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Running head: *NUP98* rearrangements in adult myeloid neoplasms

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Abstract:

Nucleoporin 98 rearrangements (*NUP98*r) are recurrent in myeloid neoplasms and are subtype-defining for acute myeloid leukemia (AML) in the World Health Organization Classification 5th edition (WHO5) and the International Consensus Classification (ICC). Identification of *NUP98r* is essential given frequency of treatment resistance and possibility of sensitivity to targeted therapies. However, *NUP98*r is often cryptic on karyotype and has over 40 described partners. Therefore, it is underdiagnosed in the absence of dedicated testing that is not routine at many practices, e.g. RNA-based next generation sequencing (NGS), *NUP98* break-apart FISH, or RT-PCR for specific *NUP98* fusions. Historically, AML with *NUP98*r has received the most attention in pediatric AML, where its incidence is highest, but has been increasingly characterized in adult AML. By contrast, the incidence and behavior of *NUP98* fusions in myelodysplastic syndromes (MDS) is less understood and based predominantly on case reports. In this study, we describe our adult institutional experience with a clinically validated anchored multiplex PCR RNA-based targeted NGS assay, explore strategies for rational use of specific testing for *NUP98*r including a proof-of-principle based on *WT1* and *FLT3*-ITD mutational status, and integrate our results with a review of the literature. In total, we identified 3 MDS and 15 AML patients with *NUP98*r as the genetic driver, including two novel fusion partners (*FGF14* and *LAMC3*), thus highlighting the utility of NGS testing to detect *NUP98* fusions. Recognition of *NUP98*r in myeloid neoplasms is crucial for accurate diagnosis and prognosis, with significant implications for therapy or enrollment in clinical trials.

Introduction:

Acute myeloid leukemia (AML) with nucleoporin 98 rearrangement (*NUP98*r) comprises one of several genetically defined AML subtypes that have been newly incorporated into both the 2022 International Consensus Classification (ICC) and the 5th edition of the World Health Organization Classification (WHO5), where a diagnosis of AML can be made with a blast count under 20%.[1, 2] AML with *NUP98*r has historically been associated with adverse clinical outcomes and chemotherapy resistance although recent preclinical models have raised the possibility of rational targeted therapy with menin inhibitors.[3] Thus, routine identification of *NUP98* rearrangement is important for clinical care of patients with AML and future improvement of risk-adapted therapy. However, the entity is underdiagnosed by many clinical practices since the rearrangements are frequently cryptic on conventional karyotype due to the location of *NUP98* at 11p15.4 near the terminal end of the short arm of chromosome 11. The *NUP98* gene, which encodes a component of the nuclear pore complex, rearranges with over 40 unique fusion partners, all involving the N-terminal end of *NUP98* and notable for partner-specific enrichments for monocytic, myelomonocytic, megakaryoblastic, or erythroid differentiation.[4] Accordingly, reliable detection across the entire spectrum of fusion partners requires complex testing modalities such as whole transcriptome RNA-seq, targeted RNA-based next generation sequencing (NGS) fusion assays with coverage of *NUP98* rearrangements, optical genome mapping (OGM), whole genome sequencing (WGS), or fluorescent in-situ hybridization (FISH) break-apart probes for *NUP98*. Of these, *NUP98* FISH is cheapest and fastest but does not identify the specific *NUP98* partner and would not detect other cryptic rearrangements that are potential drivers in the absence of *NUP98*r. By contrast, WGS is the most comprehensive and has started to become adopted clinically, with accurate risk categorization for AML and MDS including reliable detection of *NUP98*r.[5,6]

In recent years, RNA-based NGS fusion assays have enabled estimates of the prevalence of *NUP98*r in AML, ranging from 7.2-8.0% of pediatric AML and 2.5-5.0% of adult AML.[6-12] NGS has also revealed distinct co-mutational patterns, including enrichment of *FLT3*-ITD and *WT1* variants, particularly in AML with *NUP98::NSD1*. *NUP98*r has been less studied in myeloid neoplasms outside of AML. Although presumed to be exceedingly rare, their frequency may be underappreciated.[13-15] Mouse models of *NUP98::NSD1* have generated conflicting data, with one study showing almost universal transformation to AML, compared to other studies indicating a weak leukemogenic potential alone, but increased when combined with *FLT3*-ITD.[16-18] The presence of a chronic or pre-leukemic phase of *NUP98*r AML may be clinically relevant for the possibility of earlier detection and intervention.

Here, we report morphological, clinical, and molecular findings at our institution of adults with *NUP98*r AML or MDS, and explore features in our data and in public datasets which could prompt specific testing for *NUP98*r, potentially providing the basis for cost-effective strategies in clinical practices that do not employ screening for *NUP98*r. In particular, a myelomonocytic morphology and immunophenotype, *WT1* mutations in MDS, and concurrent *FLT3*-ITD and *WT1* mutations in AML, in the absence of another subtype defining genetic aberration (e.g. *NPM1),* highly enrich for myeloid neoplasms with *NUP98::NSD1* or occasionally other *NUP98* rearrangement partners.

Methods:

Nucleic acid extracted from blood, bone marrow, or extramedullary disease sites was tested by one or more of several NGS assays: (1) a clinically validated targeted RNA assay (Heme Fusion Assay (HFA);

Integrated DNA Technologies) designed principally to detect fusions through anchored multiplex PCR (AMP)[19] and performed on clinical samples as part of patient care from 2017-2024 (n=381; HFA clinical cohort) and on research samples for this study (n=7) at the Center for Integrated Diagnostics at Massachusetts General Hospital, (2) a clinically validated targeted DNA panel (Rapid Heme Panel (RHP) version 3)[20] based on NEBNextDirect (New England BioLabs) to detect single nucleotide variants, small indels, and copy number alterations and performed on clinical samples as part of patient care from 2019-2024 (n=21209; RHP cohort) at the Center for Advanced Molecular Diagnostics at Brigham and Women's Hospital, and (3) total RNA sequencing performed on research samples for this study (n=2) at the Dana-Farber Cancer Institute molecular biology core facility. The research RNA-based NGS testing was performed on nucleic acid extracted from archived cytogenetic pellets. Data was processed by default clinical pipelines (HFA, RHP) or by a custom pipeline (total RNA-seq) using adapter trimming by BBDuk, alignment to hg19 by bwa-mem, and manual analysis of bam files. *NUP98* break-apart FISH (Empire Genomics; 11p15.4) was performed on 100 interphase nuclei. Overall survival (determined from the date of first diagnosis to death from any cause) was assessed using the Kaplan-Meier method. Public RNA sequencing FASTQ files were downloaded from the Sequence Read Archive (www.ncbi.nlm.nih.gov/sra) for 2 MDS datasets (SRP149374, SRP418365) and aligned to hg19 by bwa-mem.[21, 22] Alignments were analyzed by (i) searching for select fusions via grep (restricted to alignments to partner gene regions) for exon-exon junctional sequences (30 nucleotides consisting of 15 from each exon) and their reverse complements across all possible exon combinations producing the fusion or its reciprocal as previously described, followed by manual confirmation of hits, (ii) outlier isoform analysis for aberrant expression of isoforms in select genes (*KMT2A*, *UBTF*) as previously described, and (iii) custom variant detection based on pileup data across padded coding sequence of the *WT1* gene.[23, 24] Annotations (mutations, fusions, cytogenetics, diagnoses) for the IPSS-M MDS cohort and the Leucegene AML cohort were retrieved from previously published data[24-29]; www.cbioportal.org/study/summary?id=mds_iwg_2022; data.leucegene.iric.ca/mutation; www.biorxiv.org/highwire/filestream/93175/field_highwire_adjunct_files/1/295808-2.zip). The study was conducted in accordance with the Helsinki Declaration and with approval of the institutional review boards at Dana-Farber Cancer Institute and Massachusetts General Brigham.

Results:

NUP98 rearrangements are effectively detected by AMP-based targeted RNA sequencing, revealing novel partners and a potential enrichment in high-risk MDS.

*NUP98*r was identified through the targeted RNA sequencing HFA assay in 18 patients overall (Table 1 and Figure S1), with diagnoses of AML (n=14), MDS (3), or B/myeloid mixed phenotype acute leukemia [MPAL] (1) at the time of initial *NUP98*r detection. Eight different partner genes were observed, comprising 6 established (*DDX10, HOXD13, KDM5A, NSD1, PRRX2, TNRC18*) and 2 novel (*FGF14, LAMC3*) partners. The novel partners both contained domains that form a coiled coil structure, similar to many other non-*HOX NUP98* partners.[30] *NSD1* was the most frequent partner (11/18 patients; 61.1%), with single occurrences of other partners. Fusions involving 6/8 (75%) partners (*FGF14, KDM5A, LAMC3, NSD1, PRRX2, TNRC18*) from 15/17 (88.2%) evaluated patients were cytogenetically cryptic on conventional karyotyping (Table 1), highlighting the utility of RNA-based NGS for comprehensive detection of *NUP98*r. Only one case (MPAL_1) had *NUP98* FISH performed during initial clinical workup. Nine *NUP98*r cases (50%) had a normal karyotype. Ten of the 15 (66.7%) AML/MPAL cases showed

myelomonocytic or monocytic differentiation by morphology and flow cytometry (Table 1, Figure S2), including 7/9 (77.8%) with *NUP98::NSD1* and 3/6 (50%) with non-*NSD1* partners (*TNRC18*, *DDX10*, *PRRX2*). No other subtype-defining alteration was identified by any testing modality in any of the *NUP98*r cases, including those with novel fusion partners. *KMT2A*-PTD was also absent from *NUP98*r cases, akin to mutual exclusivity reported in pediatric AML; this alteration in AML has been associated with aberrant *HOXB* expression, similar to AML with *NUP98::NSD1*, mutated *NPM1*, *DEK::NUP214*, and *UBTF*-TD, although is not subtype-defining.[6, 31, 32]

The majority of *NUP98* fusions were detected through clinical testing during the course of patient care, with clinically reported *NUP98*r in samples from 14 patients across the HFA clinical cohort, including 11/257 (4.3%) of all newly diagnosed AML patients treated at one institution (MGH) between 2017-2024 (Figure S1). The other 3 clinically identified *NUP98*r patients accounted for 2/46 (4.3%) MDS and 1/3 (33.3%) MPAL from the HFA cohort, however these test populations were subject to selection bias, particularly given the lack of clear guidelines on which MDS cases should be tested by an RNA fusion assay. Most MDS cases that were tested had high-risk features and clinical concern for AML, particularly elevated blast counts (28/46, 60.9%) and/or high to very high International Prognostic Scoring System (IPSS)-Molecular scores (29/46, 63.0%) (Table S1). No cases tested clinically by HFA from patients diagnosed with MDS/myeloproliferative neoplasm (MPN) overlap (0/24), MPN (0/49), or another myeloid neoplasm (0/5) demonstrated a *NUP98*r.

