Association of *FLT3*-internal tandem duplication length with overall survival in acute myeloid leukemia: a systematic review and meta-analysis

The most common genetic aberration in acute myeloid leukemia (AML) is the internal tandem duplication (ITD) of the FMS-like tyrosine kinase 3 (*FLT3*)-gene, leading to a variable elongation of the juxta-membrane or tyrosine kinase-1 domain of the FLT3 protein.[1] The presence of *FLT3*-ITD – especially with a high allelic ratio (AR) – is associated with poor overall survival (OS).[2] The impact of the longer length of the *FLT3*-ITD is controversial, but may be associated with more auto-phosphorylation and thereby poor survival outcomes.[3] In contrast to *FLT3*-ITD-AR measurement, *FLT3*-ITD length is independent of AML blast percentage or sampling error, and therefore is an objective and constant diagnostic variable.[4] To date, *FLT3*-ITD length has not been included as a risk factor for AML patient survival. In order to address the heterogeneous data on *FLT3*-ITD length and its association with OS, we present a systematic review and meta-analysis of adult and pediatric AML studies reporting the association between *FLT3*-ITD length and OS consisting of 2,098 *FLT3*-ITD-positive AML patients.

We performed this review according to PRISMA.[5] All relevant databases were searched for peer-reviewed studies (no conference abstracts) published from January 1, 1996 through December 31, 2021 using all possible spellings of "*FLT3*-ITD" and "Acute Myeloid Leukemia". After deduplication, two independent reviewers (DGJC and SD) screened 2,118 articles for inclusion using Rayyan (https://www.rayyan.ai/) and assessed 137 full texts for eligibility. Non–English-language articles, reviews, studies that focused on APL and studies that did not investigate the association of *FLT3*-ITD length with OS were excluded. We also excluded studies that utilized tyrosine kinase inhibitors (TKI) in treatment protocols, as these likely affect the prognostic impact of *FLT3*-ITD.[6] Disagreement was resolved through discussion between the authors. These processes yielded a total of 16 studies. Upon request, we received data of *FLT3*-ITD-positive patients from two collaborative study groups that initially did not report analyses of association of OS and *FLT3*-ITD length. This provided a total of 18 studies (*Online Supplementary Figure S1*). Selected studies were screened for bias using Quality In Prognosis Studies (QUIPS).[7]

We extracted the following data if available: number of *FLT3*-ITD patients, median age and range, number of patients with *NPM1* and *DNMT3A* mutations, white blood cell count, median *FLT3*-ITD length and range, cut-off for short and long *FLT3*-ITD length, cut-off determination method, unadjusted hazard ratio (HR) for death and 95% confidence interval (CI). In seven of 18 studies, HR for death and 95% CI were reported in the publication or raw data were available. If the HR and 95% CI were not reported, we contacted the authors up to three times to retrieve these values. If we did not receive these data, we reconstructed individual patient data from published Kaplan-Meier plots to estimate OS and the corresponding HR and 95% CI via univariable Cox regression, as previously described.[8–10] We then performed a meta-analysis of HR for death, using a random effects model with restricted maximum likelihood. We provide the pooled HR for death for adult and pediatric patients separately and combined, and the corresponding 95% CI, depicted in a forest plot. Heterogeneity between studies was evaluated using the I^2 statistic. Analyses were performed in R (version 3.6.0) with packages survival, metafor and survHE.

The eighteen studies, comprising fourteen adult and four pediatric studies, included 2,098 patients for meta-analysis. Table 1 presents the characteristics of the individual studies. Full references to each study and the risk of bias for each study are presented in the *Online Supplementary Table S1*. All but one study included *FLT3*-ITD length as binary variable to predict OS, with cut-offs for short and long *FLT3*-ITD lengths ranging from 39 to 178 base pairs (bp). The most commonly (9/18) used cut-off value was 48 bp. For the one study that included *FLT3*-ITD length as ordinal variable based on quartiles, we used the median as cut-off.[11] Six studies determined the cut-off point based on the literature, seven used the median of their study population and five studies used an 'optimal' cut-off value. We reconstructed individual patient data from Kaplan-Meier curves for seven studies. The pooled HR for death calculated within the random effects model for patients with a long *FLT3*-ITD length, compared with patients with short *FLT3*-ITD length, was 1.50 (95% CI: 1.28–1.75; I^2=26.2%) (Figure 1). Stratified for adult and pediatric AML patients, the pooled HR for death were 1.43 (95% CI: 1.23–1.67; I^2=17.5%) and 1.97 (95% CI: 1.14–3.43; I^2=57.4%), respectively.

