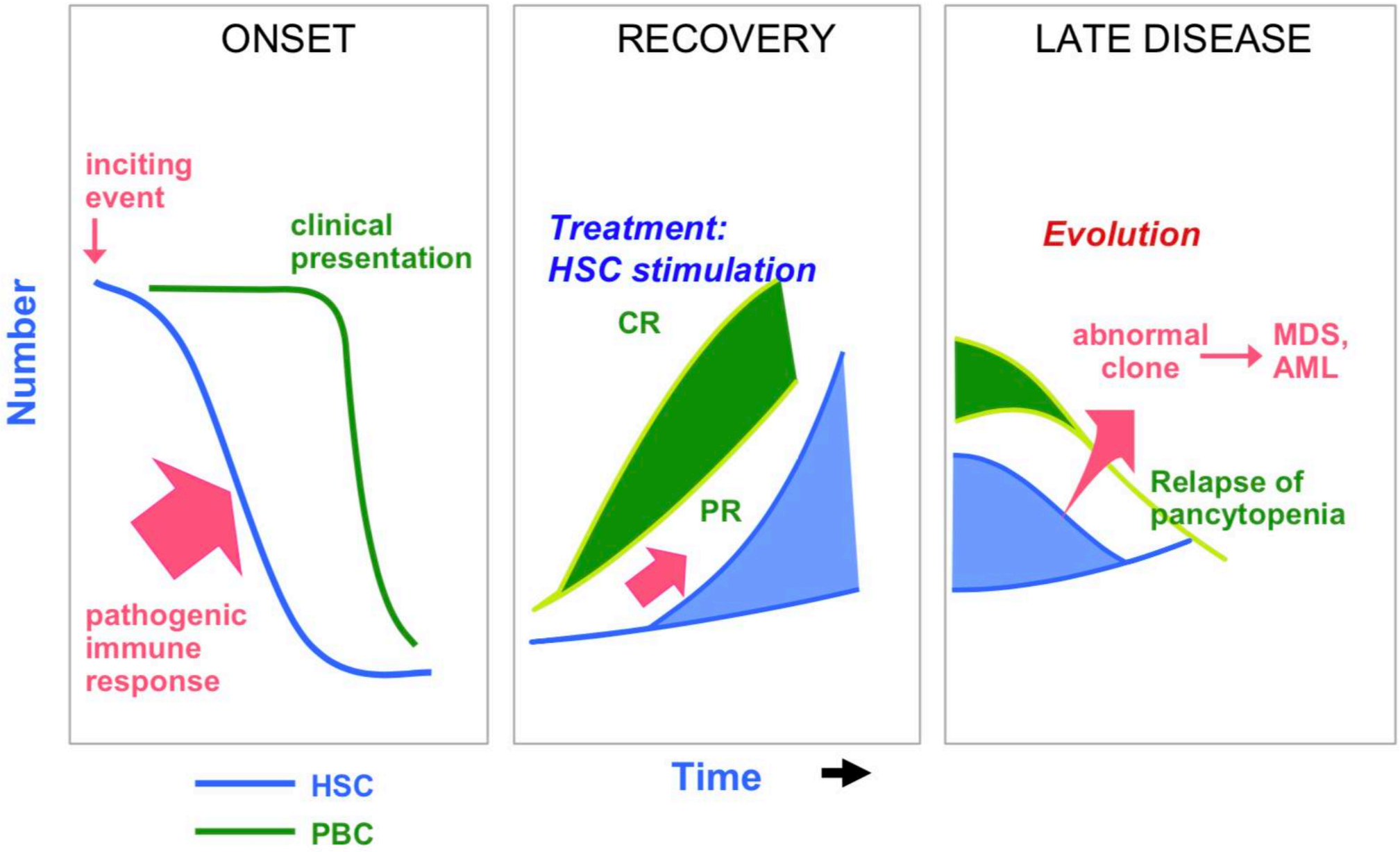
Age Distribution - By Gender



Total Number of patients: 1501

median 20 yrs

SIMPLIFIED PATHOPHYSIOLOGY OF ACQUIRED APLASTIC ANEMIA



Young NS and Maciejeski JP, N Engl J Med 2007; 336:1665-1672

Grading of severity of Aplastic Anemia:

Marrow cellularity < 30%

Severe Aplastic Anemia: Peripheral Blood: two of three values:
ANC < 500
PLT < 20.000
Reticulocytes < 1% or <20.000 (absolute)

Very Severe Aplastic Anemia: As above but ANC < 200

Non-severe (moderate) Aplastic Anemia: Marrow cellularity < 30%
ANC > 500
RBC or PLT transfusion dependent

Aplastic Anemia

Causes

Drugs, radiation, toxic agents….

Idiopathic
Hepatitis
Eosinophilic fasciitis

Telomere disease
Mutations
Fanconi anemia…

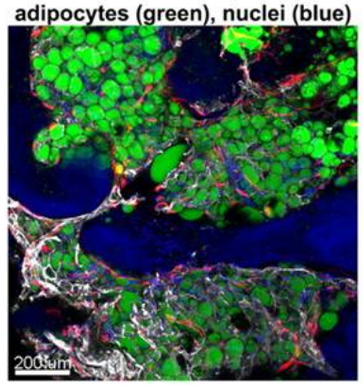
Pathophysiology

Toxic agents destroy the BM

Immune destruction

Mutations -genetics

A AA: CD34^{+}cells (red), CD146 stromal cells (white), adipocytes (green), nuclei (blue)



Takaku T et al, Blood 2010; 116: e41-e55

Replacement of the BM cells with fat —> hypoplastic BM and pancytopenia in the peripheral blood

Drugs and Chemicals Associated With Acquired Aplastic Anemia[a]

Drugs and Chemicals	Examples
Allopurinol	
Antibiotics	Chloramphenicol, streptomycin, tetracycline, methicillin, mebendazole, sulfonamides, trimethoprim/sulfamethoxazole, flucytosine
Anticonvulsants	Hydantoins, carbamazepine, phenacemide
Antidiabetes drugs	Tolbutamide, chlorpropamide
Antihistamines	Cimetidine, ranitidine, chlorpheniramine
Antiprotozoals	Quinacrine, chloroquine
Antithyroid drugs	Methimazole, methylthiouracil, propylthiouracil
Benzene	
Carbimazole	
Carbonic anhydrase inhibitors	Acetazolamide, methazolamide
Cytotoxic drugs used in cancer chemotherapy	
Estrogens	
Gold	
Insecticides	
Lithium	
Methyldopa	
Nonsteroidal anti-inflammatory drugs	Phenylbutazone, indomethacin, ibuprofen, sulindac, aspirin
D-penicillamine	
Potassium perchlorate	
Quinidine	
Sedatives	Chlorpromazine, prochlorperazine, piperacetazine, chlordiazepoxide, meprobamate, methyprylon

In most cases acquired aplastic anemia is idiopathic and no etiological factor can be found

- These are the cases where immune pathophysiology mainly exists—>
  - cytotoxic T lymphocytes appear to be functionally and phenotypically activated,
  - skewed to Th1 phenotype and are responsible for the increased IFN-γ production and subsequent Fas-mediated death of the hematopoietic stem cells in the bone marrow.

The NEW ENGLAND JOURNAL of MEDICINE

January 31, 1985
N Engl J Med 1985; 312:257-265

Circulating Activated Suppressor T Lymphocytes in Aplastic Anemia

Nicholas C. Zoumbos, M.D., Ph.D., Pedro Gascón, M.D., Ph.D., Julie Y. Djeu, Ph.D., Stephen R. Trost, B.A., and Neal S. Young, M.D.

Interferon is a mediator of hematopoietic suppression in aplastic anemia in vitro and possibly in vivo.

N C Zoumbos, P Gascon, J Y Djeu, and N S Young Authors Info & Affiliations

PNAS
January 1, 1985 | 82 (1) 188-192 | https://doi.org/10.1073/pnas.82.1.188

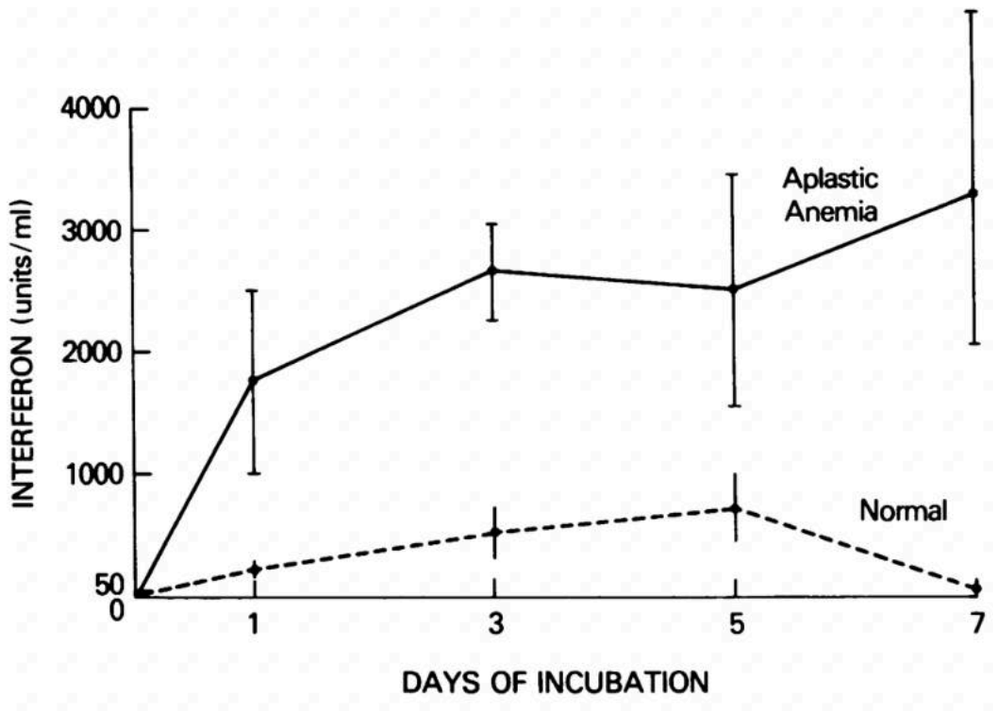
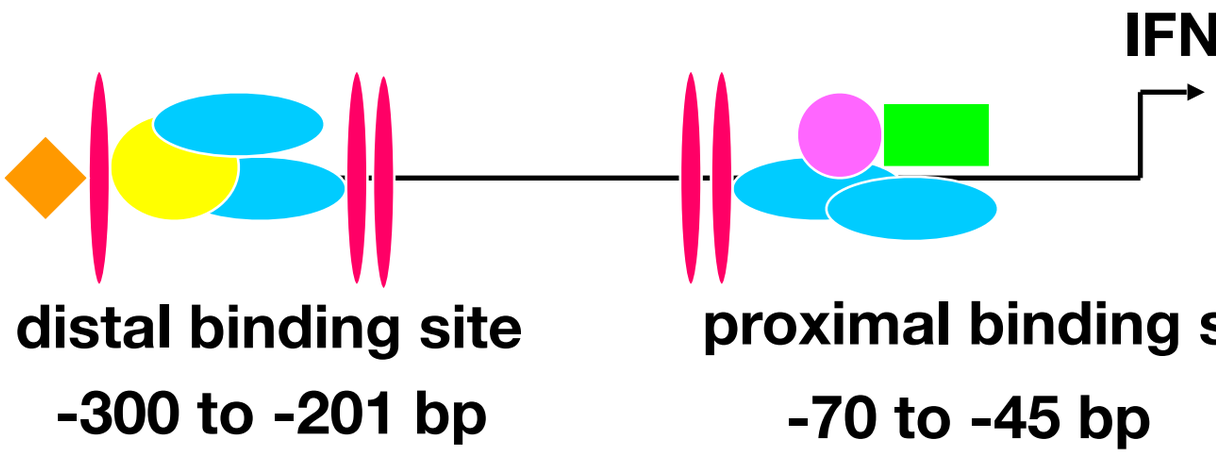


Table 2. Circulating interferon levels

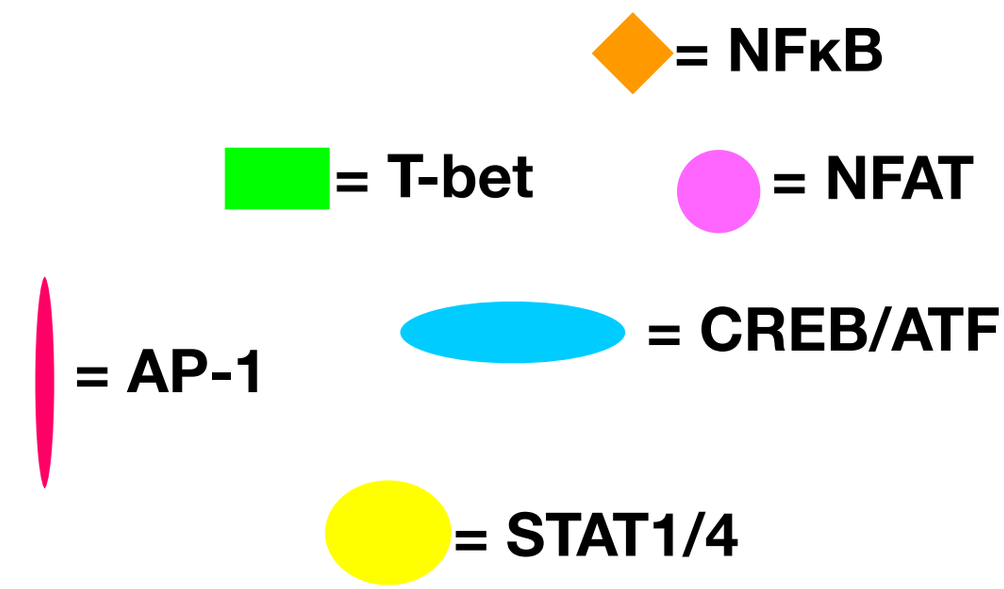
	n	Interferon, IU/ml	No. > 10 IU/ml
Aplastic anemia patients	24	87 ± 30	10/24
Normal persons	16	<10	0/16
Multiply transfused patients with hereditary diseases	18	<10	0/18

