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Clonal hematopoiesis and therapy-related myeloid neoplasms following neuroblastoma treatment

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Abstract:

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TO THE EDITOR:

Clonal hematopoiesis and therapy-related myeloid neoplasms following neuroblastoma treatment

Short title: Childhood therapy-related myeloid neoplasms

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Therapy-related myeloid neoplasms (TMN) constitute one of the most challenging complications of cancer treatment.[1] Whilst understanding of TMN pathogenesis remains fragmentary, genomic studies in adults have thus far refuted the notion that TMN simply result from cytotoxin-induced DNA damage.[2–4] Analysis of the preclinical evolution of a limited number of adult TMN have retraced the majority of cases to clonal haematopoiesis (CH) that predates cytotoxic treatment and lacks the mutational footprint of genotoxic therapies.[2–6] Balanced translocations, generally attributed to treatment with topoisomerase II inhibitors, are implicated in a minority of TMN.[1] TMN is a leading cause of premature death in childhood cancer survivors, and affects 7-11% of children treated for high-risk neuroblastoma and sarcoma.[7,8] However, the origin of pediatric TMN remains unclear. Targeted sequencing of known cancer genes detects CH in ~4% of children following cytotoxic treatment,[6,9] whereas CH is vanishingly rare in young individuals in the general population.[10,11] Moreover, to our knowledge, no cases of childhood TMN have been retraced to pretreatment CH. In light of these observations, we asked whether a broader driver landscape had eluded targeted CH screens in pediatric cancer patients and/or whether therapy-induced mutagenesis may be an under-recognised catalyst of CH and TMN in this patient group.

As proof of concept, we first applied whole genome and deep targeted sequencing of serial bone marrow and blood samples to investigate the pathogenesis of TMN arising in two children following high-risk neuroblastoma treatment. This study was approved by the National NHS Research Ethics Service (reference 16/EE/0394). Patient guardians provided written informed consent. DNA extracted from blood, bone marrow and tumors (**Table S1**) underwent whole genome and/or targeted sequencing of hematological cancer genes (**Table S2**). Mapping to the human reference genome GRCh37 and somatic variants calling were performed using an extensively validated pipeline.[12] From somatic mutations, we reconstructed phylogenetic relationships between samples using methods described previously.[12] We assessed signatures of base substitutions, as defined by their trinucleotide context, to search for evidence of therapy-related mutagenesis.

Sequencing data is accessible at the European Genome Archive (EGAD00001006423, EGAD00001006424).

Patient 1 (PD31013), a seven-year-old girl, developed therapy-related myelodysplastic syndrome (t-MDS) ten months after completing treatment for high-risk neuroblastoma (**Figure 1A**), which included induction (seven agents including cisplatin and carboplatin) followed by myeloablative chemotherapy and autologous hematopoietic stem cell transplant (HSCT).[13] Persistent thrombocytopenia developed six months after treatment completion. Bone marrow examination revealed t-MDS with del(7q) and monosomy 7 in 4/20 and 11/20 metaphases, respectively, and leukemogenic *PTPN11* G503E and *SETBP1* D868G variants. Repeat bone marrow assessment four months later identified a stable blast percentage alongside neuroblastoma relapse, to which the child succumbed.

To reconstruct TMN development, we interrogated blood or bone marrow samples from seven time points, including neuroblastoma-infiltrated marrow taken before treatment, from which we microdissected hematopoietic islands. The TMN was characterised by an increased burden of point mutations (2284) compared to *de novo* paediatric AML (median 600)(**Supplemental Figure 1**).[14] Most TMN mutations (88%) were attributed to single base substitution (SBS) signatures 31 and 35, which have been closely linked to platinum chemotherapy exposure (**Figure 1B**).[15–17] Similarly, doublet-base substitutions (DBS) clearly exhibited the imprint of platinum agents (DBS5; **Supplemental Figure 2A**).

