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Induction Response Criteria in Acute Myeloid Leukemia: Implications of a Flow Cytometric MRD Negative Test in Refractory Adults

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Running Head 'Prognostic implications of a negative flow cytometric MRD test when refractory by morphology in AML'

To the Editor:

A morphology exam incorporating a differential count of a recommended 500 nucleated bone marrow cells (Dohner*, et al* 2017) continues to be requirement for an acute myeloid leukemia patient to be classified as in complete remission by International Working Group (IWG) response criteria (Cheson*, et al* 2003). It has however become increasingly standard to perform morphology and flow cytometry in parallel to assess remission. Like morphology, flow cytometry (MFC) assays for residual leukemia will enumerate blast percentage for the 5% threshold but additionally provide greater accuracy and the possibility to discriminate leukemic from normal blasts at a sensitivity of 10^{-3} to 10^{-4} (Schuurhuis*, et al* 2018). Both approaches require training, experience and a representative bone marrow sample for reliable results and have limitations that may potentially contribute to discrepant results and inter-laboratory variation (Walter 2018). It has been suggested that a morphology assessment of response is less specific than flow cytometry when rebound normal regeneration following induction chemotherapy results in more than 5% normal blasts, (predominantly a feature of pediatric marrows). Administration of G-CSF or dysplastic changes from stressed regeneration can also add difficulty to morphological differential counts.

Outcome data from multiple previous studies applying flow cytometry to measure residual disease in morphological complete remission (Schuurhuis*, et al* 2018) have contributed to the European LeukemiaNet (ELN) recommendation that morphological CR without measurable residual disease (CR_{MRD-}) be included as a defined response endpoint for AML (Dohner*, et al* 2017). CR_{MRD-} by flow cytometry is associated with a significantly better overall survival than morphological CR/CRi for younger adults even after first induction (63% versus 52% at five years)(Freeman*, et al* 2018) as previously shown for older patients treated intensively (Freeman*, et al* 2013).

Considering the prognostic relevance of a negative MRD test (MRD-) in patients with ≥5% morphological blasts, flow cytometric measurements of persistent leukemia were compared with morphological IWG criteria in two separate pediatric trial cohorts reported over 5 years ago (Inaba*, et al* 2012, Loken*, et al* 2012). The two studies defined a positive MRD test differently (≥0.1% (Inaba*, et al* 2012) versus any MRD detected (Loken*, et al* 2012)) but in both cohorts, the subgroup with ≥5% morphological blasts but MRD- by flow cytometry post first induction had a similar outcome to those who achieved a CR_{MRD-} (Inaba*, et al* 2012, Loken*, et al* 2012). Thus, at least for pediatric bone marrows, from this data morphological blast counts add no predictive value for outcome to a negative flow cytometric MRD test. Should this also be the case for adults, then a negative flow cytometric

MRD test in a representative bone marrow sample could obviate the requirement for a morphology blast count to define CR following induction.

However, we observe that there is insufficient data in adult AML to corroborate flow cytometry being more predictive than morphology for response when morphological blast percentage exceeds the CR threshold. In particular, there has been no formal evaluation in adult trials of the outcome of patients who have discrepant refractory / MRD- results at induction response assessment. Moreover, the frequency of these discrepancies in regenerating adult bone marrow following induction chemotherapy remains uncertain but is likely to be less common than in the pediatric setting. In a retrospective analysis of mainly adult patients only 4% of BM samples classified as refractory by morphology at various treatment time-points were MRD negative by flow cytometry (Ouyang*, et al* 2015); a much lower frequency than that observed post first induction in the pediatric studies (26% (Loken*, et al* 2012) and 38% (Inaba*, et al* 2012)). We also note from the recent article by Zhou et al that in their series of 87 patients with confirmed relapse by morphology, all were MRD+ by flow cytometry(Zhou*, et al* 2017). Consequently, potential discrepancies between morphology and flow cytometry are most likely when testing for response rather than for relapse.