WT1 mutations are recurrently observed with and without *FLT3*-ITD in AML with *NUP98::NSD1* and occur in myeloid neoplasms with other *NUP98* rearrangements.

We investigated co-mutational profiles of the 14 *NUP98*r cases from the HFA cohort, as characterized by targeted DNA-based NGS testing (RHP). Consistent with prior studies, the most common co-occurring mutations at diagnosis were (i) *FLT3*-ITD in 6/14 cases (42.9%; all harboring *NUP98::NSD1*) with variant allele frequencies (VAFs) ranging from <1% to 25% and (ii) *WT1* in 6/14 cases (42.9%; 5 with *NUP98::NSD1* and 1 with *NUP98::LAMC3*) with VAFs ranging from 5.5% to 47.2%, where 4/14 (28.6%) had multiple (2-4) *WT1* mutations (Figure 1 and Table S2). All 6 cases with *WT1* mutations harbored one or more frameshift variants for a total of 12 frameshifts (versus 1 nonsense) including frameshift insertions in all cases.   Concurrent *FLT3*-ITD and *WT1* mutations were seen in 3/14 cases (21.4%). Mutations in *RUNX1*, *MYC*, *TET2*, *KRAS*, and *PTPN11* were also each seen more than once (Figure 1). Myelodysplasia related (MR) gene mutations were not identified, except in *RUNX1*, which is considered an MR gene by ICC but not by WHO5. *FLT3*-ITDs were detected exclusively in AML cases.[16, 33] By contrast, 1/2 (50%) MDS cases demonstrated multiple *WT1* mutations up to 40% VAF. *WT1* mutations also developed after the initial diagnosis in 3 cases (AML_5, AML_6, AML_7) with fusions to *TNRC18*, *NSD1*, and *DDX10*, again with one or more frameshifts in every case. Thus overall, 9/14 (64.3%) cases presented with or developed a *WT1* mutation at various stages of their clonal hierarchies.

Mutational status of *WT1* identifies candidate cases for selective testing for *NUP98*r in MDS.

We explored whether *WT1* mutations detected by up-front DNA-based NGS testing of MDS, in the absence of potential subtype-defining molecular or cytogenetic features determined from routine workups, might provide a rational strategy for initiation of HFA testing with the goal of detecting rare *NUP98*r cases. As a proof of principle, we interrogated DNA-based NGS testing across all MDS cases of the HFA cohort (n=45 with DNA NGS), revealing *WT1* mutations in 4/45 (8.9%) cases: an *NPM1*-mutated case by RHP, a *MECOM*-rearranged (*MECOM*r) case by cytogenetic studies, a case with *KMT2A*-PTD by

RHP, and the *NUP98*r case described earlier that was cryptic on karyotype and characterized subsequently by HFA. Under a hypothetical tiered approach to MDS evaluation, the latter 2 cases would be candidates for dedicated *NUP98*r testing, whereas the first 2 cases were already characterized by their initial workups. However, *NUP98*r testing of *KMT2A*-PTD cases may have limited yield, given the mutual exclusivity of *KMT2A*-PTD with *NUP98*r (and most other molecular subtypes) reported in pediatric AML and the similar absence of *KMT2A*-PTD from the *NUP98*r HFA cohort. Therefore, the hypothetical yield of the proposed tiered strategy would be 1/1 (100%) if *KMT2A*-PTD cases were deliberately excluded from further testing or 1/2 (50%) otherwise or if a clinical practice did not include detection of *KMT2A*-PTD as part of their initial workup. Of note, one *NUP98*r MDS case from the HFA cohort would have been missed by this strategy, since that case harbored only a *DNMT3A* mutation, which is widely mutated across myeloid neoplasms and thus not amenable to a molecular strategy for rationed testing.

The above *WT1* mutational rate (8.9%; 4/45) and hypothetical HFA testing rate (2.2%; 1/45 after also excluding *KMT2A*-PTD) reflected a high-risk MDS cohort. As a broader estimate of testing rate using the public IPSS-M dataset, 37/2591 (1.4%) of its MDS (WHO4) cases harbored pathogenic *WT1* mutations with an enrichment in higher risk subtypes (MDS-EB2: 17/438 (3.9%), MDS-EB1: 10/464 (2.2%)) and subsequent contribution to criteria for a proposed *AML-like* group of MDS.[25, 34] Of these 37 *WT1*-mutated MDS cases, 14 showed key alterations considered mutually exclusive with *NUP98*r: mutated *NPM1* (6), *KMT2A*-PTD (4), biallelic *TP53* mutations (1), biallelic *DDX41* mutations (1), t(6;9)(p23;q34)/*DEK::NUP214* (1), and t(3;21)(q26;q22)/*RUNX1::MECOM* (1). After their exclusion, hypothetical HFA testing might then apply to 23/2591 (0.9%) MDS cases. These 23 cases demonstrated either single missense variants (n=8 cases), single nonsense variants (3), single splice variants (2), or purely frameshifts (8 insertions only, 1 deletion only, 1 both), thus an alternative minimalistic strategy might test only the 9/2591 (0.3%) harboring an insertion frameshift. The IPSS-M cohort, however, did not have *NUP98*r status (or RNA sequencing data) to measure testing yield.

We attempted to further validate the *WT1*-based strategy by applying it to the RHP cohort (Figure 2A). Screening yielded 17 adult patients with MDS harboring *WT1* mutations, of which 7 were found to have an alteration considered mutually exclusive with *NUP98*r by either RHP or kayotype: *NPM1* mutation (5), *MECOM*-r (1), or *TP53* multi-hit (1) (Figure 2A). Cases from 6 additional patients harbored *KMT2A*-PTD, which we decided not to test further for the reasons discussed earlier. Of the remaining 4 patients, 2 had already undergone testing within the HFA clinical cohort, with 1 positive for *NUP98*r and 1 negative for any fusions by HFA. The final 2 patients underwent retrospective HFA testing for this research study, with 1 positive for *NUP98*r and 1 failing sequencing quality control metrics. Thus, the overall HFA yield for *NUP98*r within *WT1*-mutated MDS without a key driver (including *KMT2A*-PTD) was 2/3 (66.7%) in this limited dataset.

We also reanalyzed public RNA sequencing data from two adult MDS cohorts, resulting in detection of *NUP98*r in 4 more MDS cases involving 2 different partner genes (3 cases with *NUP98::NSD1*, 1 with *NUP98::HOXA9*), for cohort frequencies of 2/215 (0.9% in SRP418365) and 2/109 (1.8% in SRP149374) (Supplementary Table 4). No evidence was found for *NUP98* fusions involving 22 other partner genes that have been reported previously in MDS, CMML, or AML across predominantly adult studies (Tables 2-3: *DDX10, EMX1, FGF14, HHEX, HMGB3, HOXA11, HOXA13, HOXC13, HOXD12, HOXD13, KAT7, KDM5A, LNP1, NSD3, PHF23, PRRX1, PRRX2, PSIP1, RAP1GDS1, TLX1, TNRC18, TOP1*). We lacked knowledge of *WT1* mutational status on the DNA level to fully evaluate the *WT1*-based strategy. As a proxy, we

instead screened the RNA-seq data for expressed *WT1* loss-of-function (LOF) mutations (frameshift, nonsense, or splice site) while adopting an approach prioritizing sensitivity in order to partially offset inherent limitations posed by RNA, including variably low *WT1* expression, nonsense mediated decay of mutant RNA transcripts, splicing mutations that may not appear within mature RNA transcripts (e.g., by conferring exon skipping rather than intron retention), and shallow sequencing coverage. Our analysis detected expressed *WT1* LOF mutations in 3/4 (75%) *NUP98*r cases (3/3 *NUP98::NSD1*; 0/1 *NUP98::HOXA9*) and 15/324 (4.6%) MDS cases overall, with individual cohort frequencies of 5/215 (2.3% in SRP418365) and 10/109 (9.2% in SRP149374) (Supplementary Table 5). The higher frequency of the latter cohort was hypothesized to be a consequence of CD34+ enrichment and was associated with higher expressed VAFs, however the possibility of a component of false positives also existed. Outlier isoform analysis demonstrated 2 *KMT2A*-PTDs and 1 *UBTF*-PTD within non-*NUP98*r cases expressing *WT1* LOF mutations. After excluding *KMT2A*-PTD cases, the hypothetical yield of the *WT1*-based strategy was 1/3 (33.3% in SRP418365) and 2/10 (20% in SRP149374) but potentially could be greater, given the lack of annotations (e.g. cytogenetics) and the possibility of additional findings upon standard workups (e.g., *MECOM*r). Of note, all 3 *NUP98::NSD1* cases exhibited *WT1* frameshift insertions, similar to cases in our local cohort. Thus, the alternative strategy of using *WT1* frameshift insertions might largely maintain sensitivity while increasing specificity, with a hypothetical yield across both public cohorts of 3/4 (75%) for *NUP98*r, where the non-*NUP98*r case harbored *UBTF*-TD and would also benefit from subtyping.

