18 Future Developments: Measurable Residual Disease

Adriano Venditti, Peter J. M. Valk, Nigel H. Russell, and Sylvie D. Freeman

18.1 Introduction

The prognostic impact of MRD at different treatment time points of standard regimens has been established by numerous previous studies. Several trial groups have now tested whether MRD assessments are feasible in real time to guide treatment. Improved leukemia genomic classification combined with the clinical availability of next generation sequencing (NGS), the increasing delivery of allogeneic transplantation to high-risk patients, new therapies, and assay development all have to be incorporated into the framework of MRD testing. This presents challenges but also opportunities to extend and improve its utility in clinical practice and advancing treatment options.

A. Venditti
Hematology, Department of Biomedicine and Prevention, University Tor Vergata, Rome, Italy

P. J. M. Valk
Department of Hematology, University Medical Center, Rotterdam, the Netherlands

N. H. Russell
Haematology, Guy's and St Thomas' NHS Foundation Trust, Guy's Hospital, London, UK

S. D. Freeman (✉)
Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK
e-mail: s.freeman@bham.ac.uk

18.2 MRD-Directed Therapy: Update from Clinical Trials

The ever more expanding knowledge of the biology of acute myeloid leukemia (AML) has not only driven the discovery of novel agents with a targeted mechanism of action (Gerstung et al. 2017) but also encouraged the development of new strategies such as the "risk-adapted approach." Such a strategy is based on the assumption that the old-fashioned approach "one size fits all" should be replaced by an alternative one that counterbalances the intensity of therapeutic intervention based on the genetic characteristics of AML and its risk of relapse (Cornelissen et al. 2012). The philosophy behind this strategy consists in the attempt to preserve as much as possible a favorable cost/benefit ratio, avoiding over-treatment of patients with low-risk AML or under-treatment of those with high-risk disease. The evolving criteria of response make such a scenario even more complex. In fact, morphologic complete remission (mCR), although still representing the gold standard, provides an unfaithful picture of the quality of response (Freeman and Hourigan 2019; Schuurhuis et al. 2018). Therefore, multiparameter flow cytometry (MFC) and/or polymerase chain reaction (PCR), first applied for diagnostic purposes, have become leading techniques to explore the quality of response below the threshold of mCR, by quantifying the so-called "measurable residual disease"

C. Röllig, G. J. Ossenkoppele (eds.), *Acute Myeloid Leukemia*, Hematologic Malignancies,
https://doi.org/10.1007/978-3-030-72676-8_18

(MRD) (Schuurhuis et al. 2018). Whatever the technique applied, the prognostic role of MRD is widely recognized in several retrospective studies showing that the cumulative incidence of relapse (CIR) of patients without detectable MRD is 6–40% whereas it is 50–80% in those with MRD (Freeman et al. 2018; Ivey et al. 2016; Jongen-Lavrencic et al. 2018; Terwijn et al. 2013; Guenot et al. 2019; Hoffmann et al. 2019; Hollein et al. 2018a; Buccisano et al. 2012; Rucker et al. 2019). Indeed, the frequently observed association between MRD status and clinical outcome has led the European LeukemiaNet (ELN) to include mCR-MRD negative as a new criterion of response (Dohner et al. 2017). However, unequivocal acknowledgment of MRD as a critical tool to implement the therapeutic decision-making process requires that its role is demonstrated also in prospective studies. If the role of MRD is confirmed prospectively, it may serve as a biomarker rather than as a simple prognosticator. In this view, the perfect trial is the one randomizing patients with MRD to intensified therapy (e.g., allogeneic stem cell transplant) versus conventional therapy (e.g., multiple consolidation courses or autologous stem cell transplant). It is unlikely that such a trial will ever see the light for younger patients and, as of today, MRD-based decisions still represent a difficult task in AML. In such a complicated context, efforts are being made to explore prospectively the impact of MRD assessment in patients with AML. In the following section, we discuss the current prospective MRD-driven trials in AML and the implications of their findings.

