Supplemental Appendix

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Case Reports

Case 1 (Acute Myeloid Leukemia):

A 12-year-old female with global developmental delay presented to her pediatrician with three weeks of sore throat and one week of non-bloody, non-bilious emesis. On exam, she was noted to have splenomegaly, and a complete blood cell count revealed a total white cell count of 75,560 per microliter (7% with blast morphology), hemoglobin of 7.9 g/dL, and a platelet count of 48,000. She had noted easy bruising without petechiae or mucosal bleeding. Physical exam was notable for ecchymoses on the torso and buttocks, and a spleen extending 4 centimeters below the costal margin. She was admitted and bone marrow (BM) evaluation confirmed the diagnosis of AML (French American British subtype M4, **Figure 1A**) with a normal karyotype. She underwent induction therapy with cytarabine, daunorubicin, and etoposide. BM evaluation at the end of induction showed remission with no sign of persistent disease by flow cytometry. She completed four courses of therapy with total cumulative doses of 48 mg/m^2 mitoxantrone, 44,000 mg/m^2 intravenous cytarabine (210 mg intrathecal), 150 mg/m^2 daunorubicin, 1250 mg/m^2 etoposide, and 12,000 IU/m^2 of L-asparaginase. Since completing her course, she has had fluctuating degrees of thrombocytopenia. Eighteen months after completing therapy, her platelet count was consistently <50,000. This prompted a repeat BM evaluation which showed a hypocellular marrow with tri-lineage hematopoiesis, and no signs of malignancy or dysplasia. She has had an extensive workup for her isolated thrombocytopenia (unremarkable BM morphology and elevated immature platelet fraction) and has a working diagnosis of chronic immune

thrombocytopenia. Her platelet count has been as low as 10,000, but she has not had any bleeding complications and has not undergone any treatment.

Clinical genetics was consulted during her initial hospitalization based on her global developmental delay, hypotonia, obesity, atrial septal defect, and dysmorphic features (low hairline, thick eyebrows, low-set, dysmorphic ears, hypertelorism, short philtrum, prominent incisors with micrognathia and retrognathia, single palmar crease, and tapered fingers). Prior genetic work up for Prader-Willi and Fragile X syndromes were negative. Given her diagnosis of AML, there was concern for an overgrowth syndrome. At the time, this included Weaver (EZH2 mutation) and Cohen syndromes. After completing her therapy, whole exome sequencing was performed on the patient and both parents on the peripheral blood. Sequencing by GeneDx showed a heterozygous, *de novo* variant, R882C (2644C>T), in the *DNMT3A* gene, consistent with the diagnosis of DOS (**Figure 1B**). At the time of her DOS diagnosis, there were no reports in the literature of DOS patients with AML. She was also found to have a heterozygous variant, 456+4A>T, in the *FANCC* gene that was inherited from her father, who had no history of a hematologic malignancy or overgrowth. Genomic studies of her AML cells were performed at diagnosis and 4.4 years later while in a morphologic remission.

Case 2 (Pre-B Acute Lymphoblastic Leukemia):

A 9-year-old male with learning disabilities and epilepsy presented with several weeks of feeling unwell, bruising, and fever. At that time a complete blood cell count revealed a total white cell count of 48,700 per microliter with 92% blasts, hemoglobin of 9.5 g/dL,

and a platelet count of 28,000. BM evaluation confirmed the diagnosis of B-cell acute lymphoblastic leukemia (CD10+, CD19+, TdT+), and a diagnostic lumbar puncture showed no evidence of leukemia in the spinal fluid. His karyotyping revealed a hyperdiploid clone (62-63, X, +X, +X, -Y, +4, +5, +6, +8, +9, +10, +11, +12, +14, +16, +17, +18, +18, +21, +21, +22). His white count rose to 53,600 per microliter prior to initiating therapy and he was therefore classified as High Risk per NCI criteria. He received 4-drug induction therapy (dexamethasone, vincristine, daunorubicin, and pegaspargase), and was found to be MRD positive at the end of induction. He therefore received augmented BFM consolidation (cyclophosphamide, cytarabine, mercaptopurine, vincristine, pegaspargase, and intrathecal methotrexate) and repeat BM evaluation at the end of that cycle showed remission with an MRD negative marrow. Six years from his diagnosis, he has had no evidence of recurrence.