Mutational status of *WT1*/*FLT3*-ITD identifies candidate cases for selective testing for *NUP98*r in AML.

In AML, RNA-based NGS testing was a part of routine workups in some but not all our local institutional practices during the study period. As access to testing expands, it is likely that clinical practices will increasingly adopt either universal upfront RNA-based NGS or a tiered approach with reflex testing of all AML cases that do not have a characterized subtype after routine workup. However, in a setting of limited resources with goals of maximizing positive predictive value, we explored the utility of *WT1*/*FLT3*-ITD dual mutations in the absence of subtype-defining genetic features (by RHP or cytogenetics) as another potential rational strategy in AML for initiation of HFA testing. Screening of the RHP cohort identified 41 adult patients with AML harboring both a *WT1* mutation and *FLT3*-ITD, of which 31 were found to have a genetic abnormality considered mutually exclusive with *NUP98*r, including *NPM1* (15), *KMT2A*-PTD (7), *PML::RARA* (6), *DEK::NUP214* (1), *MECOM*r (1), and *CEBPA* bZIP domain mutation (1) (Figure 2B), of which all but *KMT2A*-PTD further enabled AML classification by the ICC or WHO5. Of the remaining patients, 3 had already undergone testing within the HFA clinical cohort, with 1 positive for *NUP98::NSD1* and 2 negative for fusions. The final 7 patients included 5 with available material for retrospective HFA testing for this research study, yielding detection of *NUP98*r in 4/5 (80%) cases (Figure S1). One of the 4 patients with successful confirmation of *NUP98*r at the AML stage also had *NUP98*r detected at an earlier MDS stage through the *WT1* screen. This case (MDS_3) progressed from MDS-MLD to MDS-IB2 together with rising peripheral blasts and relapsed quickly after transplant as AML, with emergence and outgrowth of *FLT3*-ITD across the serial samples (Figure 3A-B). FISH analysis performed for this study supported the early clonal nature of the *NUP98*r at the initial MDS timepoint (Figure 3C). Thus, the overall HFA yield for *NUP98*r within *FLT3*-ITD+/*WT1*+ AML without a mutually exclusive molecular alteration by RHP or karyotype was 5/8 (62.5%). To further characterize

the 3 cases which remained unresolved after HFA, two had available material for total RNA-sequencing, revealing *UBTF*-TD in both.

Examination of the well-characterized Leucegene AML cohort (n=452) demonstrated similar findings (Supplementary Table 6). Out of 17 AML cases positive for both *FLT3*-ITD and *WT1* mutations, 15 harbored a genetic alteration considered mutually exclusive with *NUP98*r, again with *NPM1* mutations (8) and *KMT2A*-PTD (5) as the most common, along with *PML::RARA* (1) and classic biallelic *CEBPA* mutations (1). Of the two remaining cases, one harbored *NUP98::NSD1* while the other could be considered AML-MR. Thus, the hypothetical yield of a *WT1/FLT3*-ITD strategy would be 1/2 (50%) if *KMT2A*-PTD status is determined up front. The Leucegene cohort also contained 16 additional AML cases with *WT1* mutations but lacking *FLT3*-ITD, of which 11 harbored a genetic alteration considered mutually exclusive with *NUP98*r, including *PML::RARA* (6), *NPM1* mutations (2), classic biallelic *CEPBA* mutations (1), *RUNX1::RUNX1T1* (1), and *KMT2A::AFDN* (1). After their exclusion, 5 cases remained, with two harboring *NUP98::NSD1*, two potential AML-MR and one AML-NOS. Thus, the hypothetical yield of a *WT1*-based strategy regardless of *FLT3*-ITD status would be 3/7 (42.9%) if *KMT2A*-PTD cases are excluded.

NUP98r myeloid neoplasms have an aggressive clinical course with poor outcomes even after SCT.

In our two cohorts, 11 of the 14 AML patients plus the one patient with MPAL were initially treated with induction chemotherapy (daunorubicin plus cytarabine [7+3] or vincristine, doxorubicin, methotrexate, plus cytarabine). Three of 14 patients with AML and all 3 MDS patients received hypomethylating agent (HMA)-based therapy with decitabine and venetoclax or with decitabine alone. One patient with AML died two days after starting 7+3 induction chemotherapy, and two patients failed to achieve remission, while the other 15 proceeded to hematopoietic stem cell transplant (SCT) in first complete remission (CR1). Post transplant relapse was seen in 60.0% (9/15) of patients transplanted in CR1, including MDS_3 who relapsed with AML (Figure 3). Of the remaining patients, five are in remission at 26 days, 42 days, 9.4 months, 19.6 months, and 21.3 months after SCT, and one has achieved sustained remission (98 months) after a second SCT (Table 1). In the HFA cohort, eight patients died with a mean overall survival (OS) of 14 months. The median OS of the RHP cohort was 12 months (Figure 4).

Discussion:

*NUP98*r is a rare genetic finding that is AML-defining in new classification systems but prone to under-detection without dedicated or complex testing. It portends a poor prognosis and likely requires dedicated therapeutic approaches. Here, utilizing a clinically validated targeted RNA sequencing approach, we studied the frequency of *NUP98*r in myeloid neoplasms in adult patients at two large academic centers and found 18 cases overall, including 11/257 (4.3%) of all newly diagnosed AML patients treated at one institution. In doing so, we also detected *NUP98*r in patients with MDS, uncovered novel *NUP98* fusion partners, and identified frequent co-mutations which could be leveraged to prompt dedicated testing for *NUP98*r.

In our review of the literature, less than 200 adult *NUP98*r with AML (Table 2) and far fewer adult *NUP98*r cases with other myeloid diagnoses (14 MDS, 4 CMML; Table 3) have been described to date. The frequency of *NUP98*r in MDS is difficult to estimate precisely, given the lack of large comprehensive

studies (Table 3). The most applicable study tested 101 consecutive adult MDS patients at a single institution by OGM, resulting in detection of *NUP98*r in 2/101 (2.0%) cases (1 *NUP98::NSD1*, 1 *NUP98::PRRX2*). Similarly, our reanalysis of public RNA-sequencing data from two adult MDS cohorts revealed *NUP98*r in 2/215 (0.9%) and 2/109 (1.8%) patients (3 *NUP98::NSD1*, 1 *NUP98::HOXA9*). Our study of the HFA clinical cohort revealed *NUP98*r at a slightly greater incidence in 2/46 (4.3%) adult MDS patients (*1 NUP98::NSD1, 1 NUP98::FGF14*), however this cohort was subject to non-universal testing patterns and enriched for high-risk MDS; we also identified another high-risk MDS case with *NUP98::NSD1* through our dedicated strategies. Although relatively small, these studies suggest that this genetic aberration may be more common in MDS than previously thought, particularly in high-risk patients. Of note, an older study testing only for *NUP98::NSD1* by RT-PCR detected no cases out of 193 MDS patients.[35] Finally, since *NUP98*r is AML-defining in both ICC (if $\geq$10% blasts) and WHO5 (if $\geq$5%BM/$\geq$2% blood blasts) when with increased blasts, the 2/3 of the *NUP98*r MDS reported in the literature with at least 5% blasts would now be diagnosed as AML. Therefore, screening of MDS cases will also be important to identify cases that are actually AML, if they meet the blast criteria and have *NUP98*r.

Only two *NUP98*r cases in our cohort were recognized by karyotype (11.2% of 17 evaluable; Table 1), highlighting the need for testing beyond conventional cytogenetics. Indeed, the most common *NUP98*r gene partners in adult and pediatric AML (*NSD1* and *KDM5A*) are well-known to produce karyotypically cryptic fusions. Moreover, a substantial proportion of uncommon *NUP98*r gene partners may similarly generate cryptic fusions according to recent comprehensive studies of adult AML enabled by RNA-based NGS. The largest such study reported 4 uncommon partners that were always cryptic by karyotype (*HMGB3*, *KMT2A*, *PSIP1*, and *TNRC18*), 4 that were never karyotypically cryptic (*HOXA9*, *TOP1*, *DDX10*, and *HHEX*), and one that was variably cryptic (*PRRX2*).[10] In our study, uncommon fusions involving both novel partners (*FGF14* and *LAMC3*) as well as *TNRC18* were karyotypically undetectable, versus two (*DDX10* and *HOXD13*) that were characterizable. By contrast, in a study of pediatric AML with *NUP98*r, uncommon partners were mostly detectable by conventional karyotype G-banding.[7]

Until universal screening for *NUP98*r becomes widely adopted as part of routine workups, strategies for rational test utilization are critical to ensure accurate detection of this entity. These strategies may be particularly beneficial for MDS, where guidelines for RNA-based NGS testing are lacking. We propose a tiered approach in the absence of universal screening and show here that it is possible to identify a subset of cases with high likelihood of *NUP98*r based on results from standard molecular testing. Specifically, MDS with *WT1* mutations and AML with *FLT3*-ITD and *WT1* co-mutations are enriched for *NUP98*r and thus represent candidates for follow-up dedicated testing in the absence of AML subtype defining alterations and *KMT2A*-PTD. These cases alternatively could harbor *UBTF*-TD, another high-risk alteration that is more common in pediatric MDS/AML but also occurs rarely in adults, including the 2 AML-NOS cases in our *FLT3*-ITD+/*WT1*+ AML cohort and a *WT1*+ MDS case from the public RNA sequencing data; of note, most *UBTF*-TD should eventually be detectable during up-front testing by adding *UBTF* exon 13 to DNA-based panels. Further development of strategies to detect *NUP98*r may be warranted to leverage other known features, such as its association with FAB subtypes M4 and M5 (e.g., 10/15 cases in our *NUP98*r cohort) or the high frequency of a normal karyotype (9/17 evaluable cases in our cohort).