18.3 MRD-Guided Preemptive Treatment

Studies focusing on sequential MRD detection have shown that the persistence or re-emergence (molecular relapse) of the relevant molecular marker may be detected in advance of morphological relapse, allowing therapeutic intervention before overt hematological relapse and potentially improving long-term outcome.

The updated analysis of the RELAZA-2 trial (Platzbecker et al. 2018, 2019) now provides data for 94 patients who received MRD-driven treatment with azacitidine. In patients with AML or high-risk myelodysplastic syndrome who were in remission after appropriate treatments (including allogeneic stem cell transplant), MRD positivity was defined by either molecular MRD (quantitative PCR) or as a fall in CD34+/CD117+ cell chimerism below the threshold of 80%. In the first cohort of 198 screened patients, MRD reappeared in 53 patients and they were given pre-emptive azacitidine (Platzbecker et al. 2018). This prevented relapse in 51% of patients with MRD (median follow-up of 13 months) whereas in the remaining overt hematologic recurrence did not occur until a median of 422 days. In the subsequent cohort of 41 additional patients converting to an MRD-positive test (Platzbecker et al. 2019), the authors observed that 6 months from preemptive azacitidine initiation, 25 (61%) were still in mCR; 19 had a decline of the level of MRD below the predefined threshold. The combined 94 patients had 6 months relapse-free survival of 60%. Although not randomized, the prospective RELAZA-2 trial provides evidence that an MRD-guided intervention can prolong survival in MRD-positive patients by preventing or significantly postponing disease recurrence.

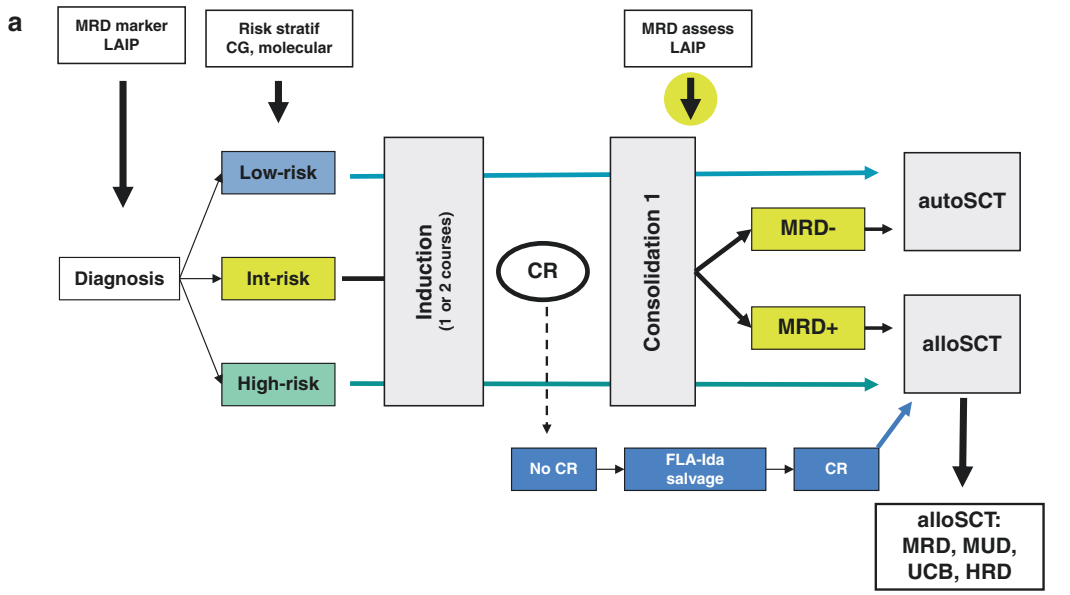
The NCRI AML17/19 trial is also evaluating whether early intervention at the time of molecular relapse improves overall survival compared to the standard of care. Patients were eligible for a monitor versus no monitoring randomization if they had an RT-qPCR molecular MRD target, that is, chimeric fusion genes generated by balanced chromosomal rearrangements or *NPM1* mutations, which collectively are present in over 50% of AML presenting in younger adults (Grimwade et al. 2016). Over 600 non-APML AML patients have entered this randomization which was made 2:1 in favor of monitoring. Patients in the monitoring arm undergo sequential BM sampling following each cycle of therapy and then 3 monthly for 2 years but can continue for longer if there is a relapse when the monitoring clock is reset. It was calculated that a total of 600 patients was sufficient to give a 90% power