This has prompted us to examine the data for discrepant morphology and flow cytometric MRD results post first induction in the three adult AML trials for which outcomes by flow cytometric MRD status in CR have previously reported by the HOVON (Terwijn*, et al* 2013) and NCRI (Freeman*, et al* 2018, Freeman*, et al* 2013) trial groups (Table 1). Flow cytometric MRD samples were more likely to be hemodilute than morphology as not prioritised for 'first pull' bone marrow. In these trials, the morphology and flow cytometric MRD results were independently reported. Only a few adults from all three trials were reported to be in CR but had ≥5% leukemic blasts by flow cytometry (n=25) (Table 1); Most (88%) survived less than 2 years.

For adults not in CR, frequencies of MRD negativity (defined as <0.1% by HOVON or as no detectable MRD by NCRI) post first induction ranged from ~10% to ~31% with the highest in the HOVON/SAKK younger adult trial which evaluated G-CSF priming in induction**.** In all three cohorts of refractory adults with MRD data, better outcomes were observed in the refractory/MRD- patients, although small numbers limit analysis. Moreover, 3 year survival rates (Table 1) were not inferior to those of patients in morphological CR post first induction (3 year OS if CR/CRi, 59% in NCRI AML17, 67% in HOVON/SAKK AML 42A, 29% in NCRI AML16 trial of older adults) and comparable to those of patients achieving CR_{MRD-} post first induction in the NCRI studies (3 year OS if CR_{MRD-}, 69% in NCRI AML17, 73% in HOVON/SAKK AML 42A, 42% in NCRI AML16).

We note that outcomes were very similar for refractory/MRD- younger adults between the NCRI and HOVON trials; this was also true for the refractory/MRD+ subgroup. Thus from the results of two independent cohorts, the predictive value of flow cytometric MRD in refractory adults appears robust and comparable despite variation in the flow cytometric MRD methodology and criteria for a positive test.

Misclassification as refractory at induction can result in unnecessary treatment. The above results substantiate in adults the ELN statement that 'remission status as assessed by flow cytometry provides a more reliable predictor of outcome than conventional morphology-based CR assessment (Dohner*, et al* 2017).

It is therefore logical to propose that a morphological differential cell count should no longer be a requirement for CR classification of induction response when a representative ('first pull') bone marrow sample is MRD negative by a validated flow cytometric MRD assay from experienced laboratories.

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Table 1

	Younger Adults				Older Adults	
	NCRI UK AML17(Freeman, *et al* 2018)		**HOVON/SAKK AML 42A**[11]		**NCRI UK AML16**[6]	
Post 1st Induction						
Patients 'Not in CR with MFC-MRD data'	N=259		N=81		N=79	
	Not in CR but MFC-MRD-	**Not in CR and MFC-MRD+**	**Not in CR but MFC-MRD-**	**Not in CR and MFC-MRD+**	**Not in CR but MFC-MRD-**	**Not in CR and MFC-MRD+**
No. (%)	25 (9.7%)	234 (90.4%)	25 (30.9%)	56 (69.1%)	21 (20.8%)	80 (79.2%)
3 year OS	61%	33%	60%	27%	35%	<10%
Median OS	57 months	16 months	Not reached with median follow-up of 31 month	14 months		
3 year CIR	26%	52%	32%	54%		
3 year RFS	63%	36%	56%	34%		
No. of CR1 transplants	8 allo	102 allo	5 allo 9 auto	22 allo 9 auto		

Patients in CR but ≥5% blasts by flow cytometry	N= 7 6 died within 2 years		N=3 1 died within 2 years		N=15 All dead by 20 months	

CR, Complete Remission by morphology; MFC-MRD, measurable residual disease by flow cytometry; MFC- MRD+, MRD positive; MFC-MRD- , MRD negative; OS, overall survival; CIR, cumulative incidence of relapse; RFS, relapse free survival; CR1 transplant, transplant in first remission.