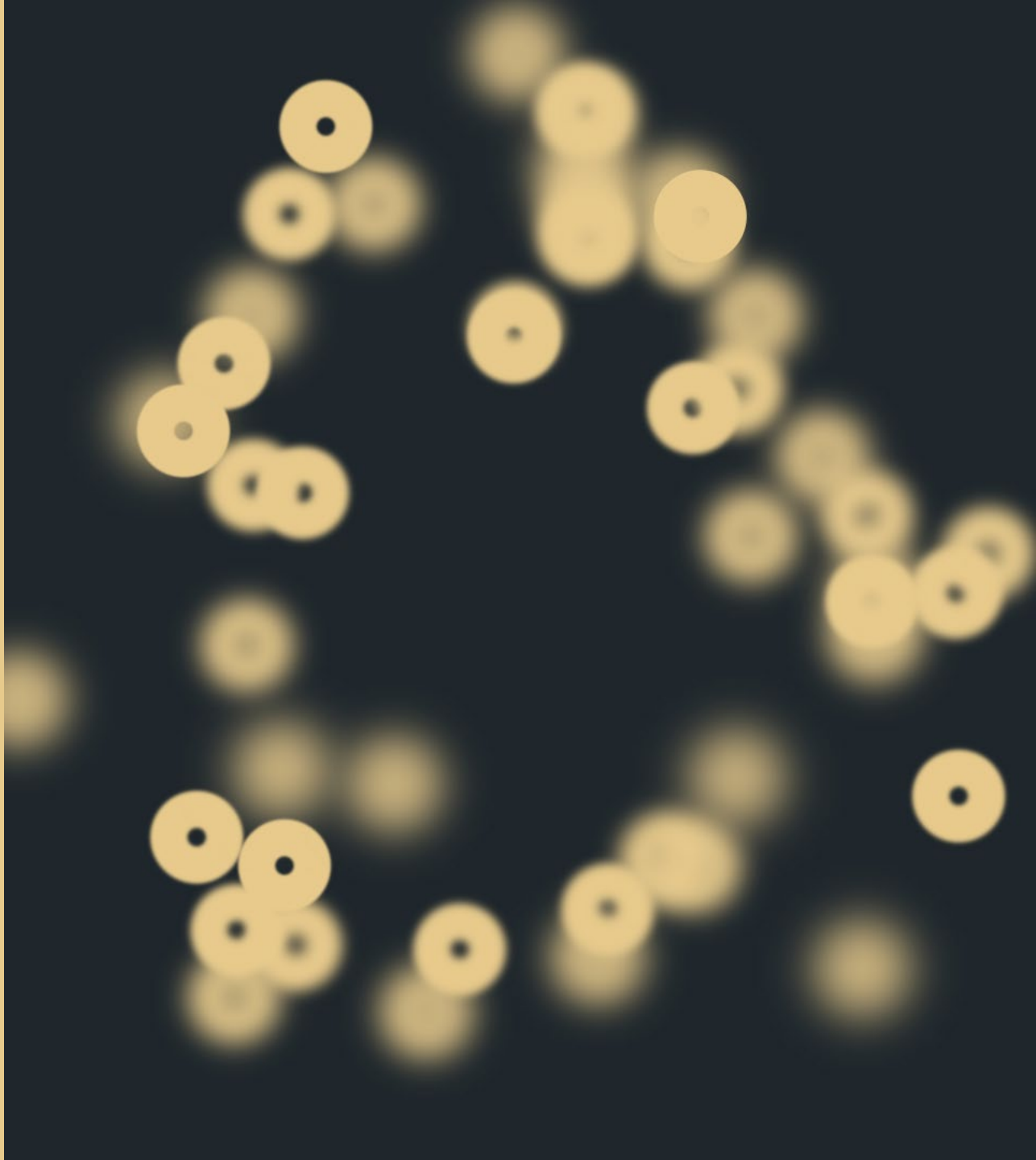
eha

# EHA-SWG Scientific Meeting on Recent Advances in the Pathogenesis and Treatment of Secondary Acute Myeloid Leukemias

Berlin, Germany
April 25-26, 2025



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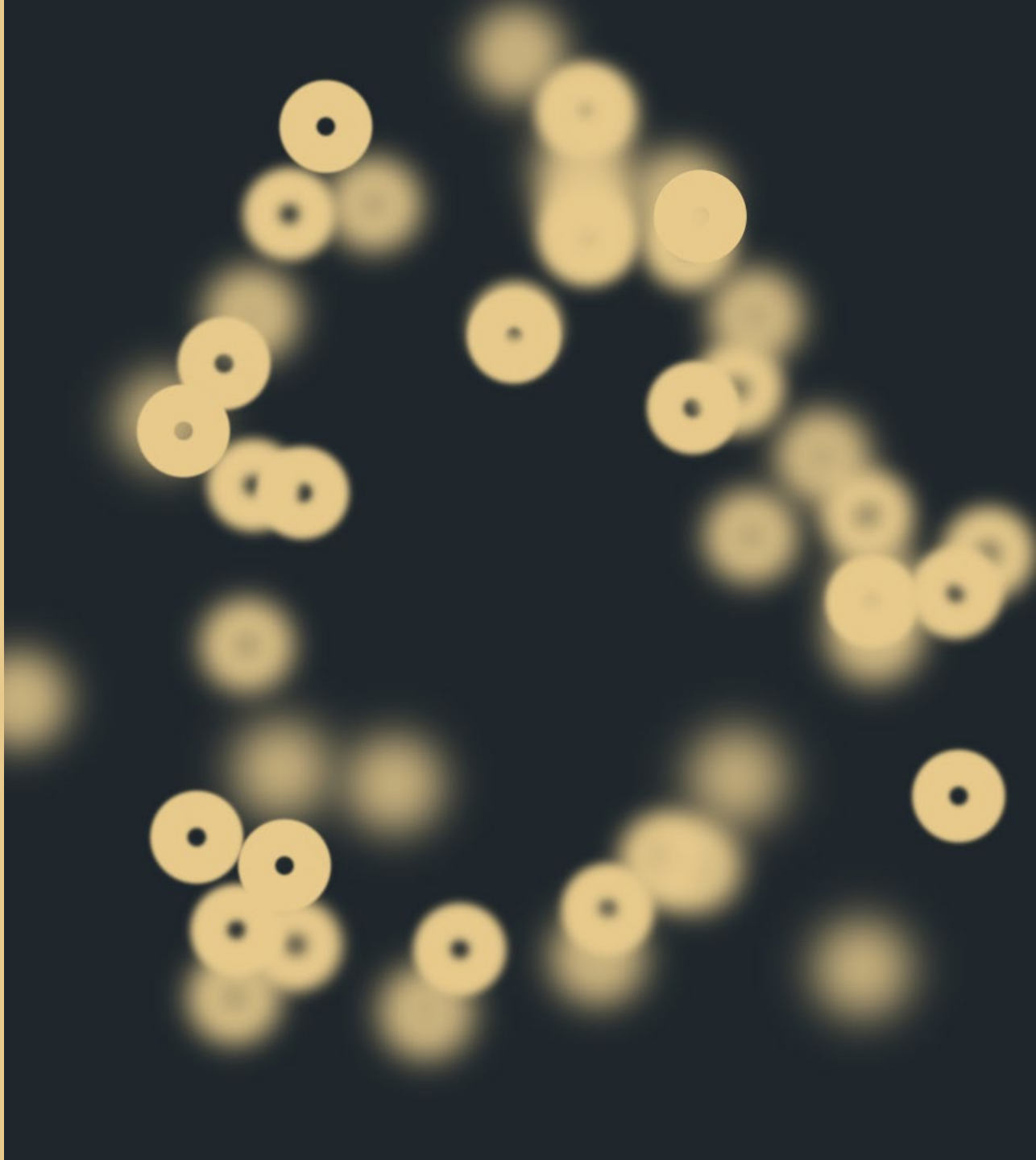
# sAML in childhood (including after gene-therapy)

Pietro Merli

Berlin, 26/04/2025



# Long term survivors of pediatric cancers

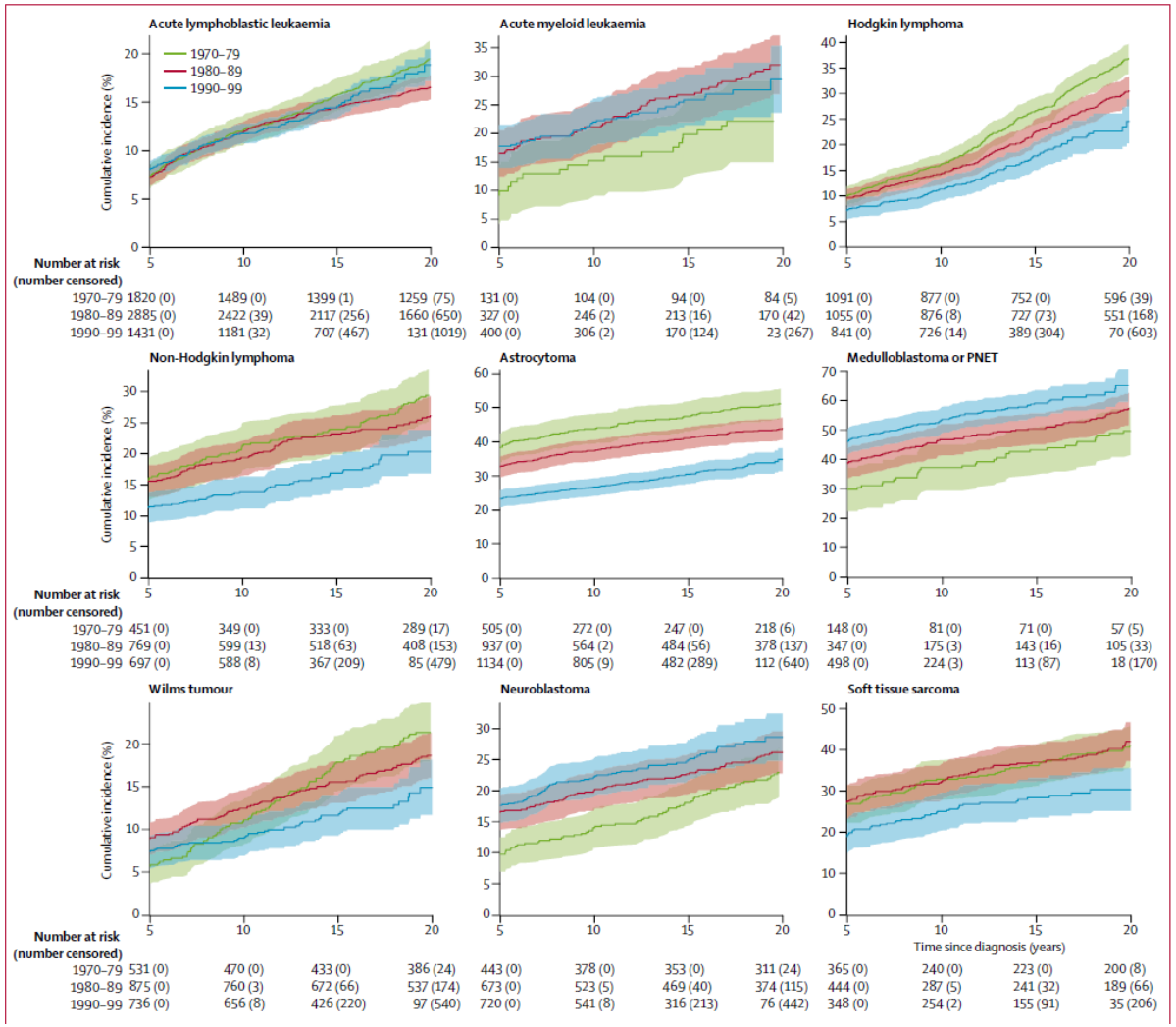
| | All survivors (n=23 601) | Diagnosis decade | | | Siblings (n=5051) |
|---|---|---|---|---|---|
| | | 1970–79 (n=6223) | 1980–89 (n=9420) | 1990–99 (n=7958) | |
| **Sex** | | | | | |
| Female | 10 947 (46%) | 2900 (47%) | 4321 (46%) | 3726 (47%) | 2643 (52%) |
| Male | 12 654 (54%) | 3323 (53%) | 5099 (54%) | 4232 (53%) | 2408 (48%) |
| **Race or ethnicity** | | | | | |
| Non-Hispanic white | 19 346 (82%) | 5533 (89%) | 7796 (83%) | 6017 (76%) | 4377 (90%) |
| Non-Hispanic black | 1500 (6%) | 241 (4%) | 577 (6%) | 682 (9%) | 151 (3%) |
| Hispanic | 1784 (8%) | 292 (5%) | 616 (7%) | 876 (11%) | 214 (4%) |
| Other | 862 (4%) | 135 (2%) | 388 (4%) | 339 (4%) | 140 (3%) |
| **Age at diagnosis, years** | | | | | |
| 0–9 | 14 811 (63%) | 3830 (62%) | 6111 (65%) | 4870 (61%) | .. |
| 10–20 | 8790 (37%) | 2393 (39%) | 3309 (35%) | 3088 (39%) | .. |
| **Age at last follow-up or death, years** | | | | | |
| <20 | 3954 (17%) | 402 (7%) | 1120 (12%) | 2432 (31%) | 419 (8%) |
| 20–29 | 9293 (39%) | 953 (15%) | 4354 (46%) | 3986 (50%) | 1591 (32%) |
| 30–39 | 7257 (31%) | 2651 (43%) | 3088 (33%) | 1518 (19%) | 1734 (34%) |
| 40–49 | 2693 (11%) | 1816 (29%) | 855 (9%) | 22 (<1%) | 1047 (21%) |
| ≥50 | 404 (2%) | 401 (6%) | 3 (<1%) | 0 (0%) | 260 (5%) |
| **Diagnosis** | | | | | |
| Acute lymphoblastic leukaemia | 6148 (26%) | 1824 (29%) | 2892 (31%) | 1432 (18%) | .. |
| Acute myeloid leukaemia | 866 (4%) | 131 (2%) | 333 (4%) | 402 (5%) | .. |
| Other leukaemia* | 303 (1%) | 74 (1%) | 105 (1%) | 124 (2%) | .. |
| Astrocytomas | 2594 (11%) | 509 (8%) | 945 (10%) | 1140 (14%) | .. |
| Medulloblastoma, PNET | 997 (4%) | 148 (2%) | 349 (4%) | 500 (6%) | .. |
| Other CNS tumours† | 645 (3%) | 79 (1%) | 206 (2%) | 360 (5%) | .. |
| Hodgkin lymphoma | 2996 (13%) | 1097 (18%) | 1057 (11%) | 842 (11%) | .. |
| Non-Hodgkin lymphoma | 1932 (8%) | 453 (7%) | 774 (8%) | 705 (9%) | .. |
| Wilms tumour | 2148 (9%) | 534 (9%) | 877 (9%) | 737 (9%) | .. |
| Neuroblastoma | 1838 (8%) | 443 (7%) | 674 (7%) | 721 (9%) | .. |
| Soft tissue sarcoma | 1162 (5%) | 365 (6%) | 448 (5%) | 349 (4%) | .. |
| Ewing sarcoma | 714 (3%) | 203 (3%) | 277 (3%) | 234 (3%) | .. |
| Osteosarcoma | 1205 (5%) | 360 (6%) | 474 (5%) | 371 (5%) | .. |
| Other bone tumours‡ | 53 (<1%) | 3 (<1%) | 9 (<1%) | 41 (1%) | .. |

Gibson TM et al., Lancet Oncol 2018



sAML in childhood (including after gene-therapy)

# Long term survivors of pediatric cancers




Gibson TM et al., Lancet Oncol 2018



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# Long term survivors of pediatric cancers