• IFN-γ is the hallmark cytokine of the Th1 immune response

• Regulation of the IFN-γ production occurs primarily at the level of transcription



IFN-γ promoter binding sites

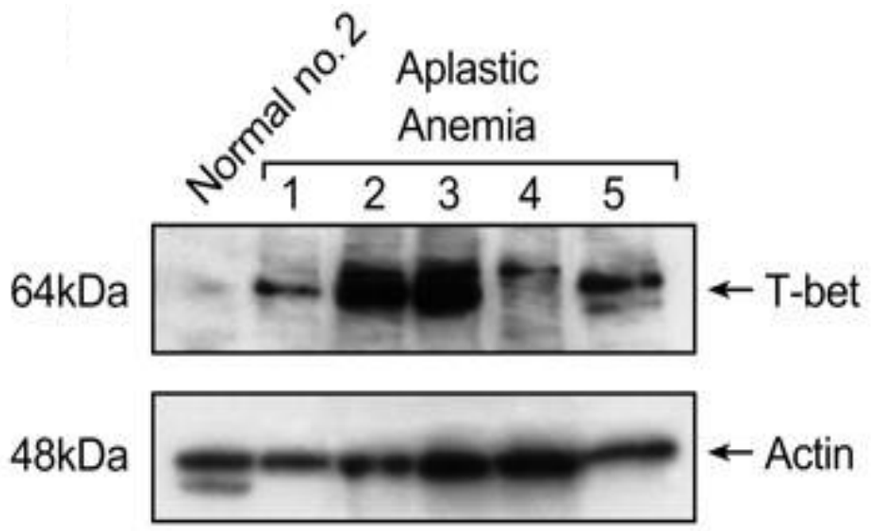


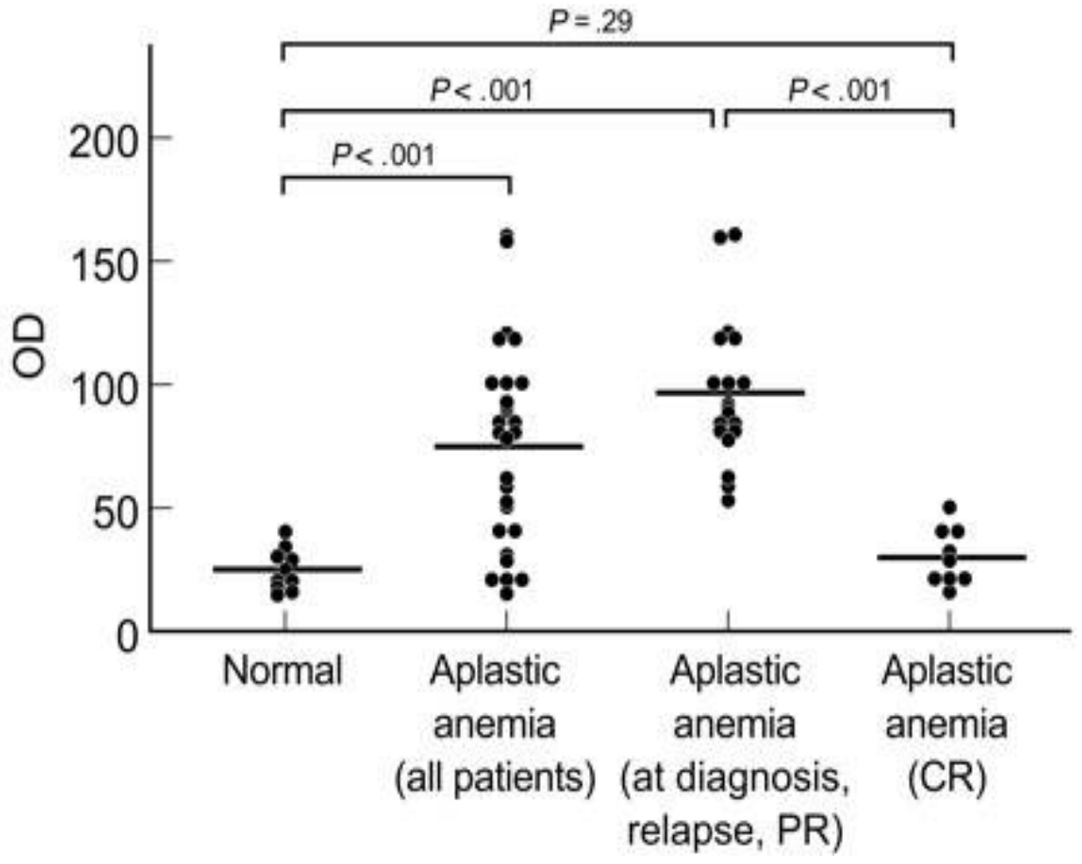
blood

IMMUNOBIOLOGY | MAY 15, 2006

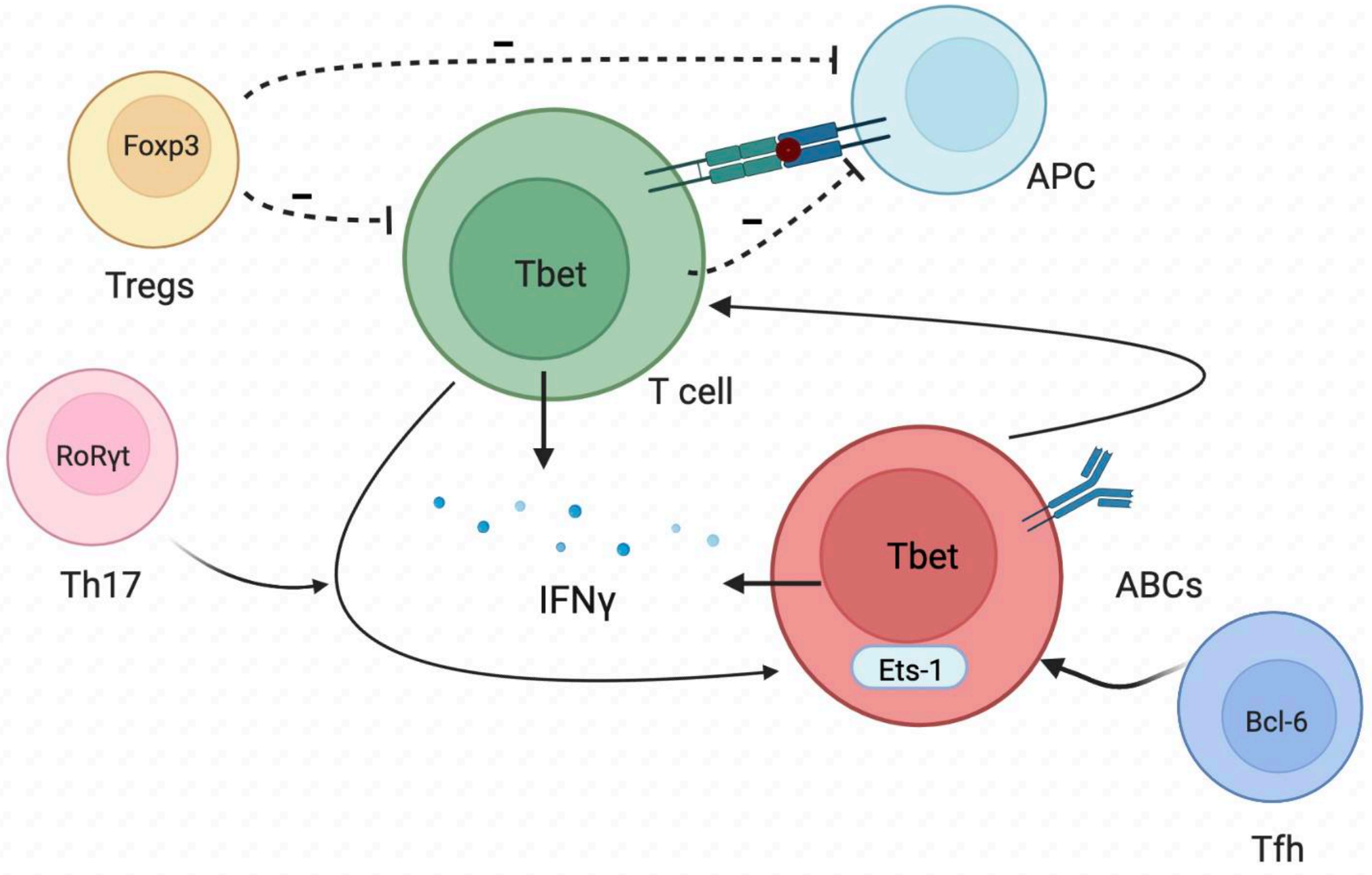
T-bet, a Th1 transcription factor, is up-regulated in T cells from patients with aplastic anemia

Elena E. Solomou, Keyvan Keyvanfar, Neal S. Young





Normal T cell activation and IFN-γ production



Solomou EE et al, unpublished data

Proposed Mechanism in Acquired Aplastic Anemia

T follicular helper

Bcl-6

???

Tbet

Age-related B cells (ABCs)

+

+

APC

Ag

ζ-chain

SAP

Prf1

Itk

Ca++

Tbet

FoxP3

Treg

T cell

IFN-γ

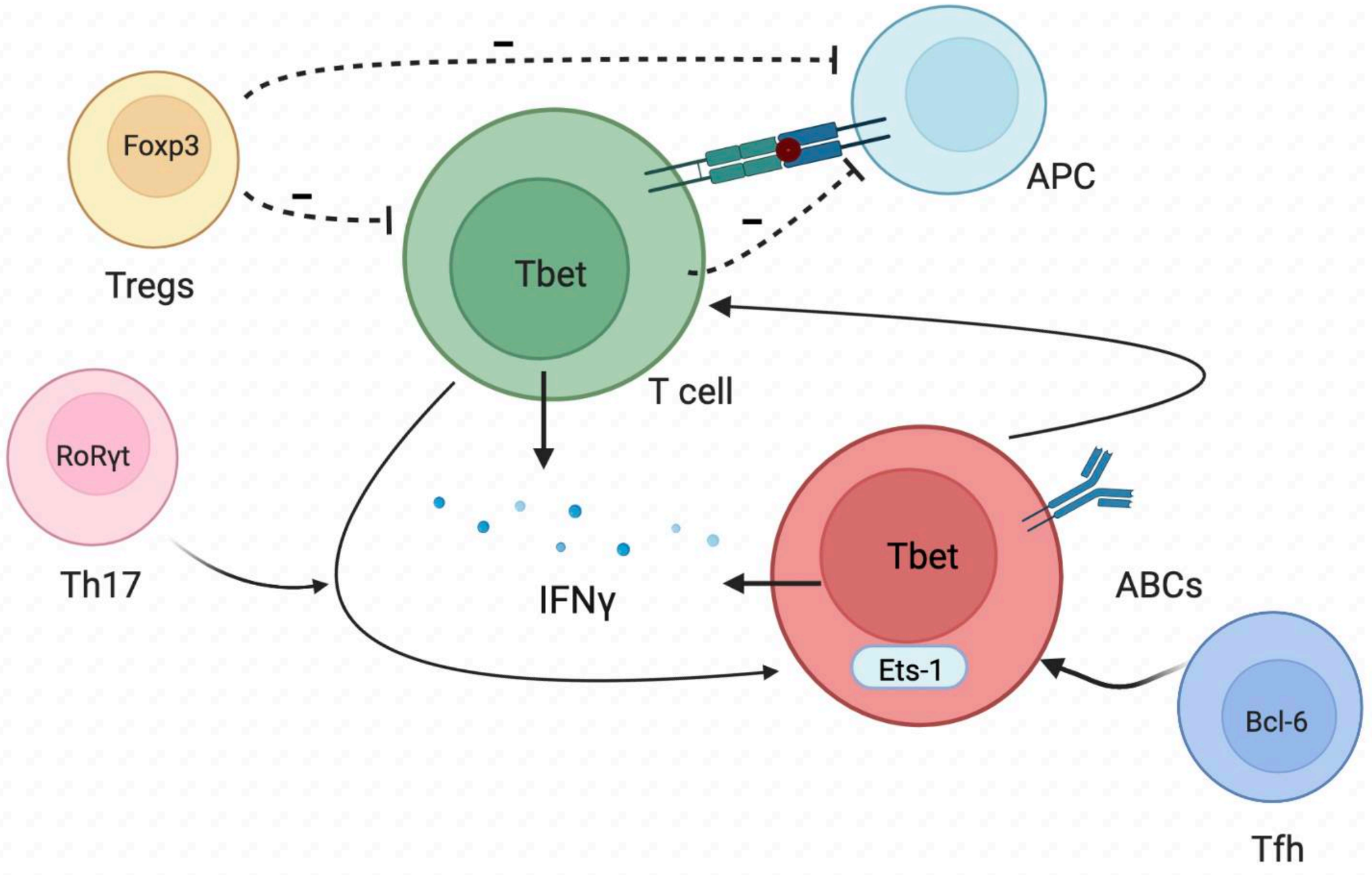
Fas-mediated apoptosis
HSC destruction

Th17

RORγt

Solomou EE

Normal T cell activation and IFN-γ production



Solomou EE et al, unpublished data

Age-associated B cells (ABCs)

- Very recently it was shown that a subpopulation of B cells also express T-bet
- These cells are characterized as age-associated B cells (ABCs) and express high levels of CD11c and CD19, they are CD21 negative and express T-bet.
- This unique population of memory B cells (T-bet+ ABCs) are found increased in patients with autoimmune diseases (i.e. systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis) and in animal models of autoimmunity.
- The role of ABCs in human autoimmune diseases is still under investigation, but data from animal models show that ABCs have a critical role in the onset and development of autoimmunity
- ABCs are responsible for autoAbs and inflammatory cytokine production (IFN-γ, TNFα, IL-17), and stimulation of T cells.