Although identification of *NUP98*r cases is critical for appropriate diagnosis and prognosis of AML, the optimal approach to *NUP98*r testing must balance cost and turnaround time with sensitivity. The most economical and fastest testing option is *NUP98* FISH, with a proposed reimbursement in the United States of $145.28 per test (CPT code 88368) and a turnaround time as short as 1-2 days but longer if run in batches/infrequently. However, since the incidence of *NUP98*r cases is less than 5% of cases of adult AML, the overall cost of universal testing for all AML patients would be quite high relative to the very low pre-test probability – the cost to the healthcare system is effectively greater than 20 times the individual FISH cost, or more than $2905.60 per each *NUP98*r case detected. In MDS, where *NUP98*r is rarer (potentially 2% of cases), universal testing would be even more costly. Selective testing, such as through *WT1* or *FLT3*-ITD/*WT1* strategies, is therefore a much better fit for *NUP98* FISH. Larger studies will be needed to better characterize yield and to further develop and optimize strategies.

On the surface, RNA sequencing appears to be more costly, with a proposed reimbursement in the United States of $2919.60 per test for a targeted RNA sequencing panel (CPT code 81455, 2025 Clinical Diagnostics Laboratory fee schedule) and a longer turnaround time (at least 4-7 days) than FISH. However, universal RNA sequencing allows for essentially 100% detection of *NUP98*r fusions, identification of gene partners, and appropriate disease subclassification. In addition, RNA sequencing approaches capture not only *NUP98*r cases but a wide spectrum of clinically important alterations that are critical for diagnosis, prognosis, and treatment of AML, some of which may also be cryptic by metaphase karyotype. Similar results may be obtained by WGS-based or whole transcriptome-based methods. On the other hand, a tiered approach with only karyotype and a DNA panel upfront would have an initial turnaround time of 3-5 days. Based on the results, reflex testing for RNA sequencing or FISH could be added. This strategy increases pre-test probability and decreases costs compared to universal testing, but results in longer turnaround times and lower sensitivity of *NUP98*r detection. Importantly, all testing algorithms are institution-specific, influenced by the availability of individual tests, testing schedules, and local logistics. Thus, testing decisions are ideally managed/supervised by pathology, as algorithmic testing in hematopathology has previously been shown to improve cost-effectiveness.[36]

*NUP98*r has consistently been associated with worse outcomes in studies of both pediatric and adult AML.[7, 11, 37-40] In our study, we observed high relapse rates even after SCT in CR1 (60% of patients). Therefore, there is a need to identify *NUP98*r at diagnosis and to develop more effective treatment strategies. In pre-clinical models, *NUP98*r AML has demonstrated sensitivity to Menin inhibition, with eviction of both *NUP98* fusion proteins and *KMT2A* (*MLL1*) from chromatin at a critical set of pro-leukemic genes.[3] Given the recent approval of Menin inhibitors for AML with *KMT2A* rearrangement and their active development for *NPM1*-mutated AML, there are several phase 1 clinical trials (e.g. NCT05326516 and NCT05453903) that also recruit patients with *NUP98*r AML.[41-44] Recent PDX mouse models of *NUP98*r have also indicated that the combination of a Menin inhibitor with a CDK4/6 inhibitor (palbociclib) or a FLT3 inhibitor (gilteritinib) has a synergistic anti-leukemic effect.[45] In addition, several alternative treatments may be promising for *NUP98*r AML. One example is Venetoclax, a BCL-2 inhibitor, which may be effective against AML with HOXA/B gene overexpression.[46, 47] Another example is dasatinib, an inhibitor of ABL and SRC family kinases, which has synergistic effects on cells with *NUP98::NSD1* and *FLT3*-ITD.[48]

In conclusion, our results indicate that AML with *NUP98*r cases are usually cytogenetically cryptic and can be missed with conventional molecular testing, such as karyotype testing, FISH for common

translocations, and myeloid-directed NGS panels looking at DNA mutations. Targeted RNA sequencing with anchored multiplex PCR or hybrid capture enrichment, whole transcriptome sequencing or other genome wide technologies, such as optical genome mapping, should be considered to detect *NUP98r* alterations. Our high-yield tiered approach could be used to perform dedicated testing in the subset of AML and MDS that are enriched for *NUP98*r, which we, like others, demonstrated to be associated with poor prognosis. *NUP98*r in fact should be specifically investigated in MDS as well, since it could lead to a change in diagnosis to AML and since the ability to detect *NUP98*r prior to leukemic transformation may allow for earlier intervention.

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Table 1: Summary of patient characteristics

Patient	WHO revised 4th ed. Diagnosis (FAB)	WHO 5th ed. Diagnosis	International Consensus Classification	BM blast (%)	PB blast (%)	Age at Dx (y)	Sex	Fusion partner	Cryptic on KT	Karyotype (KT)	Mutations*	Initial Treatment	# of SCT	# of Relapse	Outcome
AML_1	AML-NOS (M0)	AML with NUP98 rearrangement	AML with NUP98::KDM5A	65	59	68	F	KDM5A	yes	46,XY[20]	CDKN2A TP53	7+3	1	0	Dead (GVHD), 6.1 months
AML_2	AML-NOS (M5b)	AML with NUP98 rearrangement	AML with NUP98::NSD1	75	41	60	M	NSD1	yes	46,XY,del[3](p21)[cp3]/46,XY[cp20]	FLT3-ITD WT1 x4 TET2 MYC	7+3	1	0	Dead (intracranial hemorrhage), 4.5 months
AML_3	ET in Blast Phase/AML-NOS (M5a)	AML with NUP98 rearrangement	AML with NUP98::NSD1, progressing from ET	30	24	77	F	NSD1	yes	46,XX[cp20]	FLT3-ITD TET2 x3 JAK2 V617F	Decitabine + venetoclax	1	0	Alive, in remission after SCT, 21.3 months
AML_4	AML-NOS (M5a)	AML with NUP98 rearrangement	AML with NUP98::NSD1	87	76	50	M	NSD1	yes	46,XY[20]	FLT3-ITD (x2) WT1	7+3 and gilteritinib	1	1	Dead of relapsed/refractory AML, 18.3 months
AML_5	AML with mutated RUNX1 (M5a)	AML with NUP98 rearrangement	AML with NUP98::TNRC18	44	77	18	M	TNRC18	yes	46,XY[20]	RUNX1 NRAS	7+3	2	2	Dead of relapsed/refractory AML, 54.4 months
AML_6	AML-NOS (M4)	AML with NUP98 rearrangement	AML with NUP98::NSD1	84	83	42	F	NSD1	yes	46,XX[20]	FLT3-ITD	7+3 and midostarin	0	1	Dead of relapsed/refractory AML, 24.4 months
AML_7	AML-NOS (M4)	AML with NUP98 rearrangement	AML with NUP98::DDX10	20	90	65	F	DDX10	no	46,XX,del[9](q21q34),inv[11](p15q22)[20]	PTPN11	Decitabine	0	1	Dead of relapsed/refractory AML, 10.9 months
AML_8	AML with mutated RUNX1 (M0)	AML with NUP98 rearrangement	AML with NUP98::NSD1	90	82	51	M	NSD1	yes	46,XY,t7;19](q11.2;q13.2),del[11](q21q24)[10]/46,XY[10]	FLT3-ITD RUNX1	7+3	1	2	Dead of relapsed/refractory AML, 14.3 months
AML_9	Therapy-Related AML (M0)	AML with NUP98 rearrangement, post cytotoxic therapy	AML with NUP98::HOXD13, therapy-related	58	1	39	F	HOXD13	no	46,XX,t[2;11](q31;p15)[18]/46,XX[2]	None	7+3	2	1	Alive, in remission after second SCT, 98.1 months
AML_10	AML-NOS (M0)	AML with NUP98 rearrangement	AML with NUP98::LAMC3	89	97	22	M	LAMC3**	yes	46,XY,t[4;17](q21;q21),add[11](p15)[9]/48,idem,+der[4]t[4;17],+21[cp11]	WT1 KRAS	7+3	1	1	Dead of relapsed/refractory AML, 11.7 months
AML_11	AML with mutated RUNX1 (M0)	AML with NUP98 rearrangement	AML with NUP98::NSD1	79	87	24	M	NSD1	yes	46,XY[20]	WT1 (x2) FLT3-ITD (x2) RUNX1 NFE2 MYC	7+3 and midostaurin	1	0	Alive, in remission after SCT, 9.4 months
MDS_1	MDS-EB2	MDS-IB2	MDS-EB	4	0	42	F	FGF14	yes	46,XX[20]	DNMT3A	Decitabine + venetoclax	1	0	Alive, in remission after SCT, 19.6 months
MDS_2	MDS-EB2	AML with NUP98 rearrangement	AML with NUP98::NSD1	10	4.5	77	F	NSD1	yes	47,XX,+8[3]/46,XX[17]	WT1 (x2)	Decitabine	1	0	Alive, 9.0 months, day +42 after SCT
MPAL_1	Mixed-phenotype (B/myeloid) acute leukemia (M5a)	Mixed-phenotype (B/myeloid) acute leukemia	MPAL, B/myeloid, with NUP98::NSD1	98	19.5	30	F	NSD1	yes	46,X,der[X](X;5)(p22.1;q35),der[5]t[X;5]t[5;11](q35;p15.4),der[11]t[5;11](q35;p15.4)t[X;5][20].ish der[X](Xptel-,DXZ1+),der[5](D5S723/D5S721+,EGR1+,Xptel+),der[11](5'NUP98+)[5]	WT1 (x3) CEBPA KRAS PTPN11	AYA induction	1	0	Alive, 4.0 months, day +26 after HCT
AML_12	AML-NOS (M5a)	AML with NUP98 rearrangement	AML with NUP98::NSD1	83	37	48	F	NSD1	yes	46,XX[cp20]	WT1 (x2) FLT3-ITD (x2) KRAS TET2 (x2)	7+3	1	1	Dead of relapsed/refractory AML, 31.8 months
AML_13	AML-NOS (M4)	AML with NUP98 rearrangement	AML with NUP98::NSD1	87	5	82	F	NSD1	yes	46,XX[20]	WT1 FLT3-ITD	Decitabine + venetoclax	1	1	Dead of relapsed/refractory AML, 28.6 months
AML_14	AML with mutated RUNX1 (M5a)	AML with NUP98 rearrangement	AML with NUP98::PRRX2	81	77	61	M	PRRX2	N/A	N/A	WT1 FLT3-ITD (x2) RUNX1 DNMT3A	7+3	0	0	Dead of AML, 2 days
MDS_3	MDS with multilineage dysplasia	MDS with low blasts and multilineage dysplasia	MDS-NOS with multilineage dysplasia	2	0	68	F	NSD1	yes	46,XX,t[11;12](q13;p13)[4]/46,XX[16]	WT1 (x3) MYC (x2) DNMT3A	Observation	1	1	Dead of relapsed/refractory AML, 12.4 months

*Mutations includes those that appeared in subsequent bone marrow biopsies

7+3: induction with cytarabine days 1-7 and idarubicin days 1-3

AYA induction: vincristine, doxorubicin, methotrexate, cytarabine

**This same patient with NUP98::LAMC3 was previously in a workshop report (the 2017 Society for Hematopathology/European Association for Haematopathology Workshop Report (Harris, Czuchlewski et al. 2019).