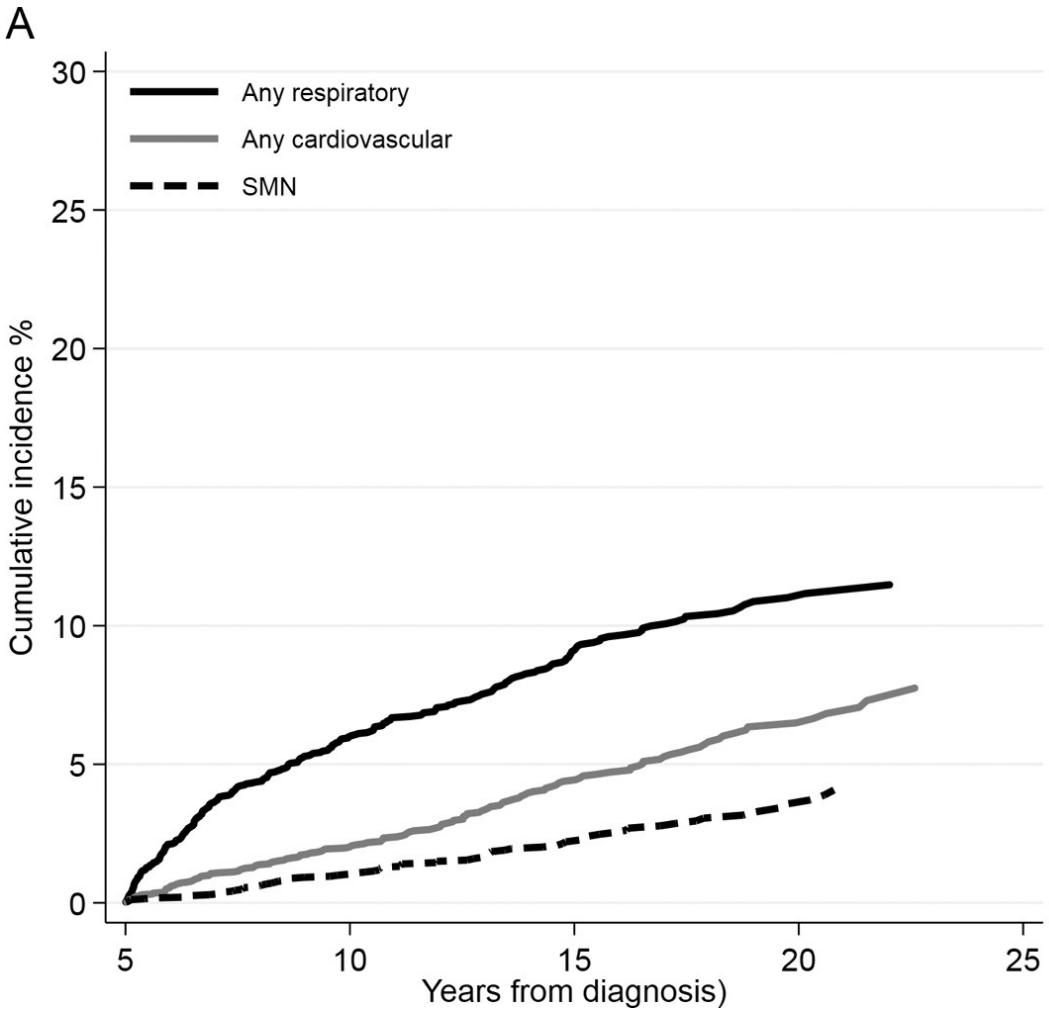
| | 1970–79 | 1980–89 | 1990–99 | 1980s *vs* 1970s (p value) | 1990s *vs* 1970s (p value) | 1990s *vs* 1980s (p value) |
|---|---|---|---|---|---|---|
| Endocrine | 5·9% (5·3–6·4) | 3·6% (3·2–3·9) | 2·8% (2·5–3·2) | <0·0001 | <0·0001 | 0·0033 |
| Thyroid nodules requiring surgery | 1·9% (1·6–2·3) | 1·2% (0·9–1·4) | 0·9% (0·7–1·1) | 0·00017 | <0·0001 | 0·10 |
| Gonadal dysfunction | 3·5% (3·1–4·0) | 1·8% (1·5–2·1) | 0·9% (0·7–1·0) | <0·0001 | <0·0001 | <0·0001 |
| Diabetes mellitus requiring insulin | 0·4% (0·2–0·5) | 0·5% (0·3–0·6) | 0·9% (0·7–1·0) | 0·35 | 0·00014 | 0·0015 |
| Second malignant neoplasms | 2·7% (2·3–3·1) | 2·4% (2·1–2·7) | 1·9% (1·6–2·2) | 0·31 | 0·0033 | 0·024 |
| Cardiovascular | 4·8% (4·3–5·3) | 5·6% (5·2–6·1) | 4·9% (4·5–5·3) | 0·018 | 0·74 | 0·023 |
| Heart failure | 0·9% (0·6–1·1) | 1·0% (0·8–1·2) | 0·8% (0·6–0·9) | 0·32 | 0·49 | 0·057 |
| Myocardial infarction | 0·6% (0·4–0·8) | 0·5% (0·4–0·6) | 0·4% (0·3–0·5) | 0·47 | 0·14 | 0·38 |
| Stroke | 1·5% (1·2–1·8) | 2·4% (2·1–2·7) | 2·0% (1·7–2·3) | <0·0001 | 0·036 | 0·032 |
| Thromboembolic disease | 2·2% (1·8–2·5) | 2·1% (1·8–2·4) | 2·0% (1·7–2·3) | 0·75 | 0·40 | 0·54 |
| Neurological | 4·8% (4·2–5·3) | 5·3% (4·9–5·8) | 4·3% (3·9–4·7) | 0·10 | 0·17 | 0·00058 |
| Memory problems | 1·7% (1·4–2·0) | 2·5% (2·2–2·8) | 2·8% (2·5–3·1) | 0·00047 | <0·0001 | 0·24 |
| Balance problems | 0·6% (0·4–0·8) | 1·0% (0·8–1·2) | 1·2% (1·0–1·4) | 0·012 | <0·0001 | 0·13 |
| Paralysis | 2·7% (2·3–3·1) | 2·2% (1·9–2·5) | 0·2% (0·1–0·3) | 0·059 | <0·0001 | <0·0001 |
| Hearing Loss | 3·0% (2·6–3·5) | 4·2% (3·8–4·6) | 5·7% (5·2–6·1) | 0·00010 | <0·0001 | <0·0001 |
| Visual impairment | 4·5% (4·0–5·0) | 4·1% (3·8–4·5) | 4·1% (3·7–4·5) | 0·34 | 0·29 | 0·91 |
| Cataracts requiring surgery | 0·8% (0·6–1·1) | 1·0% (0·8–1·2) | 1·3% (1·1–1·5) | 0·18 | 0·0040 | 0·084 |
| Blindness | 4·0% (3·5–4·5) | 3·5% (3·1–3·8) | 3·1% (2·8–3·5) | 0·11 | 0·0043 | 0·14 |
| Gastrointestinal | 2·3% (2·0–2·7) | 2·3% (2·1–2·6) | 1·5% (1·3–1·8) | 0·95 | 0·00037 | <0·0001 |
| Intestinal obstruction | 2·0% (1·7–2·4) | 1·9% (1·7–2·2) | 1·1% (0·9–1·3) | 0·64 | <0·0001 | <0·0001 |
| Hepatitis | 0·3% (0·2–0·4) | 0·4% (0·3–0·5) | 0·4% (0·3–0·5) | 0·22 | 0·28 | 0·88 |
| Musculoskeletal | 5·8% (5·2–6·4) | 4·4% (4·0–4·7) | 3·3% (2·9–3·6) | <0·0001 | <0·0001 | <0·0001 |
| Amputation | 5·1% (4·6–5·6) | 2·9% (2·5–3·2) | 1·2% (1·0–1·4) | <0·0001 | <0·0001 | <0·0001 |
| Major joint replacement | 0·8% (0·6–1·1) | 1·6% (1·4–1·9) | 2·2% (2·0–2·5) | <0·0001 | <0·0001 | 0·0015 |
| Respiratory | 0·7% (0·5–0·9) | 0·5% (0·4–0·7) | 0·8% (0·6–0·9) | 0·37 | 0·42 | 0·051 |
| Pulmonary fibrosis | 0·2% (0·1–0·3) | 0·3% (0·2–0·4) | 0·7% (0·5–0·8) | 0·21 | <0·0001 | 0·00078 |
| Renal | 0·5% (0·4–0·7) | 1·0% (0·8–1·2) | 0·9% (0·8–1·1) | 0·0010 | 0·0026 | 0·75 |
| Dialysis | 0·5% (0·3–0·7) | 0·9% (0·7–1·1) | 0·9% (0·7–1·1) | 0·0009 | 0·0026 | 0·72 |

Data are % (95% CI), unless otherwise specified.

***Table 2:*** **Cumulative incidence of grade 3–5 chronic health conditions by organ system at 15 years after primary cancer diagnosis in survivors**

Gibson TM et al., Lancet Oncol 2018

# Second malignant neoplasms (SMNs)



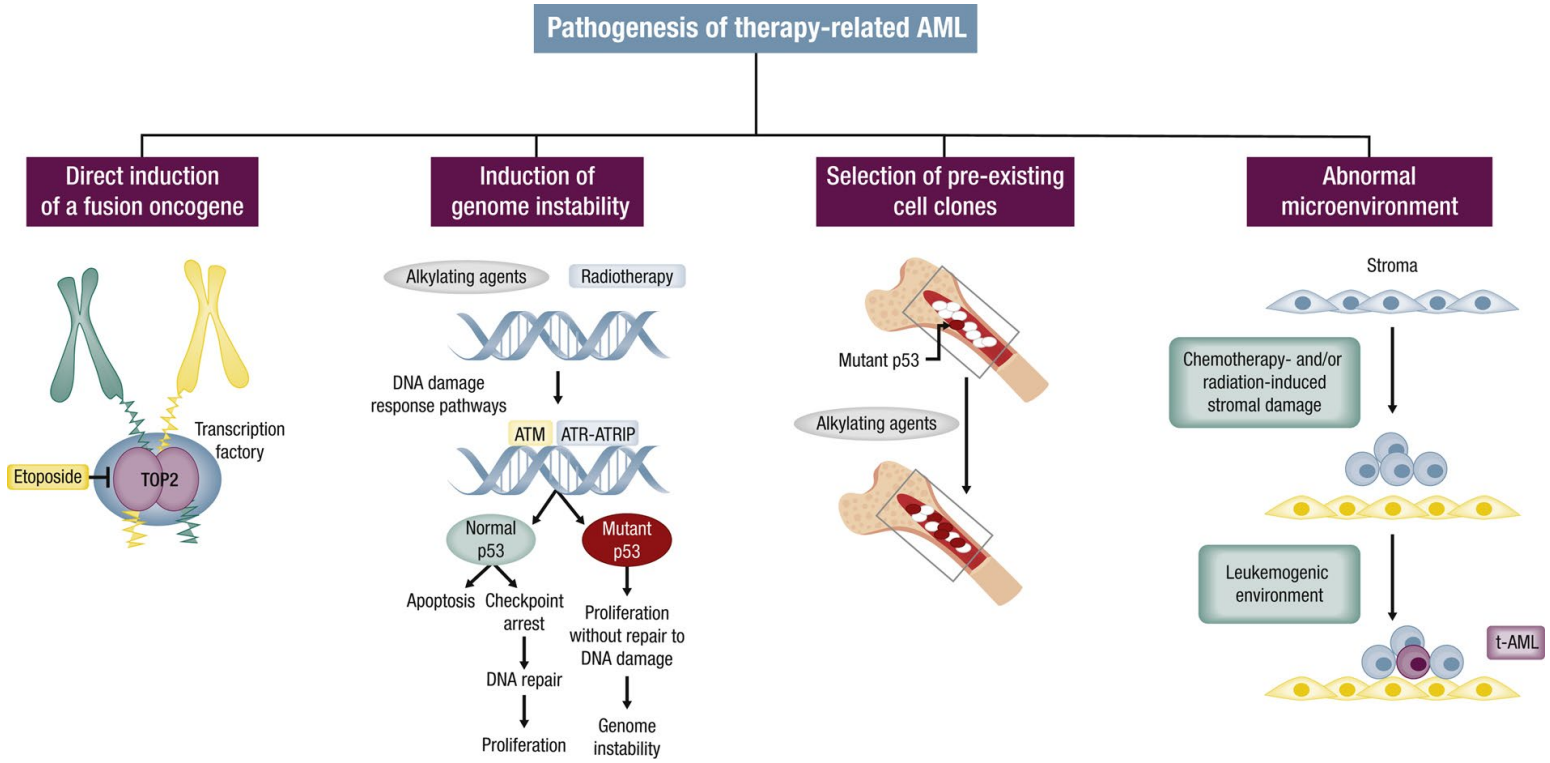

| Difference in Years | No. of Cases | % of Total SMN |
|---|---|---|
| 0–5 | 56 | 22.30 |
| 06 to 10 | 53 | 21.12 |
| 11 to 15 | 45 | 17.93 |
| 16 to 20 | 37 | 14.74 |
| 21 to 25 | 20 | 7.97 |
| 26 to 30 | 14 | 5.58 |
| 31 to 35 | 19 | 7.57 |
| >36 | 7 | 2.79 |
| Total | 251 | 100% |

| Cancer | No. of Cases | % of Total SMN |
|---|---|---|
| Thyroid | 46 | 18.33 |
| Sarcoma | 38 | 15.14 |
| Astrocytoma | 26 | 10.36 |
| Lymphoma | 24 | 9.56 |
| Salivary gland carcinoma | 18 | 7.17 |
| Melanoma | 11 | 4.38 |
| Breast | 10 | 3.98 |
| Others | 78 | 31.08 |
| Total | 251 | 100% |

# sAML

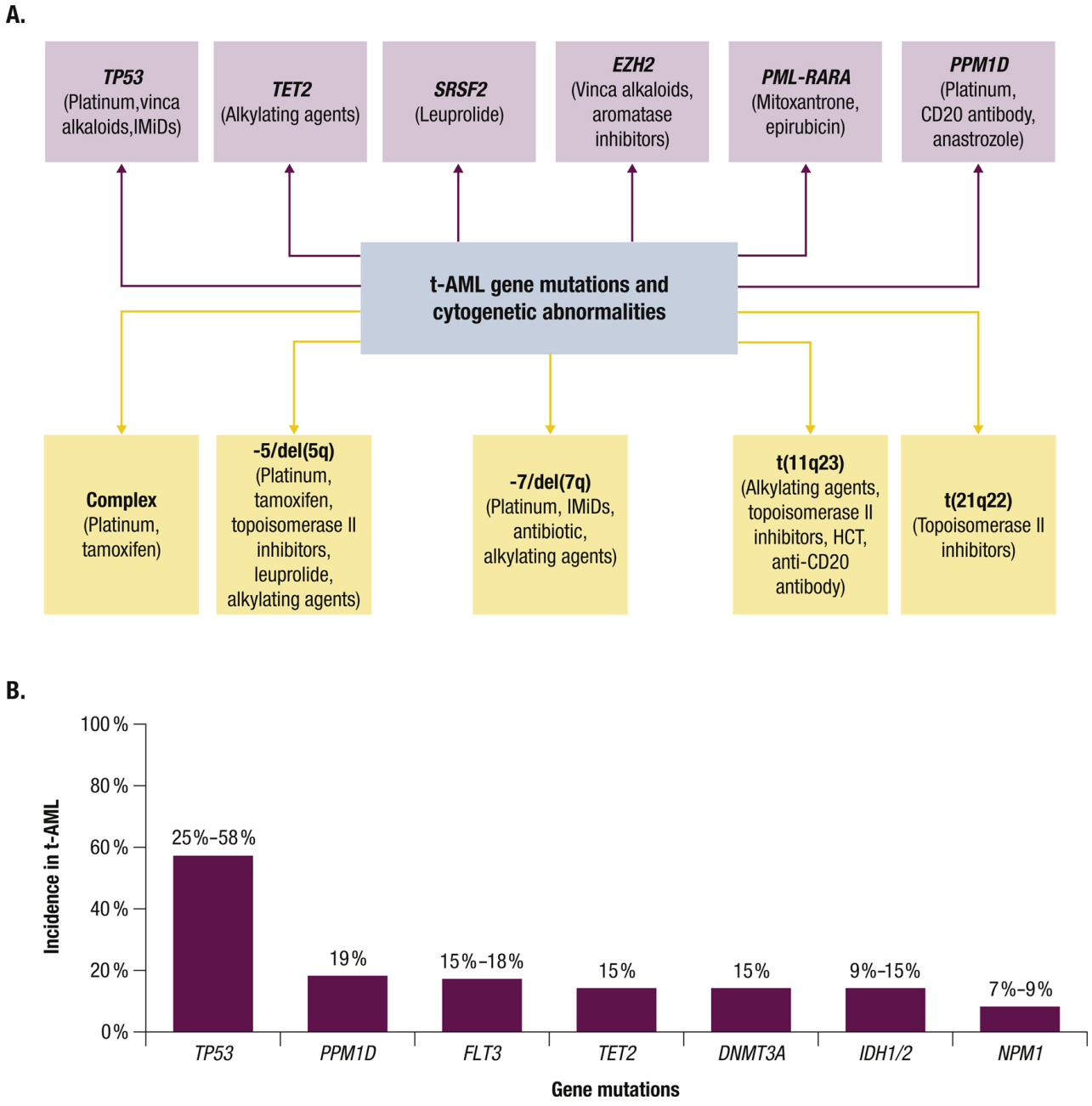
Mechanisms driving t-AML pathogenesis (non–mutually exclusive):

1) direct induction of a fusion oncogene through chromosomal translocation;
2) induction of genome instability;
3) chemotherapy or radiation-induced damage to the bone marrow creating a pro-inflammatory, pro-leukemic environment;
4) selection of pre-existing treatment-resistant hematopoietic cell clones