Age-associated B cells (ABCs)

- ABCs represent ~1% of circulating B cells, ~5% or more in pts with SLE and correlate with disease activity
- ABCs continuously differentiate from the peripheral blood B cell population. During inflammation they exit the spleen and circulate in the blood.
- ABCs differentiation requires a combination of stimuli: Engagement of BCR, and engagement of TLR7/9 and exposure to IFN-γ and IL-21.
- TLR7/9 can be stimulated by microbial infections, cellular debris, and chromatin.
- TLR7/9 is critical but not sufficient for ABCs appearance; Subsequent IFN-γ and IL-21 exposure is required that is CD40 dependent.
- T follicular helper cells are critical for the development of ABCs because they provide cytokines and stimulation of CD40.

Age-associated B cells (ABCs)

- Stimulation of B cells with antigens, Toll-like receptors and IFN-γ leads to the formation of ABCs, which in turn “talk” to the T cells and stimulate them.

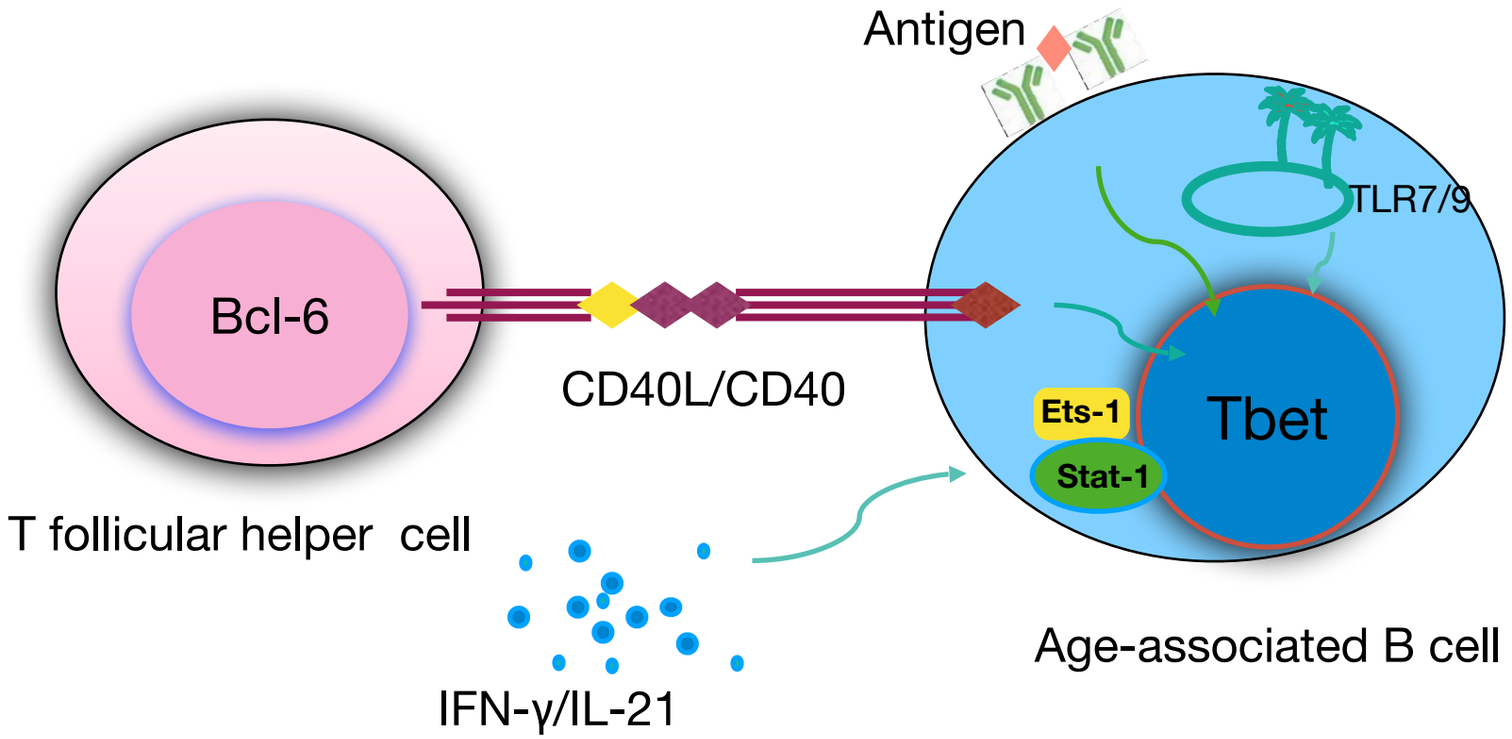
- Stimulation of T cells leads to IFN-γ production, and this IFN-γ may lead to further induction of ABCs.

- It was also proposed that by eliminating B-cell-intrinsic T-bet, T cell activation can be diminished and IFN-γ production thus be reduced.

- The molecular mechanisms that control the expansion and function of ABCs are not completely understood; IFN-γ and IL-21 can promote T-bet expression in B cells, but IL-4 antagonizes this effect. The transcription factor Ets-1 is essential along with Stat-1 for T-bet expression in B cells

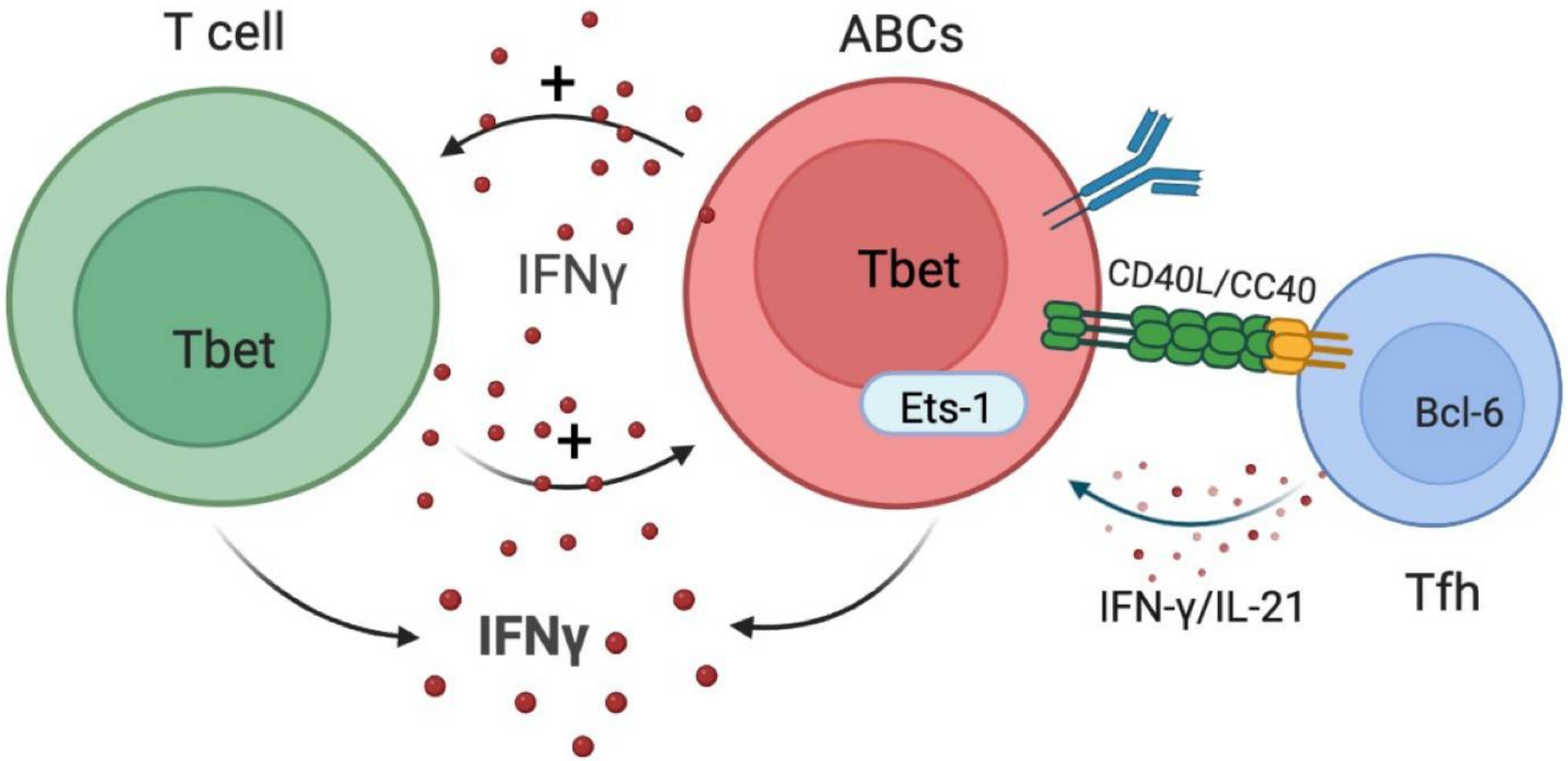
Age-associated B lymphocytes

Solomou EE et al, unpublished data



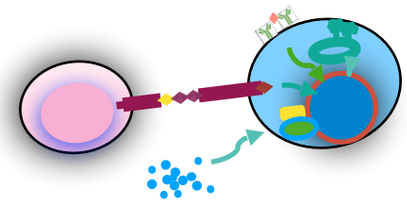
ABCs	Healthy	Autoimmune diseases
Number	Low	Increased
Localisation	Blood, spleen, bone marrow	Blood, spleen, affected organs
Function	Production of anti-viral abs and cytokines	Activation of T cells AutoAbs Inflammatory cytokines
Cytokines	IFN-γ, TNF, IL-4, IL-10, IL-17	Increased IFN-γ, TNF, IL-17, IL-6

"Cross-talk" between T cells and ABCs



Solomou EE et al, unpublished data

T-follicular Helper cells



- T-follicular helper cells represent a sub-population of CD4+helper T cells (TFH), characterized by the surface expression of CXCR5, ICOS, and PD1, the transcription factor Bcl-6, and produce mainly IL-21, but also IL-17, IL-4, and IFN-γ.

- They represent the major population that helps B cells to turn into plasma cells and produce antibodies. They are critical for the development of ABCs because they provide the essential cytokines and stimulation of CD40.

- Tfh are located in secondary lymphoid organs, including the tonsil, spleen and lymph nodes but a small population is circulating in the blood (p-TFh).

- They play a critical role in protective immunity and are also implicated in the pathogenesis of different autoimmune diseases.

- p-TFh cells have been extensively studied in the context of inflammation and autoimmunity. Patients with systemic lupus erythematosus and rheumatoid arthritis have increased p-TFh

Aim of the study

- In this study we want to explore the possible role of ABCs in patients with aplastic anemia, since their role has never been studied

- Determine the percentages of ABCs and pTfh in patients with acquired aplastic anemia. Differences in disease status?

- Investigate the mechanisms that are involved in ABCs expression in acquired aplastic anemia.

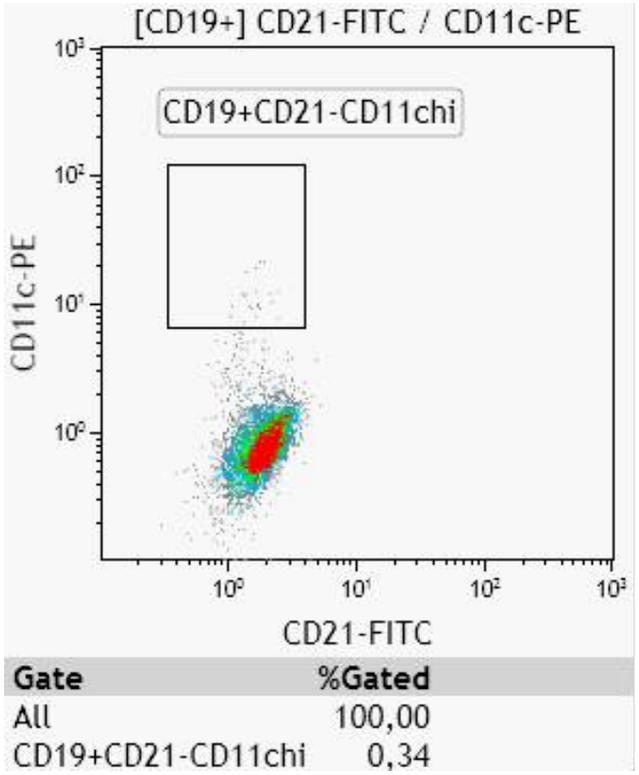
- Correlation of ABCs in acquired aplastic anemia with IFN-γ.

