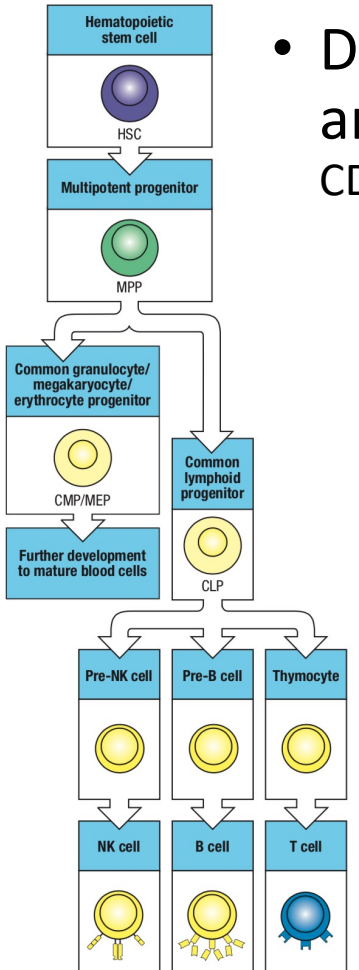
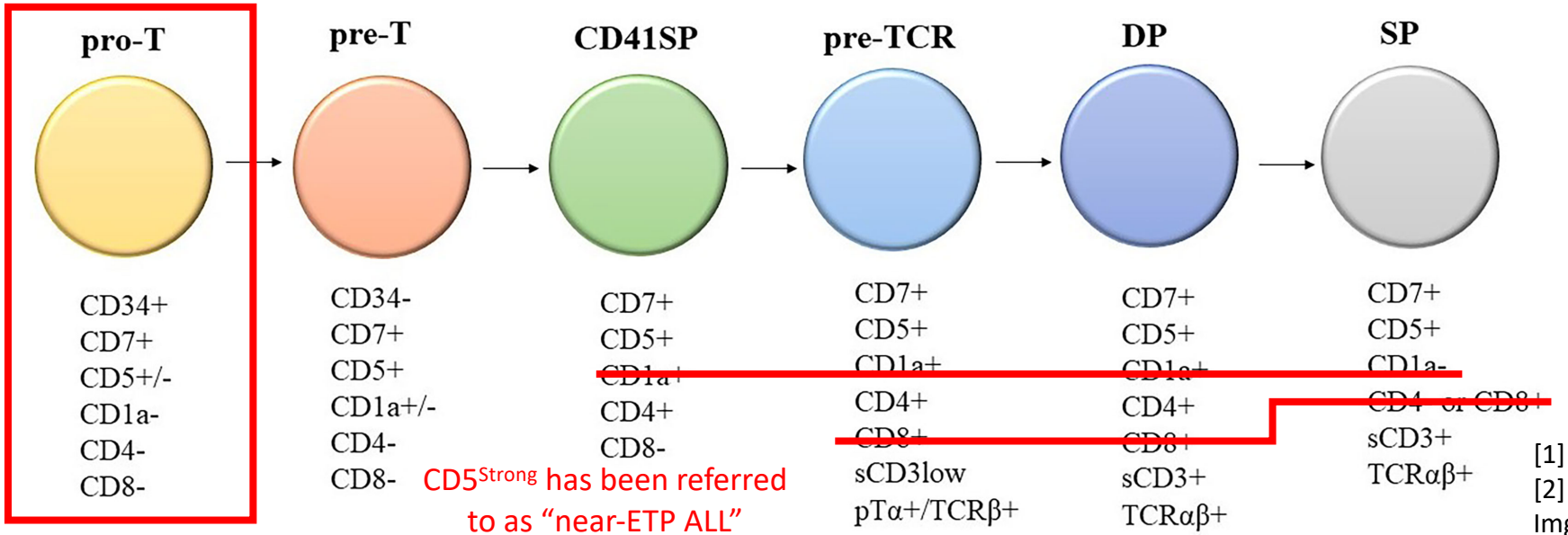
- Previously healthy 57F p/w fatigue and pallor found to have pancytopenia, lab workup unrevealing.
  - 1/3/23 BMBx at North Memorial with diagnosis of “acute leukemia” referred to UMN for further care.
  - 1/12/22 peripheral blood FISH showed extra copies of 4, 10, 17
  - 1/18/23 BMBx at UMN
    - Morphology: acute leukemia with hypercellular marrow (100%), reduced granulopoeisis and erythropoesis, absent megakaryopoeisis, **at least 33% bone marrow blasts**. Peripheral blood leukoerythroblastic with pancytopenia and **4% circulating blasts**.
    - IHC: Blasts are $CD3^{weak}$, $CD7^{weak}$, CD34+, CD56+, CD117+, TdT^{weak}, CD1a-, CD2-, CD5-, CD8-, CD20-, CD61-, CD123-, and MPO.
    - G banding no metaphases, FISH no BCR::ABL1, PCRs for FLT3 and BCR:ABL negative
    - Flow:
      - BM: 4.9% CD34+ and cCD3+ blasts that are $CD7^{dim}$, CD13+, $CD45^{dim}$, $CD56^{bright}$, sCD3-, CD14-, CD19-, CD64-, CD79a-, CD117-, cMPO-, HLA-DR-, nTdT-
      - PB: 4.1% blasts CD34+, cCD3+, $sCD7^{trace}$, $cCD7^{dim}$, $CD13^{dim}$, $CD38^{partial}$, $CD45^{dim}$, $CD56^{bright}$, $CD117^{dim}$, $CD123^{dim}$, $HLA\text{-}DR^{partial}$, $TdT^{partial}$, Negative for CD1a, CD3, CD4, CD5, CD8, CD11b, CD14, CD15, CD16, CD19, CD20, CD22, CD33, CD36, CD58
    - TCR gene rearrangement: Positive for TCR gamma gene rearrangement
    - AML NGS: JAK1 L783F and JAK3 R657Q (known/potential pathogenicity), NOTCH1 Exon 26 mut (uncertain significance)
    - **Final Diagnosis**: **ETP-ALL** based on presence of cCD3, CD7, CD13, CD4, CD117 and lack of CD1a, CD5, CD8