to detect an improvement in survival from 40 to 52.5%. The results are expected by 2022 along with analyses of Quality of Life and resource utilization.

18.4 MRD Risk-Adapted Strategies

In the recently reported GIMEMA AML1310 trial (Venditti et al. 2019), the investigators adopted a risk-adapted strategy by integrating pre-treatment prognosticators such as cytogenetics and molecular genetics with post-treatment MRD assessment (Fig. 18.1a). Adults aged 18–60 years, after induction and a first course of consolidation, were directed to an autologous or an allogeneic stem cell transplant if qualified as low- or high-risk, respectively. Intermediate risk patients were allocated to autologous or allogeneic stem cell transplant based on the MRD status after the first course of consolidation; MRD was assessed by MFC. The study showed, although in a non-randomized fashion, that delivering an allogeneic stem cell transplant to MRD-positive patients prolonged their OS and DFS to coincide with outcomes of patients without detectable MRD who received an autologous stem cell transplant. In the AML12 CETLAM trial, the Spanish investigators adopted a similar risk-adapted post-remission allocation based on genetic data and MRD (Sierra et al. 2019). MRD was determined by RT-qPCR when a suitable molecular marker was identified or MFC. After induction and a first consolidation course, patients with favorable genetics and negative MRD-test (FG-MRDneg) received 3 additional courses of consolidation, those with intermediate genetics and negative MRD-test (IG-MRDneg) 1 additional course of consolidation and then autologous or allogeneic stem cell transplant according to the local policy. In patients categorized as high-risk (HR), either by adverse genetics or positive MRD-test, allogeneic stem cell transplant was mandatory, after the first consolidation. By applying this strategy, 57 of 542 patients who were risk-allocated shifted from the favorable- or intermediate-risk genetic category to the HR one due to a positive MRD-test after the first consolidation and therefore were directed to allogeneic stem cell transplant. Four-year OS and event-free survival (EFS) of these 57 patients were 53 ± 8% and 45 ± 7%, respectively. Four-year OS of the whole series was 48% ± 2; EFS of FG-MRDneg, IG-MRDneg, and HR was 77% ± 3, 45% ± 6, and 34% ± 4, respectively (Sierra et al. 2019).

In the ongoing UK NCRI trial for younger adults, MRD assessment has been applied to improve prognostication, particularly in patients with intermediate-risk AML in first remission which has been an area where decision-making about the choice of post-remission therapy has been the most problematic. In patients <60 years, the AML17 trial showed that post-course 2 MRD measured either by RT-qPCR in *NPM1*-mutated disease or by MFC in patients who were *NPM1* wild-type (*NPM1*wt) could identify patients at very high risk of relapse (Freeman et al. 2018; Ivey et al. 2016). For *NPM1*-mutated disease, the 3-year overall survival (OS) was 24% in patients who were RT-qPCR positive for *NPM1*-mutated transcripts in the peripheral blood (PB) post-course 2 compared with 75% for those who tested negative. In a multivariate analysis that included clinical parameters and mutational profile, MRD status was the only factor to retain significance. These results are supported by the French ALFA0702 study, which also enrolled patients aged <60 years, and showed a >4 log reduction in transcript levels in the PB or bone marrow after one cycle of induction was associated with a 3-year OS of ~90% (Balsat et al. 2017). The ALFA0702 study has also shown that the poorer outcomes of MRD-positive *NPM1*-mutated patients can be improved by allogeneic stem cell transplant (SCT) in first remission (Balsat et al. 2017). In our ongoing NCRI AML19 trial, the approximately 30% of patients who are identified post-course 2 of induction as having high-risk *NPM1*-mutated AML are recommended for intensified salvage therapy randomizing FLAG-Ida versus CPX-351 followed by repeat MRD assessment before allogeneic SCT (Fig. 18.1b). The same approach is applied to patients with intermediate risk AML who lack an *NPM1* mutation (*NPM1*wt) using MFC-MRD detection.