We found the first evidence of a premalignant expansion during induction chemotherapy: a clone sharing 128 mutations with the TMN at a median variant allele frequency (VAF) of 1.5%. The majority (78%) of these mutations exhibited platinum signatures (**Figure 1B**). Targeted sequencing first detected the *PTPN11* G503E variant in the autograft, whilst there was no evidence of the *SETBP1* D868G mutation until t-MDS diagnosis (**Supplemental Figure 3A**). Of note, the sequence context of the founding *PTPN11* driver mutation confers a 99% probability of this lesion arising due

to platinum mutagenesis (**Supplemental Methods**). However, due to the sensitivity limits of copy number variant calling (**Supplemental methods**), we cannot conclusively rule out the presence of del(7q) or monosomy 7 in the autograft.[18] In addition to interrogating loci of TMN mutations, we called somatic mutations independently in each sample. This analysis revealed a second clone, separate from the TMN lineage, within the mid-induction bone marrow (**Figure 1A**). The second clone comprised 225 substitutions with a remarkable median VAF of 7.7%, again predominantly attributed to platinum agent exposure (**Figure 1B**). This clone regressed after induction treatment.

Patient 2 (PD42747), a nearly four-year-old girl, developed therapy-related acute myeloid leukemia (t-AML) six months after completing treatment for metastatic neuroblastoma (similar protocol to Patient 1; **Figure 1C**). The t-AML harbored a balanced *KMT2A-MLLT1* translocation (**Supplemental Figure 3B**), commonly attributed to topoisomerase II inhibitors, including etoposide, which she had received during induction.[1] She remains in remission eight years after allogeneic HSCT.

Samples from three time points were available for Patient 2: autograft harvest, t-AML diagnosis and t-AML remission. The t-AML harboured an elevated number of substitutions (1264) compared to *de novo* childhood AML,[14] 93% of which exhibited platinum signatures (**Figure 1D**, **Supplemental Figure 2B**). We detected t-AML variants, though not the *KMT2A* fusion, in both autograft and remission bone marrow at median VAFs of 0.9% and 1.4%, respectively. There were no other clones in the autograft or t-AML remission sample.

As aforementioned, targeted sequencing of known cancer genes identifies CH in ~4% of children following cytotoxic treatment.[6,9] The finding that both patients harboured at least one clone without a recognised driver event prompted us to extend our unbiased sequencing approach to 18 further pediatric solid tumor patients for whom blood, parental blood (to assess inherited and *de novo* germline variants), and detailed clinical information were available. For 17 patients we were also able to sequence tumor samples (**Table S1**). Included was the sister (PD31012) of Patient 1. She

had undergone treatment for low-risk infant neuroblastoma (three cycles of etoposide and carboplatin only) and did not have CH. Germline analysis revealed that the sisters' cancer predisposition may be attributable to an inherited germline pathogenic variant in *BARD1* (c.1935_1954dup, p.Glu652Valfs*69),[19] though neither had an exceptional burden of *de novo* germline mutations (**Figure 2A**).

Analysis of the extension cohort revealed one instance of CH, again lacking a recognised oncogenic mutation, in a 4-year old girl (Patient 3, PD34954) treated for relapsed bilateral neuroblastoma (which ultimately proved fatal). This clone was defined by 810 substitutions (median VAF 6.5%), 85% of which exhibited platinum signatures. Neither the two tumors from this child, nor Patient 1's tumor, bore evidence of platinum mutagenesis, despite prior exposure (**Figure 2B**). We did not detect any further cases of CH, including in the 6 other children exposed to platinum chemotherapy.

Collectively, our results reveal that the imprint of platinum agent mutagenesis dominated all clones in the three children with detectable CH, in marked contrast to CH observed in adults treated with these drugs.[5,6] The reasons for this disparity are unclear. It is conceivable that haematopoietic stem cell age impacts susceptibility to mutagenesis or capacity to survive it and continue replicating. Furthermore, all three patients with CH harboured at least one clone without a known driver mutation, corroborating evidence that knowledge of the somatic events under selective pressure is incomplete, and that 'driverless' CH cannot be accounted for by neutral drift alone.[11,20,21]