Our results indicate that AML patients with a long *FLT3*-ITD length have a moderately but statistically significantly higher risk of death, compared with patients with a short *FLT3*-ITD length. Long *FLT3*-ITD length might be associated with a higher degree of constitutive kinase

Table 1. Characteristics of individual studies included in the meta-analysis.

Author	Year	*FLT3*-ITD+ patients, N	Median bp (range)	Cut-off, bp	Cut-off selection	HR for death	Data source
	Adult						
Stirewalt et al.	2006	47	39 (15-153)	≥40 *vs.* <40	median	1.65	a
Kusec et al.	2006	86	70 (30-100)	≥70 *vs.* <70	median	0.31	b
Gale et al.	2007	260	48 (15-231)	≥48 *vs.* <48	median	1.39	a
Schiller et al.	2012	39	45 (3-144)	≥45 *vs.* <45	median	2.60	a
Blau et al.	2012	60	61 (21-203)	>61 *vs.* <61	median	0.86	a
Schlenk et al.	2014	323	NA (15-195)	≥48 *vs.* <48	literature	1.32	c
Koszarska et al.	2014	68	39 (6-210)	≥48 *vs.* <48	literature	1.92	a
Kim et al.	2015	73	50 (16-150)	≥70 *vs.* <70	'minimum *P* value'	2.19	a
Liu et al.	2019	89	39 (6-90)	≥39 *vs.* <39	median	2.45	b
Zhang et al.	2020	81	51 (18-207)	>69 *vs.* <69	'minimum *P* value'	1.58	b
Schlenk et al.	2020	99	48 (18-240)	≥48 *vs.* <48	literature	1.15	d
Cucchi et al.	2021e	133	43 (3-186)	≥48 *vs.* <48	literature	1.66	b
Cucchi et al.	2021f	126	46 (-3-178)	≥48 *vs.* <48	literature	1.08	b
Engen et al.	2021	111	51 (15-132)	≥50 *vs.* <50	'minimum *P* value'	1.9	c
Castaño-Bonilla et al.	2021	161	48 (3-201)	≥48 *vs.* <48	median	0.84	c
	Pediatric						
Meshinchi et al.	2008	77	52 (15-174)	≥49 *vs.* <49	'minimum *P* value'	2.29	c
Gamis et al.	2014	190	48 (3-210)	≥48 *vs.* <48	Literature	1.34	d
Manara et al.	2017	54	NA (18-126)	≥48 *vs.* <48	Literature	1.20	a
Cucchi et al.	2018	21	39 (6-103)	≥48 *vs.* <48	'minimum *P* value'	4.70	d

[a]Hazard ratio (HR) and 95% confidence interval (CI) based on reconstructed individual patient data from Kaplan-Meier curves. [b]HR and 95% CI provided in published manuscript. [c]HR and 95% CI provided on request. [d]HR and 95% CI based on individual patient data (provided by the authors). [e]Results provided separately for the Dutch-Belgian Cooperative Trial Group for Hematology-Oncology (HOVON)/Swiss Group for Clinical Cancer Research (SAKK) HOVON 102 AML/SAKK 30/09 trial. [f]Results provided separately for the HOVON 132 AML/SAKK 30/13 trial. ITD: internal tandem duplication; NA: not available; OS: overall survival; bp: base pair; N: number of *FLT3*-ITD-positive acute myeloid leukemia (AML) patients in study.

activation leading to a more aggressive phenotype.[3] Alternatively, long *FLT3*-ITD length may be a surrogate for ITD localization in the tyrosine kinase domain rather than in the juxta-membrane domain,[1] which is associated with drug resistance and inferior OS. However, Liu *et al.*[3] suggest an association independent of ITD localization, since the authors observed poor OS in AML patients with long *FLT3*-ITD within the juxta-membrane domain. We explored other explanatory variables that correlate with survival in the *Online Supplementary Table S2*. These variables were spread evenly across 'short' and 'long' *FLT3*-ITD patients – only white blood cell count appeared to be elevated in patients with long *FLT3*-ITD.