Early T-precursor (ETP) ALL diagnosis

- Described in 2009[1] as a subset of T-ALL with poor outcomes
  - Defined by flow cytometry: cytoplasmic CD3+, CD7+, CD5 weak, CD8–, CD1a–, and positive for one or more myeloid stem cell antigens (E.g. CD34, CD117, CD13, CD11b, HLADR, CD65, etc.)
    - Incorporated into WHO 2016[2] as a provisional entity based on this immunophenotype.
    - Can be distinguished from MPAL, T/myeloid by a lack of MPO expression





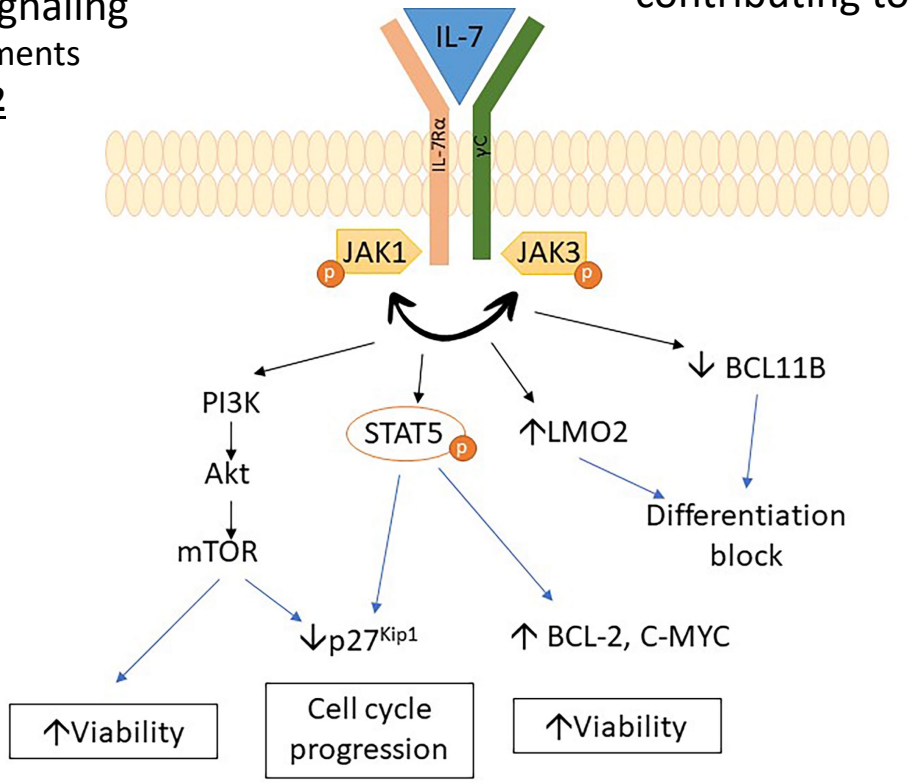
$CD5^{Strong}$ has been referred to as "near-ETP ALL"

[1] Lancet Oncol 2009; 10:147.
[2] Blood 2016; 127:2391.
Img Front. Oncol 2021. 11:750789

Mutational profile

- ETP-ALL[1]
  - 15% NOTCH1 mutations
  - Infrequent CDKN2A/B mutations
  - Mutations more commonly found in AML (**FLT3**, DNMT3A, RUNX1, RAS)
  - Aberrant **IL7R/JAK/STAT** signaling
    - BCL11B 14q32 rearrangements
    - Higher expression of **BCL2**
    - MEFC2/LMO2/LYL1
  - KMT2A/HOXA
  - PRC2/EZH2