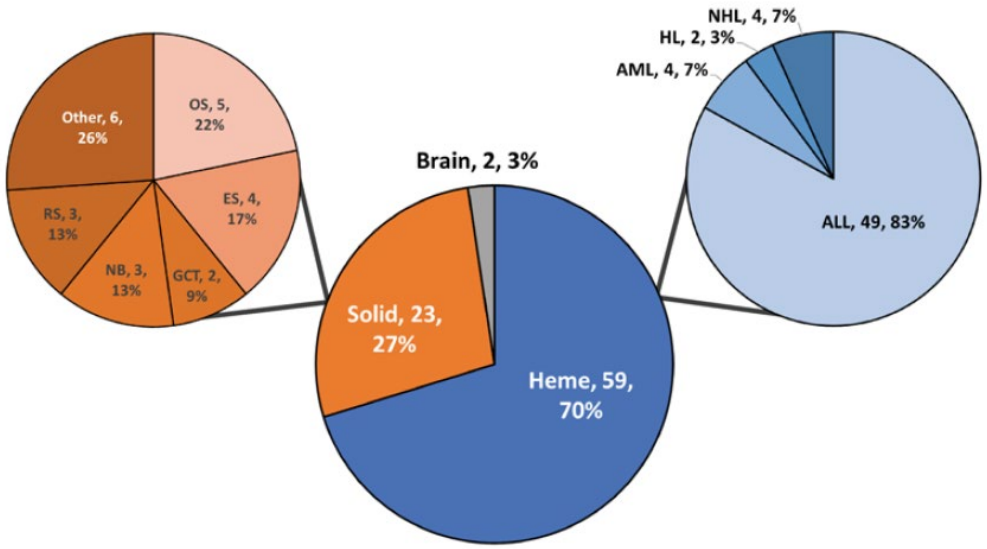

Heuser, 2016; McNerney et al., 2017

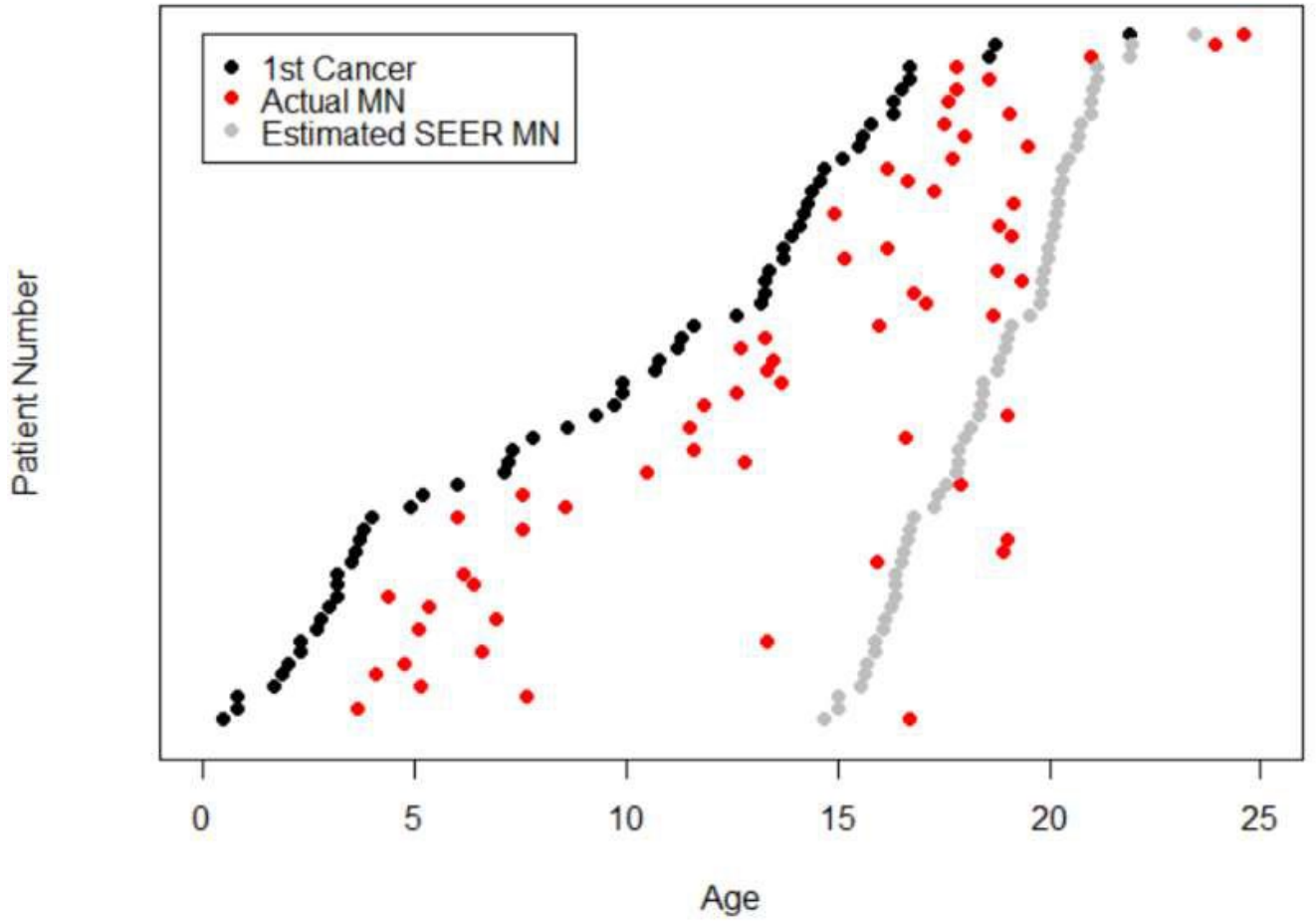
# Mutational landscape



# Pediatric Landscape

- 84 pediatric tMN cases (tMDS: n = 28, tAML: n = 56)
- whole exome, whole genome, and/or RNA sequencing

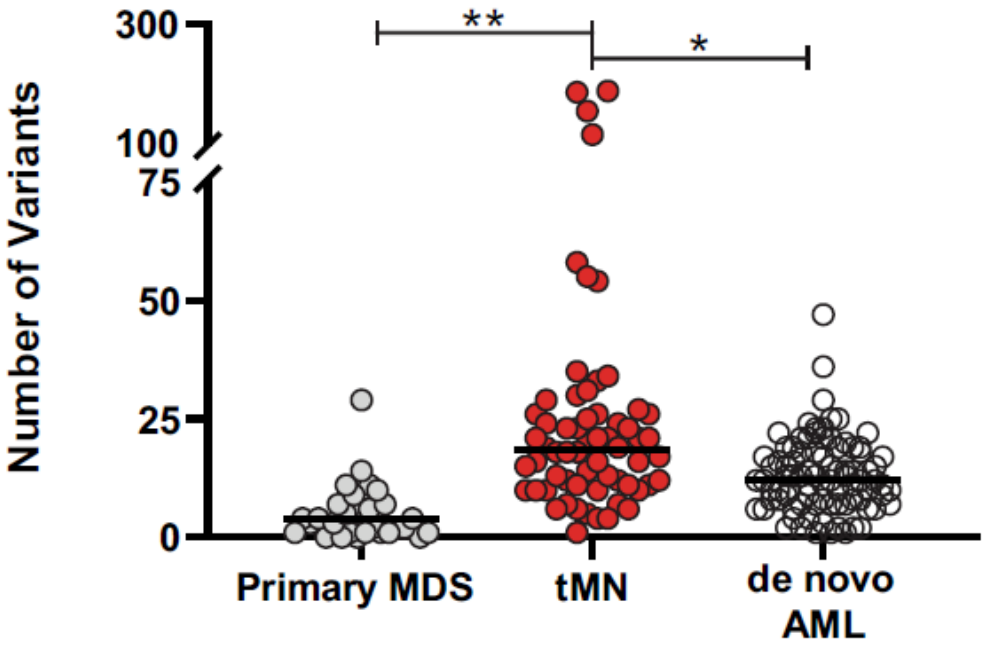


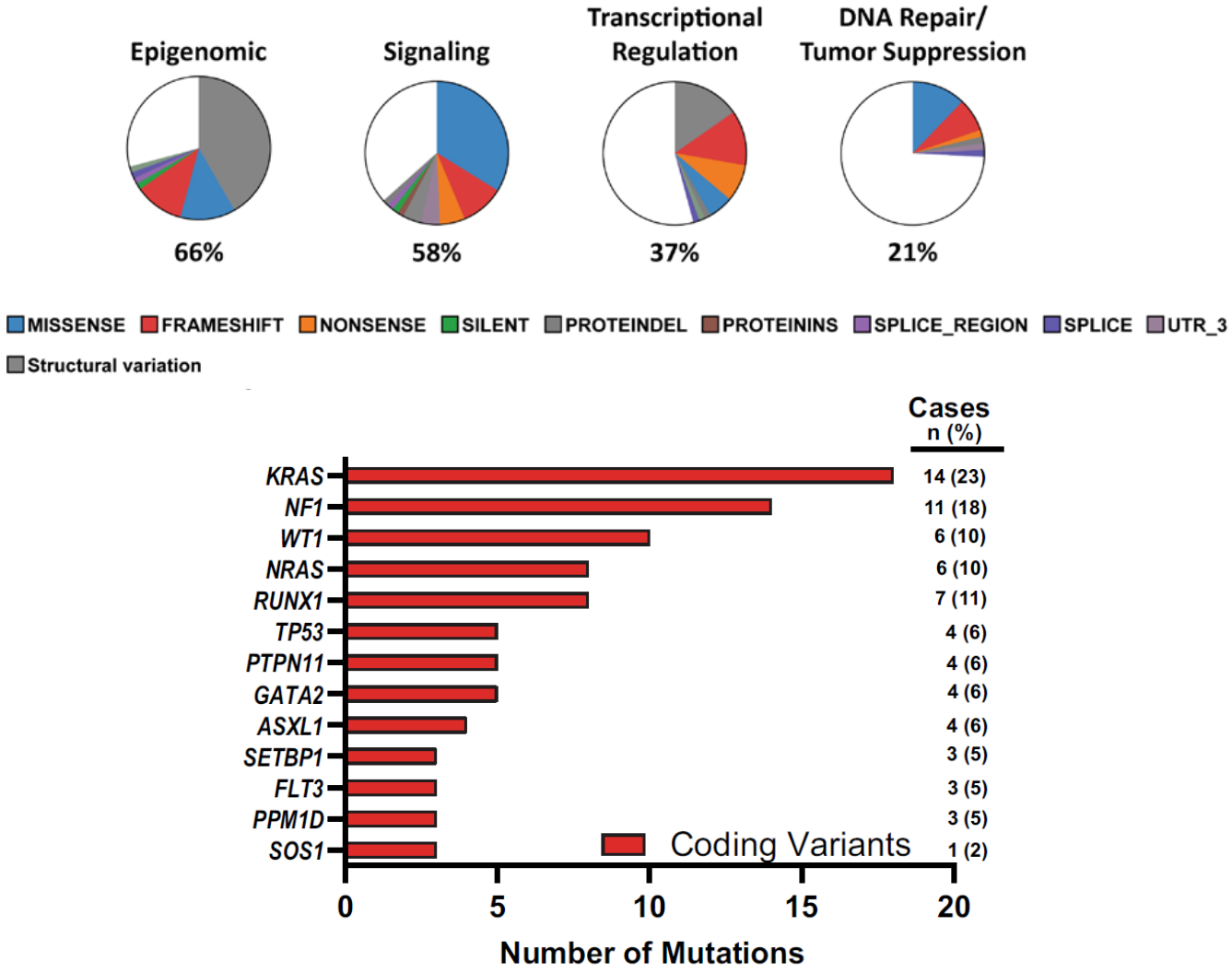






Schwartz JR et al., Nat Commun 2021

# Pediatric Landscape



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# Cancer predisposition syndromes

13 of 84 patients (15%, 95% exact binomial CI: 8.5–25.0%) had germline alterations; more common in tMN than the published prevalence of 8.5–10% in other groups of children with cancer

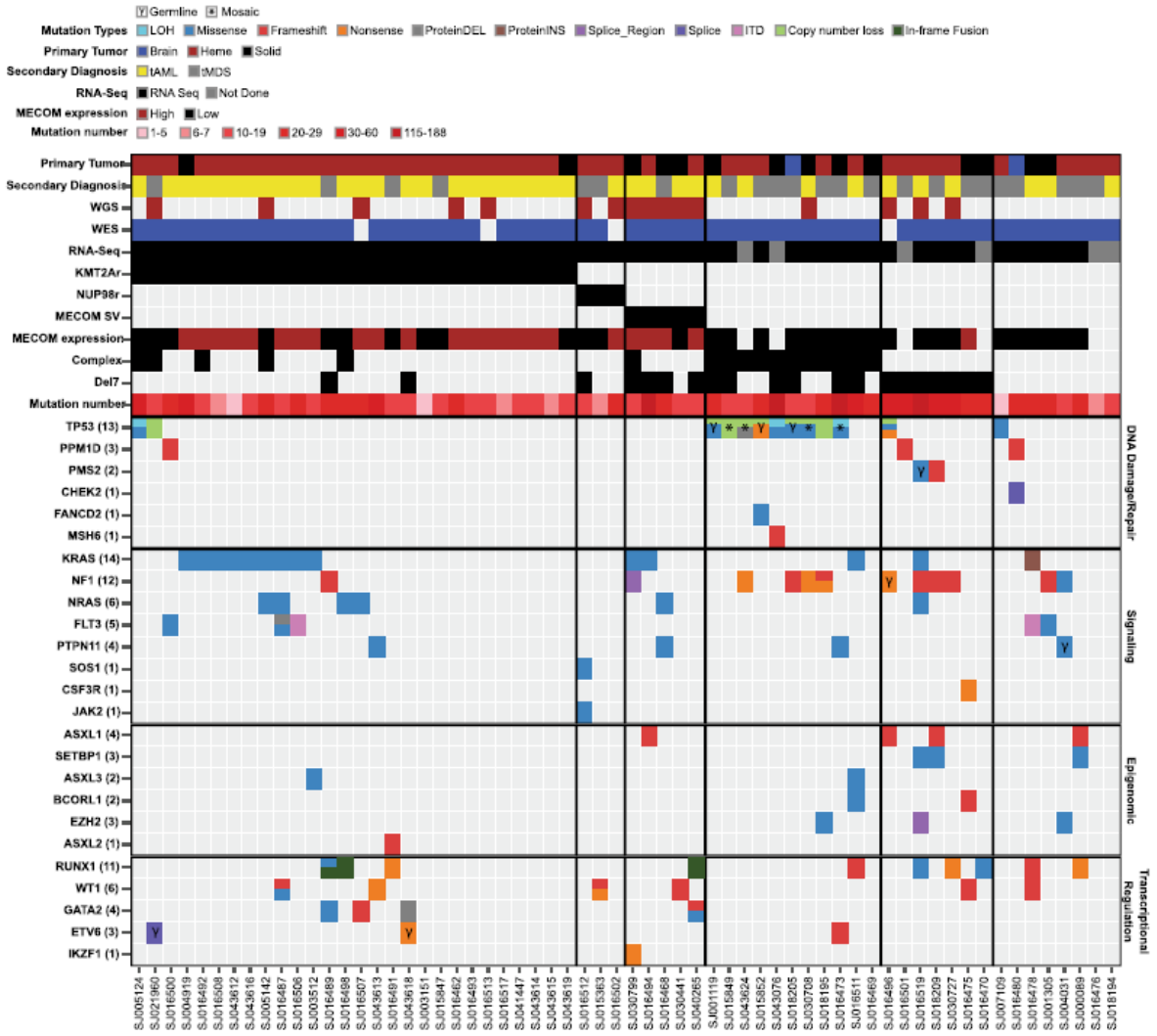
**Table 2 Pathogenic and Likely Pathogenic Germline Variants Present in the Pediatric tMN Cohort.**