Methods

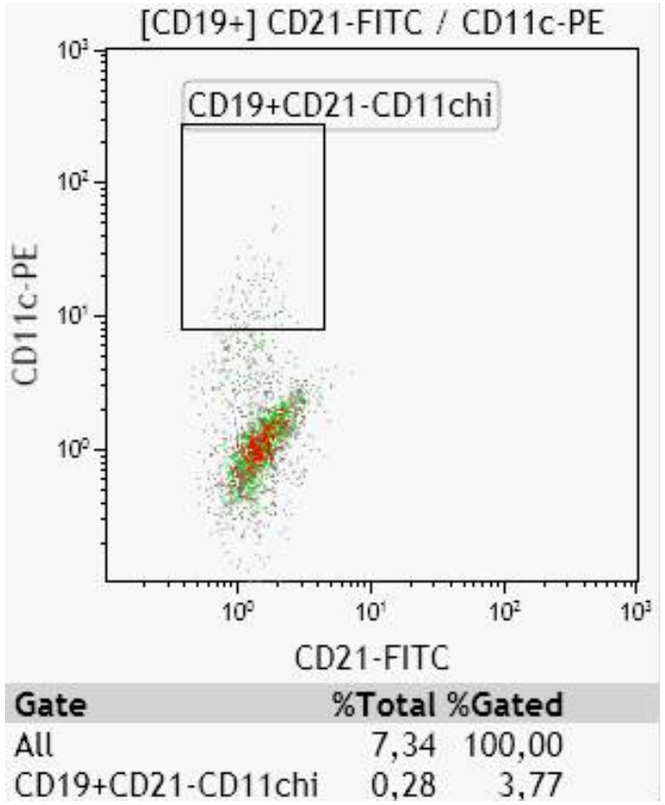
- Severity of aplastic anemia was defined based on standard criteria.
- We isolated peripheral blood mononuclear cells (PBMCs) from patients with aplastic anemia (n=21, age 8-50 years, 7 patients were children/adolescent) and 20 healthy, age-matched controls.
- Written informed consent was obtained from all study subjects or their legal guardians/parents.
- Cells were stained with the surface markers CD11c, CD19 and CD21, and intracellular Tbet for ABCs, and subsequently analyzed using flow cytometry .
- For the TFh cells CXCR5+ICOS+CD4+ T helper cells (p-TFh) are the circulating component of TFh.

Results

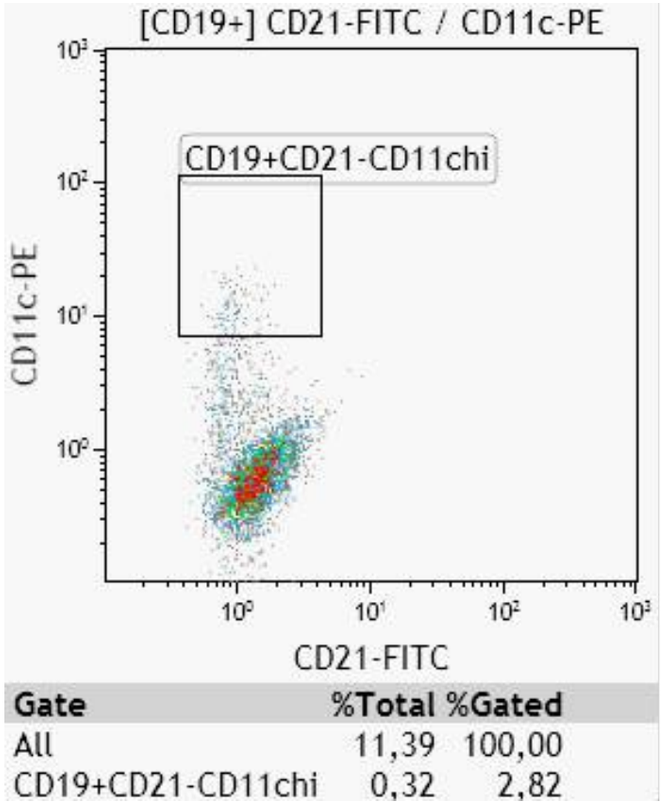
- Patients with aplastic anemia at presentation showed increased numbers of circulating ABCs compared to healthy controls (**2.71** ± 0.39% vs **0.43** ±0,09% respectively, p=0.022)



Healthy Control



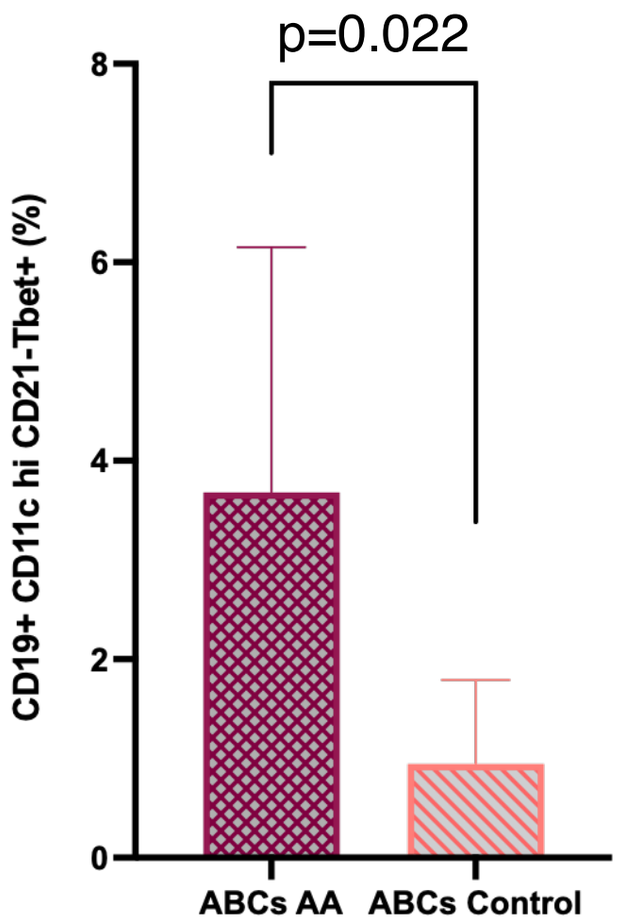
Aplastic Anemia #1



Aplastic Anemia #2

Solomou EE et al, unpublished data

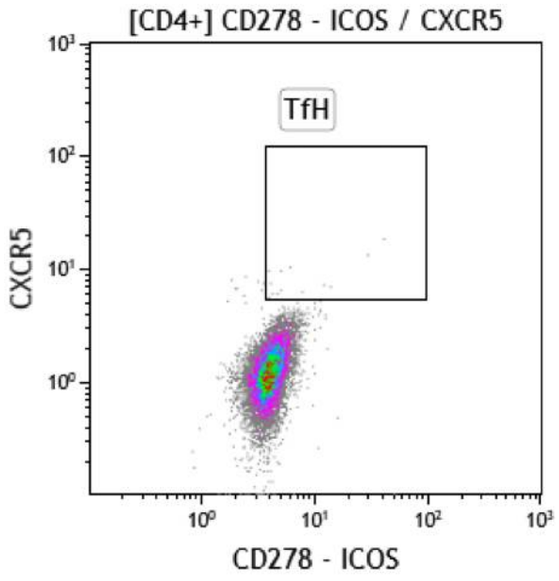
Increased Age-associated B cells in patients with Aplastic Anemia

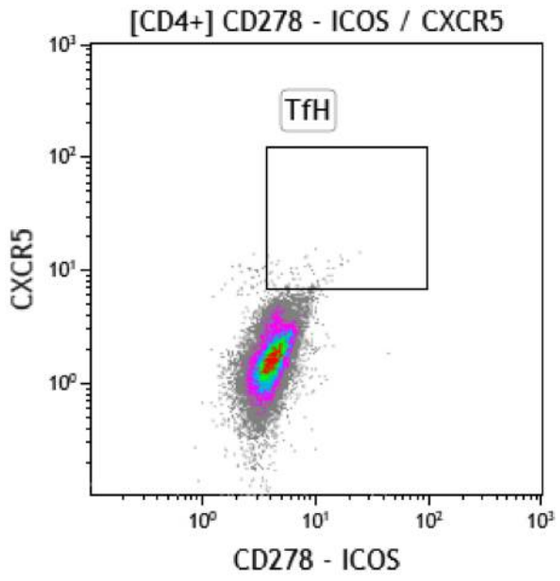


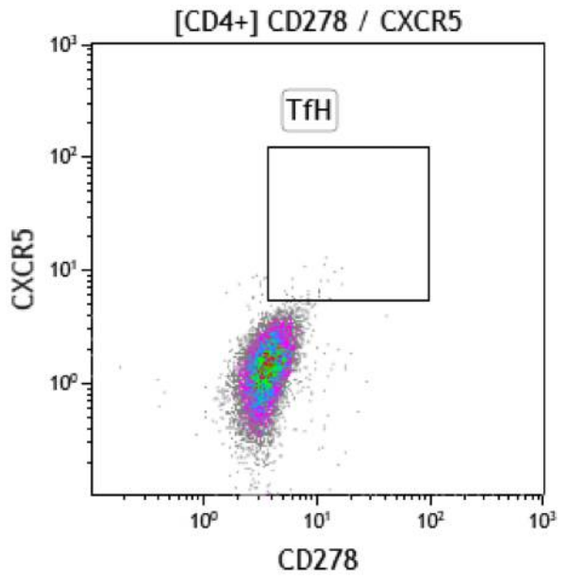
•Patients with aplastic anemia have increased ABCs compared to healthy controls (**2.71** ± 0.39% vs **0.43** ±0,09% respectively, p=0.022)

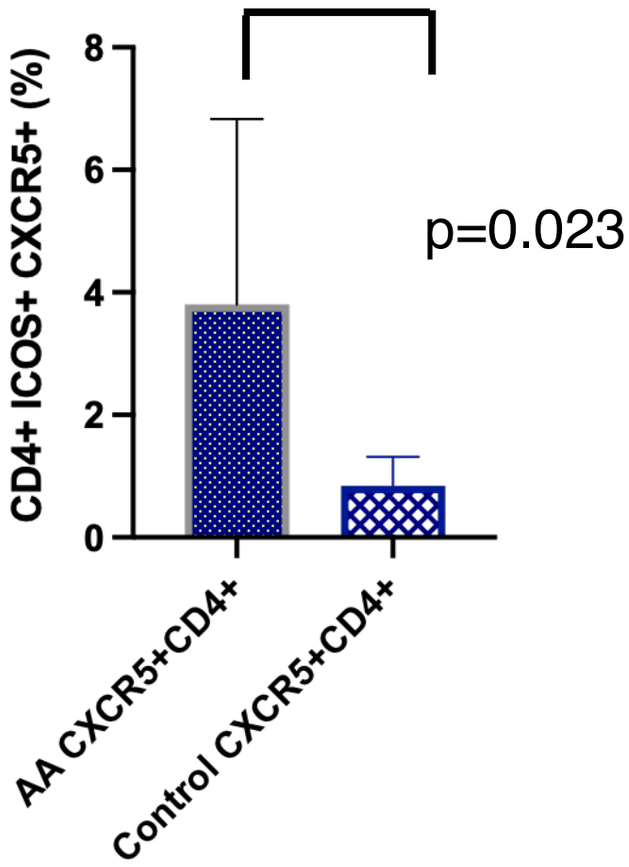
Solomou EE et al, unpublished data

Increased Follicular helper T cells in patients with Aplastic Anemia









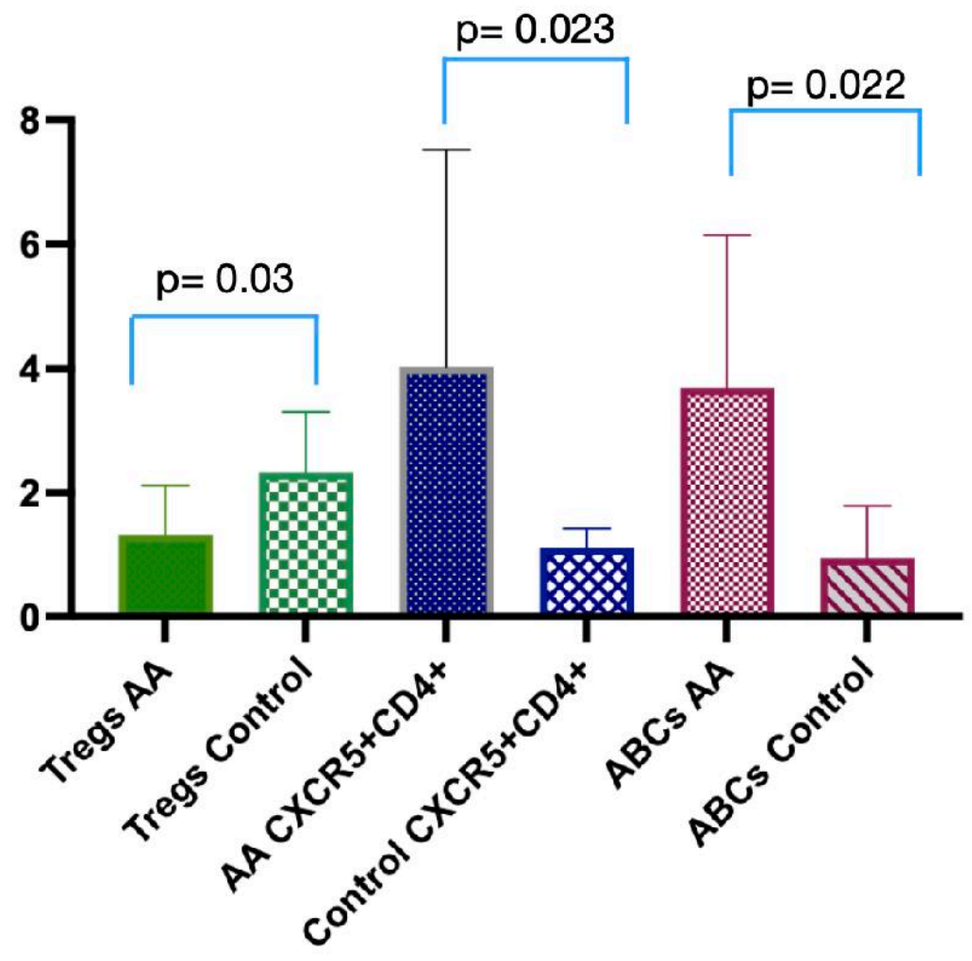
Patients with aplastic anaemia have increased pTfh compared to healthy controls (**3.03** ± 0.55% Vs **1.3** ± 0.52% respectively, p=0.023

Solomou EE et al, unpublished data

Increased TFh in patients with Aplastic Anemia

- Patients with aplastic anemia showed **increased** numbers of **ABCs** and **pTfh** compared to healthy controls.