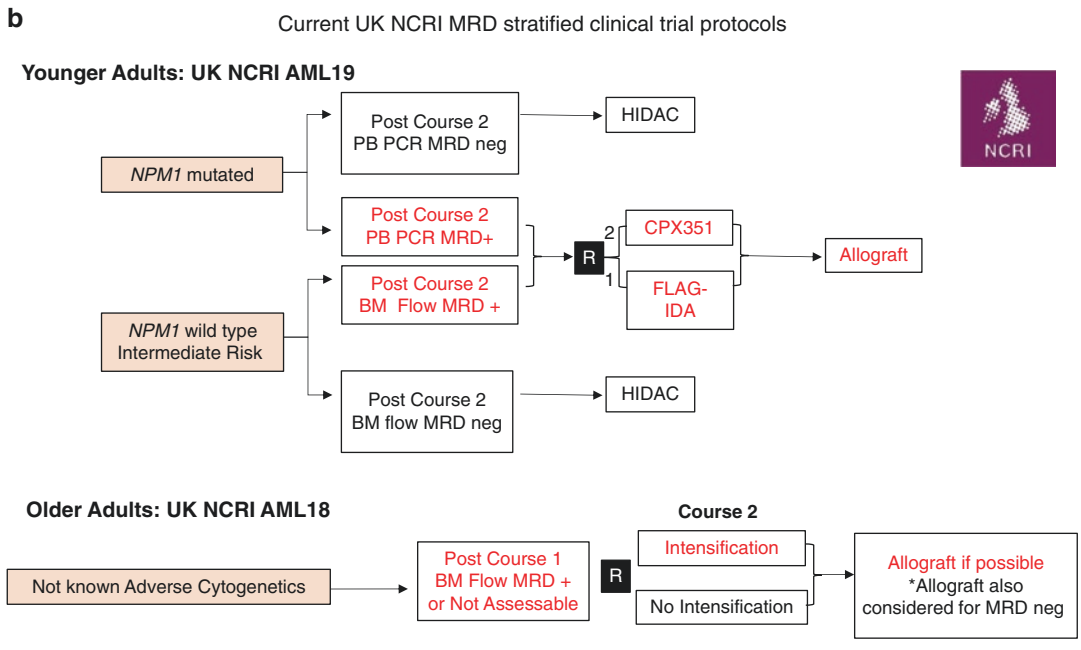


Fig. 18.1 Examples of MRD risk-adapted strategies implemented in clinical trials. (**a**) GIMEMA AML1310 trial. *LAIP* leukemia associated phenotype, *CG* cytogenetic, *CR* complete remission, *MRD* minimal residual disease, *autoSCT* autologous stem cell transplant, *alloSCT* allogeneic stem cell transplant, *MRD* matched related donor, *MUD* matched unrelated donor, *UCB* umbilical cord blood, *HRD* haploidentical related donor, *FLA-Ida* Fludarabine-Arabinoside-Idarubicin. (**b**) NCRI (UK, Denmark, New Zealand) AML19 and AML18 Trials: role of MRD-directed intensification. (**c**) HOVON132 AML/SAKK 30/13 study: role of MRD after induction cycle II