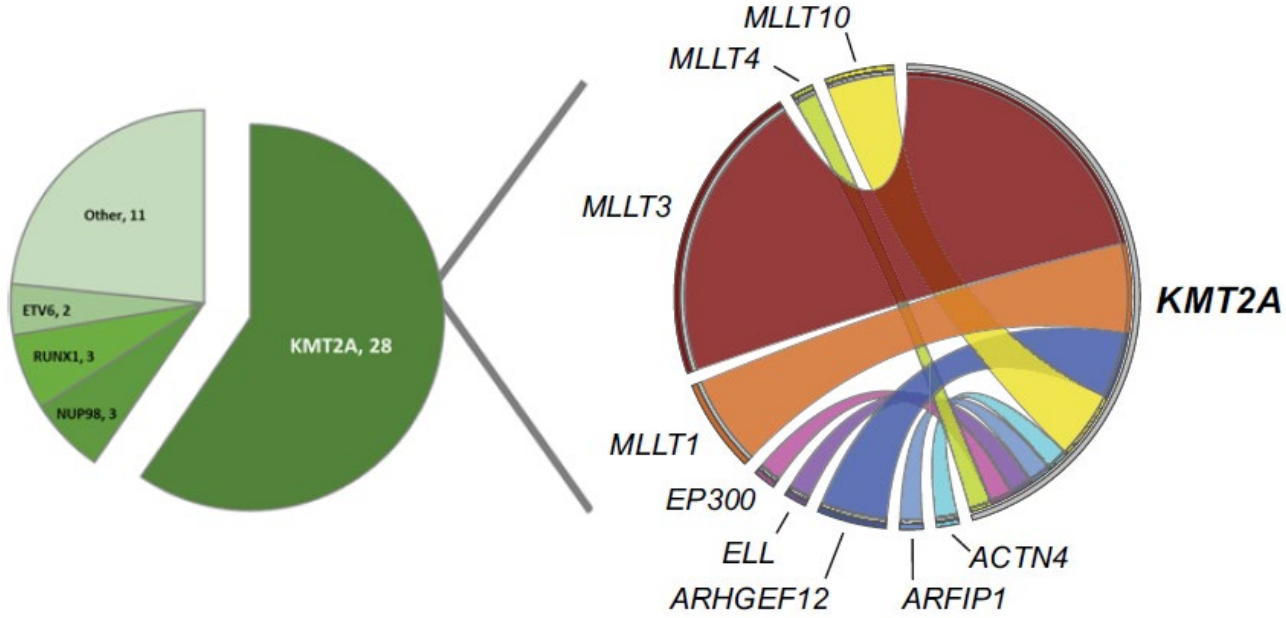
| Case | 1° Diagnosis | 2° Dx | Gene | RefSeq accession | Mutation type | Amino acid change | VAF | REVEL score | ACMG classification (criteria) |
|---|---|---|---|---|---|---|---|---|---|
| SJ016504 | NHL | tAML | *ARID2* | NM_152641 | nonsense | p.R1272X | 0.53 | | LP (PVS1, PM2) |
| SJ016509 | ALL | tMDS | *CREBBP* | NM_004380 | missense | p.R1446C | 0.35 | 0.952 | LP (PS2, PM2, PP3) |
| SJ043618 | ALL | tAML | *ETV6* | NM_001987 | nonsense | p.R359X | 0.56 | | P (PVS1, PS3, PM2, PP1) |
| SJ021960 | ALL | tMDS | *ETV6* | NM_001987 | frameshift | p.N386fs | 0.30 | | P (PVS1, PS3, PM2) |
| SJ004031 | ALL | tMDS | *EZH2* | NM_001203247 | missense | p.R685H | 0.43 | 0.907 | LP (PM2, PP2, PP3) |
| SJ016496 | ALL | tAML | *NF1* | NM_000267 | nonsense | p.R2496X | 0.50 | | P (PVS1, PM2, PP1) |
| SJ016519 | ALL | tAML | *PMS2* | NM_000535 | missense | p.S46I | 0.34 | 0.939 | LP (PS3, PP1, PM3, PP3) |
| SJ004031 | ALL | tMDS | *PTPN11* | NM_002834 | missense | p.S502L | 0.39 | 0.976 | LP (PM1, PM2, PP2, PP3) |
| SJ043615 | ALL | tAML | *RPL22* | NM_000983 | splice | E40_E3splice | 0.44 | | LP (PVS1, PM2) |
| SJ016463 | Osteosarcoma | tMDS | *TP53* | NM_000546 | missense | p.R337C | 0.58 | 0.715 | P (PS3, PM1, PM2, PP2, PP3) |
| SJ001119 | Osteosarcoma | tAML | *TP53* | NM_000546 | missense | p.R337L | 0.58 | 0.765 | P (PS3, PM1, PM2, PM5, PP3) |
| SJ015852 | ALL | tMDS | *TP53* | NM_000546 | nonsense | p.W53X | 0.52 | | P (PVS1, PM2, PP4) |
| SJ018205 | Anaplastic Astrocytoma | tMDS | *TP53* | NM_000546 | missense | p.H179Y | 0.50 | 0.948 | P (PS2, PS3, PM1, PM2, PP1, PP3) |
| SJ016486 | ALL | tAML | *TRIP11* | NM_004239 | frameshift | p.Q1367fs | 0.40 | | LP (PVS1, PM2) |



Schwartz JR et al., Nat Commun 2021

# Pediatric Landscape



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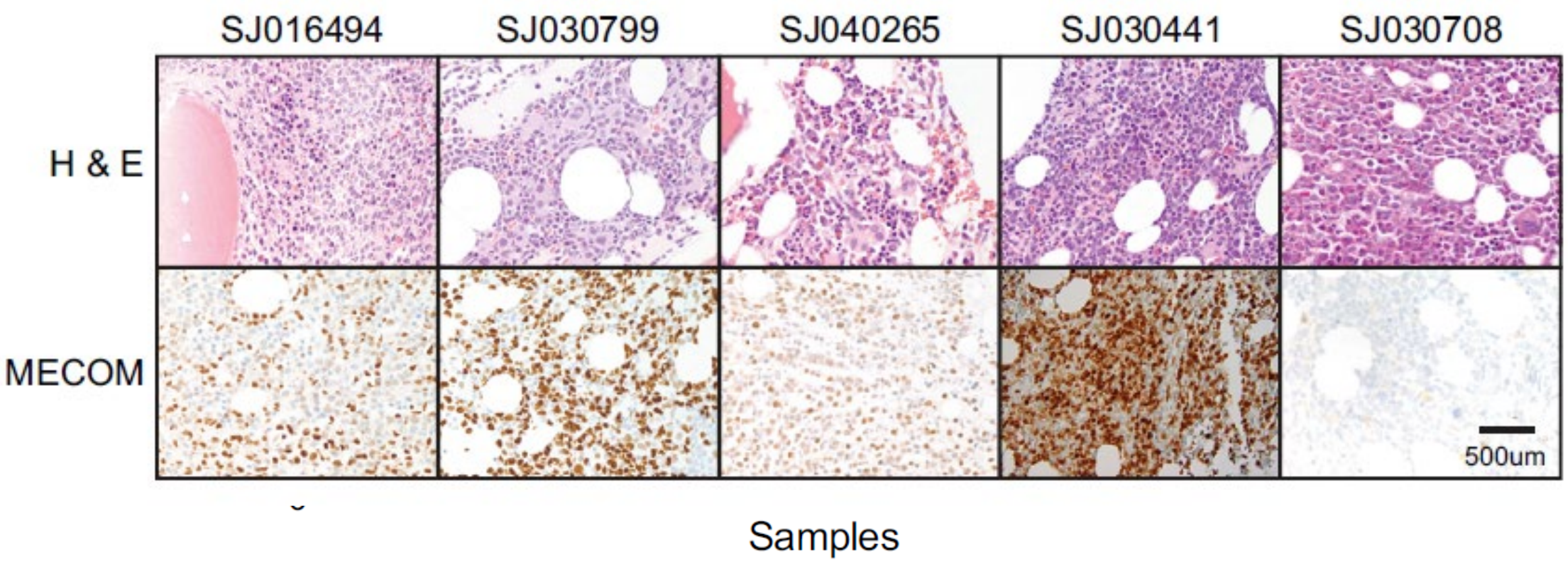
# Focus on *MECOM*

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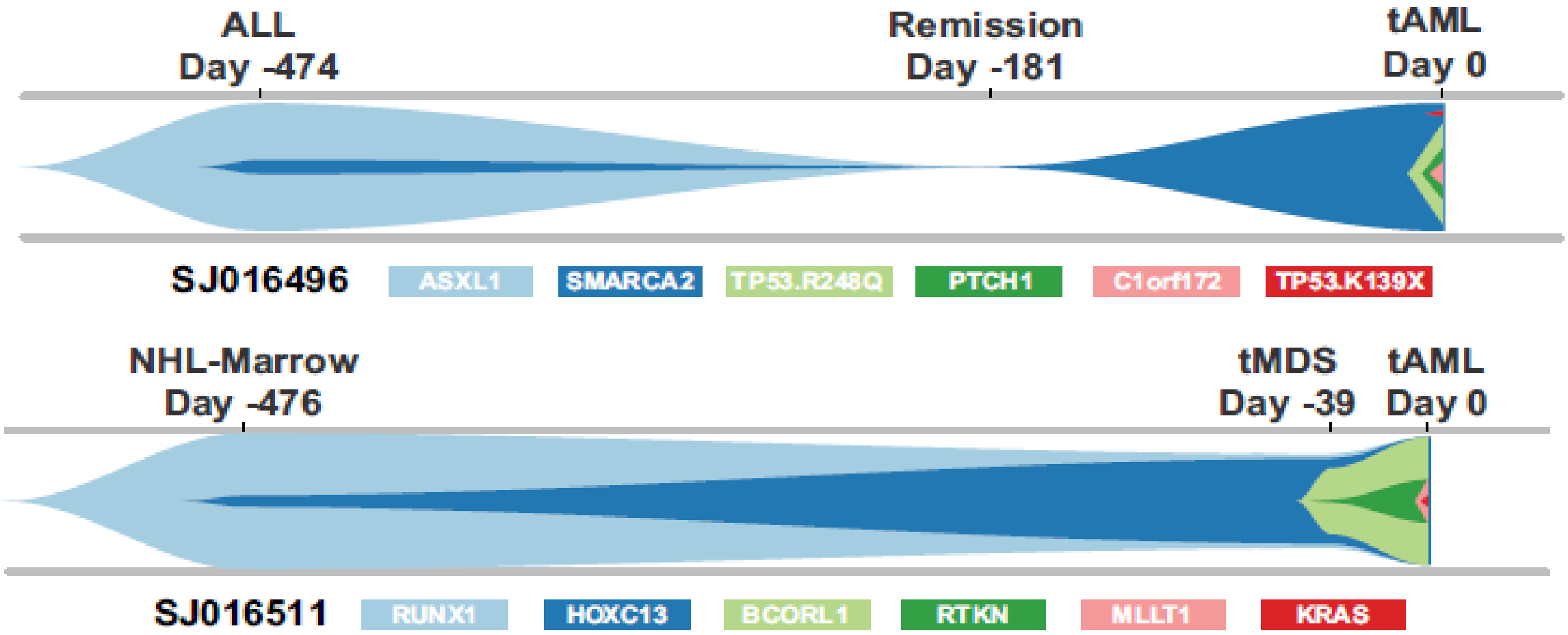
# Clonal evolution of pediatric tMN






Schwartz JR et al., Nat Commun 2021

# Pediatric Landscape - conclusions

- KMT2Ar are the most common driver alterations in this pediatric tMN cohort along with Ras/MAPK pathway mutations. Somatic TP53 alterations were also frequent, but these mutations appeared to arise after chemotherapy, unlike adult tMN;
- MECOM overexpression is frequent, and in some of these cases the overexpression was driven by enhancer hijacking;
- pediatric tMN-defining variants arise most commonly as a consequence of cytotoxic therapy, and that these malignant clones can be identified, on average, >1 year before morphologic evidence of neoplasm.
- unlike adults with tMN, scarce evidence of pre-existing minor tMN clones;
- rare cases of lineage switch disease rather than true secondary neoplasms;



Schwartz JR et al., Nat Commun 2021

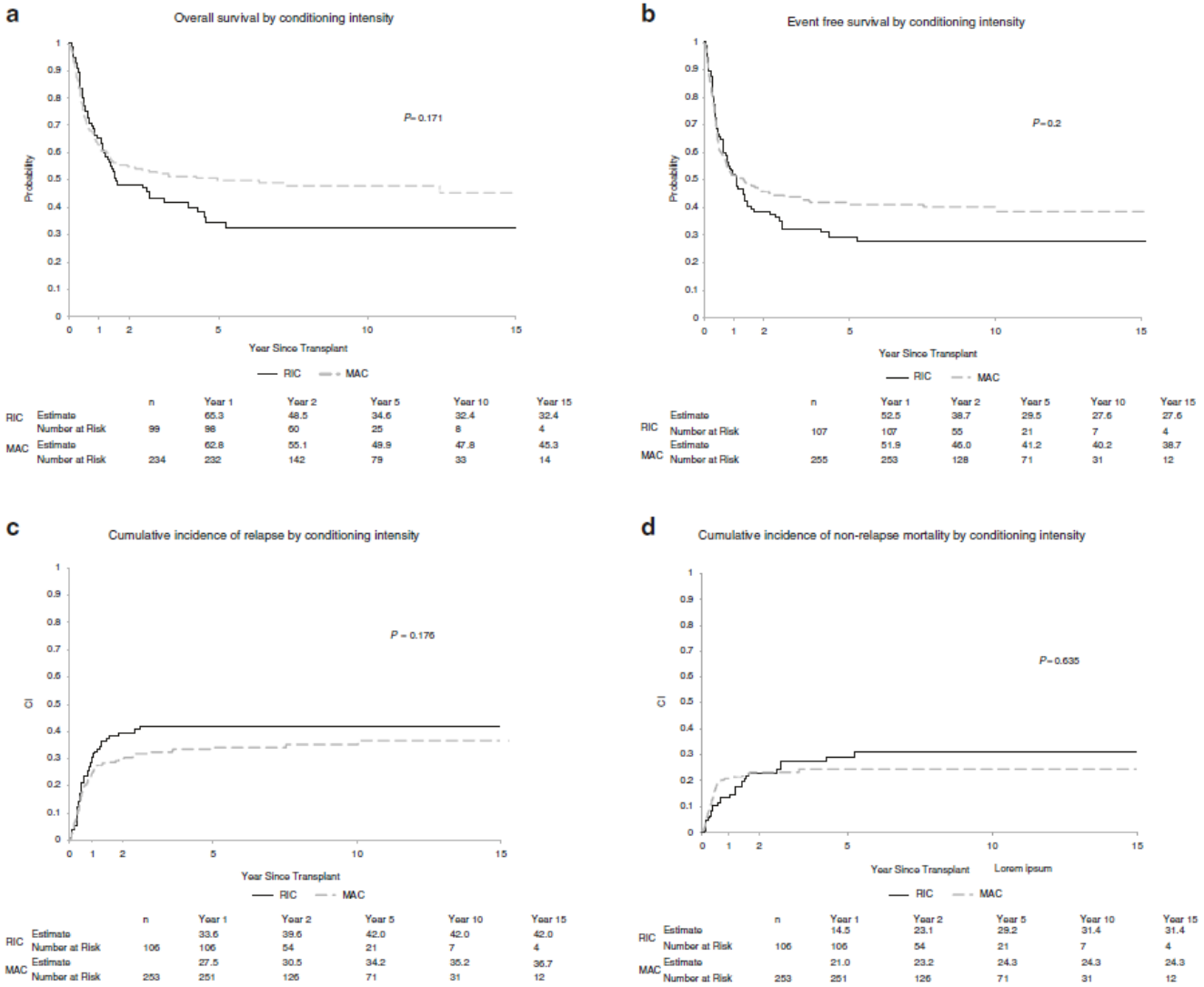
# Treatment - HSCT

- 401 patients from 54 centers
- Retrospective analysis
- US, Europe, Mexico, Australia
- tMDS or tAML who were aged 21 years or younger at the time of HCT and who received transplants
- between 1995 and 2017
- Therapy-related myeloid neoplasms were defined according to the 2017 World Health Organization (WHO) criteria
- Patients with known inherited genetic predisposition disorders (like Fanconi anemia, or Li-Fraumeni syndrome) were not included in this study.

| | | | | | |
|---|---|---|---|---|---|
| *Primary diagnosis* | | | | | 0.130 |
| ALL | 81 (20.2) | 62 (22.7) | 17 (13.8) | 2 (40.0) | |
| AML | 13 (3.2) | 8 (2.9) | 5 (4.1) | 0 (0.0) | |
| Other malignant heme disorder[b] | 39 (9.7) | 23 (8.4) | 16 (13.0) | 0 (0.0) | |
| Solid/brain tumor | 156 (38.9) | 104 (38.1) | 50 (40.7) | 2 (40.0) | |
| Unknown | 112 (27.9) | 76 (27.8) | 35 (28.5) | 1 (20.0) | |
| *Diagnosis at HCT* | | | | | 0.104 |
| tMDS | 261 (65.1) | 175 (64.1) | 82 (66.7) | 4 (80.0) | |
| tAML | 122 (30.4) | 93 (34.1) | 29 (23.6) | 0 (0.0) | |
| Unknown | 18 (4.5) | 5 (1.8) | 12 (9.8) | 1 (20.0) | |
| *Age at HCT in years* | | | | | 0.270 |
| Mean ± SD | 12.6 ± 5.0 | 12.4 ± 5.0 | 13.0 ± 5.0 | 10.2 ± 5.0 | |
| Median (range) | 12.9 (1.2–21.0) | 12.5 (1.2–21.0) | 13.5 (2.8–21.0) | 10.1 (4.8–15.7) | |
| Unknown | 1 (0.2) | 0 (0.0) | 0 (0.0) | 1 (20.0) | |
| *Time from tMDS/tAML to HCT in months* | | | | | 0.097 |
| Mean ± SD | 6.5 ± 8.7 | 5.9 ± 8.3 | 7.5 ± 9.4 | 18.0 ± 9.6 | |
| Median (range) | 3.9 (0.3–67.2) | 3.8 (0.3–67.2) | 4.4 (0.9–60.7) | 22.9 (7.0–24.1) | |
| Unknown | 7 (1.7) | 3 (1.1) | 2 (1.7) | 2 (40.0) | |
| *Donor type* | | | | | <0.001 |
| MSD | 103 (25.7) | 77 (28.2) | 25 (20.3) | 1 (20.0) | |
| MUD | 115 (28.7) | 78 (28.6) | 35 (28.5) | 2 (40.0) | |
| MMRD | 49 (12.2) | 21 (7.7) | 28 (22.8) | 0 (0.0) | |
| MMUD | 28 (7.0) | 22 (8.1) | 6 (4.9) | 0 (0.0) | |
| Cord | 73 (18.2) | 55 (20.2) | 18 (14.6) | 0 (0.0) | |
| Unknown | 33 (8.2) | 34 (12.5) | 13 (10.6) | 2 (40.0) | |
| *Graft source* | | | | | 0.006 |
| BM | 223 (55.6) | 160 (58.6) | 61 (49.6) | 2 (40.0) | |
| PBSC | 101 (25.2) | 56 (20.5) | 44 (35.8) | 1 (20.0) | |
| Cord | 73 (18.2) | 55 (20.2) | 18 (14.6) | 0 (0.0) | |
| Unknown | 4 (1.0) | 2 (0.7) | 0 (0.0) | 2 (40.0) | |
| *Cytogenetic category* | | | | | 0.138 |
| MLL rearrangement | 69 (17.2) | 54 (19.8) | 15 (12.2) | 0 (0.0) | |
| Monosomy 7 | 67 (16.7) | 44 (16.1) | 23 (18.7) | 0 (0.0) | |
| Normal/trisomy 8 | 26 (6.5) | 18 (6.6) | 6 (4.9) | 2 (40.0) | |
| Random aberrations | 65 (16.2) | 42 (15.4) | 23 (18.7) | 0 (0.0) | |
| Structurally complex karyotype | 36 (9.0) | 20 (7.3) | 16 (13.0) | 0 (0.0) | |
| Unknown | 138 (34.4) | 95 (34.8) | 40 (32.5) | 3 (60.0) | |

