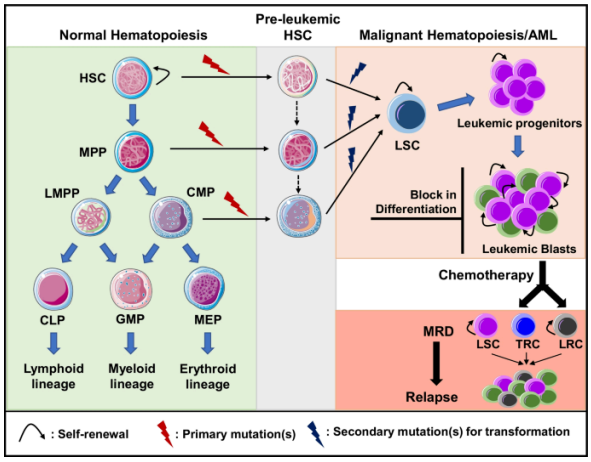
Structural changes in pediatric AML

Background

- AML is marked by an accumulation/proliferation of immature white blood cells termed **"blasts" (Long et al.).**



- **Pediatric patient survival has remained around 75%** despite recent research advances
- Most structural and mutational alterations in pediatric AML impact transcription factors or other proteins directly related to transcription
- scRNA-seq profiling shows that regardless of subtype, leukemic cells from pediatric patients at diagnosis, remission, and relapse all cluster separately (**Lambo et al.**).
- **Immunotherapy is not currently a treatment option** for AML patients (**Tettamani et al.).**
    - It is difficult to identify AML restricted antigens, and thus typical immunotherapeutic approaches would lead to hematopoietic toxicity.
    - Blast cells are immune evasive:

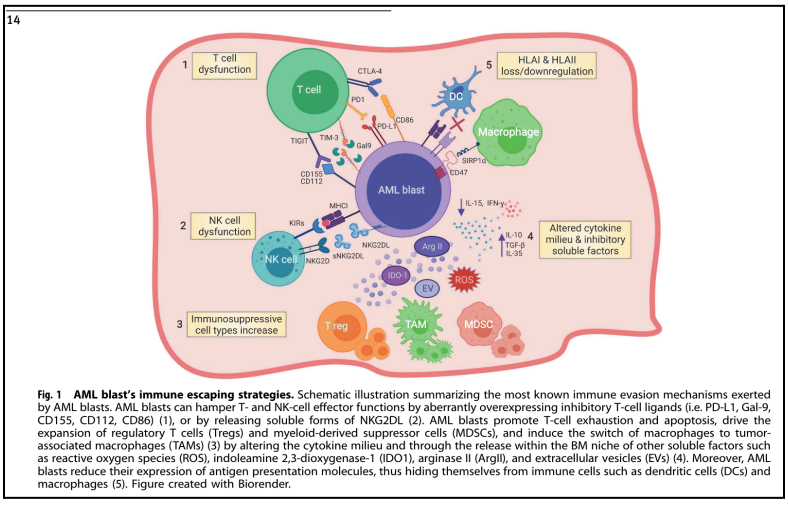
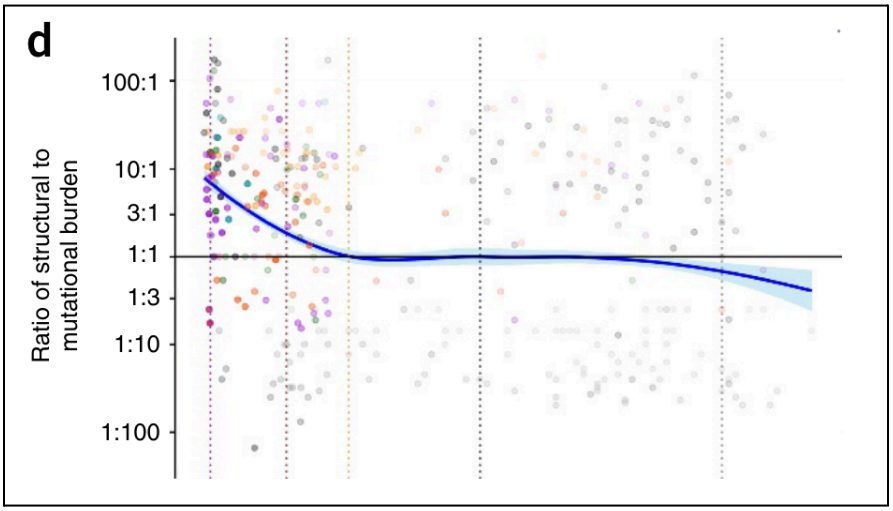


