## Abstract: P449

# Title: TARGETED RNA SEQUENCING FREQUENTLY IDENTIFIES HIGH-RISK FUSION GENES IN YOUNGER ADULTS WITH OTHERWISE INTERMEDIATE-RISK AML AND ISOLATED FLT3-ITD

**Abstract Type: Poster Presentation**

**Topic: Acute myeloid leukemia - Biology & translational research**

**Background:**

Acute myeloid leukemia (AML) is a molecularly heterogeneous disease with diverse genetic alterations influencing prognosis and treatment outcomes. Patients with AML and *FLT3* internal tandem duplication (ITD) without a co-occurring *NPM1* mutation are now classified in the ELN intermediate risk group. This genotype has previously been associated with the presence of fusion genes such as *NUP98::NSD1* or *DEK::NUP214* which may be cytogenetically cryptic and confer a very poor prognosis. The prevalence of these and other related lesions in this group of patients is unknown and may be underestimated by standard diagnostic methods. Their identification may be useful both to inform treatment and to provide a target for molecular MRD monitoring.

**Aims:**

To identify rare or crytic fusions in patients with AML and *FLT3* ITD without a co-occurring *NPM1* mutation using targeted RNA sequencing.

**Methods:**

The UK NCRI AML17 and AML19 studies enrolled younger adults (generally aged <60y) with newly diagnosed AML into a series of intensive chemotherapy randomisations. All patients underwent central testing for *FLT3* and *NPM1* mutations by PCR and had cytogenetic evaluation in accredited regional laboratories. Patients with *FLT3* ITD but without *NPM1* mutations or a defining cytogenetic abnormality underwent screening for cryptic fusions by RT-PCR (for *NUP98::NSD1* and *DEK::NUP214*) and targeted RNA sequencing using the Illumina TruSight Fusion Panel which targets 507 genes reported to be involved in rearrangements in haematological or solid tumours.

**Results:**

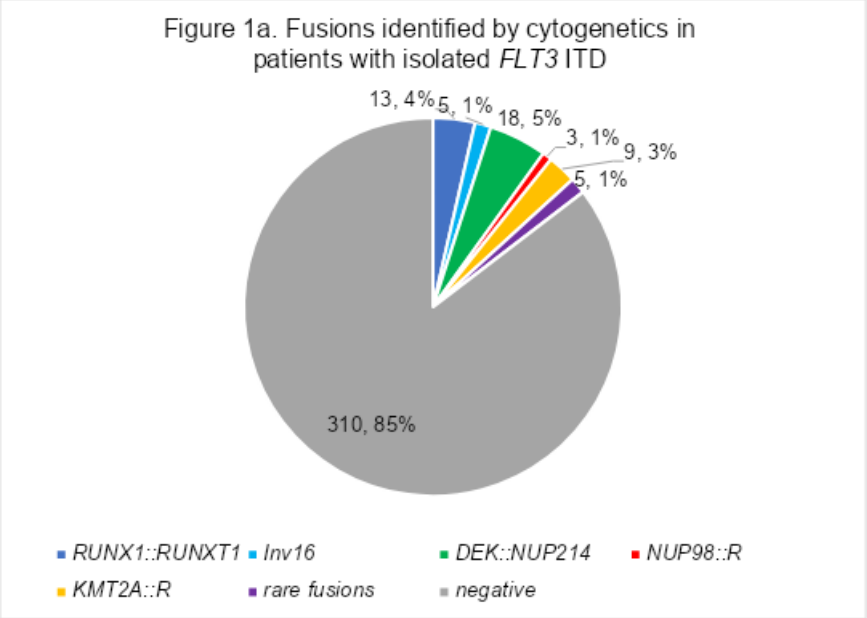
AML17 and 19 recruited a total of 5068 patients between April 2009 and November 2020 including 360 (7%) patients with *FLT3* ITD and unmutated *NPM1*. Routine cytogenetic assessment identified a fusion gene in 50/360 (14%) of these patients (figure 1a) including *DEK::NUP214* (n=18, 5%) *RUNX1::RUNX1T1* (n=13, 4%), *KMT2A::R* (n=9, 3%) *CBFB::MYH11* (n=5, 1%), *NUP98::NSD1* (n=3, <1%), and the rare fusions *NPM1::MLF1 (n*=3, <1%) and *RUNX1::MECOM* (n=2, <1%). Molecular monitoring by RT-PCR was carried out for *RUNX1::RUNX1T1*, *KMT2A::R* and *CBFB::MYH11*.