Sharma A et al., Bone Marrow Transplant 2021

# Treatment - HSCT




| | | n | Year 1 | Year 2 | Year 5 | Year 10 | Year 15 |
|---|---|---|---|---|---|---|---|
| RIC | Estimate | | 65.3 | 48.5 | 34.6 | 32.4 | 32.4 |
| | Number at Risk | 99 | 98 | 60 | 25 | 8 | 4 |
| MAC | Estimate | | 62.8 | 55.1 | 49.9 | 47.8 | 45.3 |
| | Number at Risk | 234 | 232 | 142 | 79 | 33 | 14 |

| | | n | Year 1 | Year 2 | Year 5 | Year 10 | Year 15 |
|---|---|---|---|---|---|---|---|
| RIC | Estimate | | 52.5 | 38.7 | 29.5 | 27.6 | 27.6 |
| | Number at Risk | 107 | 107 | 55 | 21 | 7 | 4 |
| MAC | Estimate | | 51.9 | 46.0 | 41.2 | 40.2 | 38.7 |
| | Number at Risk | 255 | 253 | 128 | 71 | 31 | 12 |

| | | n | Year 1 | Year 2 | Year 5 | Year 10 | Year 15 |
|---|---|---|---|---|---|---|---|
| RIC | Estimate | | 33.6 | 39.6 | 42.0 | 42.0 | 42.0 |
| | Number at Risk | 106 | 106 | 54 | 21 | 7 | 4 |
| MAC | Estimate | | 27.5 | 30.5 | 34.2 | 35.2 | 36.7 |
| | Number at Risk | 253 | 251 | 126 | 71 | 31 | 12 |

| | | n | Year 1 | Year 2 | Year 5 | Year 10 | Year 15 |
|---|---|---|---|---|---|---|---|
| RIC | Estimate | | 14.5 | 23.1 | 29.2 | 31.4 | 31.4 |
| | Number at Risk | 106 | 106 | 54 | 21 | 7 | 4 |
| MAC | Estimate | | 21.0 | 23.2 | 24.3 | 24.3 | 24.3 |
| | Number at Risk | 253 | 251 | 126 | 71 | 31 | 12 |

Sharma A et al., Bone Marrow Transplant 2021

# Treatment - HSCT

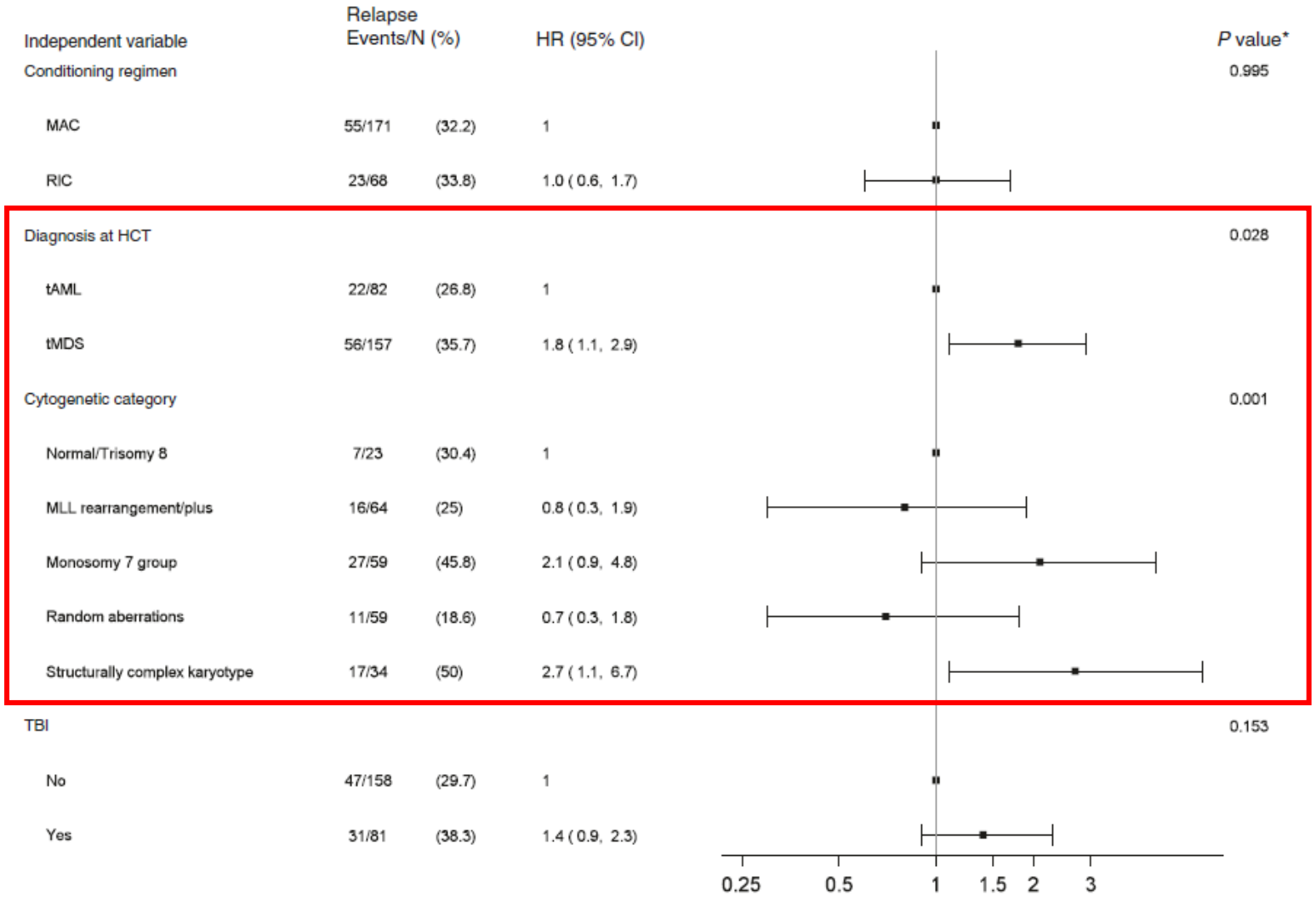
| The primary cause of death | Conditioning intensity | | | |
|---|---|---|---|---|
| | MAC *N* (%) | RIC *N* (%) | Unknown *N* (%) | Total *N* (%) |
| Total number of deaths | 151 | 81 | 3 | 235 |
| Treatment-related | 58 (38.4) | 17 (20.9) | 1 (33.3) | 68 (32.3) |
| Acute GVHD | 7 (4.6) | 1 (1.2) | 0 (0.0) | 8 (3.4) |
| Chronic GVHD | 8 (5.3) | 4 (4.9) | 0 (0.0) | 12 (5.1) |
| Graft rejection or failure | 6 (4.0) | 0 (0.0) | 0 (0.0) | 6 (2.6) |
| Infection[a] | 11 (7.3) | 5 (6.2) | 0 (0.0) | 16 (6.8) |
| Organ failure (not due to GVHD or infection) | 20 (13.3) | 6 (7.4) | 0 (0.0) | 26 (11.1) |
| Pulmonary complications | 6 (4.0) | 1 (1.2) | 1 (33.3) | 8 (3.4) |
| Malignancy[b] | 3 (2.0) | 7 (8.6) | 0 (0.0) | 10 (4.3) |
| Relapse/persistence/progression of disease | 70 (46.4) | 37 (45.7) | 1 (33.3) | 108 (46.0) |
| Other | 2 (1.3) | 1 (1.2) | 0 (0.0) | 3 (1.3) |
| Unknown | 18 (11.9) | 19 (23.5) | 1 (33.3) | 38 (16.2) |

*GVHD* graft-versus-host disease, *MAC* myeloablative conditioning, *RIC* reduced-intensity conditioning.
[a]Infection (isolation of an organism leading to sepsis/organ failure with no other ascertainable cause of death in the previous 7 days)
[b]Malignancy refers to a malignancy unrelated to the therapy-related myeloid neoplasm diagnosis.

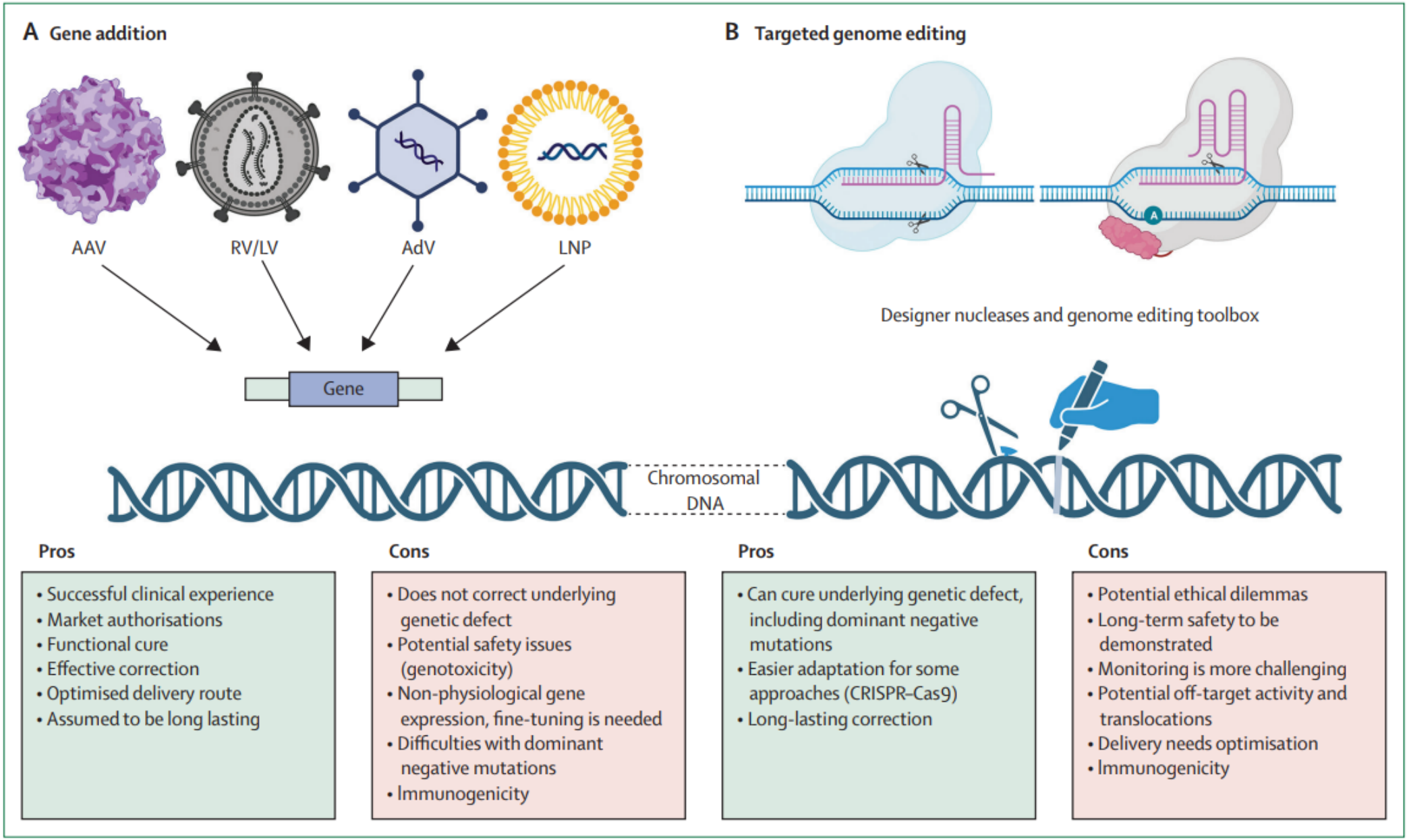


Sharma A et al., Bone Marrow Transplant 2021

# Treatment - HSCT




Sharma A et al., Bone Marrow Transplant 2021




Schambach A et al., Lancet 2024

# Gene therapy for blood and metabolic disorders

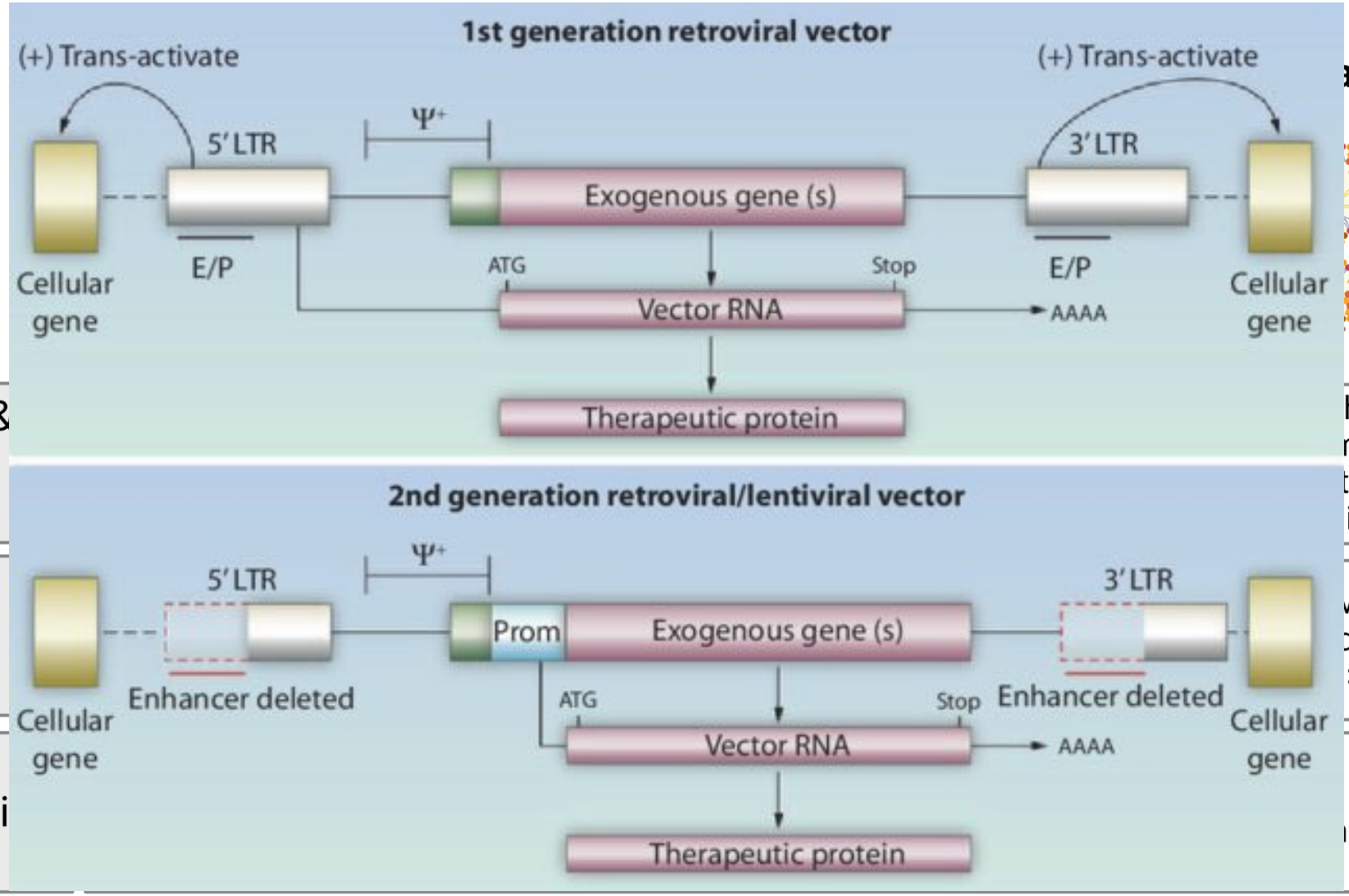
## Gene therapy-based treatments are currently under development for a range of hematological, immunological and metabolic disorders

1. β-thalassemia
2. Sickle cell disease
3. Hemophilias
4. Primary immune deficiencies, AID
5. Metabolic disorders (ALD, MLD and MPS)
6. Hematological malignancies (leukemias and lymphomas)

ALD, adrenoleukodystrophy; MLD, metachromatic leukodystrophy.