He was referred to clinical genetics at the age of 2 years for dysmorphic features and global developmental delay. He was born at 32 weeks of gestation by emergency LSCS following spontaneous onset of labor. His birth weight was 6lbs 1oz. He required resuscitation at delivery and went to special care for 3 weeks. He had CPAP for 3 days. He fed very poorly. He had a prolonged seizure requiring admission to PICU at 3 years of age. He had only one seizure with fever prior to that. He is on Valproate. He now only has the occasional seizure every 6 to 12 months. His epilepsy is now well controlled with absence seizures remaining. He has recurrent respiratory infections and apnea, which have improved with the dexamethasone given as part of his chemotherapy. His sleep pattern is very poor. At 2 years of age, he had made very slow developmental

progress: could stand in the standing frame and roll from his belly to his back and was reportedly trying to crawl. By the age of 7 years and 1 month he had profound developmental disability. He has never walked alone. He has no language apart from the occasional ‘ma ma’ and ‘ba ba’. A brain MRI showed a thin corpus callosum with a loss of deep white matter, large lateral and third ventricles and several small arachnoid cysts in the temporal region.

He has overgrowth: at age 2 his growth parameters were: occipital frontal circumference 53 cm (98th percentile), height 97.2cm (above the 99.6th percentile), and weight 16.75kg (99.6th percentile). He has dysmorphic features including a round face, heavy horizontal eyebrows, narrow palpebral fissures with down-slanting eyes and a prominent chin. He was recruited for next generation sequencing as part of the 100,000 Genomes’ England research project[1] which revealed a *de novo DNMT3A* variant (R882H, 2645G>A), consistent with diagnosis of DOS.

Case 3 (T- Lymphoblastic Lymphoma/ Leukemia)

First reported in a description of patients with variants in a different gene, CTLC[2], prior to the description of DOS and again in a case report of DOS patients[3], a 6-year-old female with known DOS and history of ganglioneuroblastoma presented with cervical lymphadenopathy and low-grade fevers. She was initially treated by an otolaryngologist with steroids for a mediastinal mass with pleural effusion. Thoracentesis was done and she was transferred to the oncology service. A complete blood cell count at the time of transfer revealed a total white cell count of 7,300 per microliter, hemoglobin of 8.3 g/dL, and a platelet count of 235,000. Lymph node and BM biopsies confirmed the diagnosis

of T-lymphoblastic lymphoma/leukemia (BM involvement <25%). She was treated with four-drug induction therapy (dexamethasone, vincristine, daunorubicin, and pegaspargase) but at the end of induction still had active disease (persistently positive PET scan). Nelarabine was added for consolidation (along with cyclophosphamide, cytarabine, mercaptopurine, vincristine, and pegaspargase) which induced a remission. However, she relapsed during maintenance therapy with a mature T cell leukemia (same TCR rearrangement as at initial diagnosis, immature T-lymphoblastic lymphoma). She underwent three courses of salvage chemotherapy (cyclophosphamide, etoposide, and bortezomib; high dose methotrexate; and daratumumab, dexamethasone, and venetoclax), none of which induced remission. She was transitioned to palliative care and died less than 2 years from her initial lymphoma diagnosis.

Her ganglioneuroblastoma was first diagnosed at 18-months-old with a posterior mediastinal mass and elevated vanillylmandelic and homovanillic acids (126 mg/g creatinine and 100 mg/g creatinine, respectively). She underwent resection and pathology showed a differentiated ganglioneuroblastoma with favorable features (low mitosis-karyorrhexis index, favorable histology, non-amplified *NMYC* and no loss of heterozygosity at 1p or 11q) and no BM involvement. She was monitored with serial metiodobenzylguanidine (MIBG) scans and found to have a new liver lesion 5 months later. She was treated with 2 cycles of chemotherapy (carboplatin, etoposide, cyclophosphamide and doxorubicin) and did well for nearly three years, when there was a significant increase in size of the tumor. She underwent surgical tumor debulking. While there was significant residual tumor, it was negative by MIBG scan and her VMA

and HVA levels remained within normal limits; she did not receive any further treatment prior to her lymphoma diagnosis 16 months later.

The patient's *DNMT3A* variant (R882H, 2645G>A) was first discovered when she was 5 years old on a comprehensive solid tumor sequencing panel sent on her ganglioneuroblastoma, with a variant allele frequency of 0.45, consistent with a germline mutation. These results were confirmed with whole exome sequencing of the peripheral blood of the patient; her parents were both negative for this mutation (blood samples). *DNMT3A*^{R882H} was therefore assumed to represent a *de novo* germline mutation in one of the parents. An additional heterozygous *de novo* mutation in the *CLTC* gene was identified as well (D913fs*59, 2737_2738dupGA). She was seen by genetic counselors, who found her to have clinical features consistent with DOS (esotropia, central hypothyroidism, hypotonia, macrocephaly, down slanting palpebral fissures, flattened nasal bridge, upturned nose, global developmental delay, height 98th percentile, weight 87th percentile, and head circumference 85th percentile).