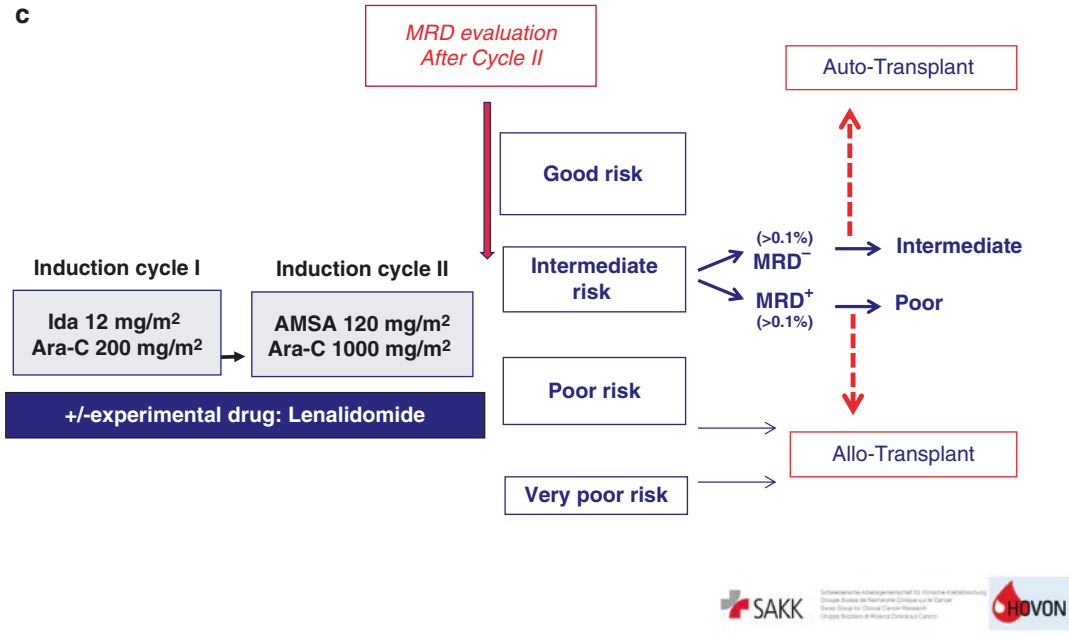


Fig. 18.1 (continued)

In a study which globally involved 2450 NCRI AML17 trial patients, post-course 2MFC-MRD positivity, which was detected in about 30% of *NPM1*wt intermediate risk patients, predicted a significantly poorer survival (5-year OS, 33 vs. 63% for MRD– patients) and a high probability of relapse when MRD level was ≥0.1% (3-year cumulative incidence of relapse, 89%) (Freeman et al. 2018). Furthermore, transplant benefit was more apparent in patients with MRD+ (HR, 0.72; 95% CI, 0.31 to 1.69) than those with MRD− (HR, 1.68 [95% CI, 0.75–3.85]) (Freeman et al. 2018). As a consequence, MFC-MRD assessment was implemented in the NCRI AML19 trial to stratify otherwise intermediate risk *NPM1*wt patients as high risk and eligible for the same high-risk randomization as high-risk *NPM1*-mutated AML (Fig. 18.1b).

Finally, the results of the HOVON 132 AML/SAKK 30/13 clinical trial are now available (Löwenberg et al. 2021). The trial was closed to further recruitment last year, and the final analysis showed that with an MRD guided approach, MRD status after cycle 2 lost prognostic value in intermediate-risk AML in the risk-adjusted treatment context. The trial design was reminiscent of the GIMEMA study, with a post-induction-2 stratification of patients belonging to the intermediate-risk genetic category based on the level of MRD, assessed by MFC and mutant *NPM1* (Fig. 18.1c). The GIMEMA, NCRIAML17/19, and HOVON132 AML/SAKK 30/13 trials are coincident in their selection of time point for MRD assessment and subgroup deemed to benefit the most from such a determination. Their experience demonstrates the feasibility of MRD assessment after 2 courses of chemotherapy (1 Induction and 1 consolidation or 2 induction courses) to help planning tailored post-remission programs for adults belonging to the intermediate-risk category, at least in the frame of specifically designed trials. In addition, the results of the AML12 CETLAM trial point to the hypothesis that MRD status also has a role in guiding post-remission management of low-risk patients.