Scott CT, DeFrancesco L. *Nat Biotechnol* 2016;34(6):600–7. Tani K. *Int J Hematol* 2016;104(1):42–72. Clément F, et al. *Stem Cell Investig* 2017;4:67.
Beitelshees M, et al. *Discov Med* 2017;24(134):313–22.

# Vectors




articles

| | | |
|---|---|---|
| 1 | Expression & Genomic integration | h level<br>n expression<br>t expression<br>ic integration |
| 2 | Transduction Packaging capacity | viding and<br>dividing cells<br>> 10 kb |
| 3 | Immunogenici | Low<br>unogenicity |



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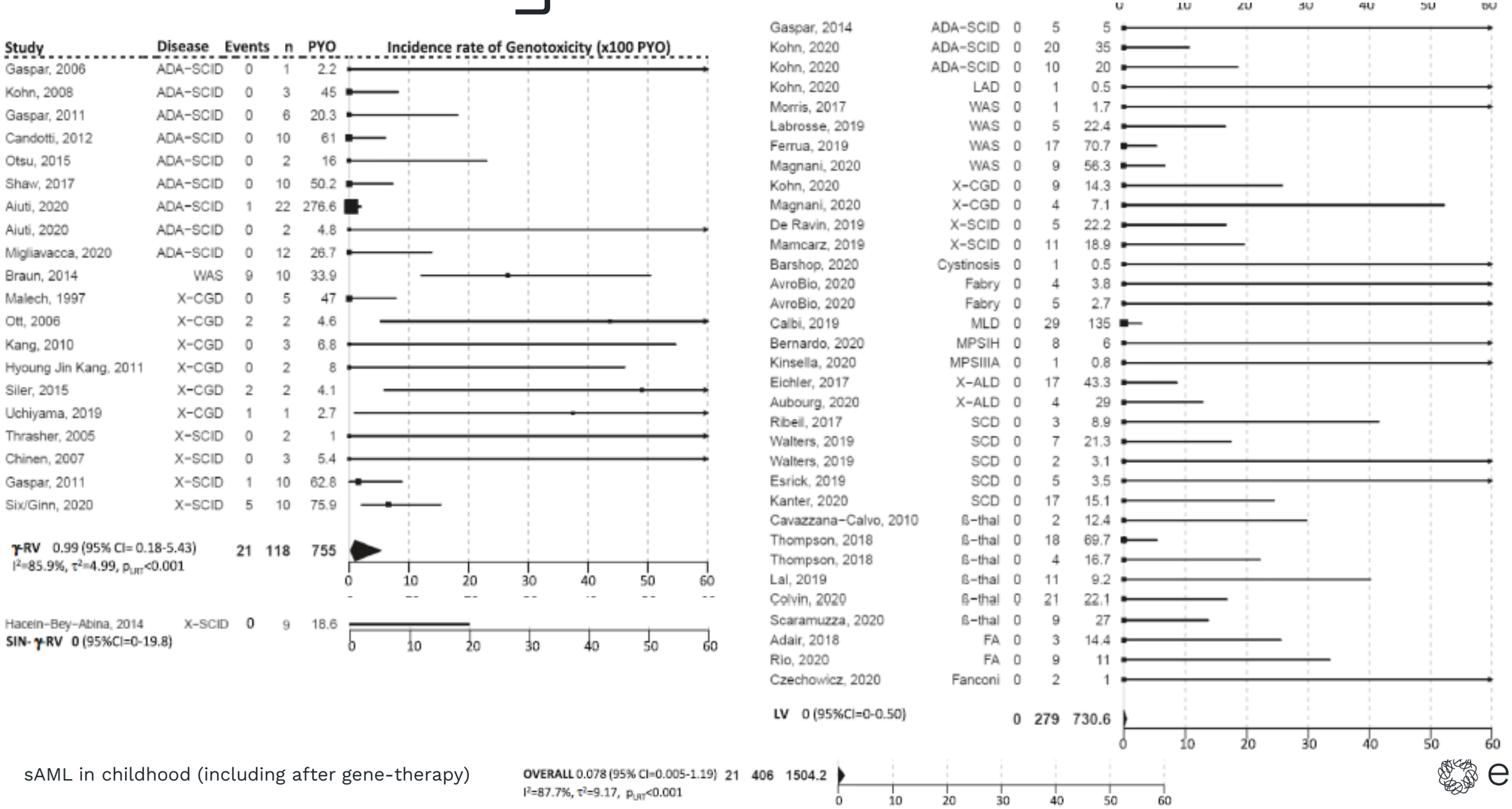
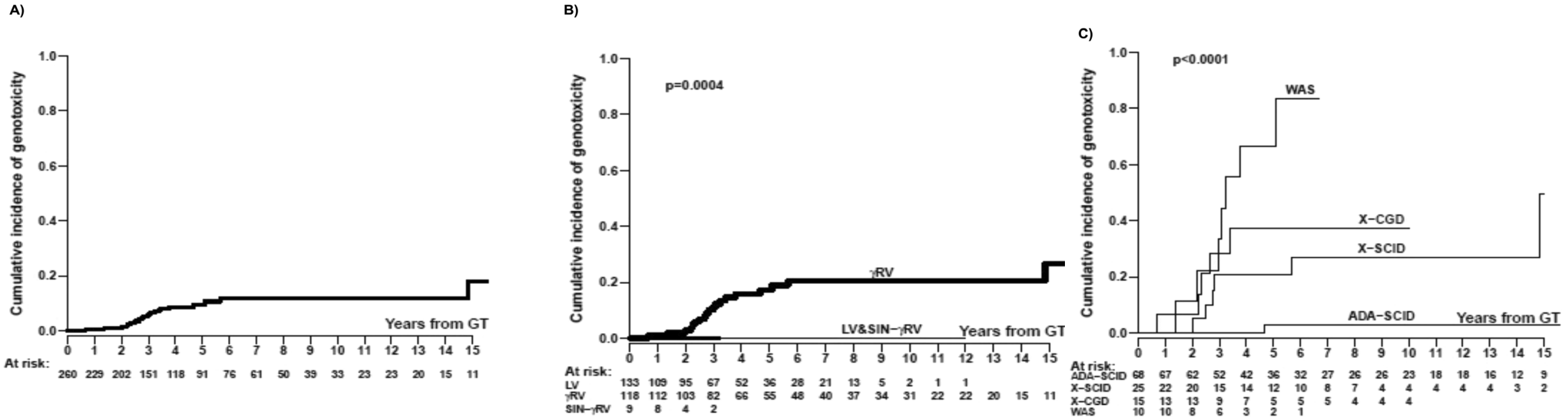
# Genotoxicity of different vectors



# Genotoxicity of different vectors






Tucci F et al., Nat Commun 2022

# Cases of AML in SCD enrolled in LentiGlobin Program

| Patient:<br>Date of Diagnosis: | SCD Pt 1<br>2018 | SCD Pt 2<br>2021 |
|---|---|---|
| Age | 45 y/o | 25 y/o |
| Study/Group | HGB-206/Group A | HGB-206/Group A |
| Time from Dosing to Diagnosis | 3 years | 5.5 years |
| Presenting Diagnosis | MDS (progressed to AML) | AML |
| Genetics | Monosomy 7; RUNX1, PTPN11 | Monosomy 7; RUNX1, PTPN11 |
| Relevant Findings | No vector in blast cells | Vector detected in blasts close to VAMP-4 |
| Busulfan AUC (µM x min) | 3460 | 4084 |
| Reference | Hsieh Mh, et al. Blood Adv 2020 | Goyal S, et al. N Engl J Med 2022 |

# Case 1

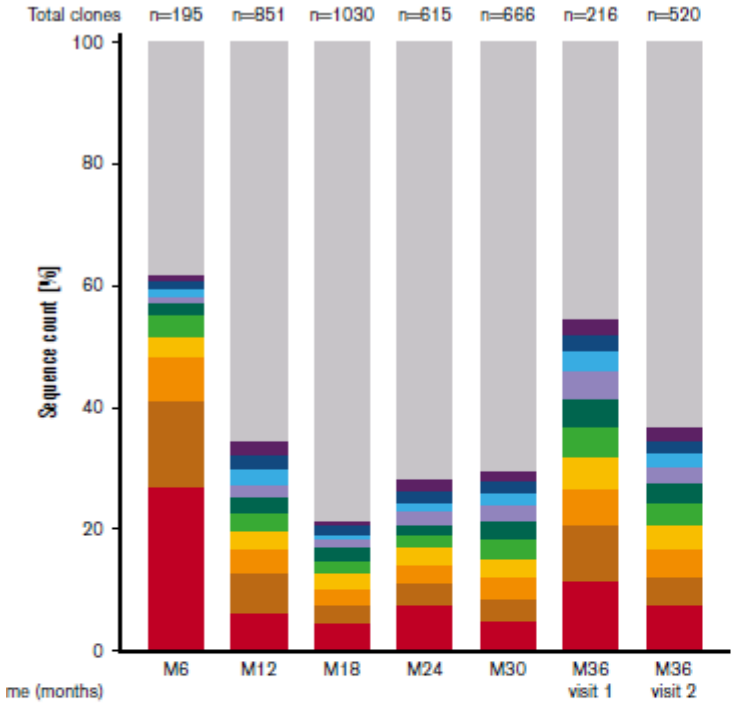



**Table 1. VCN analysis of CD34$^+$ and CD34$^-$ cells post-MDS diagnosis and post-AML recurrence**

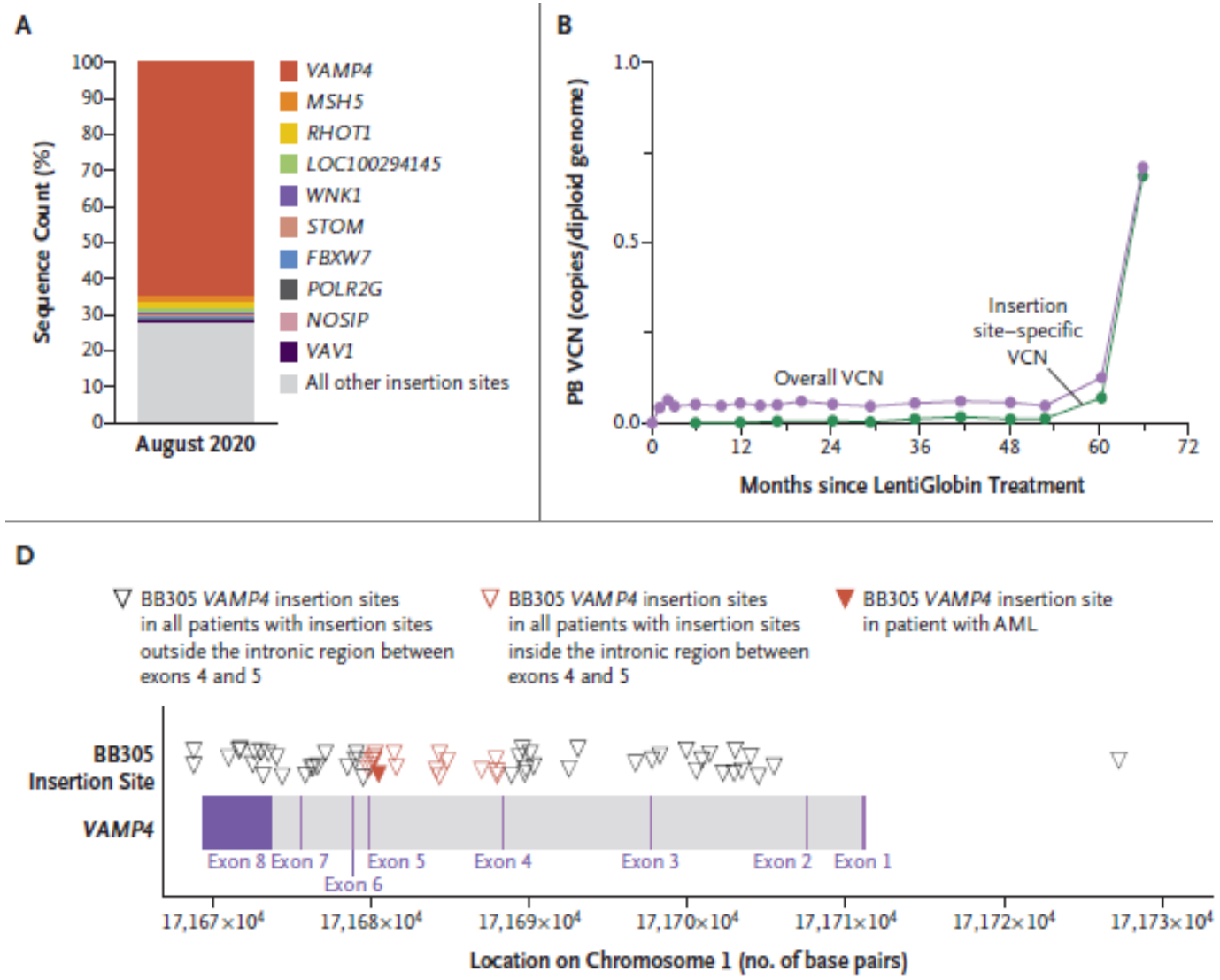
| | Post-MDS diagnosis | | Post-AML recurrence* |
|---|---|---|---|
| **Sample** | **Purity, %** | **VCN, c/dg** | **VCN, c/dg** |
| **BM** | | | |
| Unsorted BM sample | n/a | 0.14 ± 0.0 | nd |
| CD34$^-$ selected | 98 | 0.21 ± 0.03 | <LOQ |
| CD34$^+$ selected for myeloblasts | 93 | 0.02 ± 0.01 (<LOQ) | <LOQ |
| **PB** | | | |
| Unsorted PB sample | n/a | 0.10 ± 0.0 | nd |
| CD34$^-$ selected | 99 | 0.07 ± 0.01 | <LOQ |
| CD34$^+$ selected for myeloblasts | 53 | 0.08 ± 0.01 | <LOQ |

| Rank | Month 6 | 12 | 18 | 24 | 30 | 36 visit 1 | 36 visit 2 |
|---|---|---|---|---|---|---|---|
| Top1 | DIP2B | BAT2 | TMEM217 | PHACTR4 | USP48 | TARBP1 | LARS |
| Top2 | MEGF8 | CASC3 | LIN9 | STPG1 | CPSF7 | UBAP2L | SKAP1 |
| Top3 | NUP93 | RABEP1 | MAP4 | RPTOR | RAPGEF6 | C15orf38-AP3S2 | GK2 |
| Top4 | TMEM121 | TMEM87A | PTPRA | PBX3 | C6orf10 | TMEM65 | YWHAB |
| Top5 | SSH3 | UBE4B | HELZ | TBC1D5 | PAAF1 | MIR548AG2 | LOC100996351 |
| Top6 | TULP3 | STXBP3 | MFSD11 | MIR5195 | PBX3 | TNRC6C | RAB7A |
| Top7 | TBCD | RUNX3 | NET1 | EYA3 | LOC102546299 | HCG27 | HLA-B |
| Top8 | ZSWIM5 | DDX31 | XRN2 | IP6K1 | MAPK1 | SUV39H1 | C6orf10 |
| Top9 | DDX60 | QPRT | PHACTR4 | VMP1 | KANSL1 | KMT5B | AXIN1 |
| Top10 | SEC14L1 | PITPNB | ARID3A | BRCC3 | CHD9 | ATRX | RPA2 |
| Other mapp. IS | 185 | 841 | 1020 | 605 | 656 | 206 | 510 |



Hsieh Mh, et al. Blood Adv 2020

# Case 2






Goyal S, et al. N Engl J Med 2022

# Cases of AML in SCD enrolled in LentiGlobin Program

| Patient:<br>Date of Diagnosis: | SCD Pt 1<br>2018 | SCD Pt 2<br>2021 |
|---|---|---|
| Treatment | 5-azacytadine + decitabine<br>7+3<br>Cladribine + HD-ARAC | 3 induction cycles |
| Allogeneic HSCT | YES<br>PT/Cy haplo<br>(Flu-Mel-TBI) | haplo |
| Outcome | Relapse 6 months after HSCT | Relapse 3 months after HSCT<br>Died of disease progression |

Hsieh Mh, et al. Blood Adv 2020
Goyal S, et al. N Engl J Med 2022

# LentiGlobin Program

| Cohort characteristics | Group A (n=7) | Group B (n=2) | Group C (n=35[a]) |
|---|---|---|---|
| **Pre-collection transfusion regimen** | Optional | Required | Required |
| **HSC Source** | Bone Marrow | Bone Marrow | Plerixafor-mobilization and apheresis |
| **Conditioning AUC Target, µM*min per dose[b]** (Median AUC achieved) | 4,000 (4,747) | 5,000 (5,136)[c] | 5,000 (4,829) |
| **Manufacturing Process** | Original | Original/Refined[d] | Refined |
| **Total Cell Dose, x10$^6$ CD34+ cells/kg** (Median total CD34+, CD34$^{hi}$ LT-HSPCs) | Low (2.1, 1.6) | Medium (2.7[c], NA) | High[e] (6.9, 5.7[f]) |
| **Transduction Efficiency** (Median DP VCN [c/dg], Median % Transduced) | Low (0.7, 27.7) | High (3.1[c], 77.4[c]) | High (3.7, 80.3) |



Goyal S, et al. N Engl J Med 2022


# Leukemia after gene therapy for sickle cell disease: insertional mutagenesis, busulfan, both, or neither

Richard J. Jones[1,*] and Michael R. DeBaun[2,*]

[1]Sidney Kimmel Cancer Center at Johns Hopkins, Johns Hopkins University, Baltimore, MD; and [2]Vanderbilt-Meharry Sickle Cell Disease Center of Excellence, Vanderbilt University Medical Center, Nashville, TN

- Several lines of evidence suggest an alternative explanation for events in the trial, including that SCD population studies show an increased relative, but a low absolute, risk of AML/ MDS.
- We propose a new hypothesis: after gene therapy for SCD, the stress of switching from homeostatic to regenerative hematopoiesis by transplanted cells drives clonal expansion and leukemogenic transformation of preexisting premalignant clones, eventually resulting in AML/MDS

# Gene Therapy for X-ALD

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Hematopoietic Stem-Cell Gene Therapy for Cerebral Adrenoleukodystrophy

Florian Eichler, M.D., Christine Duncan, M.D., Patricia L. Musolino, M.D., Ph.D., Paul J. Orchard, M.D., Satiro De Oliveira, M.D., Adrian J. Thrasher, M.D., Myriam Armant, Ph.D., Colleen Dansereau, M.S.N., R.N., Troy C. Lund, M.D., Weston P. Miller, M.D., Gerald V. Raymond, M.D., Raman Sankar, M.D., Ami J. Shah, M.D., Caroline Sevin, M.D., Ph.D., H. Bobby Gaspar, M.D., Paul Gissen, M.D., Hernan Amartino, M.D., Drago Bratkovic, M.D., Nicholas J.C. Smith, M.D., Asif M. Paker, M.D., Esther Shamir, M.P.H., Tara O'Meara, B.S., David Davidson, M.D., Patrick Aubourg, M.D., and David A. Williams, M.D.