- Additionally, these patients also showed **decreased** numbers of **regulatory T cells** compared to healthy controls (**0,74**±0,27% vs **2,25**±0,70% respectively, p=0.03).



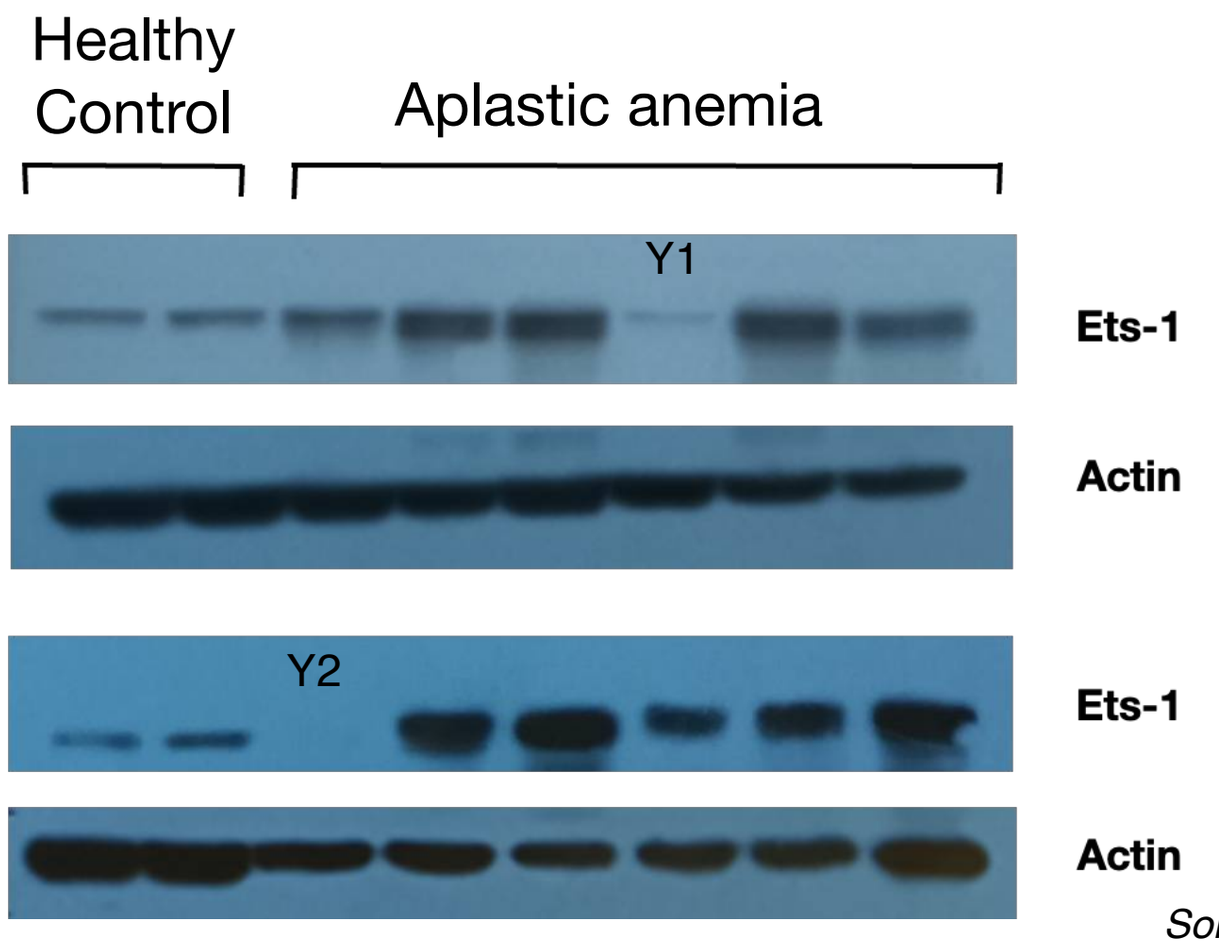
Solomou EE et al, unpublished data

- To further explore the mechanism involved in the increased expression of these two cell populations in AA patients, we examined the intracellular Ets-1 protein levels.

- AA patients showed statistically significant increased Ets-1 protein levels compared to controls (western blot).

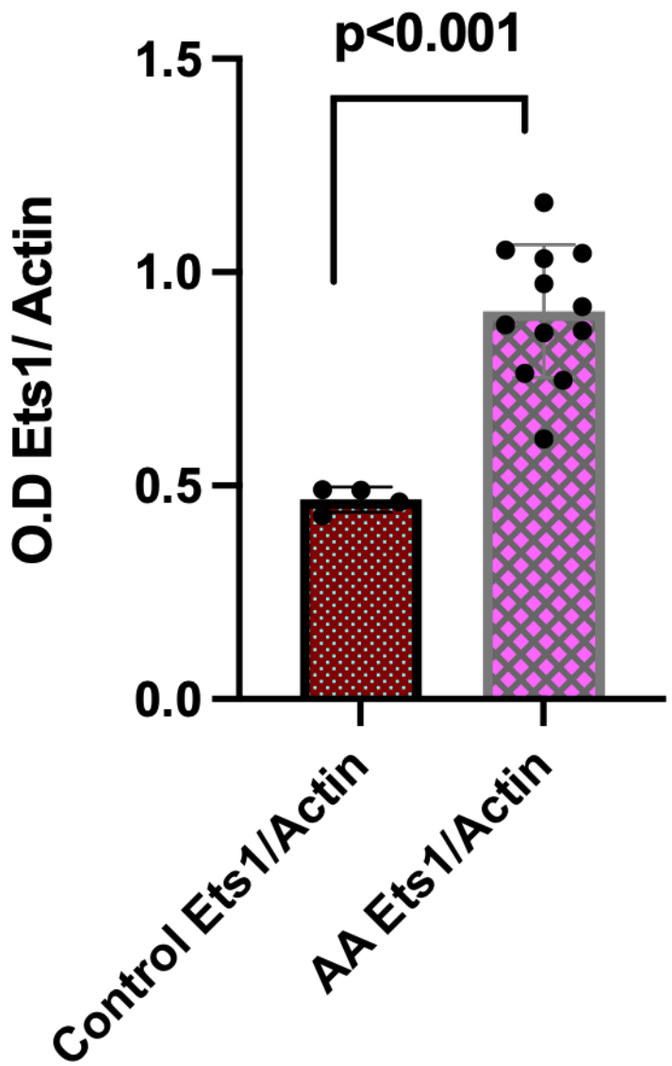
- Ets-1 protein levels correlated with the increased ABCs counts. Stat-1 protein levels were comparable between patients and controls, as previously described

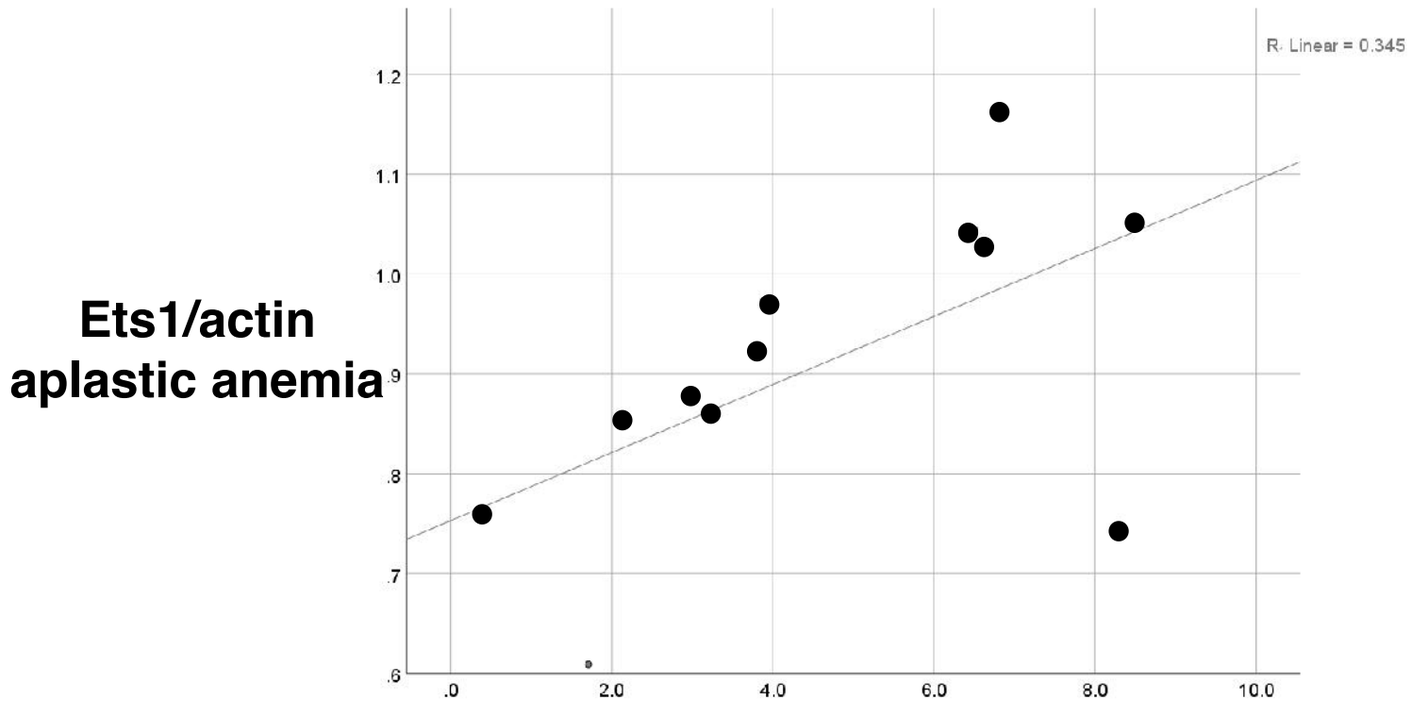
* Y1 and Y2: Aplastic anemia patients in remission



Solomou EE et al, unpublished data

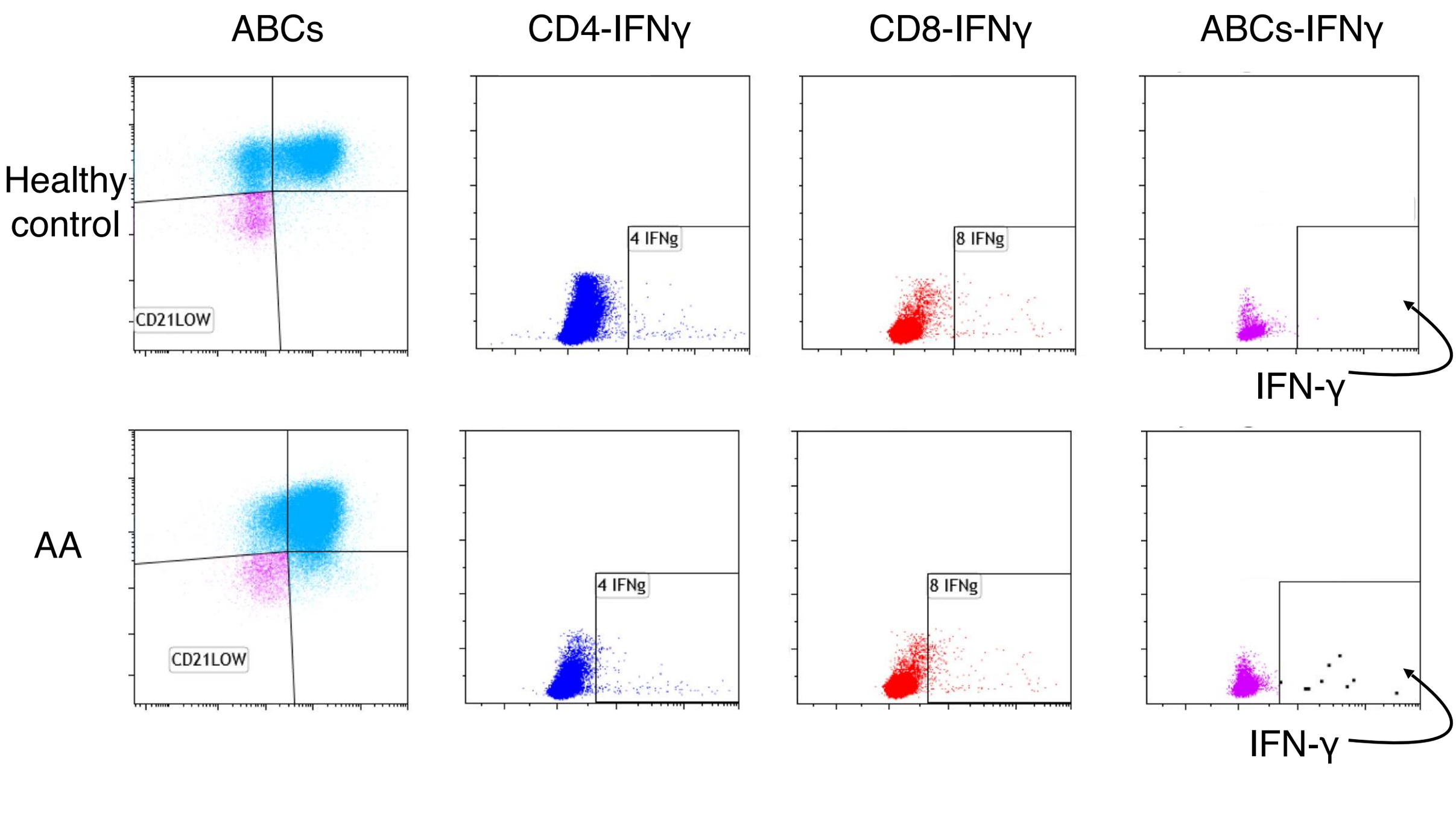
The increased protein levels of Ets-1 correlate with the increased % of ABCs observed in patients with aplastic anaemia