In the two TMN patients, the preponderance of platinum signatures in nascent pre-malignant clones, high TMN mutation burdens and 99% probability that Patient 1's founding driver mutation arose due to therapy-related mutagenesis contrast sharply with findings in adult TMN.[2–6] Together, these results suggest that the role of therapy-related mutagenesis in pediatric TMN may extend beyond the rare generation of balanced translocations linked to topoisomerase II inhibitors.[1,22,23]

The overall survival benefit of high-dose chemotherapy with autologous HSCT is unclear for several pediatric cancers, including neuroblastoma,[24] and TMN is a leading cause of non-relapse mortality for these patients.[7,8] The only factor clearly associated with improved survival in childhood TMN is a shorter time between diagnosis and allogeneic HSCT.[25] In both our TMN patients, pre-leukemic clones predated myeloablative treatment and pervaded the autograft. Pre-transplant CH is emerging as a biomarker of TMN and non-relapse mortality risk in adult autograft patients.[26] This raises the possibility that early detection of pediatric CH, with or without known leukemogenic drivers, may inform personalised autograft decisions, enable earlier TMN diagnosis and improve outcomes. Unbiased, systematic evaluation of the true frequency and prognostic implications of pediatric therapy-related CH is needed to determine any role for screening in clinical practice.

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Authorship: S.B., M.M. and G.C. designed the experiment. T.H.H.C., S.B., G.C., W.L., J.I. and E.M. analyzed data. T.R., A.L.G., M.M., J.A., T.R.W.O. G.A.A.B., E.D., M.G. and J.N. curated samples, provided pathology input and/or performed experiments. M.T. provided clinical genetics expertise. A.S. provided statistical input. S.B. and G.C. wrote the manuscript with input from all authors. S.B. and M.M. directed the study.

Conflict of Interest Disclosures: The authors declare no competing financial interests.

References

1. McNerney ME, Godley LA, Le Beau MM. Therapy-related myeloid neoplasms: when genetics and environment collide. *Nat. Rev. Cancer*. 2017;17(9):513–527.
2. Wong TN, Ramsingh G, Young AL, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. *Nature*. 2015;518(7540):552–555.
3. Ok CY, Patel KP, Garcia-Manero G, et al. TP53 mutation characteristics in therapy-related myelodysplastic syndromes and acute myeloid leukemia is similar to de novo diseases. *J. Hematol. Oncol.* 2015;8:45.
4. Takahashi K, Wang F, Kantarjian H, et al. Preleukaemic clonal haemopoiesis and risk of therapy-related myeloid neoplasms: a case-control study. *Lancet Oncol.* 2017;18(1):100–111.
5. Pich O, Cortes-Bullich A, Muiños F, et al. The evolution of hematopoietic cells under cancer therapy. *bioRxiv*. 2020;
6. Coombs CC, Zehir A, Devlin SM, et al. Therapy-Related Clonal Hematopoiesis in Patients with Non-hematologic Cancers Is Common and Associated with Adverse Clinical Outcomes. *Cell Stem Cell*. 2017;21(3):374–382.e4.
7. Kushner BH, Cheung NK, Kramer K, Heller G, Jhanwar SC. Neuroblastoma and treatment-related myelodysplasia/leukemia: the Memorial Sloan-Kettering experience and a literature review. *J. Clin. Oncol.* 1998;16(12):3880–3889.
8. Bhatia S, Krailo MD, Chen Z, et al. Therapy-related myelodysplasia and acute myeloid leukemia after Ewing sarcoma and primitive neuroectodermal tumor of bone: A report from the Children's Oncology Group. *Blood*. 2007;109(1):46–51.
9. Bolton KL, Ptashkin RN, Gao T, et al. Cancer therapy shapes the fitness landscape of clonal hematopoiesis. *Nat. Genet.* 2020;52(11):1219–1226.
10. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* 2014;371(26):2488–2498.
11. Zink F, Stacey SN, Norddahl GL, et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood*. 2017;130(6):742–752.
12. Coorens THH, Treger TD, Al-Saadi R, et al. Embryonal precursors of Wilms tumor. *Science*. 2019;366(6470):1247–1251.
13. Ladenstein R, Pötschger U, Valteau-Couanet D, et al. Interleukin 2 with anti-GD2 antibody ch14.18/CHO (dinutuximab beta) in patients with high-risk neuroblastoma (HR-NBL1/SIOPEN): a multicentre, randomised, phase 3 trial. *Lancet Oncol.* 2018;19(12):1617–1629.
14. Bolouri H, Farrar JE, Triche T Jr, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions. *Nat. Med.* 2018;24(1):103–112.
15. Boot A, Huang MN, Ng AWT, et al. In-depth characterization of the cisplatin mutational signature in human cell lines and in esophageal and liver tumors. *Genome Res.* 2018;28(5):654–665.
16. Pich O, Muiños F, Lolkema MP, et al. The mutational footprints of cancer therapies. *Nat. Genet.* 2019;51(12):1732–1740.
17. Alexandrov LB, Kim J, Haradhvala NJ, et al. The repertoire of mutational signatures in human cancer. *Nature*. 2020;578(7793):94–101.
18. Sevilla J, Querol S, Molines A, et al. Transient donor cell-derived myelodysplastic syndrome with monosomy 7 after unrelated cord blood transplantation. *Eur. J. Haematol.* 2006;77(3):259–263.
19. Pugh TJ, Morozova O, Attiyeh EF, et al. The genetic landscape of high-risk neuroblastoma. *Nat. Genet.* 2013;45(3):279–284.
20. Watson CJ, Papula A, Poon YPG, et al. The evolutionary dynamics and fitness landscape of clonal haematopoiesis. *Cold Spring Harbor Laboratory*. 2019;569566.
21. Pich O, Reyes-Salazar I, Gonzalez-Perez A, Lopez-Bigas N. Discovering the drivers of clonal hematopoiesis. *bioRxiv*. 2020;