As discussed above, the best trial is the one that randomizes MRD-positive patients to intensified therapy against continuing conventional therapy. The current UK NCRI AML18, which is designed for patients >60 years without known adverse risk cytogenetics and fit for intensive

chemotherapy, has such a design (Fig. 18.1b). Patients entering the trial have centralized testing for an MFC-MRD target (identified in over 90% of patients). Following a first induction course of DA chemotherapy plus gemtuzumab, BM samples are assessed for remission status and MFC-MRD. Patients not in remission or who are MRD positive are randomized between continuing standard chemotherapy as course 2 or intensified therapy with the addition of cladribine to DA or a FLAG-Ida regimen. In the MRD + ve arm, MRD is reassessed following count recovery. As of writing 493 patients have entered this randomization. The rationale was based on the findings of our previous NCRI AML16 trial in this age group which demonstrated that MRD negativity (inducible in 51% of patients in remission after one cycle of intensive chemotherapy) was associated with a significantly better 3-year survival (42 vs. 26% in MRD-positive patients) (Freeman et al. 2013). Of course, treatment intensification may not result in MRD negativity or improve survival as these patients have already demonstrated chemorefractoriness to standard induction therapy and intensification may adversely affect treatment-related mortality. What is desirable is a less toxic targeted approach to treat MRD and indeed such an approach using a combination Venetoclax and low dose cytarabine may be highly effective in *NPM1*-mutated older adults remaining MRD positive by RT-qPCR after intensive chemotherapy (Tiong et al. 2019).

18.5 Managing Pre-transplant MRD

The ELN AML working party consensus statement, by adopting a dynamic risk-assessment approach including MRD determination, recommends that allogeneic stem cell transplant should be favored when the risk of relapse exceeds 35–40% and when the projected disease-free survival is expected to improve by at least 10% (Cornelissen et al. 2012). Based on this, it appears that allogeneic stem cell transplant represents the optimal option to offer in the situation of MRD positivity since it reduces relapses (Cornelissen et al. 2012). However, some retrospective studies reported that being MRD positive before allogeneic stem cell transplant had a negative impact on post-transplant outcome, regardless of the intensity of the conditioning regimen that was delivered (Araki et al. 2016; Walter et al. 2015). Indeed, patients who were MRD positive before allogeneic stem cell transplant had outcomes comparable to those transplanted with active disease (Araki et al. 2016; Hourigan et al. 2016). A large meta-analysis (Buckley et al. 2017), including 19 retrospective studies published between 2005 and 2016, confirmed that pre-transplant MRD positivity was associated with a shorter duration of leukemia-free survival and OS and higher rates of CIR. The unfavorable effect of pre-transplant MRD positivity took place irrespective of detection method, conditioning intensity, and patient age. These experiences are sometimes used as an argument not to transplant "pre-transplant MRD positive patients." Therefore, the question is whether a consolidative allogeneic stem cell transplant remains a valid option also for this category of patients or should alternative strategies be pursued? A retrospective analysis of 547 patients enrolled in the HOVON/SAKK protocols demonstrated that all AML risk-categories benefited from allogeneic stem cell transplant; however, the absolute benefit was greater in pre-transplant MRD-positive than MRD-negative patients (Versluis et al. 2017). The authors assumed that the graft vs leukemia potential was equally effective in MRD-positive as well as MRD-negative patients. In a prospective, non-randomized trial of 137 patients with t(8;21), Zhu et al. (2013) distinguished high-risk (*RUNX1-RUNX1T1* transcript reduction <3 logs after second consolidation course) from low-risk (*RUNX1-RUNX1T1* transcript reduction >3 logs after second consolidation course) individuals. Of 69 high-risk patients, 40 received allogeneic stem cell transplant and 29 additional courses of chemotherapy or autologous stem cell transplant. Patients who received allogeneic stem cell transplant had a significantly lower CIR and superior OS and DFS as compared to those not allocated to allogeneic stem cell transplant. In spite of the non-randomized treatment allocation,