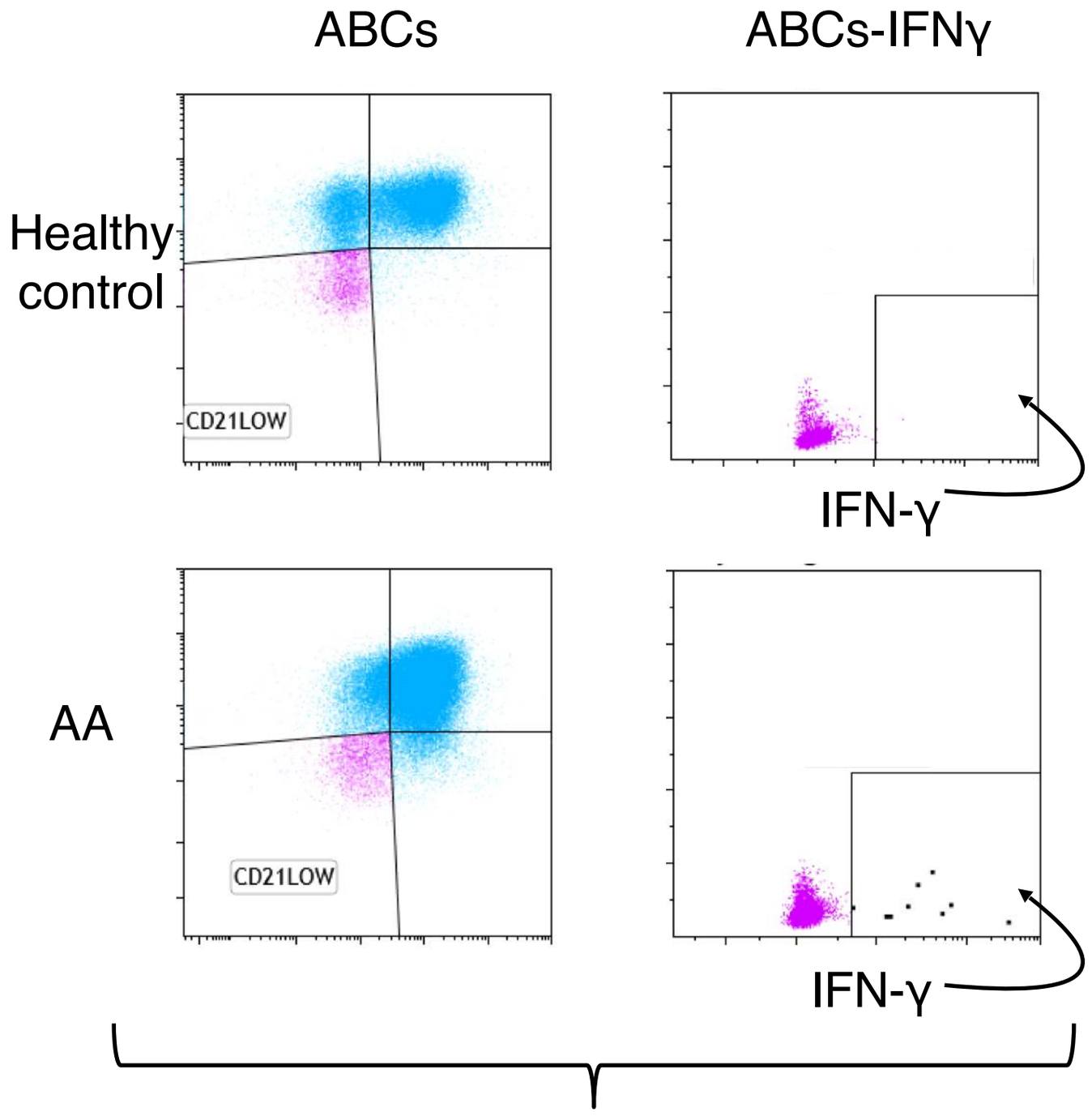
Solomou EE et al, unpublished data

ABCs from patients with aplastic anemia show increased IFN-γ levels compared to the healthy controls - Unstimulated cells



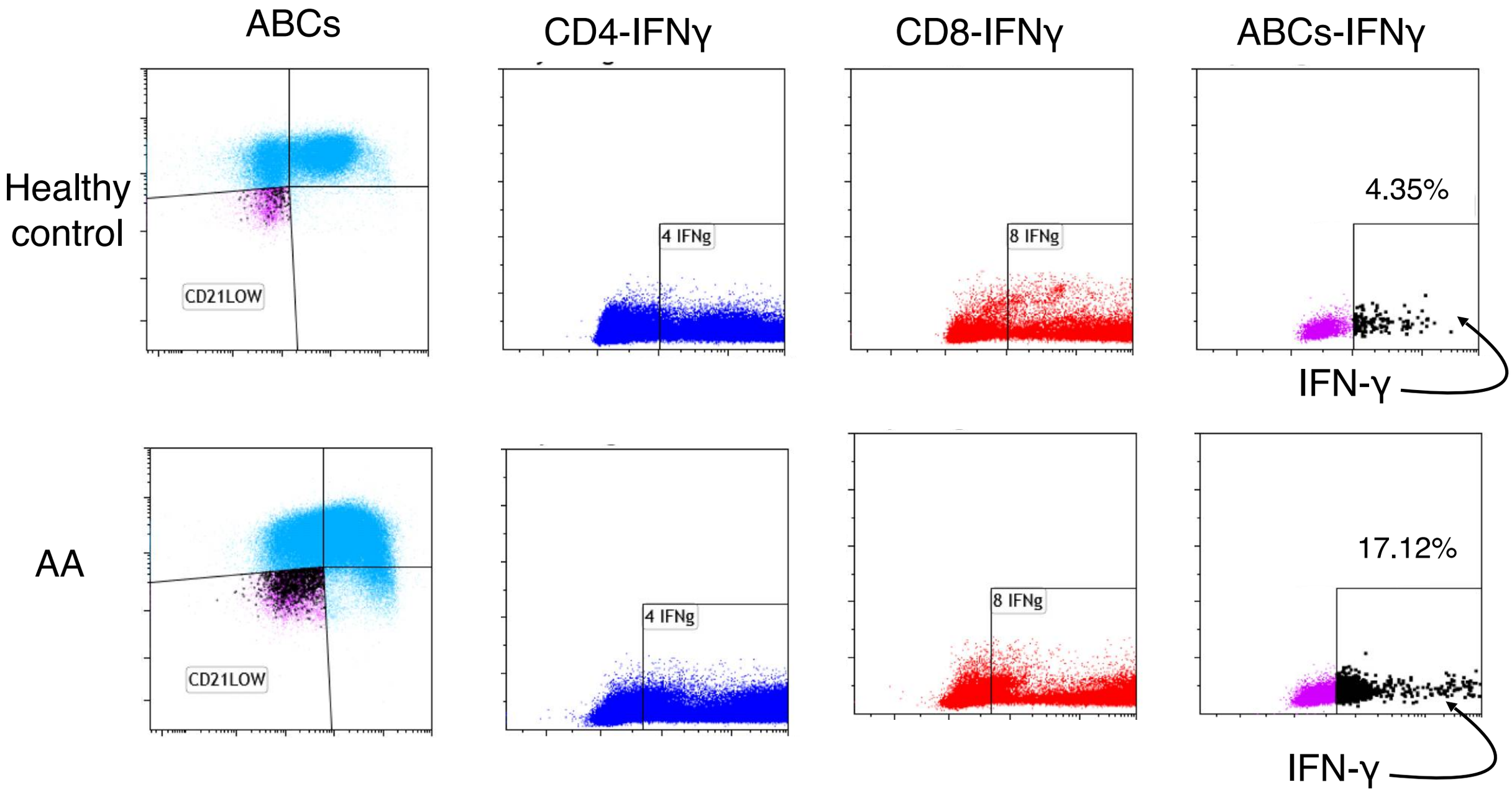
Solomou EE et al, unpublished data

ABCs from patients with aplastic anemia show increased IFN-γ levels compared to the healthy controls - Unstimulated cells



Unstimulated

ABCs from patients with aplastic anemia show increased IFN-γ levels compared to the healthy controls - Stimulated cells with PMA+ionomycin



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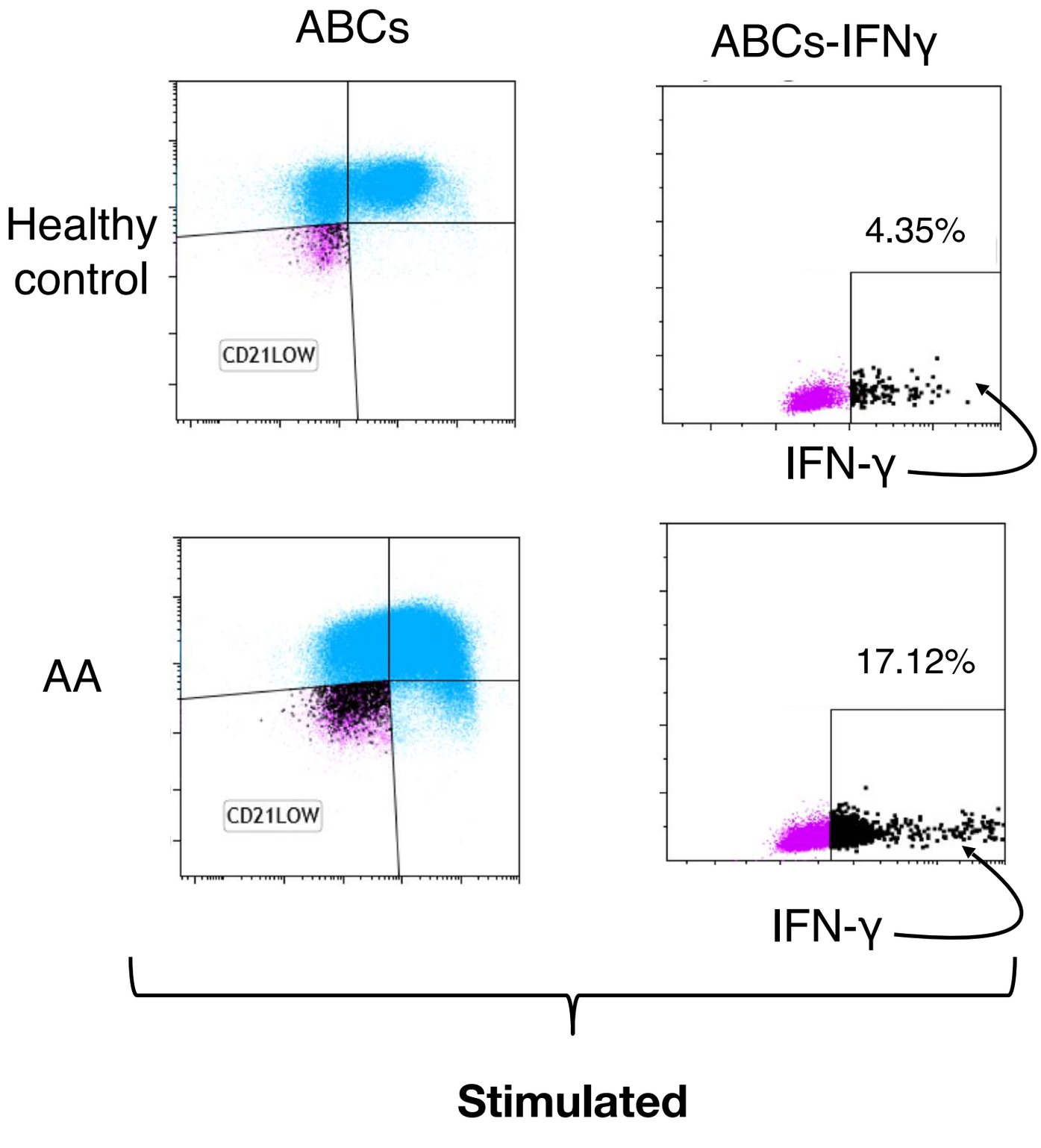
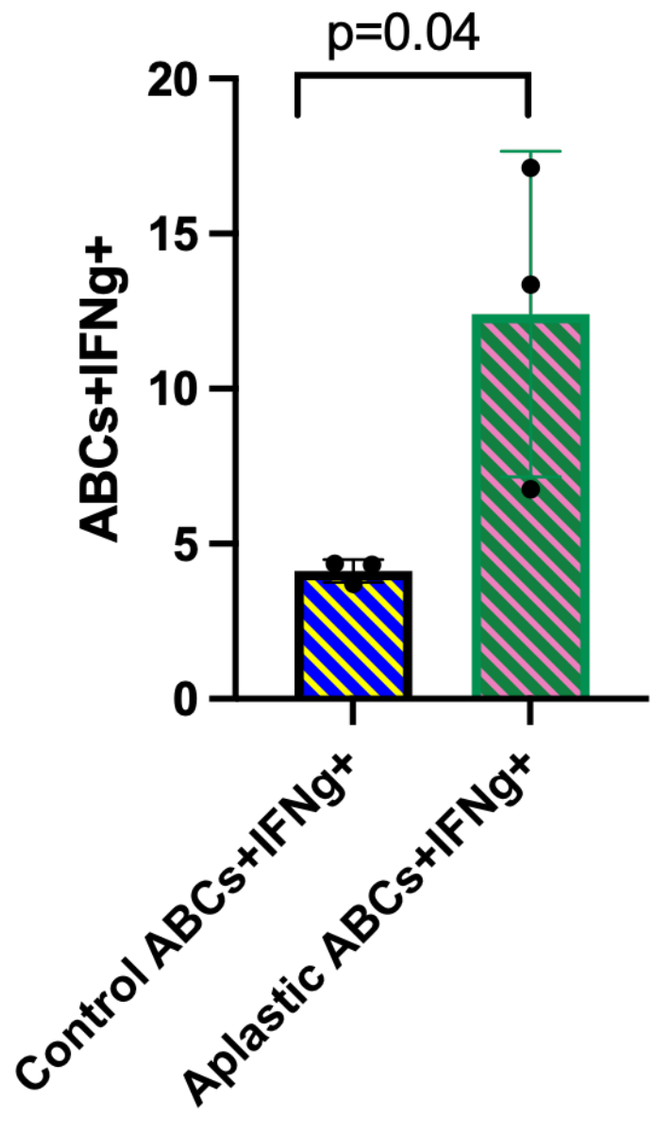


ABCs from patients with aplastic anemia show increased IFN-γ levels compared to the healthy controls in stimulated cells with PMA+ionomycin



Solomou EE et al, unpublished data

Conclusion

- Our results show for the first time that aplastic anemia patients show expanded ABCs compared to age-matched control subjects, and seem to be related to disease status.