22. Megonigal MD, Cheung NK, Rappaport EF, et al. Detection of leukemia-associated MLL-GAS7 translocation early during chemotherapy with DNA topoisomerase II inhibitors. *Proc. Natl. Acad. Sci. U. S. A.* 2000;97(6):2814–2819.
23. Krumbholz M, Bradtke J, Stachel D, et al. From initiation to eradication: the lifespan of an MLL-rearranged therapy-related paediatric AML. *Bone Marrow Transplant.* 2015;50(10):1382–1384.
24. Berthold F, Boos J, Burdach S, et al. Myeloablative megatherapy with autologous stem-cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high-risk neuroblastoma: a randomised controlled trial. *Lancet Oncol.* 2005;6(9):649–658.
25. Locatelli F, Strahm B. How I treat myelodysplastic syndromes of childhood. *Blood*. 2018;131(13):1406–1414.
26. Gibson CJ, Lindsley RC, Tchekmedyian V, et al. Clonal Hematopoiesis Associated With Adverse Outcomes After Autologous Stem-Cell Transplantation for Lymphoma. *J. Clin. Oncol.* 2017;35(14):1598–1605.

Figure Legends

Figure 1. Development of clonal hematopoiesis and therapy-related myeloid neoplasms in Patients 1 and 2. (A) Patient 1's neuroblastoma treatment course in parallel with the emergence of clonal hematopoiesis (clones 1 and 2 denoted in blue and grey, respectively) and the progression of clone 1 to therapy-related myelodysplastic syndrome (t-MDS, denoted in orange and red). Sequencing coverage (X) is indicated for whole genome (WGS) and targeted sequencing (TS). For Patient 1 (panel A), targeted sequencing coverage is reported at the *PTPN11* G503E locus. The median variant allele frequency (VAF) of mutations defining each clone is indicated as a percentage value next to their respective circle. Black circles indicate that no evidence (i.e. no significant enrichment of mutant reads) of the clone in question was found at that time point. (B) The mutational spectra defining Patient 1's clone 2 (light blue and dark blue circles), clone 2 (grey circle) and t-MDS (red and orange circles) is defined by the number of single base substitutions (SBS, y-axis) per trinucleotide context (x-axis). SBS spectra characteristic of platinum agent-induced mutagenesis are shaded yellow. The pie charts to the right of each mutational spectra plot indicate that the majority of the SBS at all time points are accounted for by platinum agent-associated mutational signatures (SBS31 and SBS35), with the small remainder of mutations attributed to clock-like mutational processes associated with ageing (SBS1 and SBS5) and oxidative stress (SBS18). (C) Patient 2's neuroblastoma treatment timeline in parallel with the progression of clonal hematopoiesis (light blue circle) to acute myeloid leukemia (AML, red circle), with persistence of residual clonal hematopoiesis after t-AML remission (dark blue circle). As in panel A, the median VAF of mutations defining each clone is indicated as a percentage value. *t-AML treatment, prior to allogeneic transplant, comprised “ADE” (cytarabine, daunorubicin, etoposide) and “FLAG-IDA” (fludarabine, high dose cytarabine, idarubicin, granulocyte-colony stimulating factor) chemotherapy. (D) The mutational spectra defining Patient 2's clonal hematopoiesis and t-AML is shown in the same manner as for Patient 1 in panel B.

Figure 2. *De novo* germline and somatic mutations in clonal hematopoiesis, therapy-related myeloid neoplasms and solid tumors in childhood cancer patients. (A) The VAF distribution (upper plot) and single nucleotide variant burden (lower plot) of *de novo* germline mutations, solid tumours, clonal hematopoiesis (CH) and therapy-related myeloid neoplasms (TMN) in 20 pediatric oncology patients. Samples are grouped by individual. The patient’s solid tumor diagnosis is indicated in the upper left corner of the respective bar plot. Asterisks denote patients who had been exposed to platinum chemotherapy at the time of sampling. (B) Mutational signature profile of *de novo* germline, CH, TMN and solid tumor mutation for the three individuals with clonal hematopoiesis, demonstrating the preponderance of platinum-agent mutational signatures SBS31

and SBS35 in CH and TMN. SNV, single nucleotide variant; DNM, *de novo* germline mutation; CH, clonal hematopoiesis; TMN, therapy-related neoplasm; NB, neuroblastoma; OS, osteosarcoma; WT, Wilms tumor; ES, Ewing sarcoma; IFS, infantile fibrosarcoma; RMS, rhabdomyosarcoma; URT, unclassifiable renal tumor.
* indicates prior exposure to platinum chemotherapy at the time of sampling.