Fig. 1 AML blast's immune escaping strategies. Schematic illustration summarizing the most known immune evasion mechanisms exerted by AML blasts. AML blasts can hamper T- and NK-cell effector functions by aberrantly overexpressing inhibitory T-cell ligands (i.e. PD-L1, Gal-9, CD155, CD112, CD86) (1), or by releasing soluble forms of NKG2DL (2). AML blasts promote T-cell exhaustion and apoptosis, drive the expansion of regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), and induce the switch of macrophages to tumor-associated macrophages (TAMs) (3) by altering the cytokine milieu and through the release within the BM niche of other soluble factors such as reactive oxygen species (ROS), indoleamine 2,3-dioxygenase-1 (IDO1), arginase II (ArgII), and extracellular vesicles (EVs) (4). Moreover, AML blasts reduce their expression of antigen presentation molecules, thus hiding themselves from immune cells such as dendritic cells (DCs) and macrophages (5). Figure created with Biorender.

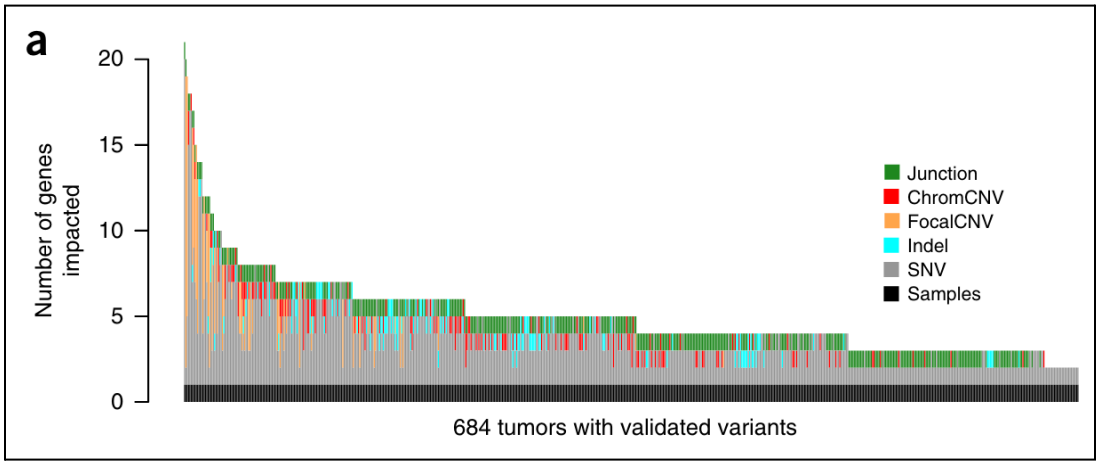
- Example mechanisms include decreased antigen presentation and immune checkpoint marker expression, leading to an exhausted T cell phenotype

Structural vs. mutational alterations

- Genomic aberrations are markedly different between pediatric and adult AML (**Bolouri et al.)**
- The ratio of structural mutational alterations decreases with age (meaning that **structural alterations are much more common in pediatric AML**)



- In the Meschinci lab's nature paper (**Bolouri et al.)**, pediatric samples with the greatest number of genes with impacted function harbored more Focal CNVs:



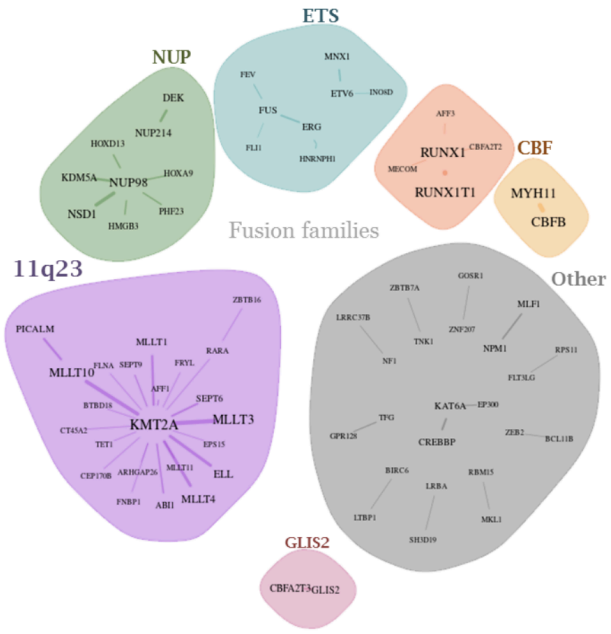
Focal Deletions

- **MBNL1 and ZEB2** are commonly deleted and co-deleted in pediatric AML **(Bolouri et al.)**.
    - Both deletions are associated with significant increase in other cytogenetic variations
    - Characteristics of samples with **co-deletions:**
        - more recurrent mutations
        - 50% also exhibited KMT2A-MLLT3 fusions
- **ELF1**
    - Transcriptional regulator of MEIS1

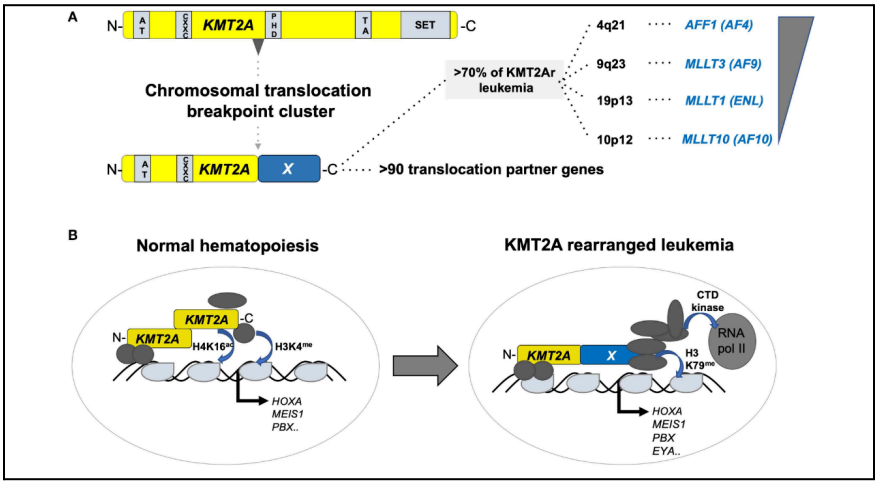
Fusions

- Typically occur early in leukemic transformation
- **Common fusions in pediatric AML**:

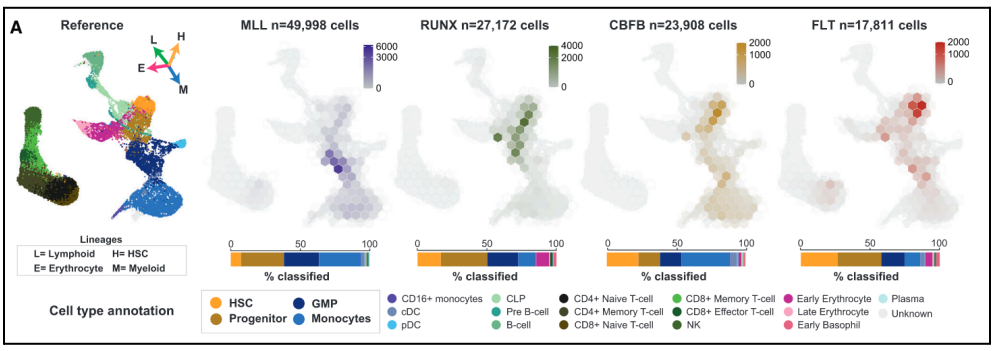
Figure S17. Summary view of the key fusion classes in pediatric AML. Each colored region represents a fusion family. Descriptive labels are written adjacent to each family. The fusion partner genes for each family are indicated by their HGNC symbols. The lines connecting gene symbols indicate fusion partners. The thickness of each line reflects the frequency of the observed fusion.