The heterogeneous dichotomization among the included studies makes it arduous to decide what 'short' and 'long' *FLT3*-ITDs are. This variation may be a source of bias, introduced by approaches to select cut-offs for short and long *FLT3*-ITD lengths to achieve a 'minimum *P* value.[12] We therefore performed a sensitivity analysis to assess the potential impact of this bias. Restricting our analysis to studies with the most commonly used cut-off point of 48 bp, the pooled HR for death was 1.35 (95% CI: 1.16–1.57; I^2=0.61%). When we included studies with a cut-off of 43–51 bp, this was 1.47 (95% CI: 1.24–1.75; I^2=30.85%). This indicates that our results are consistent regardless of the exact definition of 'long' and 'short'. Meta-regression analysis including the cut-off value as continuous explanatory variable did not show a significant effect estimate (HR [per bp] -0.01; 95% CI: -0.03 to 0.02; *P*=0.63), indicating that varying cut-offs do not explain data heterogeneity. Although we caution against merely dichotomizing *FLT3*-ITD length and would

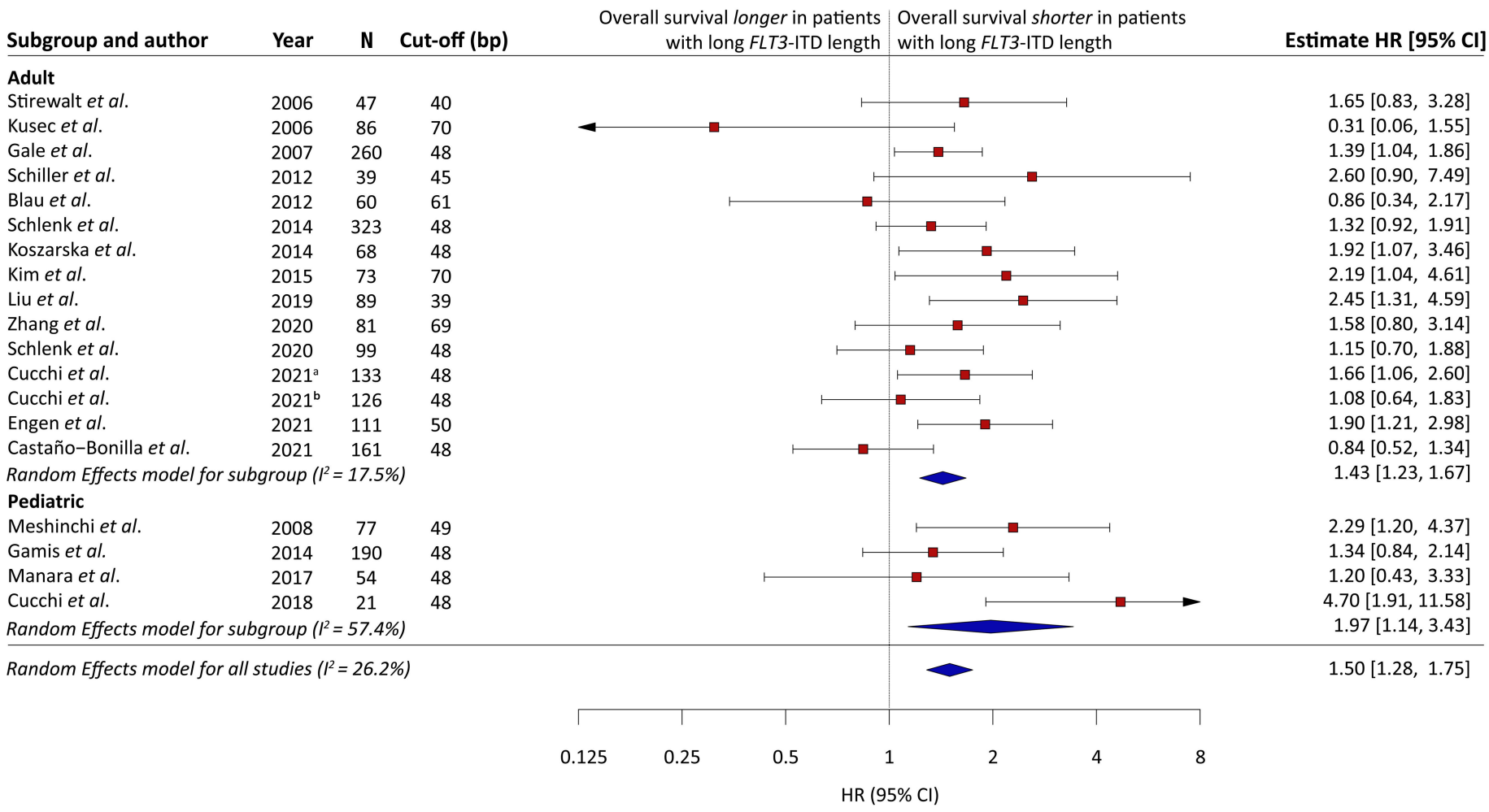

Figure 1. Meta-analysis of studies reporting *FLT3*-internal tandem duplication length and overall survival in acute myeloid leukemia patients. For Cucchi *et al.*[4] results are provided separately for (a) the Dutch-Belgian Cooperative Trial Group for Hematology-Oncology (HOVON)/Swiss Group for Clinical Cancer Research (SAKK) HOVON 102 AML/SAKK 30/09 trial and (b) the HOVON 132 AML/SAKK 30/13 trial. N: number of *FLT3*-ITD-positive acute myeloid leukemia (AML) patients in study; HR; hazard ratio for death; 95% CI: 95% confidence interval; ITD: internal tandem duplication; Cut-off: cut-off value used for group comparison of short and long *FLT3*-ITD lengths, reported in base pairs (bp).

recommend exploring various functional relations (e.g., linear, splines) between *FLT3*-ITD length and OS, a cut-off of 48 bp appears useful in clinical practice.

Our study has several limitations. First, we could only systematically investigate the univariable effect of *FLT3*-ITD length, since multivariable analyses were provided in only four of the 18 included reports.[3,4,13,14] In three of these four reports, *FLT3*-ITD length remained a relevant prognostic factor in multivariable analysis with *FLT3*-ITD-AR,[13] *FLT3*-ITD-AR, sex and cytogenetic risk[3] and *FLT3*-ITD-AR, *NPM1*, *TP53* and *CEBPα* mutations and cytogenetic risk.