Figure 1

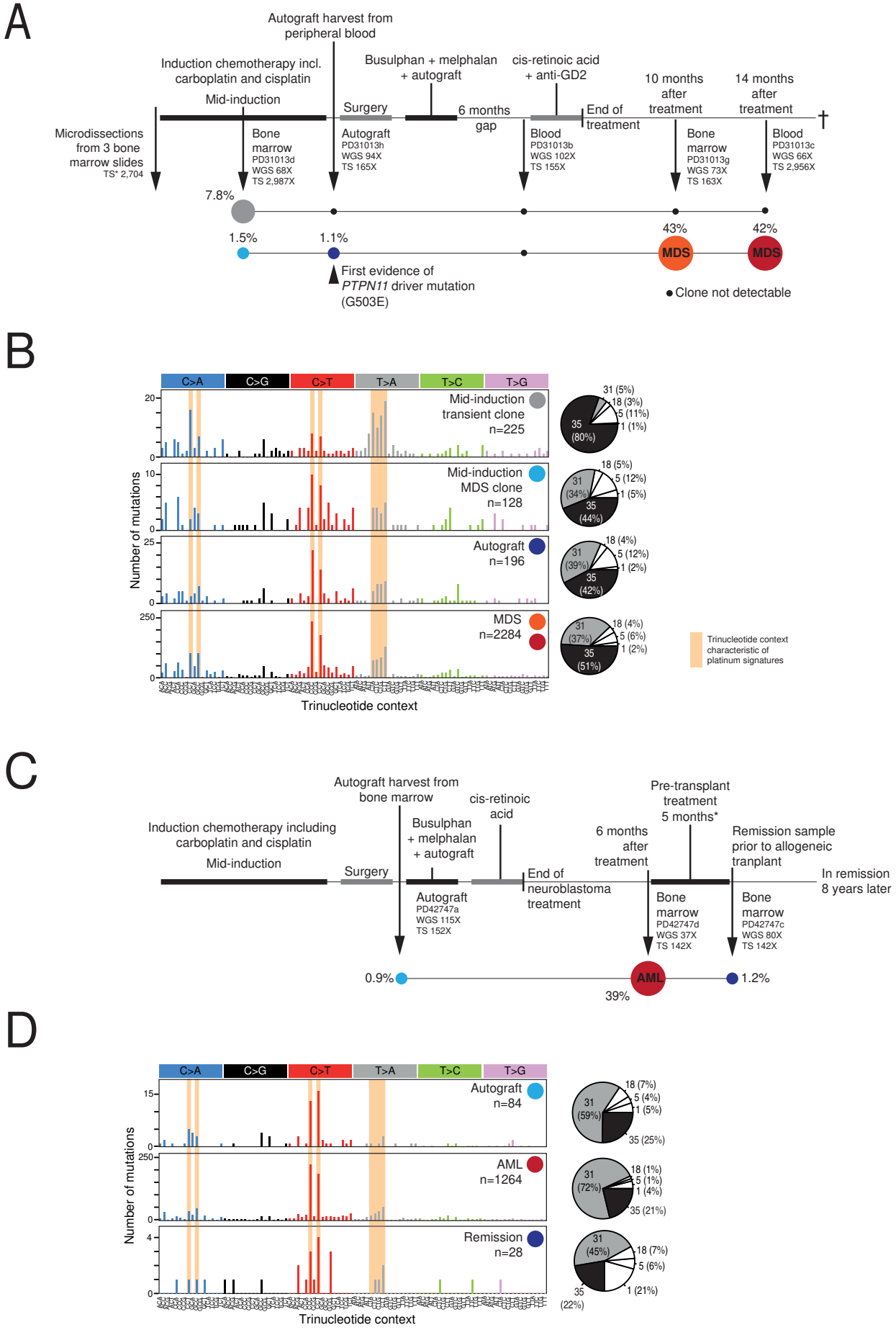