Case 4 (Chronic Cytopenias)

A 5-year-old female initially presented to the hematology service for symptoms of gum bleeding, fatigue, and loose stools. A complete blood cell count at that time revealed a total white cell count of 3,730 per microliter, hemoglobin of 12.5 g/dL, and a platelet count of 131,000 with an absolute neutrophil count of 600. No treatment was given, but she was monitored closely with serial blood counts. Her white count has fluctuated from 600 to 4,400 per microliter, with an Absolute Neutrophil Count (ANC) as low as 102/uL

(average 662) (**Supplementary Figure 1A,B**). Her hemoglobin averaged 12.3 g/dL (range: 9.9 to 13.7) and her platelet count averaged 115,000 (range: 35,000 to 164,000) (**Supplementary Figure 1C, D**). In addition to her lab abnormalities, on physical exam she was noted to have tall stature, prominent upper central incisors, macrocephaly, round face, heavy and horizontal eyebrows, and narrow palpebral fissures. Her medical history was notable for tricuspid valve prolapse with mild regurgitation, right heart enlargement, and a small atrial septal defect, hypermobility, a history of seizures, developmental delay with mixed receptive-expressive language disorder, attention deficit hyperactivity disorder, and anxiety. She had next generation sequencing sent on her peripheral blood that showed a heterozygous Y528* mutation in *DNMT3A* (later confirmed with a buccal swab), consistent with DOS. Given the association of *DNMT3A* with adult AML, she has been monitored with serial CBCs twice yearly as well as annual bone marrow biopsies since her DOS diagnosis. Her initial bone marrow biopsy at the age of 5 showed 90% cellularity with trilineage hematopoiesis, and no signs of malignancy.

At the age of 8 she had worsening of her cytopenias, with her ANC reaching a nadir of 102/uL, hemoglobin to 9.9 and platelets to 35,000. At this time, she required hospital admission for fever in the setting of severe neutropenia. Otherwise, her symptoms have been mild, with occasional mouth sores. She had an extensive workup for her neutropenia (ceruloplasmin, copper, homocysteine, and vitamin C levels, cytogenetics, myelodysplastic syndrome FISH probe panel, telomere length testing, chromosome breakage analysis and PNH testing), all of which was unremarkable. During her

hospitalization for fever and neutropenia, she was given two doses of granulocyte colony-stimulating factor (G-CSF), which induced an appropriate response with her ANC increasing to 1892. While her initial bone marrow aspirate showed 90% cellularity, it has since dropped to 50%; pathologic review revealed dysplastic megakaryocytes and erythrocytes in several serial aspirates (**Supplementary Figure 1E**), suggestive of a Myelodysplastic Syndrome. To identify any clonal abnormality, she had whole exome sequencing performed on two occasions, first on a bulk sample of the buffy coat from a peripheral blood draw during a period of neutropenia (**Supplemental Figure 1B**, ANC 232 per microliter) and the second from purified neutrophils from the peripheral blood 12 days after G-CSF treatment (**Supplemental Figure 1B,** ANC 1157 per microliter), both were compared to a buccal swab sample. Three and four non-overlapping low VAF somatic variants of uncertain significance were called in the bulk sample and purified neutrophils, respectively (**Supplemental Table 2**), which were not consistent with a clonal process. She is currently being evaluated for an allogeneic stem cell transplant. Since she does not have a diagnosis of MDS at this time, her case is not included in the count of hematopoietic malignancies in DOS patients.

Case 5 (Acute Myeloid Leukemia)

A male with known DOS (*DNMT3A* variant I310F) was diagnosed with AML at age 20 years. Molecular characterization of his AML showed a normal karyotype with an *NPM1* mutation and *FLT3*-TKD, in addition to his germline *DNMT3A*^{I310F}. After 2 cycles of chemotherapy, he was in morphologic remission with molecular minimal residual disease (based on the VAF of the *NPM1* mutation). He underwent a matched unrelated

donor hematopoietic stem cell transplant. He had mild acute skin graft versus host disease which has resolved. He remains transfusion dependent but is overall doing well without signs of relapse 2.5 years later.