Table 2: AML literature review, adult only

Institution	Year	No. of patients		Screening method	Fusion partner	Age (median)	Sex	FAB subtype	Cytogenetics	Mutations	Outcome	Reference
		Total	Fusion									
National Taiwan University Hospital, Taipei, Taiwan	2009	493 AML	11	t(7;11)(p15;p15) only	HOXA9	31 (range 23-59)	18.2% male	91% M2, 9% M4	t(7;11)(p15;p15) in all	36.3% with WT1, 27.3% with FLT3-ITD, 18.2% with KRAS, 18.2% with NRAS	13.5 mo OS, 6 mo RFS	PMID: 19225539
Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands	2011	808 AML	10	NSD1 only	10 NSD1		40% male	10% M2, 30% M4, 60% M5	90% normal, 10% with inv(5)	90% with FLT3-ITD, 30% with WT1	4-year pOS 11%, 4-year pEFS 0%	PMID: 21813447
			1	11 p15 aberration on karyotype	1 DDX10	range 15-77 of entire cohort	male	M6	+8, inv(11)(p15;q22)	NRAS	EFS 13.9 mo, OS 22.3 mo	
Cancer Genomic Atlas Research Network	2013	200 AML	3	Comprehensive	3 NSD1	Entire cohort: 55.0±16.1	Entire cohort: 54% male	N/A	N/A	N/A	EFS 0.54, OS 0.81	PMID: 23634996
Hannover Medical School, Hannover, Germany	2013	504 AML, 193 MDS	7 AML, 0 MDS	NSD1 only	NSD1	34 (range 23-48)	43% male	14.3% M1, 28.6% M2, 57% M4	71.4% normal, 14.3% with del(9q), 14.3% with inv(3)(q21q26) and monosomy 7	71.4% with FLT3-ITD, 28.6% with WT1, 14.3% with NRAS	43% complete response rate. OS and RFS no difference compared to NUP98::NSD1-neg patients	PMID: 22929522
Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China	2013	17 AML with t(7;11)(p15;p15)	16	t(7;11)(p15;p15) only	HOXA9	34 (range 18-67)	23.5% male	52.9% M2, 23.5% M4, 17.6% M5b, 6% M6	t(7;11)(p15;p15) in all, 6% with trisomy 8	6.7% with FLT3-ITD	OS 8 months, DFS 4 months	PMID: 23800796
MLL Munich Leukemia Laboratory, Munich, Germany	2013	378 AML	8	NSD1 only	NSD1	42.3 (range 20.9-71.4)	50% male	62.5% M1, 25% M2, 12.5% M4	12.5% with ins(5;11)(q35;p13 p15)	75% with FLT3-ITD, 50% with WT1	1.8 mo EFS	PMID: 22945772
Shanghai Institute of Hematology, Shanghai, China	2022	655 AML	18	Comprehensive	6 HOXA9, 6 NSD1, 2 PRRX2, 1 HMGB3, 1 HOXA11, 1 HOXD12, 1 TNRC18	Entire cohort: 48 (IQR 34-60)	Entire cohort: 51.8% male	N/A	N/A	N/A	N/A	PMID: 36442087
					TNRC18	54	M	M4	Trisomy 8	NRAS, IDH1, SMC1A	dead	
					HOXD12	16	M	M4	t(2,11)(q31;p15)	EZH2, PTPN11, RUNX1	alive	
Oregon Health & Science University, Portland, OR, USA	2022	316 AML	8 + 1 from another institution	Comprehensive	N/A (FISH break apart probe)	49 (24-77)	44.4% male	11.1 M0, 44.4 M1, 44.4 M4, 11.1 M5	33.3% normal, 44.4% with 11p15 rearrangement, 22.2% with del(5q), 33.3% with del(7q)	62.5% with WT1, 50% with KRAS, 37.5% with CEBPA, 37.5% with FLT3-ITD, 25% with IDH2, 25% with PTPN11	13 mo OS	PMID: 35258401
Ningbo First Hospital, Ningbo, China	2023	11 AML	11	NSD1 only	NSD1	30 (14-59)	45% male	N/A	81.8% normal, 9.1% with trisomy 8, 9.1% with del 9q13	63.6% with FLT3-ITD, 27.3% with WT1, 27.3% with CEBPA	1yr OS 54.5-68.6%	PMID: 36751862
Hokkaido University Hospital, Sapporo, Japan	2023	97 FLT3-ITD-positive AML	6	NSD1 only	NSD1	55 (21-61)	67% male	33.3% M1, 66.7% M4	83.3% normal, 16.6% with trisomy 8	FLT3-ITD in all by screening criteria), 50% with WT1	2yr OS 53.5%, 2-year RFS 33.3%, 83% induction failure rate	PMID: 37465857
Erasmus Medical Center - Sophia Children's Hospital, Rotterdam, the Netherlands	2023	825 adult AML, 2235 pediatric AML	13 adult	Comprehensive	Adult: 11 NSD1, 1 TOP1, 1 RAP1GDS1	N/A	N/A	N/A	Entire cohort: 18.8% of NUP98::NSD1 have trisomy 8	Entire cohort: 74% have FLT3-ITD and 42% have WT1 in NUP98::NSD1; 25% have WT1 in NUP98::X	Entire cohort: NUP98::NSD1 17% EFS and 36% OS; NUP98::X 35% EFS and 35% OS; overall NUP98r 35% OS	PMID: 36815378
Josep Carreras Leukaemia Research Institute, Barcelona, Spain	2024	291 AML	8	Comprehensive	5 NSD1, 1 EMX1, 1 KMT2A, 1 KDM5A	45 (range 31-67)	87.5% male	12.5% M0, 12.5% M1, 25% M4; 50% MRC	62.5% normal, 37.5% with chr 11 aberration	62.5% with FLT3-ITD, 62.5% with WT1, 25% with CEBPA	5/8 died	PMID: 38536941
Shanghai Jiao Tong University School of Medicine	2024	1099 adult and pediatric AML	48 adult	Comprehensive	22 NSD1, 10 HOXA9, 4 PRRX2, 2 HMGB3, 2 TOP1, 2 KDM5A, 1 TNRC18, 1 HOXA11, 1 HHEX, 1 DDX10, 1 PSIP1, 1 KMT2A	43 (range 19-79)	46.6% male	16.7% M2, 37.5% M4, 14.6% M5, 6.3% t-AML; 6.3% secondary-AML	47.8% normal, 19.6% with t(7;11)(p15;p15), 13.0% with trisomy 8, 10.9% with complex karyotype	54.2% with FLT3-ITD, 31.3% with WT1	NUP98::NSD1 8.0% CR rate and 65.2% 4yr OS; overall 37.3% CR rate; non-NUP98::NSD1 66.7% 4yr OS	PMID: 38744828
Yonsei University College of Medicine, Severance Hospital Seoul, Korea	2024	260 AML	13 (10 with data)	Comprehensive	4 NSD1, 1 HOXA9, 1 DDX10, 1 TOP1, 1 PHF23, 1 HMGB3, 1 HOXC13	35.5 (range 20-74)	50% male	N/A	2/10 normal, 1 with trisomy 8, 1 with t(7;11)(p15;p15), 1 with complex karyotype	6/10 with FLT3-ITD, 1 with WT1, 3 with NRAS, 1 with DNMT3A, 1 with RAD21, 1 with RUNX1	6/10 in CR, 3 died, 1 lost to follow-up	PMID: 39158088
Oregon Health & Science University, Portland, OR, USA	2024	746 AML	15	Comprehensive	6 NSD1, 1 KDM5A, 1 DDX10, 1 HMGB3, 1 PSIP1, 1 HOXC13, 1 TLX1 (HOX11), 1 HHEX, 2 N/A	52 (range 18-77)	67% male	13.3% M0, 46.7% M1, 33.3% M4, 6.7% M5; 20% t-AML	11/15 cryptic; 7 normal 3 with complex karyotype	10/15 with FLT3-ITD, 6 with WT1, 3 with KRAS, 2 with mono-allelic CEBPA, 2 with TET2, 2 with IDH2, 2 with PTPN11,	6/15 died, 3 alive with relapsed/persistent disease, 3 alive in CR	PMID: 39701595