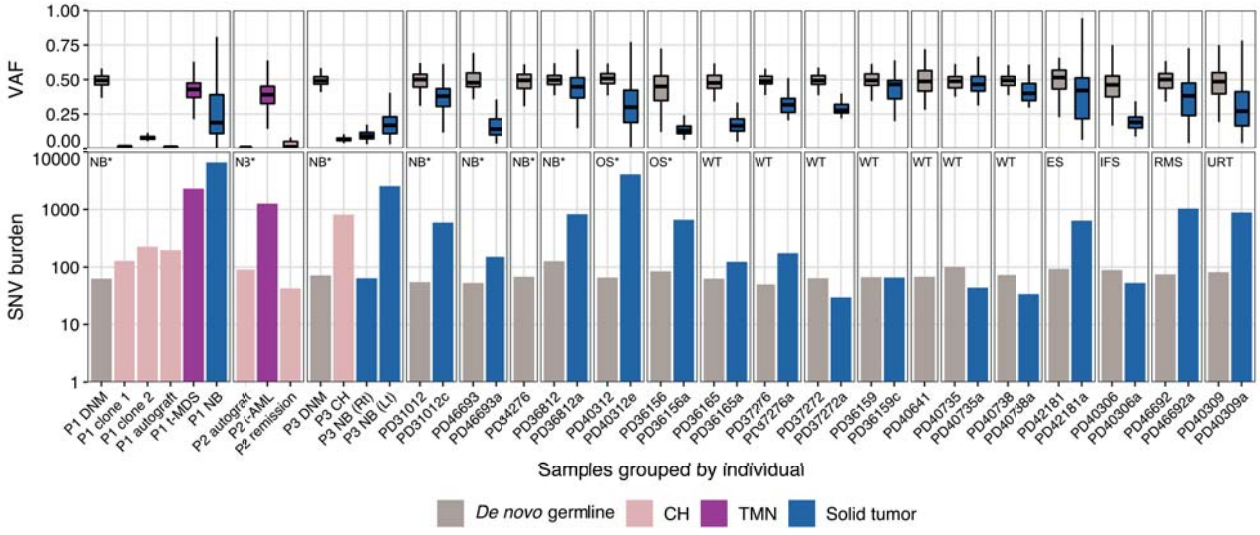