- Patients with aplastic anemia show increased numbers of pTFh along with the increased % of ABCs

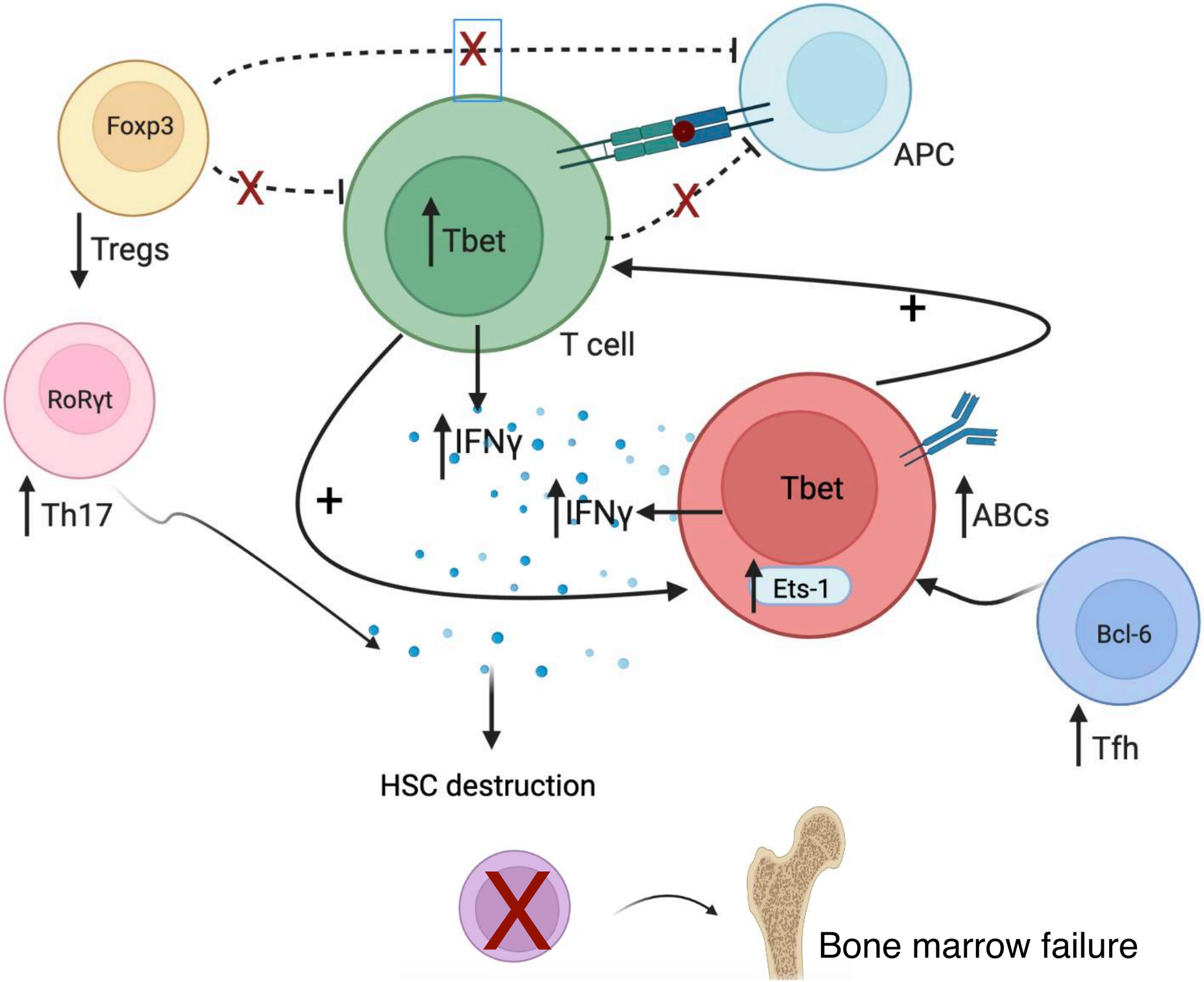
- Increased levels of the transcription factor Ets-1, that is essential for Tbet expression in ABCs in aplastic anemia patients

- The ABCs show increased IFN-γ, mainly after stimulation compared to the healthy controls

- All data suggest that the increased pTfh and ABCs observed in patients with aplastic anemia participate in the increased IFN-γ levels observed in these patients and subsequent destruction of the hematopoietic stem cells

- The development of agents that specifically target T-bet+ cells or pTfh could possibly be used as novel therapeutic agents for AA but also for other autoimmune diseases

Proposed Mechanism of IFN-γ production in Acquired Aplastic Anemia ???



193 JAK 1/2 Inhibition Preserves Hematopoietic Progenitor and Stem Cells, Prevents Aplasia, Inhibits Pro-Inflammatory Cytokines, and Prolongs Survival in Murine Immune Bone Marrow Failure

Program: Oral and Poster Abstracts
Type: Oral
Session: 508. Bone Marrow Failure: Acquired: Biology
Hematology Disease Topics & Pathways:
Research, Acquired Marrow Failure Syndromes, Translational Research, Bone Marrow Failure Syndromes, drug development, Diseases, Therapies

Saturday, December 10, 2022: 2:00 PM

Emma M. Groarke, MD[1], *Xingmin Feng, PhD*[1], *Nidhi Aggarwal, BS*[2], *Ash Lee Manley*[3*], *Zhijie Wu, MD*[1*], *Shouguo Gao, PhD*[1*], *Bhavisha A. Patel, MD*[1], *Jichun Chen, PhD*[1] *and Neal S. Young, MD*[1]

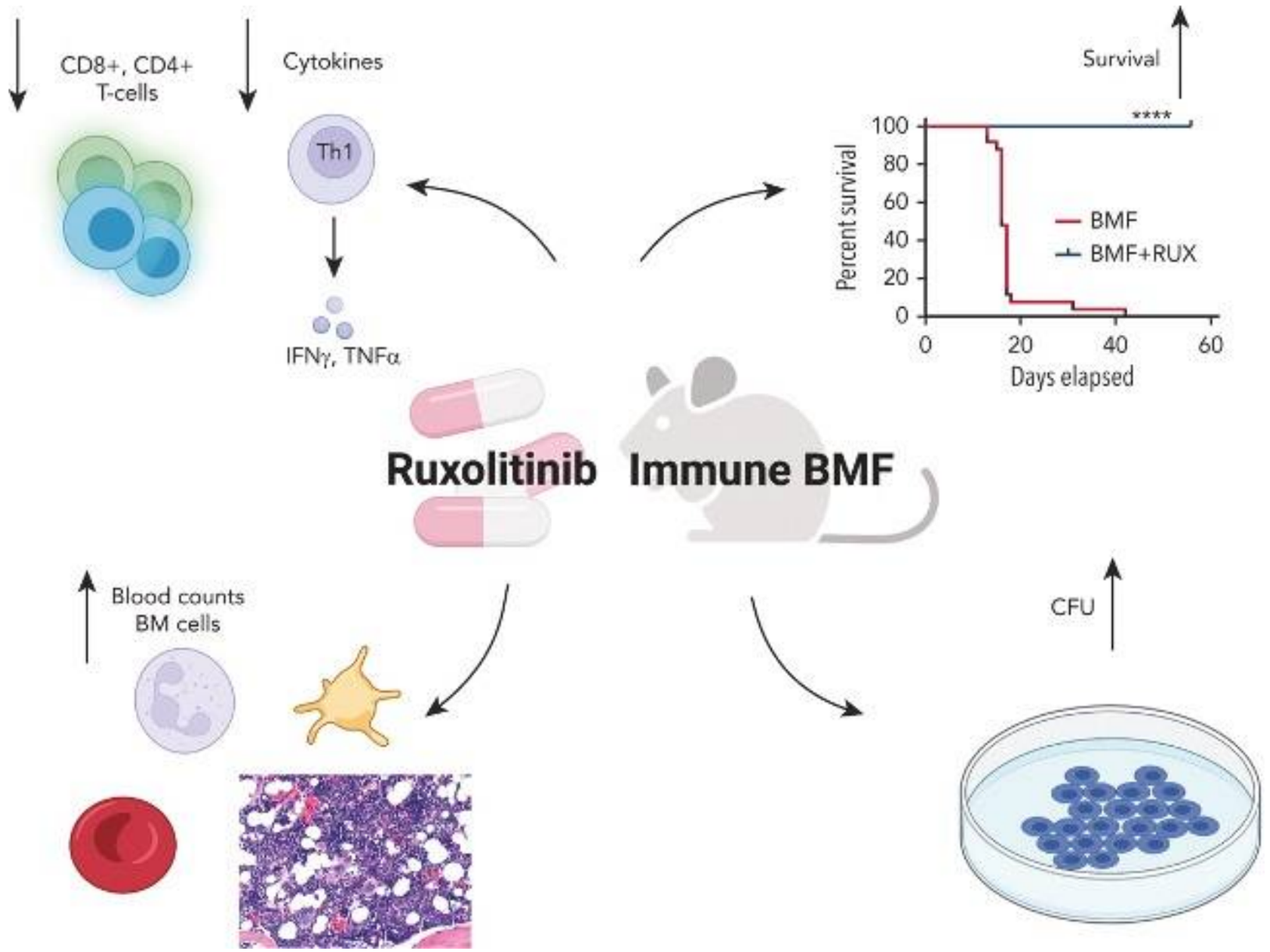
blood

HEMATOPOIESIS AND STEM CELLS | JANUARY 5, 2023

Efficacy of JAK1/2 inhibition in murine immune bone marrow failure

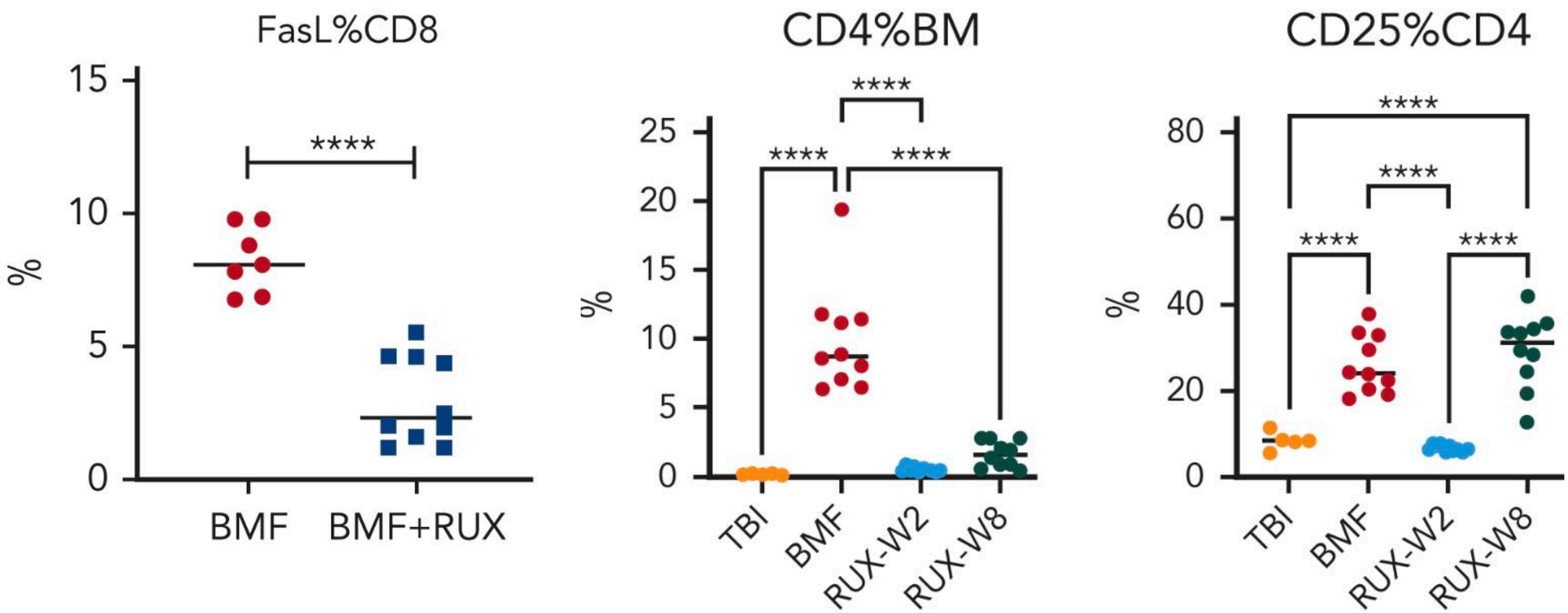
Emma M. Groarke, Xingmin Feng, Nidhi Aggarwal, Ash Lee Manley, Zhijie Wu, Shouguo Gao, Bhavisha A. Patel, Jichun Chen, Neal S. Young

Efficacy of JAK1/2 inhibition in murine immune bone marrow failure



Emma M. Groarke,et al. Efficacy of JAK1/2 inhibition in murine immune bone marrow failure, Blood, Jan 5th, 2023

- Ruxolitinib prophylaxis prevents and ruxolitinib therapy treats murine immune aplastic anemia.
- Ruxolitinib inhibits T-cell infiltration and activation and suppresses bone marrow cell apoptosis.