Table 3: MDS and MDS/MPN literature review, adult and pediatric

Institution	Year	No. of patients Total	Fusion	Detection method	Fusion partner	Age	Sex	Blasts	Cytogenetics	Mutations	Outcome	Reference
National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan	1997	52 MDS, 11 t-MDS	1 MDS, 1 t-MDS	Karyotype and Southern blot	DDX10	7, 4	1 male, 1 female	N/A	inv(11)(p15q22)	N/A	N/A	PMID:9166830
Saitama Cancer Center Hospital, Saitama, Japan	1999	98 t-AML or t-MDS	2 t-MDS	Karyotype, Southern blot, and RT-PCR	1 possible HOXB 1 unknown	8, 15	1 female, 1 male	N/A	1 with t(11;17)(p15;q21) 1 with add(11)(p15)	N/A	Died 34 mo, died 24 mo	PMID:10502319
Akita University School of Medicine, Akita, Japan	1999	1 CMML, 1 MDS-EB2	1 CMML, 1 MDS-EB2	Southern blot and RT-PCR	HOXA9	45, 69	1 male, 1 female	8.4%, 4.5% with Auer rods	t(7;11)(p15;p15)	N/A	Both progressed to AML within 1 year	PMID:10583265
Roswell Park Cancer Institute, Buffalo, NY, USA	1999	2 t-MDS	2	Southern blot and RT-PCR	TOP1	13, 15	1 female, 1 male	>2%	2 with t(11;20)(p15;q11.2)	N/A	N/A	PMID:10556215
University Hospital, Lund, Sweden	2002	1 t-MDS	1	RT-PCR for NUP98::TOP1	TOP1	60	female	12% with Auer rods	t(10;20;11)(q24;q11;p15)	N/A	Alive in remission after induction and consolidation	PMID:11979559
The Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan	2002	1 CMML	1	Southern blot and RT-PCR	HOXA13	45	male	2%	t(7;11)(p15;p15)	N/A	Relapsed 6 mo after induction, died 14 mo after diagnosis	PMID:11830496
University of Perugia, Perugia, Italy	2004	1 MDS-EB1	1	RT-PCR for NUP98::NSD1	NSD1	65	male	3% in peripheral blood	add(11)(p15)	N/A	Died 4 mo	PMID:15382262
Centre Hospitalier Universitaire Necker-Enfants Malades, Paris, France	2006	71 patients with 11p15 abnormality	1 t-MDS	FISH and RT-PCR	DDX10	55	female	N/A	t(11;11)(p15;q22)	N/A	N/A	PMID:16467868
			1 t-MDS		DDX10	50	male	N/A	inv(11)(p15q22)	N/A	N/A	
			1 CMML		HOXA9	45	male	N/A	t(7;11)(p15;p15)	N/A	N/A	
			1 CMML		HOXA9	31	male	N/A	t(7;11)(p15;p15)	N/A	N/A	
			1 MDS		LNP1/NP3	3	female	N/A	t(3;11)(q12.2;p15)	N/A	N/A	
			1 MDS		NSD1	65	male	N/A	add(11)(p15)	N/A	N/A	
			1 t-MDS		NSD3	70	female	N/A	t(8;11)(p11;p15),add(16)(q22)	N/A	N/A	
			1 t-MDS		TOP1	4	female	N/A	t(11;20)(p15;q11)	N/A	N/A	
Cedars Sinai Medical Center, Los Angeles, CA, USA	2007	1 t-MDS	1	FISH	PRRX1	74	male	3.5%	t(1;11)(q23;p15)	N/A	AML progression 8 mo, Died 10 mo	PMID:17889707
Shimane University Hospital, Izumo, Shimane, Japan	2009	1 t-MDS	1	Southern blot and RT-PCR	NSD3	60	male	0%	t(8;11)(p11;p15),del(1)(p22p32)	N/A	AML progression 12 mo, Died 23 mo	PMID:19380029
Kobe University Graduate School of Medicine, Kobe, Japan	2012	1 MDS-EB2	1	FISH and RT-PCR	PSIP1	64	female	9.2%	t(9;11)(p22;p15)	N/A	AML progression and died 7 mo	PMID:22103895
Hannover Medical School, Hannover, Germany	2013	193 MDS	0	RT-PCR for NUP98::NSD1	--	--	--	--	--	--	--	PMID:22929522
Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan	2019	1 CMML	1	RT-PCR	KAT7 (HBO1)	69	female	15%	t(11;17)(p11.5;q21)	N/A	AML progression and died 3 mo	PMID:30944097
Benioff Children's Hospital University of California San Francisco, San Francisco, CA, USA	2022	1 JMML	1	RNA seq	NSD1	2	female	5%	44,X,-X,del(9)(q13q22),-16(1),46,XX(20)	NRAS p.Gly12Val (8%)	Alive, NUP98::NSD1 not detected, 5 months after HCT	PMID:32815876
The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA	2022	101 MDS	2	Optical genome mapping	1 PRRX2, 1 NSD1	Entire cohort: 72 (25-92)	Entire cohort: 71% male	N/A	Cryptic	N/A	N/A	PMID:35915143
Sunkyunkwan University School of Medicine, Seoul, Korea	2024	15 patients with 11p15 translocation	1 t-MDS	FISH and/or RT-PCR	DDX10	50	female		inv(11)(p15q22)	N/A	Died 5 mo	PMID:39344146
			1 CMML-2		HOXA9	37	female		t(7;11)(p15;p15)	N/A	AML progression 28 mo, Died 32 mo	
			1 MDS-EB2		PSIP1	55	female		t(9;11)(p22;p15)	N/A	AML progression 4 mo, Died 12 mo	

Figure legends:

Fig 1: Co-mutation plot of the Heme Fusion Assay and Rapid Heme Panel (RHP) cohorts at initial diagnostic bone marrow biopsy. Pathogenic mutations identified in each *NUP98*r case are represented by blue boxes. If multiple mutations occur in the same gene, the number of concurrent mutations is indicated within the blue box. *RHP cohort, †novel fusion partner.

Fig 2. Mutually exclusive genetic alterations in MDS with *WT1* mutations and AML with *WT1* and *FLT3*-ITD mutations. A) Adult MDS cases with *WT1* mutation in the Rapid Heme Panel database (n=17), labeled with detected genetic driver. NOS: no genetic driver detected. *1 case sample with no known genetic driver failed QC metrics on Heme Fusion Assay. B) Adult AML cases with *WT1* mutation and *FLT3*-ITD in the Rapid Heme Panel database (n=41), labeled with detected genetic driver. AML-MR: myelodysplasia-related, by ICC; NOS: no genetic driver detected. Of note, 2 of the 3 AML-NOS cases subsequently underwent total RNA-sequencing, and both were found to harbor UBTF-TD.

Fig 3. MDS_3 progression to AML. A) Summary of aspirate blast count and mutations present in BM biopsies for this patient at the time of MDS with multilineage dysplasia (MDS-MLD), MDS with excess blasts/increased blasts 2 (MDS-IB2), and AML diagnoses. B) Representative histology of BM cores from MDS-IB2 and final AML biopsies (HE, 60x magnification). C) FISH testing on the MDS-IB2 biopsy using *NUP98* (11p15.4) break-apart probe; two representative interphase nuclei each showing one intact *NUP98* signal and one split 3' green signal (t) and 5' red signal (c), indicating a rearrangement.

Fig 4: Survival curve of the Heme Fusion Assay clinical cohort and the Rapid Heme Panel cohort. Mean overall survival was 14 months and 12 months, respectively.

Fig 1: Co-mutation plot

Gene	AML_11	AML_2	AML_4	MPAL_1	AML_10	MDS_2	AML_8	AML_3	AML_6	AML_5	AML_7	AML_1	MDS_1	AML_9	*AML_14	*AML_12	*AML_13	*MDS_3
WT1	2	4	■	3	■	2									■	2	2	3
FLT3-ITD	2	■	2				■	■	■						2	■	■	
RUNX1	■						■			■					■			
MYC	■	■																2
TET2		■						3								2		
NRAS										■								
KRAS				■	■											■		
DNMT3A													■		■			■
NFE2	■																	
CEBPA				■														
JAK2								■										
PTPN11				■							■							
TP53												■						
CDKN2A												■						
Fusion gene	NSD1	NSD1	NSD1	NSD1	LAMC3†	NSD1	NSD1	NSD1	NSD1	TNRC18	DDX10	KDM5A	FGF14†	HOXD13	PRRX2	NSD1	NSD1	NSD1