[4] However, due to the limited, heterogenous, and potentially preferential reporting of association analyses, it remains unclear whether *FLT3*-ITD length has additional prognostic value over the *FLT3*-ITD-AR, and whether this also depends on the presence of other prognostic factors, such as the *FLT3*-ITD insertion site.[1] We therefore call for broader and homogeneous reporting of statistical tests to reproduce and facilitate further research. Second, our results may only apply to patients treated in regimens without *FLT3*-TKI. Few studies report a lack of association of numerical variation of *FLT3*-ITD with survival outcomes in regimens containing *FLT3*-TKI, suggesting that FLT3-TKI overcome its adverse impact.[6,15,16] *FLT3*-TKI might, however, specifically improve survival in patients with high *FLT3*-ITD-AR.[6] Therefore, numerical variation of FLT3-ITD may remain important to inform selection of patients most benefitting from TKI. We suggest analyses of numerical variation of *FLT3*-ITD in the context of other prognostic factors using data from studies such as the RATIFY, QuANTUM-First, ADMIRAL and QuANTUM-R trials.[17] Finally, our study may be subject to publication bias since researchers are less likely to report or publish a lack in prognostic value, an issue inherent to any such meta-analysis. However, our extensive personal enquiries for additional data and advanced techniques to reconstruct individual patient data when only graphical data were available, allowed for inclusion of additional studies and patients. Altogether, this minimized the risk of publication bias and this was confirmed in a funnel plot.

In conclusion, in this meta-analysis, long *FLT3*-ITD length was associated with a moderately but statistically significantly higher HR for death compared with short *FLT3*-ITD length. Prospective analysis of its prognostic value in the context of contemporary treatment protocols,[17] the *FLT3*-ITD-AR, insertion site and other molecular aberrations,[18] is essential to refine risk stratification protocols for AML in the years to come.

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Contributions

DGJC conceived and supervised the research. JC and JJWMJ provided critical feedback on the research methodology. SD and DGJC performed the search and collected data. JKH, SM and KD provided additional clinical data. DGJC, TBP and JvR performed statistical analysis. TBP and DGJC wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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Data-sharing statement

Most data are derived from publicly available sources. Aggregated and analyzed data will be made available on reasonable request.

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