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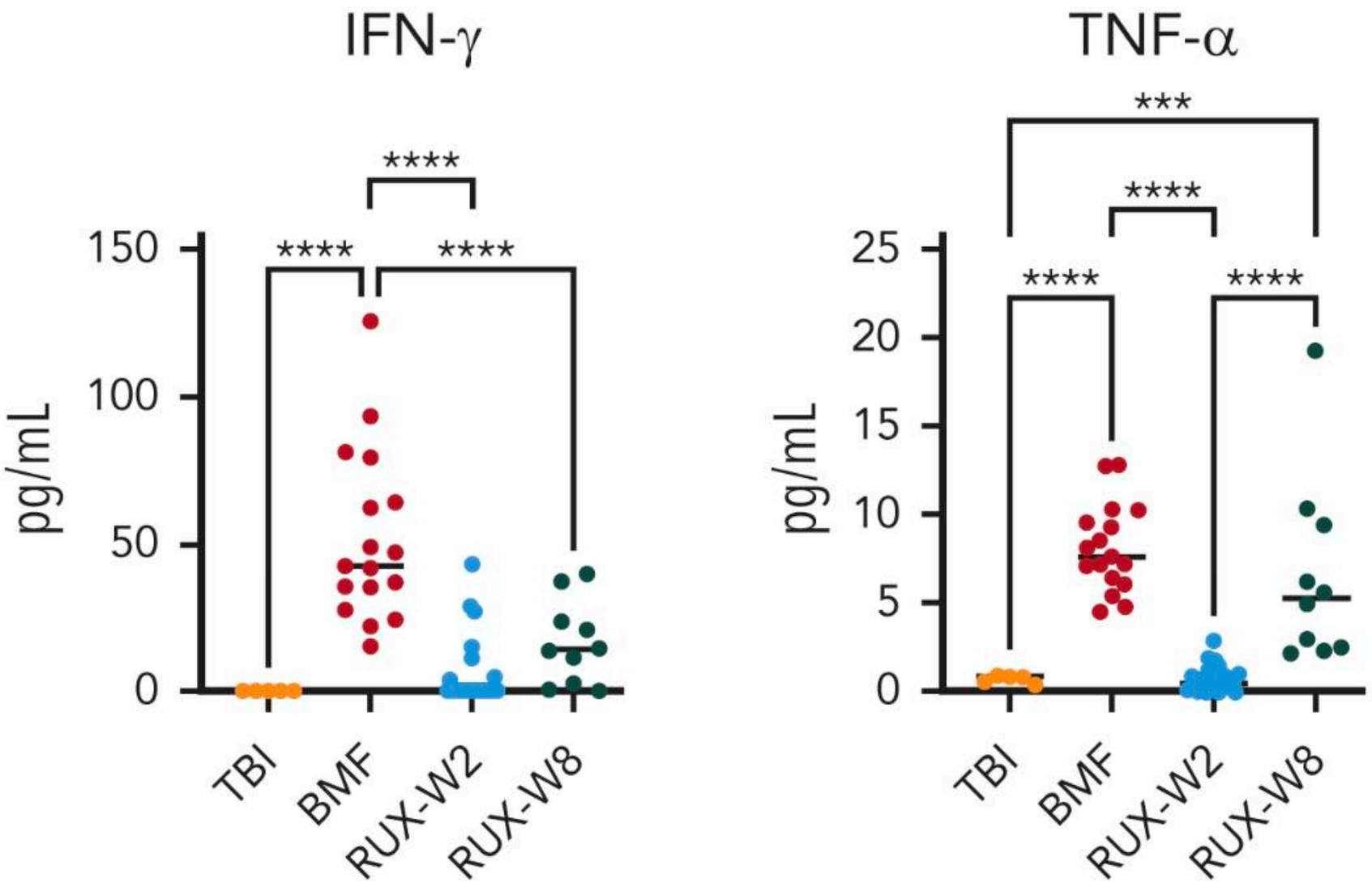

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Novel treatment options-clinical trials…..

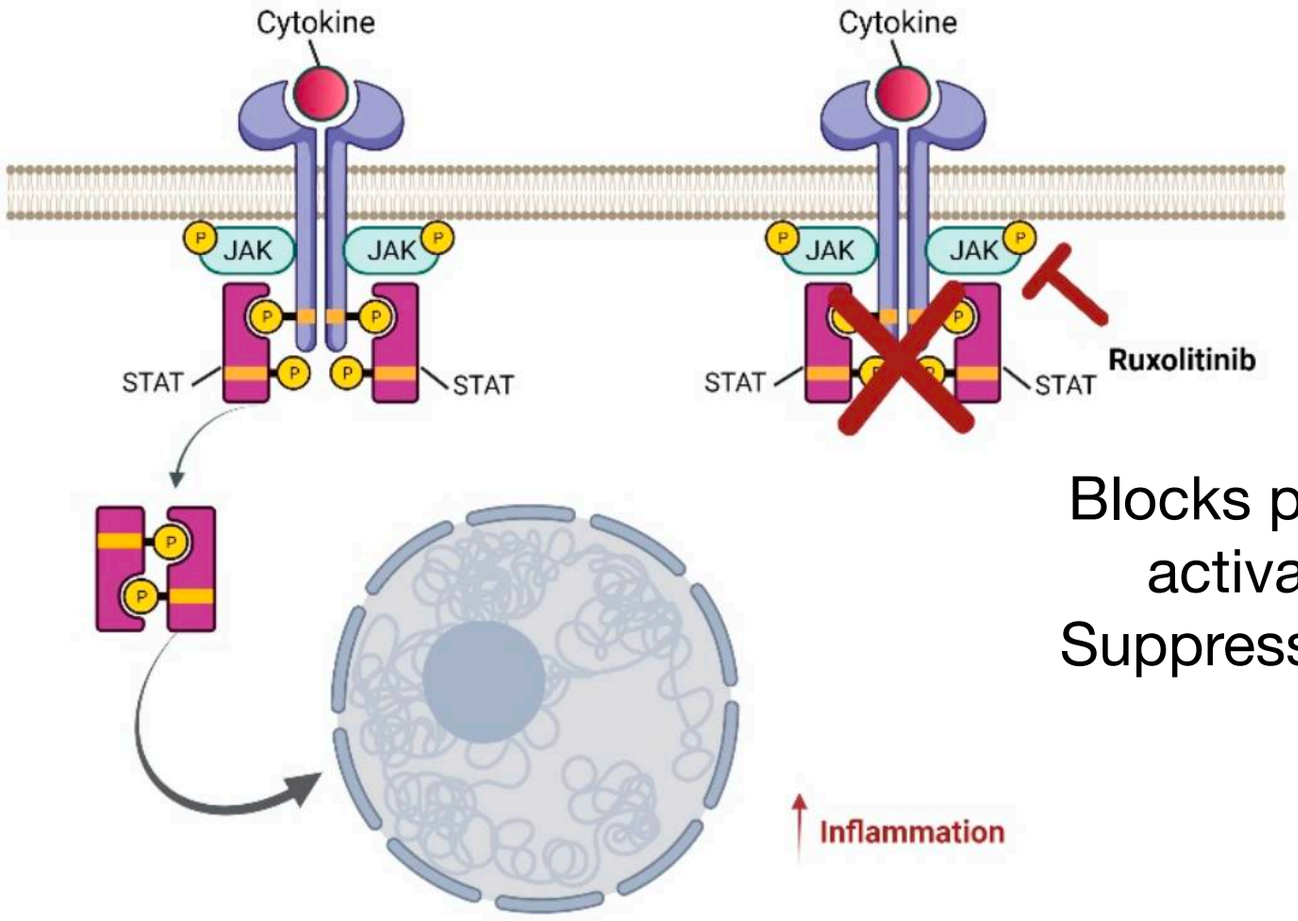
- Ruxolitinib: As mono therapy in moderate AA, low risk hMDS, LGL, PRCA

- Ruxolitinib + eltrombopag + CsA: In SAA, treatment-naive (no ATG!!!)

- Ruxolitinib + CsA for remission, plus sirolimus for clonal deletion

Goal: Oral, pre-emptory Rx with Ruxolitinib, Eltrombopag and CsA

JAK1/2 and ruxolitinib



Blocks phosphorylation and activation of STAT —> Suppression of inflammation

Figure 2. Schematic representation of ruxolitinib's effect on inflammation induced by the JAK–STAT signal transduction pathway. Ruxolitinib, administered during inflammation, blocks the receptors and triggers the reduction of STAT phosphorylation and, consequently, decreases hyperinflammation and cytokine storm. Created with BioRender.com (accessed on 16 March 2022).

Marcuzzi A et al, Pharmaceuticals 2022, 15, 374

Ruxolitinib

- Ruxolitinib in steroid-refractory GVHD (adults and children)

- Ruxolitinib also has an antiviral action against COVID19, HIV and Epstein–Barr viruses. In vitro experiments have shown that ruxolitinib is able to block viral replication in lymphocytes and macrophages, perhaps reducing the phosphorylation of STAT proteins, and inhibit the reactivation of latent HIV-1.

- Clinical evidence in the pediatric population has demonstrated the significant preventive anti-inflammatory capacity of ruxolitinib when administered at a minimum dosage (2.5 mg twice daily); at the molecular level, this condition corresponds to a partial blockage of JAK receptors, which limits the hyper-inflammation associated with STAT phosphorylation

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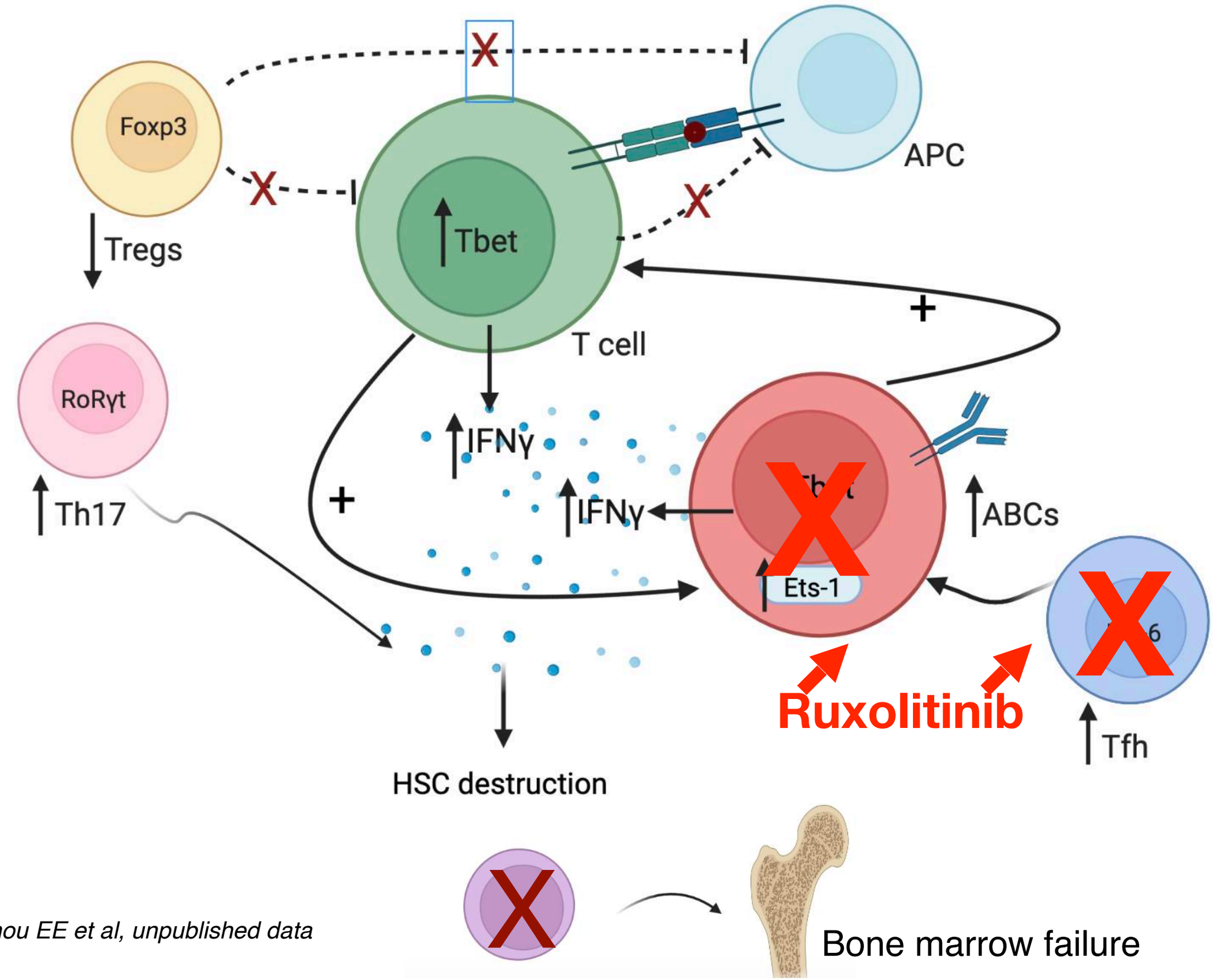

Solomou EE et al, unpublished data

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