- **KMT2A-r**
  - Oncogenic mechanism **(Mercher et al.)**:

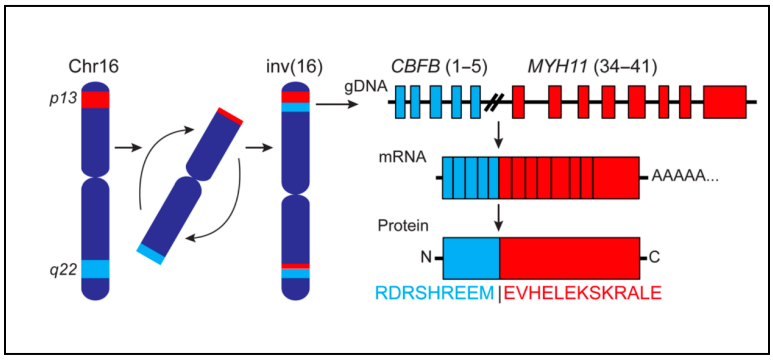


    - *"Functionally, it is currently thought that KMT2A fusions transform HSPCs by recruitment of a large super elongation protein complex (SEC) that activates transcription of target genes via directly influencing elongation by the RNA polymerase II (RNA-pol II). In addition, KMT2A fusion proteins also recruit the DOT1L histone 3 lysine 79 (H3K79me) methyltransferase that positively regulates expression of critical target genes"*
  - **RAS mutations are observed more frequently in KMT2a-rearranged pediatric AML**
    - NRAS more commonly mutated in pAML than KRAS
    - This combination is associated with poor survival
    - CLEC2A may be an attractive immunotherapeutic target in this subtype of pediatric AML **(Kirkey et al.)**
  - **Mesothelin may be a valid CAR-T target in KMT2A-rearranged AML (Le et al.)**
    - Demonstrated efficacy in cell lines and patient xenographs
  - **Cell type composition analysis shows that KMT2A-r AML malignant cells are more mature than other subtypes (Lambo et al.):**



- **RUNX1-RUNX1T1: t(8;21)**
  - Fusion is associated with **good prognosis, but poor prognosis upon relapse**
    - 16 gene relapse signature identified using DEseq, K-means clustering, and ML **(Wallace et al.)**:
      - PRDM8, RARG, CSF1, PDE4B, CCL3, NAB1, WHRN, SGSM2, LPIN3, CHARLIE1B, SATB1, ZNF441, ZNF521, AL451123.1, TSPYL4 and B3GNT5
  - This fusion is mutually exclusive from GATA2 mutations and CEBPA mutations
  - Malignant cells are **more similar to progenitor/early GMP cells** (**Lambo et. al**)

- **CBFB-MYH11: inv(16)/t(16;16)**
  - Occurs in 12% of pediatric AML patients
  - **Structure of type A fusion (Biernacki et al.)** :



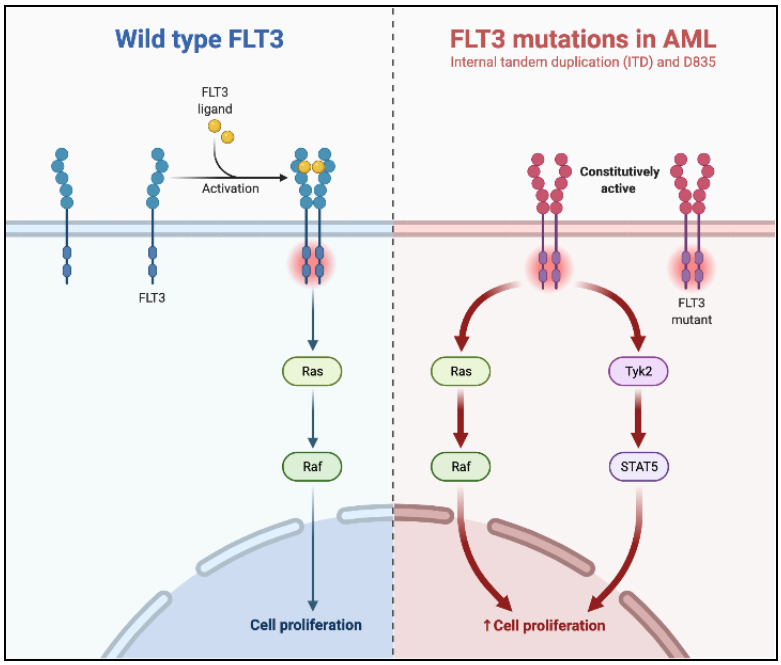
  - When co-occurring with RUNX1-RUNX1T1, termed **core-binding factor AML (CBF AML)**
  - Could potentially be targeted with neoantigen-specific T-cell therapies **(Biernacki et. al)**
    - shared among patients, so would not be patient-specific
    - Biernacki et. al showed that CD8s with CBFB-MYH11 neoantigen-specific TCRs were capable of leukemic cell killing in vitro
  - 20 gene relapse signature **(Peplinski et al.)**:
    - CBWD3, DRAP1, GNL3LP1, MAF1, GRAMD1C, MAGED2, TICAM1, MPP1, NBPF11, RAB13, RN7SL329P, HAL, TLK2P2,