Case 6 (Hodgkin Lymphoma)

A male with *DNMT3A* variant Y735C was diagnosed with Hodgkin Lymphoma at age 27 years. His disease was treatment refractory, which led to an autologous hematopoietic stem cell transplant 2 years after diagnosis; the patient continues to do well 3.5 years later. He was diagnosed with DOS after his transplant.

Case 7 (Essential Thrombocytosis)

The patient was a female diagnosed with essential thrombocytosis (ET) at age 34 years. Her ET was effectively managed with hydroxyurea and aspirin. She was diagnosed with DOS 2 years after the ET diagnosis, when a germline *DNTM3A*^{R882H} variant was discovered. While her ET was well controlled, she died due to complications of a COVID-19 infection.

Case 8 (Acute Myeloid Leukemia)

This male was the first described case of AML in a patient with DOS at 15 years of age, as described by Hollink, *et, al*.[4] At presentation, his AML was classified as FAB type M5, with an abnormal karyotype (47, XY, +8, der(11)del(11)(p11p13)del(11)(q2?1q2?4)[18]/47, XY, +8, -11, -14, -7, +mar1, +mar2, +mar3[2]), and his AML sample had a cooperating *PTPN11* mutation (T73I) with AML

panel testing. He was treated with standard of care chemotherapy, per the original report, and did not require a stem cell transplant. He has had mild thrombocytopenia and has been in continuous remission for 8 years.

Case 9 (Acute Myeloid Leukemia)

First reported in the second cohort of DOS patients to be described by Tatton-Brown, *et, al*.[5], this patient presented with FAB type M4 AML at age 12. Her cytogenetics showed an abnormal karyotype (46, XX, del(9)(q13q22)). She was treated with standard of care chemotherapy and consolidation and did not require a stem cell transplant. No additional information regarding her cooperating mutations is available. She continues to be in remission 12 years after her initial AML diagnosis.

TARGET: PARBTV

This patient was a 17-year-old male diagnosed with standard risk FAB subtype M1 AML, and CNS negative for AML. His WBC count at diagnosis was 247.1 per microliter (95% with blast morphology), and no cytogenetic abnormalities were reported. The patient was treated per COG AAML0531 (but did not receive gemtuzumab) and remained MRD positive at the end of course 1 (28% AML cells by flow) and course 2 (6.9% AML cells by flow). It is not clear from the TARGET records whether he received a stem cell transplant, but he died 444 days post-diagnosis. Whole genome sequencing through TARGET revealed a germline *DNMT3A*^{R882H} mutation (VAF 0.52 in germline (bone marrow-derived fibroblast) sample, 0.48 in the presentation sample and 0.46 in

the persistent disease sample) and an *IDH2*^{R172K} mutation in both leukemia samples tested.

TARGET: PARZIA

A 16-year-old female was diagnosed with standard risk FAB subtype M0 AML, and CNS was negative for AML cells. WBC count at diagnosis was 2.4 per microliter (blasts were not reported), and 7 of 20 metaphases showed trisomy of chromosome 10. She was treated per COG AAML0531 (and did receive gemtuzumab), and remained MRD positive at the end of course 1 (68%) and course 2 (53%). She never achieved a CR and died 77 days post-diagnosis. Whole genome sequencing performed as part of TARGET found a germline *DNMT3A*^{N501S} mutation (VAF 0.31 in germline (bone marrow-derived fibroblast), 0.52 in the presentation sample, and 0.50 in the persistent disease sample); no AML-associated somatic point mutations were detected in either AML sample. Amplification of chromosome 10 was verified in both AML samples tested.

Supplementary Methods

Methods

For previously unpublished cases, families self-reported through the Tatton-Brown Rahman Syndrome Community.[6] Parents gave informed consent for their child's case to be anonymized and described in the literature, and where appropriate, analyzed by whole genome or exome sequencing (Washington University IRB approved protocol #201011766). Bone marrow aspirate slides for Case 1 and 4 were provided by the treating physicians and were imaged on an Olympus BX53 microscope with oil

immersion at 100x magnification, and pictures were taken with an Olympus DP-72 camera.

Whole Genome Sequencing

We performed whole genome sequencing with mean coverage of 60x on bone marrow and 40x for matched buccal swab cells to identify somatic variants from Case 1. The full pipeline with all parameters is described in a CWL pipeline stably linked here: (https://git.io/JRFnM). Briefly, reads were aligned to the reference sequence build GRCh38 using BWA-MEM[7] version 0.7.15. Somatic variants were called using four tools: GATK (20644199) Mutect2 version 4.1.2.0 (23396013), Strelka (30013048) version 2.9.9, Varscan (22300766) version 2.4.2, Pindel (19561018) version 0.2.5b8, then filtered, merged, and annotated with the Ensembl Variant Effect Predictor (27268795). Copy number changes between tumor and matched normal samples were defined with copycat (https://github.com/chrisamiller/copyCat).