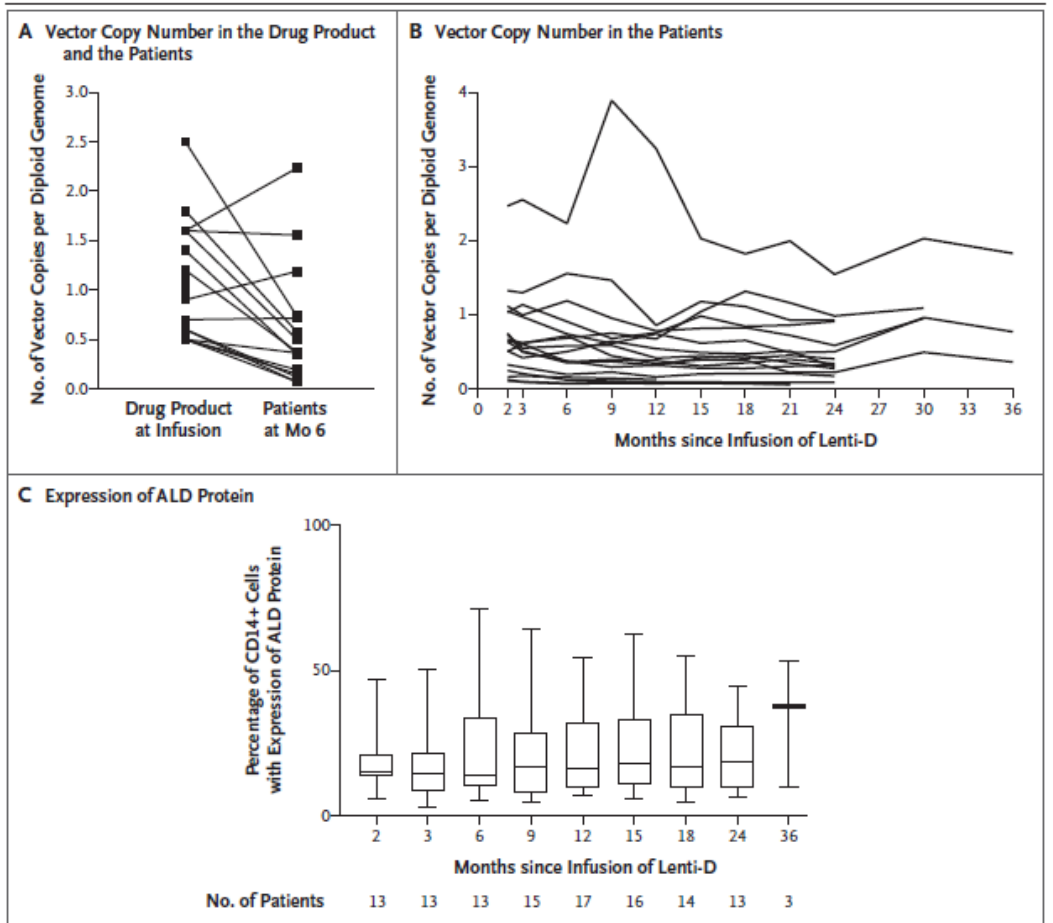



**Figure 1. Vector Copy Number and Expression of ALD Protein.**

Panel A shows the vector copy number in the Lenti-D drug product at infusion and in the peripheral blood for each of the 17 patients at 6 months after infusion. Panel B shows the vector copy number in the peripheral blood for each of the 17 patients at various time points after infusion. Panel C shows the expression of ALD protein in CD14+ cells in the peripheral blood at various time points after infusion; the horizontal lines in the boxes are median percentages, the top and bottom of the boxes are interquartile ranges, and the I bars are minimum and maximum percentages.



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# Gene Therapy for X-ALD

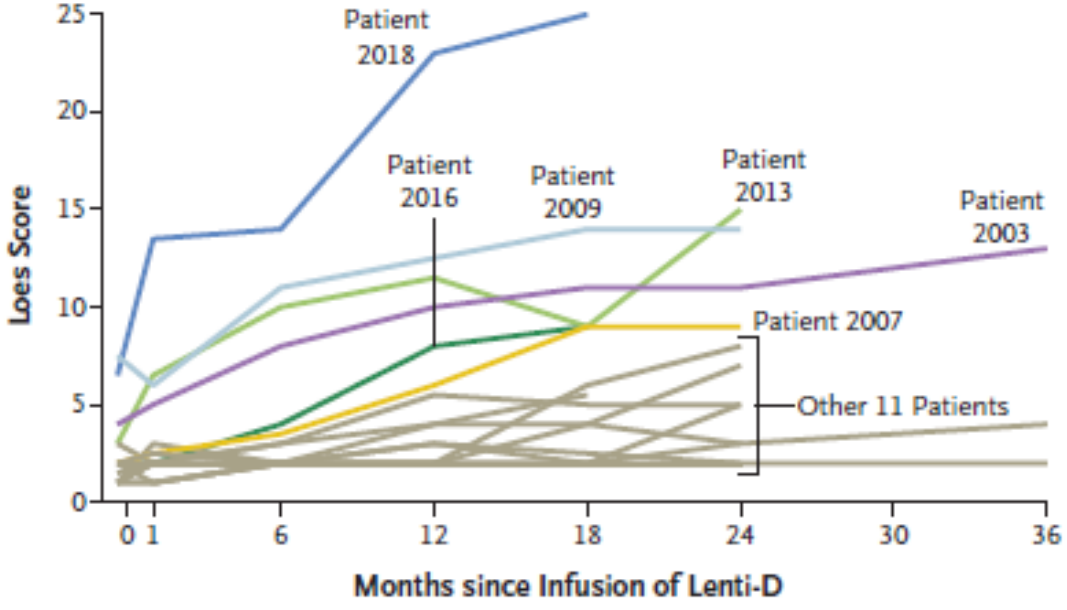



**Figure 3. Extent of Lesions on MRI.**

Shown are the Loes scores for each of the 17 patients at various time points after the infusion of the Lenti-D drug product. The Loes scores range from 0 to 34, with higher scores indicating an increased extent of lesions on magnetic resonance imaging (MRI). A score of 0.5 or less is considered to be normal.

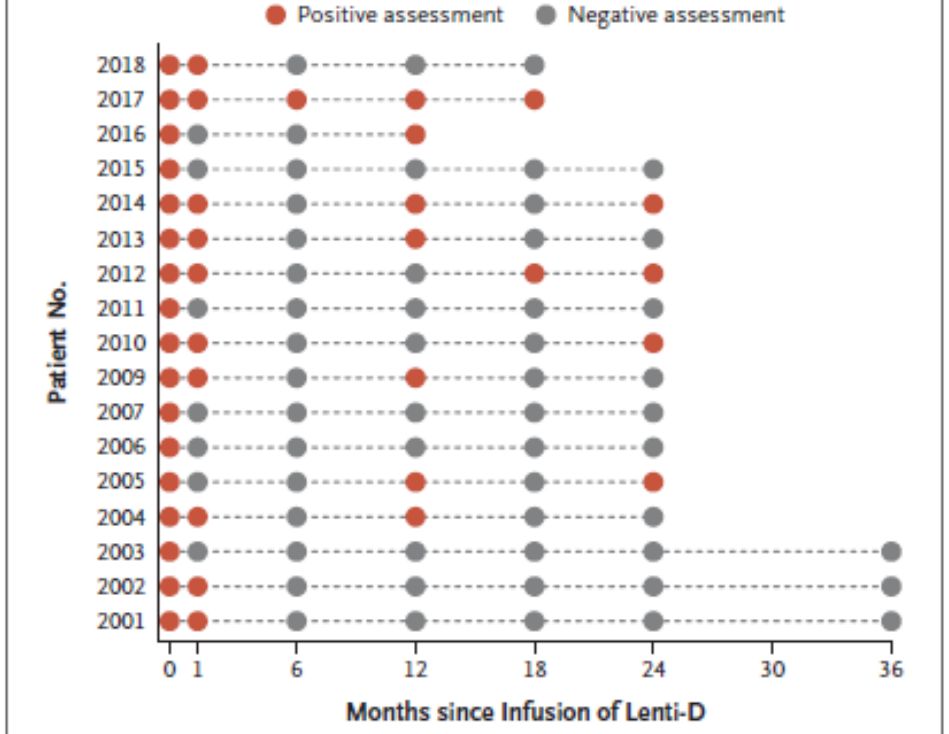
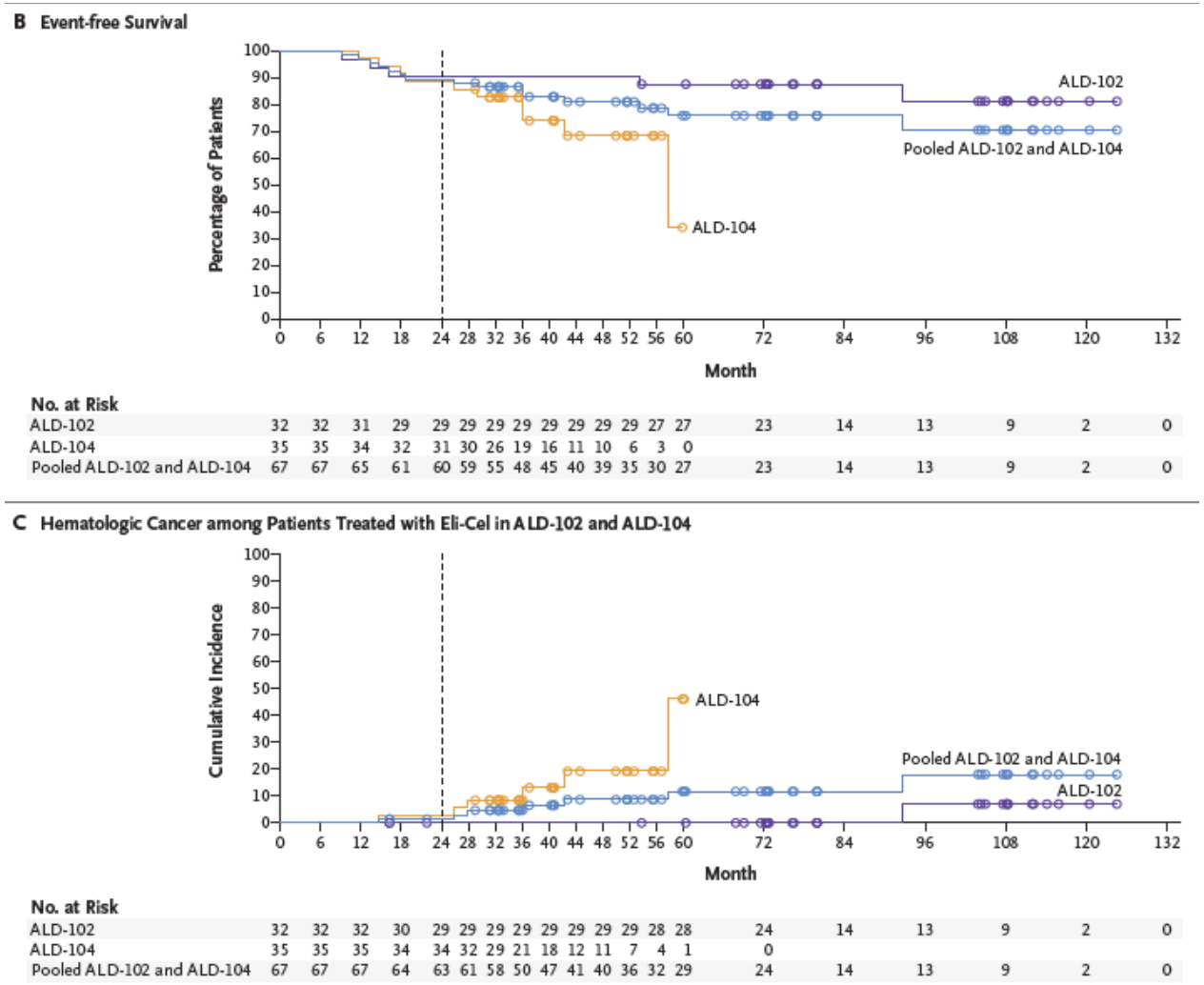



**Figure 4. Gadolinium Enhancement on MRI.**

Shown are the results of assessments for gadolinium enhancement on MRI for each of the 17 patients at various time points after the infusion of the Lenti-D drug product. Gadolinium enhancement on reemergence after initial resolution was uniformly more diffuse than the enhancement seen at

# Gene Therapy for X-ALD



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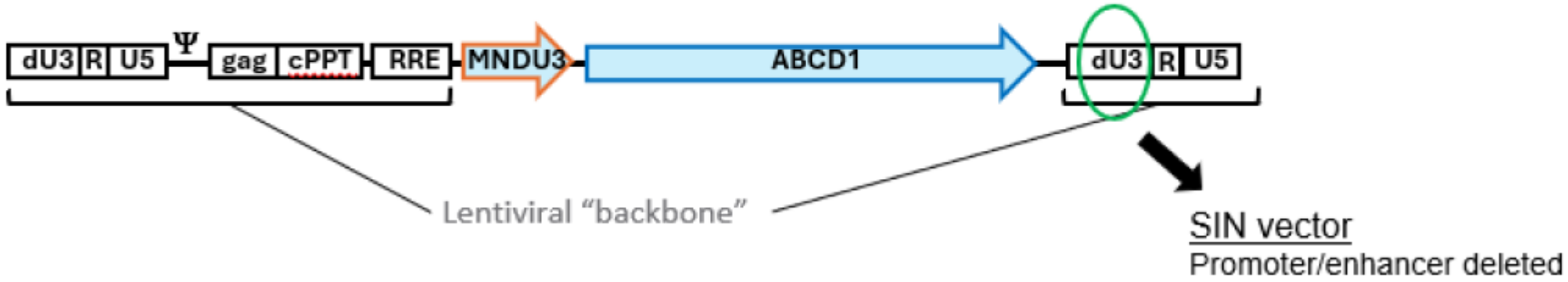
# Gene Therapy for X-ALD

Ψ Packaging symbol;  RRE, Rev responsive element; cPPT, central polypurine tract.