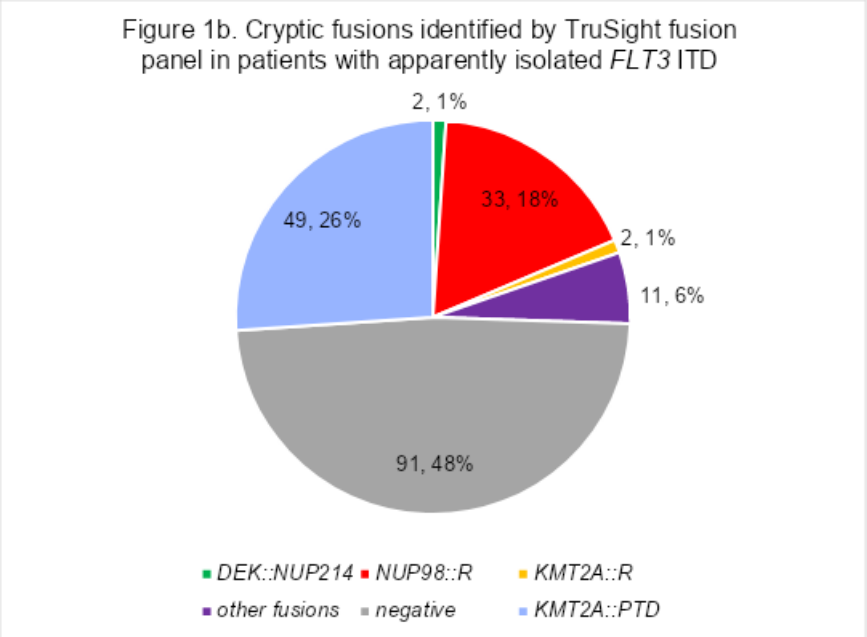
Of the remaining patients with apparently isolated *FLT3* ITD, diagnostic RNA samples were available from 188/310 (61%) and cytogenetically cryptic fusion genes were identified in 48/188 patients (26%, figure 1b) comprising *NUP98::R* in 33/188 (18%) (*NUP98::NSD1* (n=31), *NUP98::PHF23* (n=1), *NUP98::KDM5A* (n=1)), *DEK::NUP214* in 2/188 (1%), *KMT2A::R* in 2/188 (1%) and other fusions in 11 patients (*PML::RARA* (n=2), *NPM1::HAUS1* (n=2), *ZEB2::BCL11B, ETV6::MIR100HG, NCOA2::KAT6A, DDX10::SKA3, ZNF592::NUTM1, ETV6::MECOM and RUNX1::YPEL5)*. Molecular monitoring by RT-PCR was possible for *NUP98::NSD1*, *NUP98::KDM5A, DEK::NUP214, KMT2A::R* and *ETV6::MECOM. KMT2A* partial tandem duplications (PTD) were identified in a further 46 patients (24%).*

*FLT3* ITD positive patients harbouring a cryptic fusion showed a reduced survival trend compared to *FLT3* ITD positive patients without a cryptic fusion (median survival 15.62 vs 24.36 months, HR 0.68-1.47, p= 0.0871).

**Summary/Conclusion:**

We observed a surprisingly high prevalence of high-risk cryptic fusion genes in patients with apparently isolated *FLT3*ITD, with direct implications for treatment and disease monitoring. Targeted RNA sequencing or other methods for detecting cytogenetically cryptic rearrangements should be considered for younger adults with apparently isolated *FLT3*ITD.




**Keywords:** Acute myeloid leukemia, Gene fusion, Molecular cytogenetics, High risk