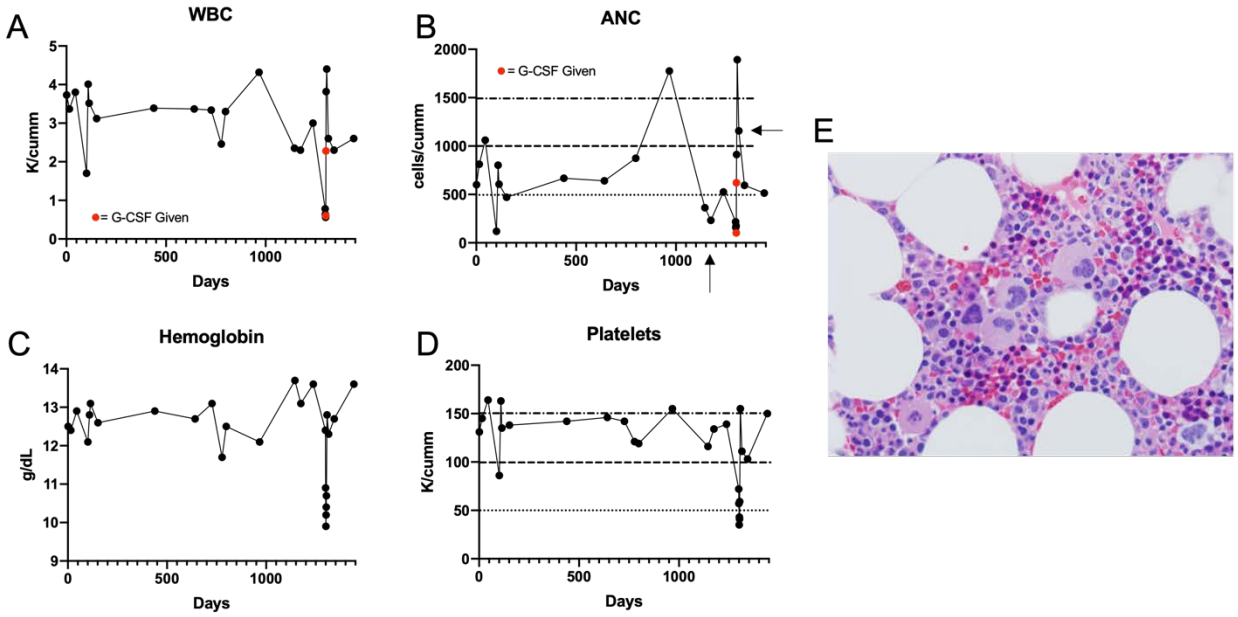
Whole exome sequencing

We performed whole exome sequencing with mean coverage of >200x on peripheral blood and 195x for matched buccal swab cells to identify somatic variants from Case 4 at two timepoints. The first analysis was done on isolated buffy coat from the peripheral blood (218x coverage). For the second analysis (after G-CSF administration) neutrophils (CD3-, CD14-, and CD16+) and T cells (CD3+, germline control) were purified from peripheral blood via fluorescence-activated cell sorting on a Beckman Coulter MoFlo cell sorter using antibodies from BD Biosciences (huCD3Clone UCHT1