Fig 2

A) Adult MDS cases in RHP database with *WT1* mutation
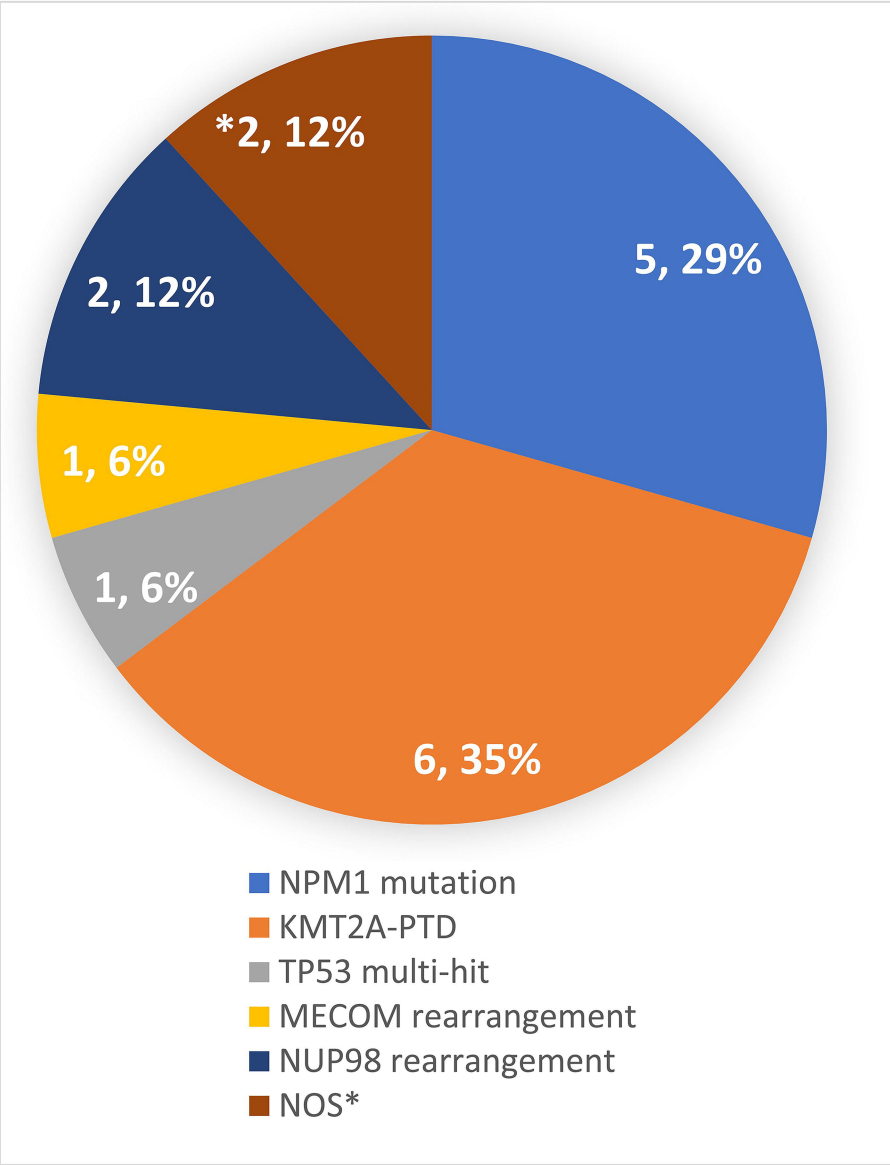
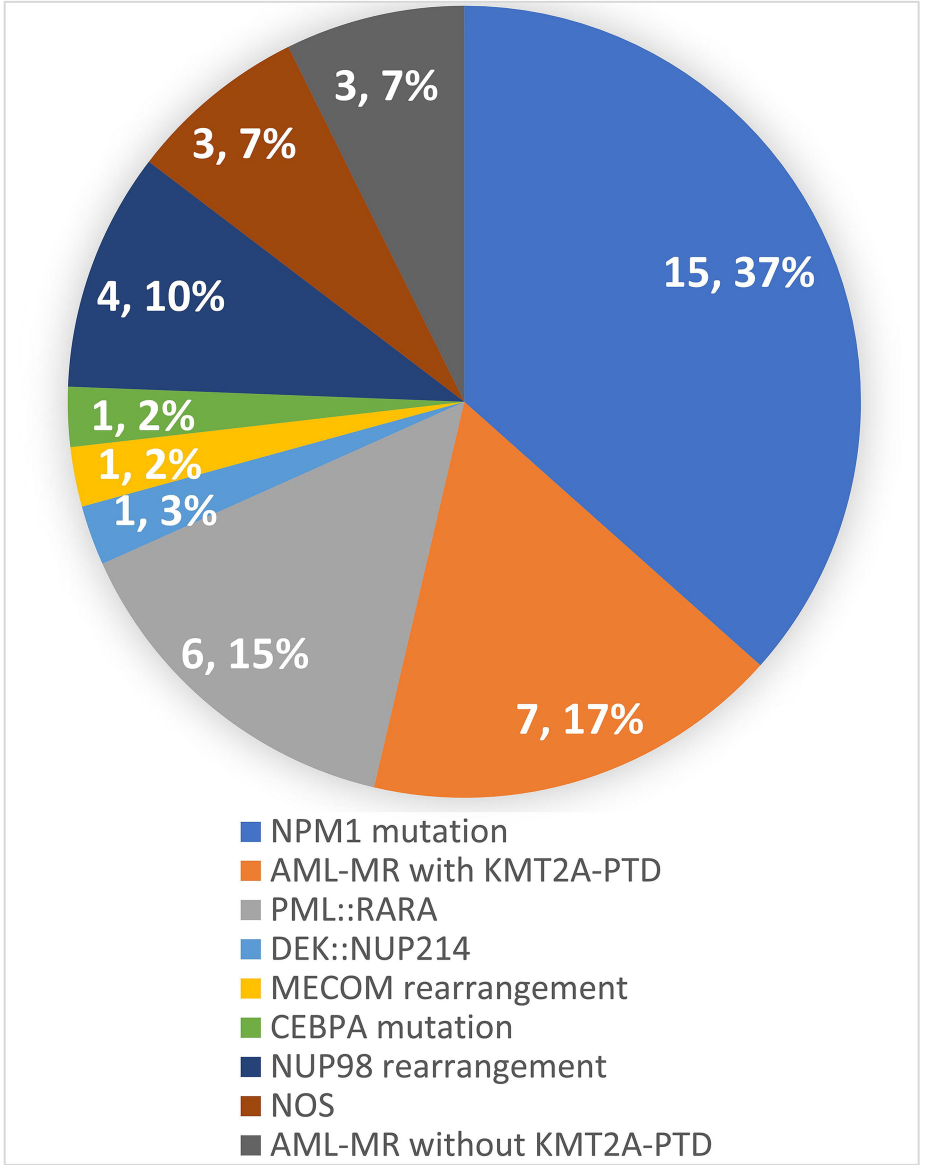B) Adult AML cases in RHP database with *WT1* mutation and *FLT3*-ITD

Fig 3: MDS_3 progression to AML

A)

Time	day 0	day 41	day 231	day 451
BM diagnosis	MDS-MLD	MDS-IB2	AML	AML
BM blast %	2%	8%	83%	60%
DNMT3A p.R749C	43.4%	56%	Unknown	43.8%
WT1 p.R380Vfs*	9.5%	13.2%	Unknown	
WT1 p.K141*	7.3%	21.9%	Unknown	37.7%
WT1 p.R462W	7%	5.7%	Unknown	
WT1 p.S381Gfs*			Unknown	48.6%
MYC p.P74L	5.5%	2.9%	Unknown	
MYC p.P72S	3.6%	3.6%	Unknown	
MYC p.P74Q	0.9%	16.7%	Unknown	
FLT3-ITD (18 bp)	0.1%	0.5%	Unknown	
FLT3-ITD (84 bp)		1.5%	Unknown	23.3%
NRAS p.G12D				42.4%
NUP98r detection	HFA	FISH		HFA

C)

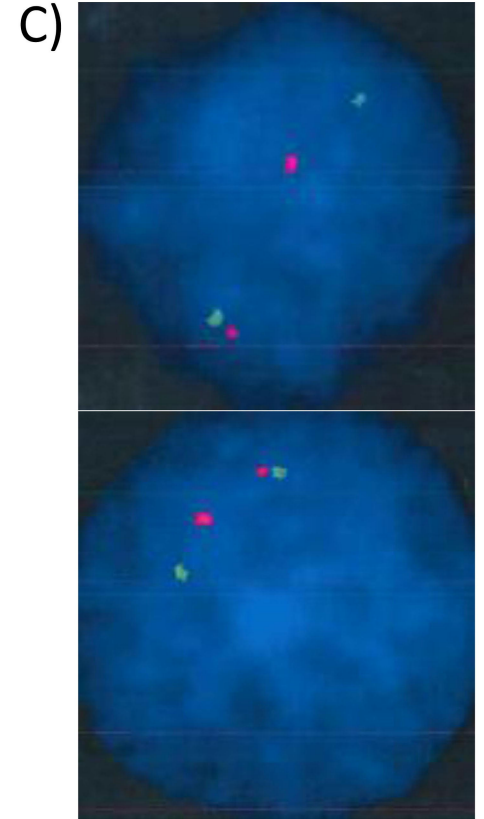B)