A
Autograft harvest from peripheral blood
Induction chemotherapy incl. carboplatin and cisplatin
Mid-induction
Surgery
Busulphan + melphalan + autograft
6 months gap
cis-retinoic acid + anti-GD2
End of treatment
10 months after treatment
14 months after treatment
Microdissections from 3 bone marrow slides
TS* 2,704
Bone marrow PD31013d WGS 68X TS 2,987X
Autograft PD31013h WGS 94X TS 165X
Blood PD31013b WGS 102X TS 155X
Bone marrow PD31013g WGS 73X TS 163X
Blood PD31013c WGS 66X TS 2,956X
7.8%
1.5%
1.1%
43%
42%
MDS
MDS
First evidence of PTPN11 driver mutation (G503E)
Clone not detectable
B
C>A
C>G
C>T
T>A
T>C
T>G
Mid-induction transient clone n=225
Mid-induction MDS clone n=128
Autograft n=196
MDS n=2284
Number of mutations
Trinucleotide context
Trinucleotide context characteristic of platinum signatures
C
Autograft harvest from bone marrow
Induction chemotherapy including carboplatin and cisplatin
Mid-induction
Surgery
Busulphan + melphalan + autograft
cis-retinoic acid
End of neuroblastoma treatment
6 months after treatment
Pre-transplant treatment 5 months*
Remission sample prior to allogeneic tranplant
In remission 8 years later
Autograft PD42747a WGS 115X TS 152X
Bone marrow PD42747d WGS 37X TS 142X
Bone marrow PD42747c WGS 80X TS 142X
0.9%
AML
39%
1.2%
D
C>A
C>G
C>T
T>A
T>C
T>G
Autograft n=84
AML n=1264
Remission n=28
Number of mutations
Trinucleotide context

Figure 2

A


VAF
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0.50
0.25
0.00
SNV burden
10000
1000
100
10
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N3*
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ES
IFS
RMS
URT
P1 DNM
P1 clone 1
P1 clone 2
P1 autograft
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P3 NB (RI)
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PD31012
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PD36159
PD36159c
PD40641
PD40735
PD40735a
PD40738
PD40738a
PD42181
PD42181a
PD40306
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PD46692
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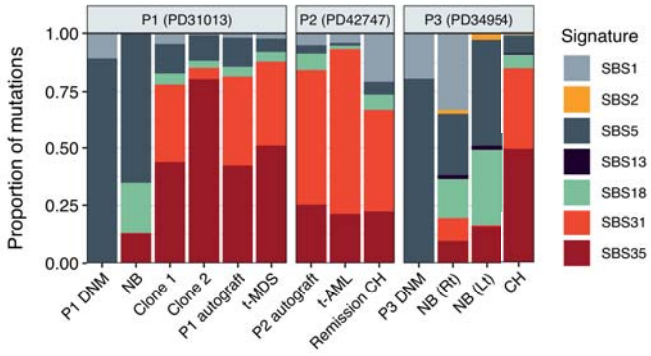
PD40309
PD40309a
Samples grouped by individual
De novo germline
CH
TMN
Solid tumor

B


P1 (PD31013)
P2 (PD42747)
P3 (PD34954)
Proportion of mutations
1.00
0.75
0.50
0.25
0.00
Signature
SBS1
SBS2
SBS5
SBS13
SBS18
SBS31
SBS35
P1 DNM
NB
Clone 1
Clone 2
P1 autograft
t-MDS
P2 autograft
t-AML
Remission CH
P3 DNM
NB (RI)
NB (LI)
CH