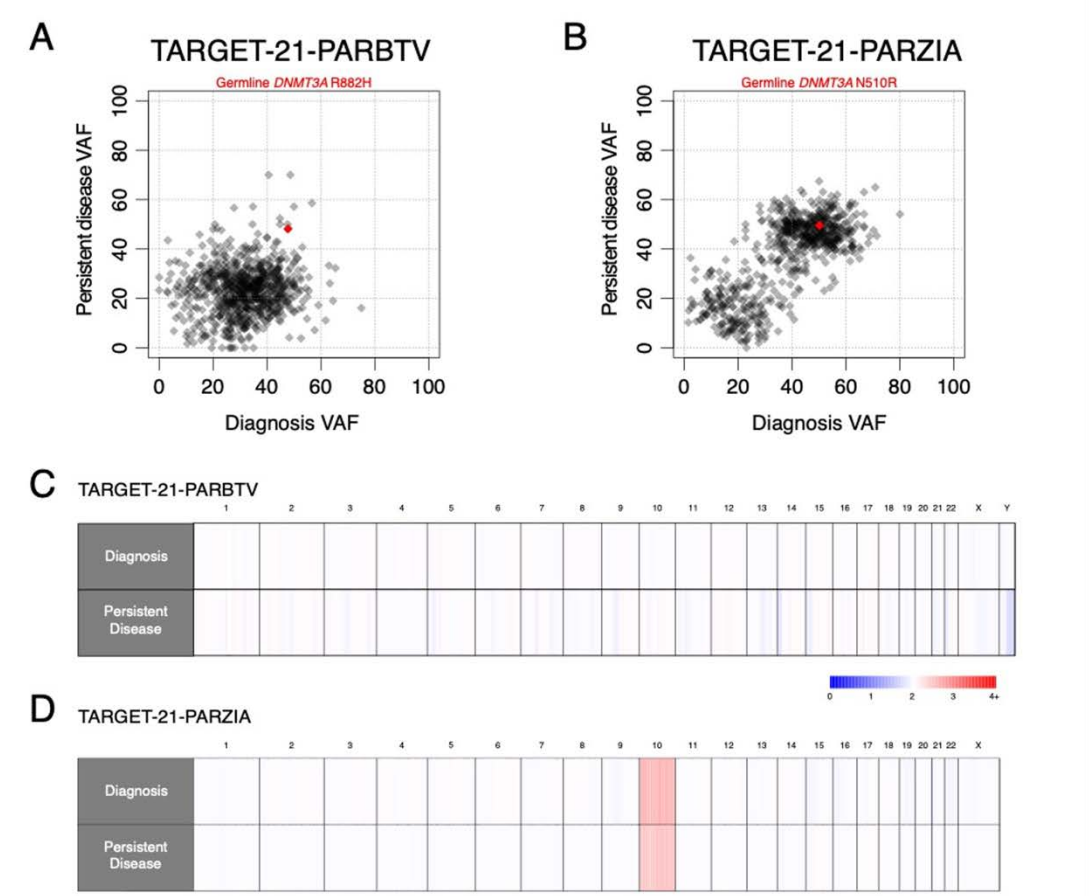
and huCD16 Clone 3G8) and BioLegend (huCD14 Clone M5E2). Neutrophils were sequenced at 290x coverage and T cells at 368x coverage. Samples were submitted to the McDonnell Genome Institute for analysis via CLIA-compliant exome sequencing and analysis for somatic mutations.

The data used for this analysis are available at NCBI dbGaP: https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000159.v11.p5. Data from the TARGET study are available at https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000465.v2.p1. Information regarding the TARGET study can be found at http://ocg.cancer.gov/programs/target.

Supplemental Figures



Supplemental Figure 1



Supplemental Figure 2

Supplemental Figure 1. Case 4 Characterization. A-D. Serial blood cell counts were monitored over a 3.5-year time period with **(A)** total white blood cell count (WBC), **(B)** absolute neutrophil count (ANC), **(C)** hemoglobin, and **(D)** platelet count . The patient received 2 doses of G-CSF, the timing of which are indicated in red for the WBC **(A)** and ANC **(B)**. The timing of the sample procurement for sequencing is indicated by arrows (vertical arrow- buffy coat sequenced, horizontal arrow- purified neutrophils). The cutoffs for mild, moderate, and severe neutropenia **(B)** and thrombocytopenia **(D)** are indicated with dotted lines. **E.** Bone marrow aspirate showing mild

dysmegakaryopoiesis and decreased cellularity without any leukemic blasts, overall bone marrow cellularity estimated to be 50%. 100x magnification.

Supplemental Figure 2. TARGET whole genome analysis. A-B. VAFs of the diagnosis and persistent disease samples, for PARBTV **(A)** and PARZIA **(B)**. The germline DNMT3A variants for each patient are highlighted in red. **D.** Copy number plots, showing no copy number alterations observed in PARBTV **(C)** and an amplification of chromosome 10 in PARZIA **(D)**.

Supplemental Tables

Supplemental Table 1. Whole Genome Sequencing Variant Calls from Case 1

Supplemental Table 2. Whole Exome Sequencing Variant Calls from Case 4

Supplemental Table 3. Non-DOS, Germline DNMT3A Variant AML Cases

Supplemental Table 4. Whole Genome Sequencing Variant Calls from TARGET Case PARBTV

Supplemental Table 5. Whole Genome Sequencing Variant Calls from TARGET Case PARZIA

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