MDS-IB2 (day 41)   AML (day 451)
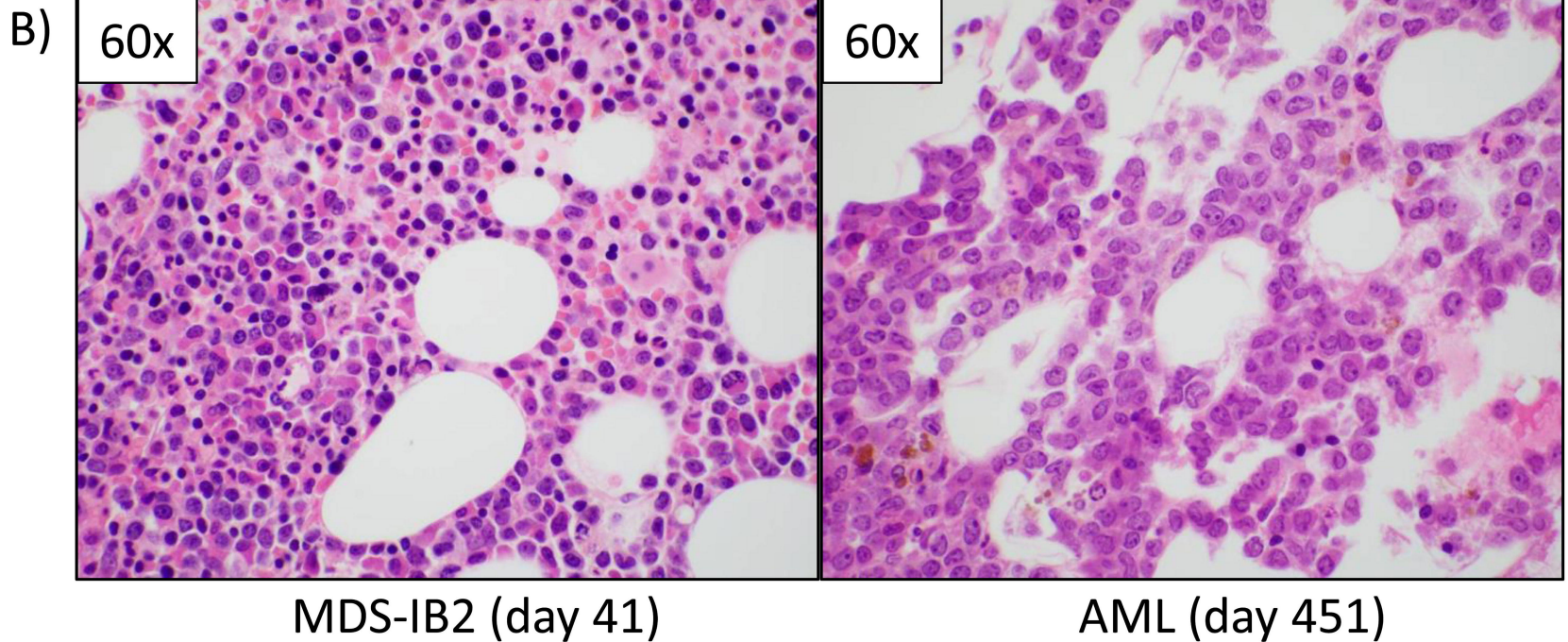
Fig 4: Overall survival
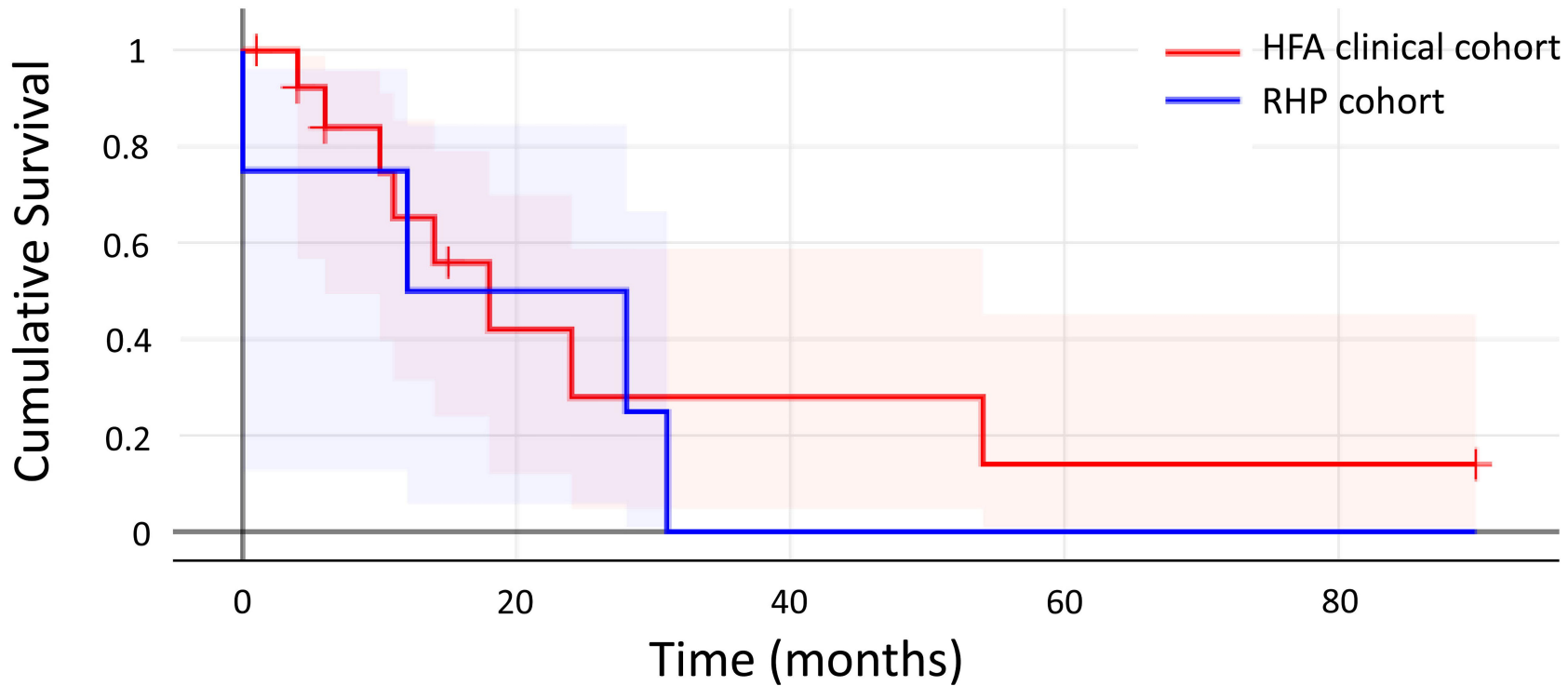
Supplemental Fig 1: HFA and RHP cohorts and *NUP98r* results

```
HFA cohort (n=381)
        │
        ▼
   NUP98r (n=14)
```

AML (n=11/257)	MDS (n=2/46)	B/myeloid MPAL (n=1/3)
6 NSD1	1 NSD1	1 NSD1
1 DDX10	1 FGF14	
1 HOXD13		
1 KDM5A		
1 TNRC18		
1 LAMC3		

```
RHP cohort (n=21209)
        │
        ▼
FLT3 ITD+ WT1+ AML (n=41), WT1+ MDS (n=17)
        │
        ▼
KMT2A PTD-, NPM1-, PML::RARA-
DEK::NUP214-, MECOMr-, CEBPA-
(n=10 AML, 7 MDS) [1 shared patient]
        │
        ▼
Available cytogenetic pellets
sufficient for retrospective testing
and not part of HFA cohort
(n=5 AML, 1 MDS) [1]
        │
        ▼
NUP98r
(n=4 AML, 1 MDS) [1]
```

AML (n=4/5)	MDS (n=1/1)
3 NSD1	1 NSD1
1 PRRX2	
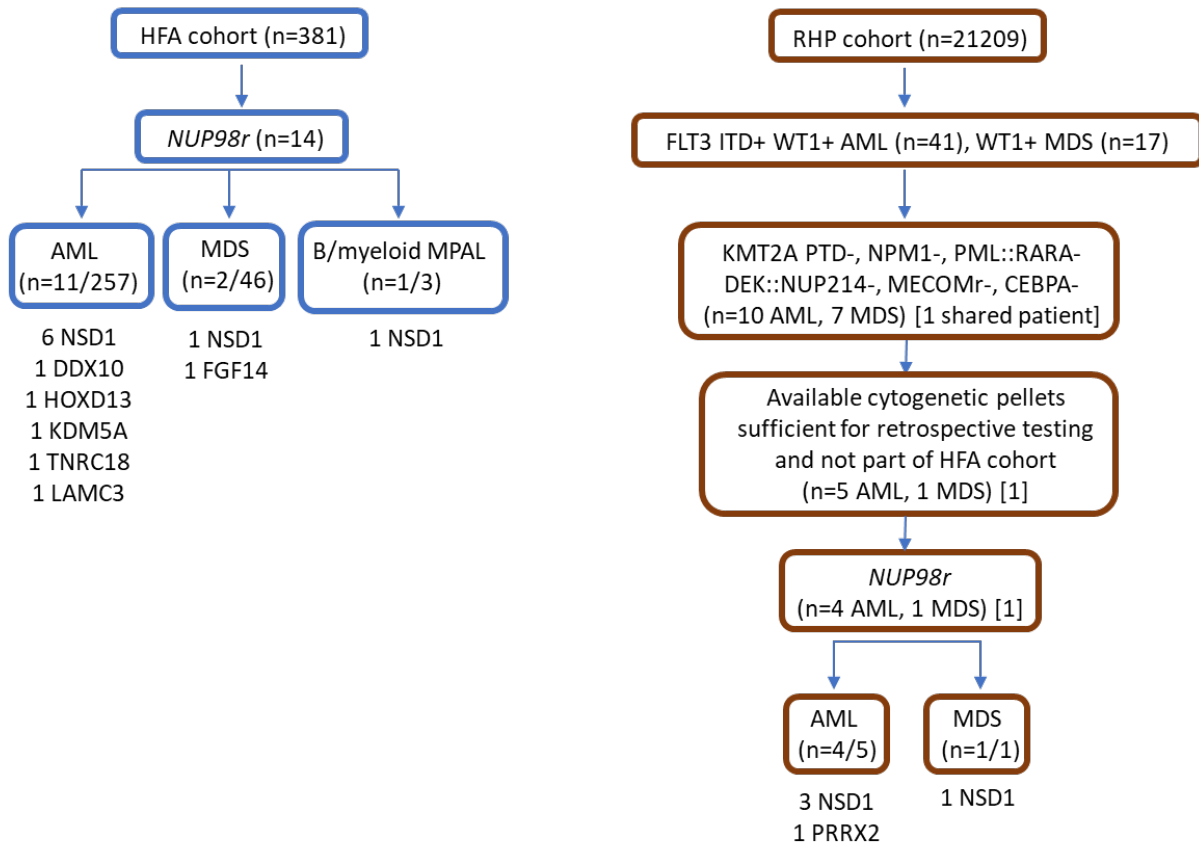
Supplemental Fig 2: *NUP98*r AML with monocytic differentiation

Supplemental Figure Legends

Supplemental Fig 1: Schematic of the Heme Fusion Assay (HFA) and Rapid Heme Panel (RHP) cohorts and *NUP98*r results.

Supplemental Fig 2. *NUP98*r AML with monocytic differentiation. Representative images of monocytic differentiation by morphology of A) bone marrow core biopsy (Hematoxylin and Eosin stain, 1000x), B) bone marrow aspirate smear (Wright-Giemsa stain, 1000x), and C) peripheral blood smear (Wright stain, 1000x), as well as flow cytometric analysis in D) showing monocytic blasts (blue population) expressing CD64 (largely), CD11C (dim) and CD11b (largely) and negative for CD14; mature monocytes are shown as the green population.

Supplemental Table Legends **See Excel File**

Supplemental Table 1: International Prognostic Scoring System-Molecular (IPSS-M) scores of MDS cases in the Heme Fusion Assay (HFA) clinical cohort

Supplemental Table 2: All mutations detected in cases from the HFA and Rapid Heme Panel (RHP) cohorts at the initial bone marrow biopsy, including transcript ID and variant allele fraction

Supplemental Table 3: Breakpoints of *NUP98* and the partner genes, including transcript, exon, and coordinates

Supplemental Table 4: *NUP98* fusions detected in public MDS RNA-sequencing data

Supplemental Table 5: *WT1* loss of function mutations expressed in public MDS RNA-sequencing data

Supplemental Table 6: Leucegene AML samples with *WT1* mutations +/- *FLT3*-ITD