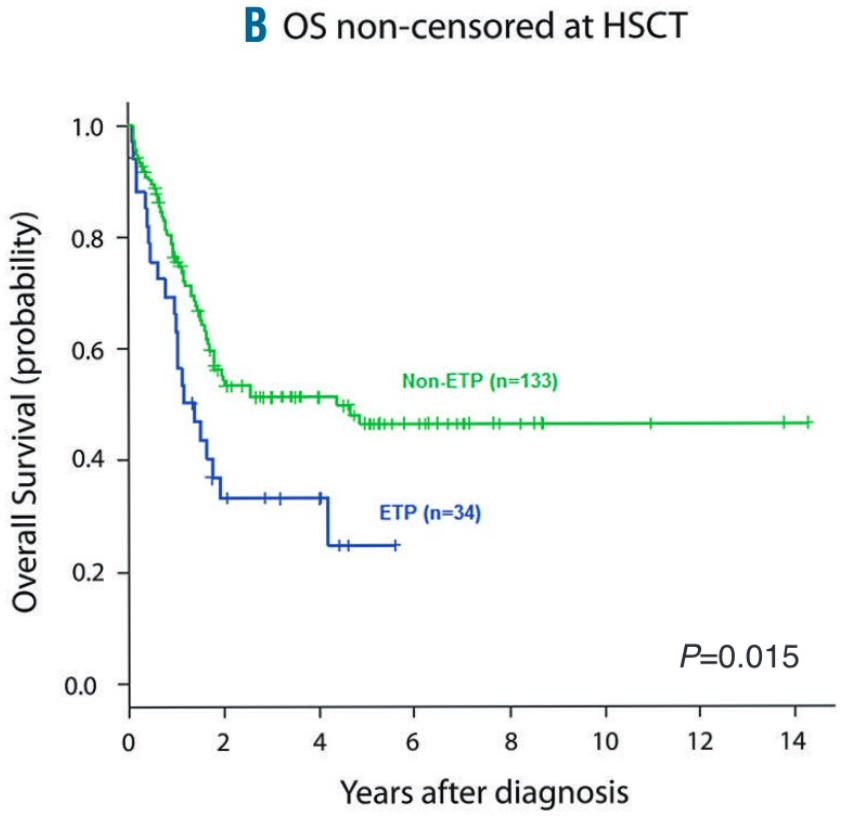
- Non-ETP-ALL [2]
  - >50% NOTCH1 mutations
  - >50% CDKN2A/B mutations (del9p21)
  - >100 mutations identified, typically in low frequencies in combinations of 10 or more contributing to leukemogenesis



[1] Front. Oncol 2021. 11:750789
[2] Blood 2017; 129:1113.

Treatment and outcomes in ETP-ALL

- Frontline treatment with standard Ph(-) ALL regimens
  - Asparaginase-based regimens (GRALL, CALGB 10403, PETHEMA, etc) vs HyperCVAD
  - Nelarabine added to frontline therapy in pediatrics
    - No benefit frontline in adults thus far[1]
  - Allogenic HSCT in CR1, ideally MRD-
- Higher rates of induction failure/MRD positivity, lower PFS/OS
  - Risk-adapted treatment intensification appears negate this in pediatric ETP-ALL, but not in adult thus far[2]
  - E.g. in the PETHEMA ALL-HR-2003 and ALL-HR-11 cohorts (n=167) 20% had ETP-ALL[3]
    - CR: 76% vs 94%
      - CR with induction 1 (VCR/Daun/ASP/Pred): 56% vs 89%
      - CR with induction 2 (FLAG-Ida): **20%** vs 5%
    - MRD at Day 35
      - MRD <0.1%: 35% vs 82%
      - MRD <0.01%: 15% vs 63%
    - HSCT: **70%** vs 21%
    - 4 year survival probability: 33% vs 51%



[1] Blood 2021; 138:366.
[2] Front. Oncol 2021. 11:750789
[3] Haematologica 2020; 105:e294.

MRD+, 1º refractory, or relapsed disease

- AML regimens e.g. FLAG-Ida Case reports in R/R; PETHEMA LAL-19 using frontline for ETP-ALL
- Nelarabine +/- chemotherapy ?risk of resistance (higher SAMHD1 expression in precursors)
- Bortezomib + chemotherapy Pediatric data in R/R
- Targeted treatment strategies
  - BCL2 inhibition: Venetoclax + chemotherapy or HMA or navitoclax (phase 1 data with 60% CR)
  - JAK inhibition: Ruxolitinib ineffective as monotherapy, frontline combination therapy RCT ongoing
  - FLT3 inhibition case reports combining sorafenib with chemotherapy
  - NOTCH1/PI3K combination NOTCH1 monotherapy ineffective, RNAseq data suggests due to PI3K escape
  - Cell surface marker targeting
    - CD33 (~60%) – Gemtuzumab Ozogomycin
    - CD38 (~80%) – Daratumumab case reports, Phase 2 ongoing
    - CD123 (~40%) – Flotetuzumab Phase I/II ongoing
  - CAR-T
    - CD7 CAR with CD7 knockout Phase I ongoing
    - CD5 CAR Phase I ongoing, but CD5 low by definition