CD151, XRCC6, HIST1H2AM, MSANTD3-TMEFF1, CDCA4, LRRC8A, and IPCEF1.

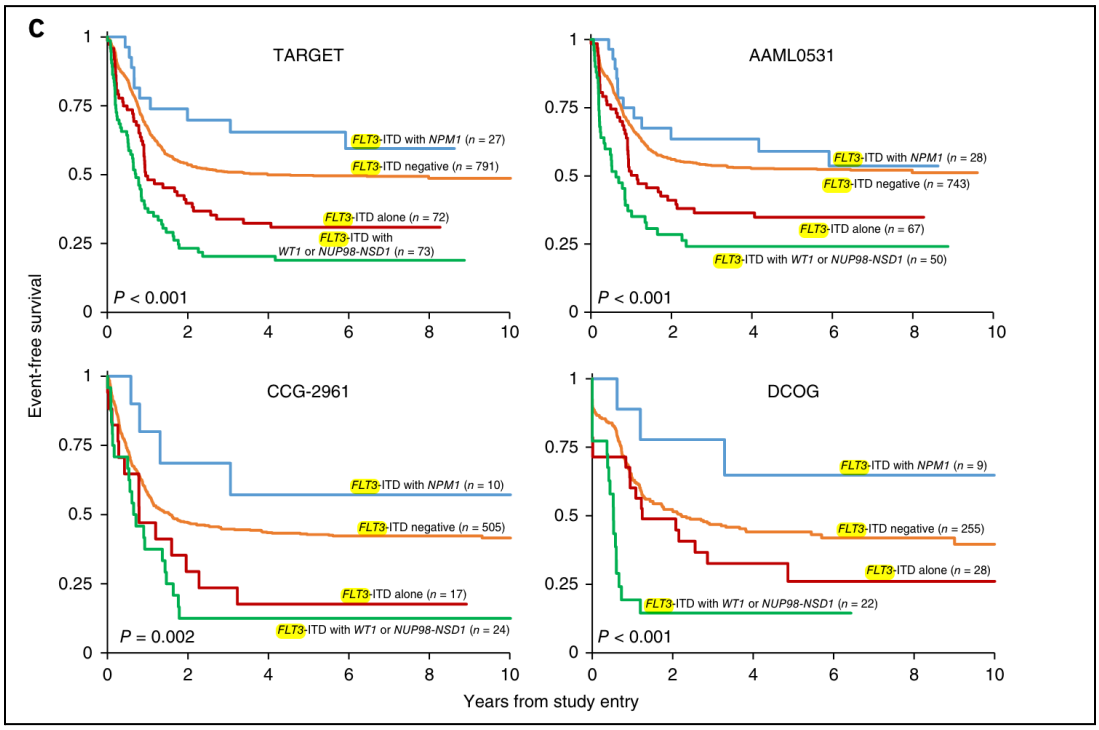
- **NUP98-NSD1:**
  - NUP98: nuclear pore complex component
    - The nuclear pore complex that contains NUP98 can act as an intranuclear transcription scaffold (***Matsukawa et. al)***
  - more common in child pAML than infant pAML

Internal Tandem Duplications

- **FLT3-ITD**
  - This internal duplication occurs at similar rates in both pediatric and adult AML
  - FLT3 is a receptor tyrosine kinase
  - Internal tandem duplications allow FLT3 tyrosine kinases to become constitutively active (meaning ligand presence is not necessary for the activation of downstream signaling pathways)



- Poor patient outcome when accompanied by WTI mutations or NUP98-NSD1 fusions.
- Good patient outcomes when accompanied by NPM1 mutations. **(Bolouri et al.)**



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