# Gene Therapy for X-ALD

**Table 1. Selected Baseline and Treatment Characteristics of Presented Patients.***

| Characteristic | Patient 3 | Patient 46 | Patient 36 | Patient 44 | Patient 54 | Patient 33 | Patient 61 | Patients without Hematologic Cancer (N = 60) |
|---|---|---|---|---|---|---|---|---|
| | | | | *Value* | | | | *Median (range)* |
| Age at eli-cel infusion — yr | 5 | 11 | 13 | 10 | 9 | 6 | 7 | 6 (4–14) |
| History of blood disease | No | No | No | No | No | No | No | |
| Baseline blood count† | | | | | | | | |
| Hemoglobin — g/dl | 11.7 | 13.7 | 12.8 | 14.9 | 13.1 | 12.8 | 10.2 | 13.5 (10.5–15.7) |
| White cells — $\times 10^{-9}$/liter | 6.9 | 4.7 | 3.2 | 7.29 | 4.8 | 8 | 6 | 6.7 (3.5–15.7) |
| Platelets — $\times 10^{-9}$/liter | 347 | 245 | 405 | 336 | 165 | 243 | 157 | 303 (191–492) |
| Mobilization regimen | G-CSF | G-CSF and plerixafor | G-CSF and plerixafor | G-CSF and plerixafor | G-CSF and plerixafor | G-CSF and plerixafor | G-CSF and plerixafor | |
| Conditioning regimen | Busulfan–cyclophosphamide | Busulfan–fludarabine | Busulfan–fludarabine | Busulfan–fludarabine | Busulfan–fludarabine | Busulfan–fludarabine | Busulfan–fludarabine | |
| Estimated average area under the plasma busulfan concentration–time curve per day — min × μmol/liter | 4729 | 4995 | 5586 | 5282 | 5473 | 5640 | 5160 | 4970 (3478–5695) |
| VCN in drug product — c/dg | 1.6 | 1.3 | 1.8 | 1.2 | 1.4 | 3.1 | 1.1 | 1.2 (0.5–2.7) |
| Total cells in drug product — $\times 10^{-6}$/kg of body weight | 6 | 5.7 | 12.1 | 15.1 | 9.6 | 22.8 | 7.7 | 12.0 (5.0–38.2) |
| Lentiviral vector cells in drug product — % | 62 | ND | 70 | 45 | 60 | 84 | 41 | 47 (19–74) |
| Platelet engraftment — days after drug infusion | 37 | 106 | 104 | 24 | 21 | 34 | 58 | 29 (14–108) |
| Neutrophil engraftment — days after drug infusion | 37 | 14 | 12 | 12 | 15 | 13 | 17 | 13 (11–41) |

# Gene Therapy for X-ALD

**Table 2. Bone Marrow Findings and Hematologic Cancer Diagnosis and Treatment.***

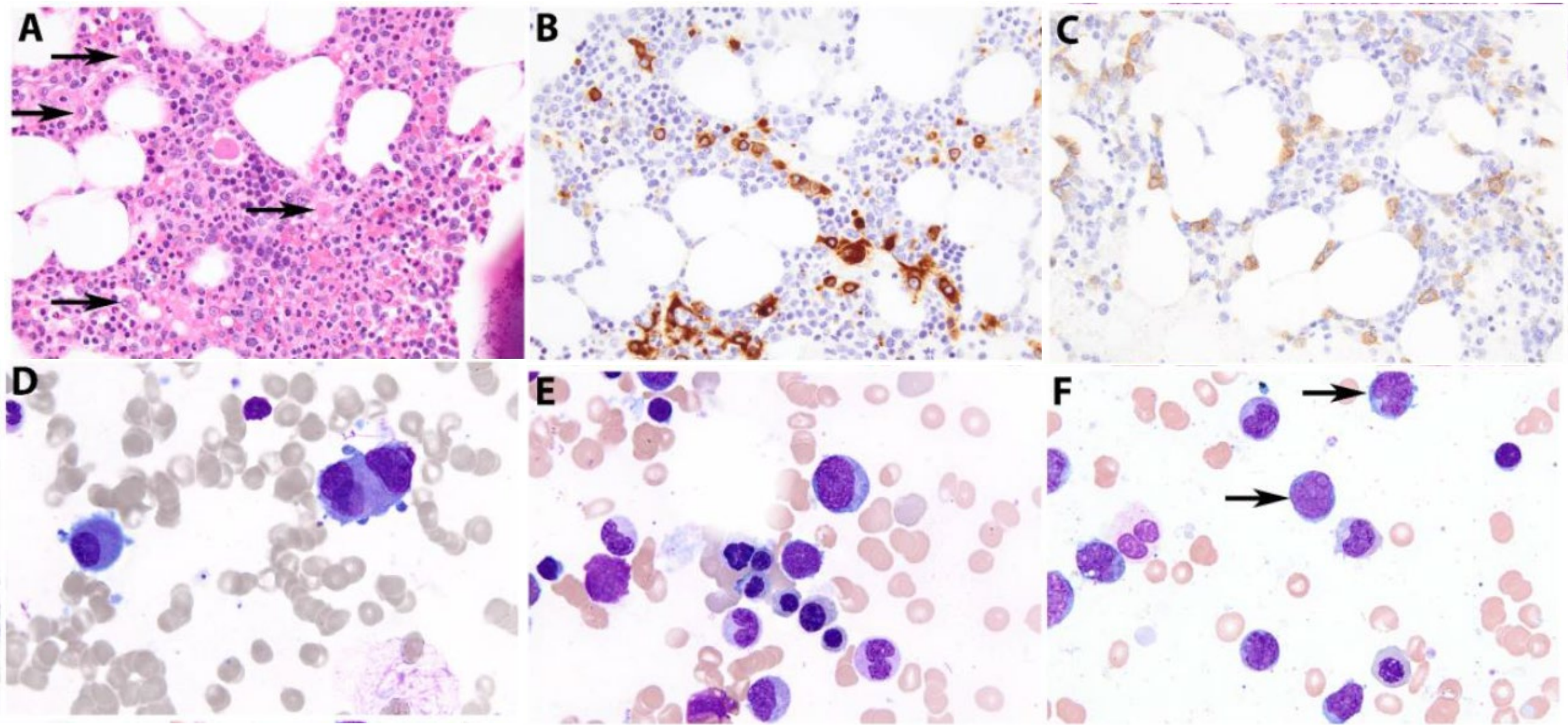
| Characteristic | Patient 3 | Patient 46 | Patient 36 | Patient 44 | Patient 54 | Patient 33 | Patient 61 |
|---|---|---|---|---|---|---|---|
| **Bone marrow findings** | | | | | | | |
| Bone marrow cell morphologic characteristics | Mo 92:<br>Mild hypocellularity (60%)<br>Increased myeloid–erythroid ratio and left-shifted myeloid maturation<br>Blasts, 15%<br>Trilineage dysplasia present including abundant micromegakaryocytes | Mo 12:<br>Moderate hypocellularity (40–50%);<br><10% Atypical megakaryocytes†<br>Blasts, <5%<br><br>Mo 14 and 18:<br>15% Hypocellularity with progressive megakaryocytic dysplasia including micromegakaryocytes | Mo 26:<br>Normocellularity (80%), with trilineage hematopoietic maturation<br>Dysplastic megakaryocytes<br>Blasts, 1% | Mo 42:<br>Mild hypocellularity (50–60%) with dysplastic megakaryocytes<br>Myeloblasts, 8%, showing abnormal coexpression of CD7 | Mo 28:<br>Myelodysplasia with 18% blasts | Mo 57:<br>AML with myelomonocytic features<br>48–65% blasts<br>Normocellular bone marrow (80–90%) | Mo 36:<br>Diminished cellularity<br>Presence of a group of myeloid blast cells (7%), including a blast cell with an Auer body consistent with myeloid MDS |
| Chromosome and karyotype analysis | Normal | Presumed germline aberration at chromosome 14 | Normal | Normal | Monosomy 7, 80% | Normal | NA |
| MDS FISH | Normal | Normal | Normal | Normal | NA | Normal | NA |
| Targeted deep sequencing with RHP | Somatic mutations in *KRAS* c.35G>C (p.G12A) at 14% VAF, and *NRAS* c.35G>A (p.G12D) at 3% VAF and *JAK2* c.2696T>C (p.I899T), VUS at 48% VAF | Germline VUS in *CDKN2A* c.168C>G (p.S56R) at a VAF of 41% | No somatic mutations in the genes screened | Pathogenic *WT1* c.1142C>A (p.S381‡) at 39% VAF and a VUS in *CDKN2B* c.34G>A (p.G12S) at 38% VAF | Mutation in *RUNX1* c.508+1_508+3delGTAinsAG (splice site) at 4% VAF | Somatic mutation in *KRAS* c.35G>A (p.G12) at 14.6% VAF (206x consensus coverage) | Mutation in *RUNX1* c.496 C>G (p.R166G) at 8.7% VAF (922x consensus frequency) |

# Gene Therapy for X-ALD

**Table 2. Bone Marrow Findings and Hematologic Cancer Diagnosis and Treatment.***

| Characteristic | Patient 3 | Patient 46 | Patient 36 | Patient 44 | Patient 54 | Patient 33 | Patient 61 |
|---|---|---|---|---|---|---|---|
| Age at diagnosis — yr | 12 | 12 | 15 | 13 | 11 | 11 | 10 |
| Diagnosis | MDS-EB | MDS-ULD | MDS-ULD | MDS-EB | MDS-EB | AML | MDS |
| Time of diagnosis — mo since eli-cel infusion | 92 | 14 | 26 | 42 | 28 | 57 | 36 |
| Pretransplantation therapy | Cytoreductive therapy | NA | NA | Cytoreductive therapy | Cytoreductive therapy | Cytoreductive therapy and chemotherapy | NA |
| Transplantation therapy | Myeloablative conditioning and allogeneic HSCT | Myeloablative conditioning and allogeneic HSCT | Myeloablative conditioning and allogeneic HSCT | Myeloablative conditioning and allogeneic HSCT | Myeloablative conditioning and allogeneic HSCT | NA | NA |
| Donor type | Unrelated mismatch cord donor | Parent haplotransplant | Parent haplotransplant | Sibling haplotransplant | Parent haplotransplant | NA | NA |
| Relative time of allo-HSCT — mo | 95 | 19 | 29 | 45 | 31 | NA | NA |
| Relative time of post–allogeneic HSCT bone marrow investigation — mo | 96 | 21 | 31 | 52§ | 33 | NA | NA |
| Post–allogeneic HSCT bone marrow findings | 100% donor cells; morphologic, immunophenotypic, and molecular remission | 100% donor cells; flow cytometry, FISH, and karyotype, normal; no mutations detected with RHP | 100% donor cells; flow cytometry and morphologic analyses, normal | >97% donor cells,§ trace recipient; morphologic analyses, normal; cytogenetics, normal; MRD, negative | 100% donor cells | NA | NA |
| Current status | Mo 120: alive, free of MFD, MDS resolved | Mo 43: alive, free of MFD, MDS resolved | Mo 49: died from GVHD | Mo 52: alive, free of MFD, MDS relapsed (MRD, negative at last follow-up)§ | Mo 37: alive, MDS resolved | Mo 61: alive, free of MFD, AML unresolved | Mo 37: alive, MDS unresolved |

# Gene Therapy for X-ALD



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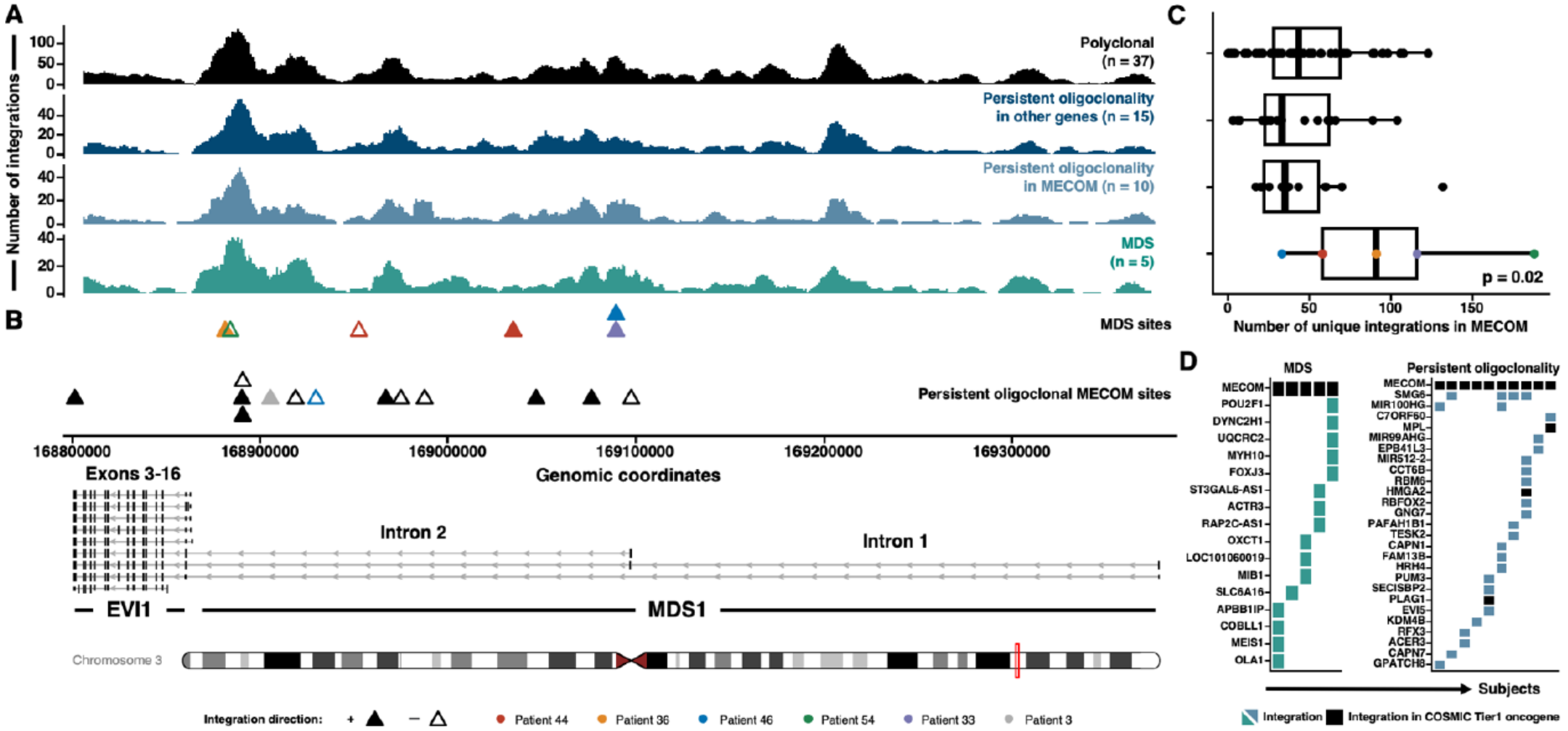
# Integration: focus on *MECOM*



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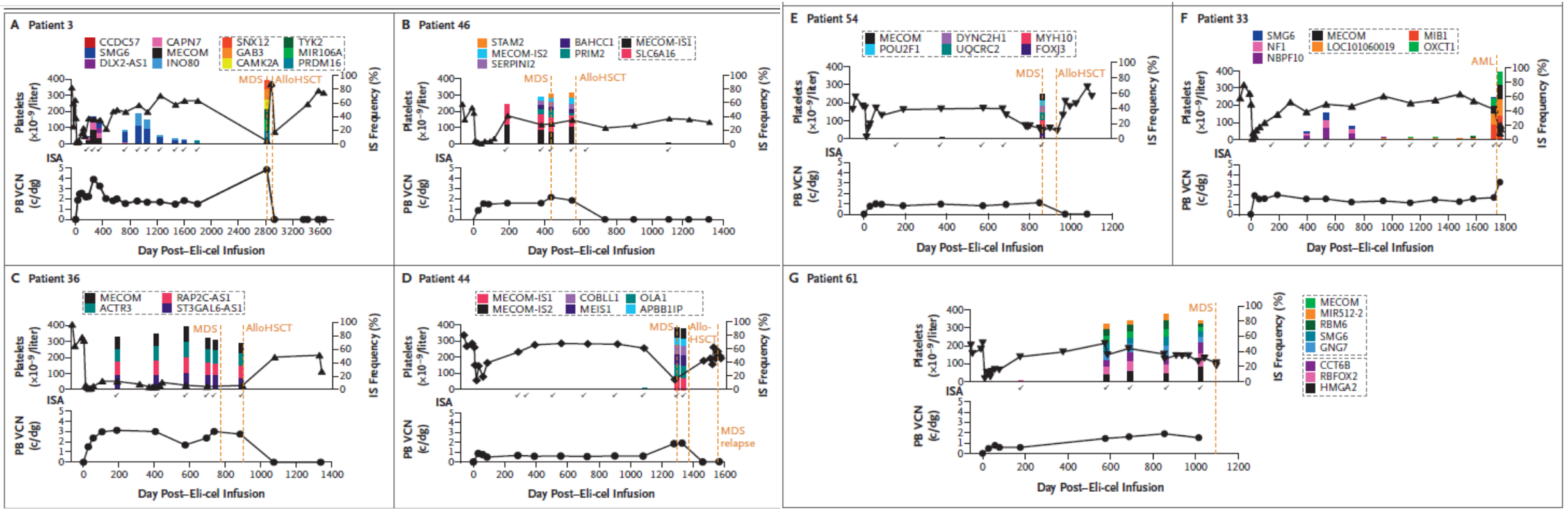
# Insertion site analysis



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# Conclusions

- sAML remains an unmet medical need also in pediatric patients, despite continuous optimization of frontline therapies;
- Genetic landscape differs from that of adult cases;
- Genomic screening approaches may be able to identify at risk patients prior to tMN development;
- Although the safety of gene therapy approaches has greatly increased, careful follow-up and continuous monitoring is needed, especially in view of future marketing of new productes.