the results of the trial suggest the potential survival advantage of a risk-adapted strategy, even in patients who were pre-transplant MRD positive. In fact, subjects who received treatments different from those scheduled according to their risk status did worse than patients who received the assigned treatment. Thol et al. (2018) demonstrated that error-corrected NGS-MRD can be applied in mCR before allogeneic stem cell transplant and that it is highly predictive. In competing risk analysis, CIR of pre-transplant MRD-positive patients was significantly higher than in pre-transplant MRD-negative ones. The authors suggested that NGS-MRD may be a very useful tool to help refining transplant and post-transplant management of patients with AML. A paradigmatic example of NGS potential was recently published by Hourigan and coworkers (2020). The authors investigated whether modulation of the intensity of conditioning regimen could reduce the risk of relapse in patients who were pre-transplant MRD positive. Pre-conditioning blood samples collected from adult patients in mCR were tested by NGS-MRD, looking for the 13 most commonly mutated genes in AML. Patients were randomly assigned to myeloablative conditioning (MAC) or reduced-intensity conditioning (RIC). No difference in terms of CIR and OS was observed between MAC and RIC patients, who were pre-transplant NGS-MRD negative. Among those who were pre-transplant NGS-MRD positive, 3-year CIR and OS were significantly improved in MAC versus RIC patients (19 vs. 67%, $p < 0.01$ and 61 vs. 43%, $p = 0.02$). This study provides evidence that MAC rather than RIC improves the outcome of pre-transplant MRD-positive patients, consistent with previous retrospective EBMT data (Gilleece et al. 2018). Altogether, these studies lend support to the hypothesis that the mere presence of MRD should not be an absolute obstacle to the delivery of an allogeneic stem cell transplant. In this view, a relevant question raises as to whether the burden of MRD is a critical factor influencing the post-transplant outcome. Theoretically, the higher the levels of MRD the greater the required neutralization from "graft vs leukemia" (GVL). Leung et al. (2012) observed that CIR and OS of a series of pediatric patients worsened proportionally to the increasing levels of pre-transplant MRD, with patients categorized as "high positive" (MRD > 1%) having the highest CIR and shortest OS. Buccisano et al. (2017) reported a very similar experience in a series of 81 pre-transplant MRD-positive adult patients. Allogeneic stem cell transplant conferred a statistically significant survival advantage to patients with "low burden MRD" (MRD < 1%). Moreover, in the NCRI AML17 trial only higher levels of pre-transplant *NPM1* mutant MRD had an adverse effect on post-transplant outcomes of *NPM1* mutated patients who were *FLT3*-ITD negative at diagnosis (Dillon et al. 2020). Prospective studies using comparable assays would help further address this issue. If a green light is given to the decision to transplant "pre-transplant MRD positive" patients, the question is how to potentiate the antileukemic effect of allogeneic stem cell transplant. Delivery of additional cytotoxic therapy before allogeneic stem cell transplant appears questionable. MRD persistence reflects most probably a condition of leukemia chemoresistance. Therefore, provision of cytotoxic therapy appears not the right approach and could be even detrimental. Relapses and/or toxicities can occur, interfering with the subsequent transplant procedure. However, the availability of new agents has paved the way for potential intervention on MRD status to overtake its prognostic role. The timely use of these new agents appears a critical factor for a successful control/eradication of MRD. In the RATIFY study (Stone et al. 2017), delivery of allogeneic stem cell transplant in first mCR was associated with a superior survival advantage in patients randomized in the midostaurin plus chemotherapy arm. This observation suggests that midostaurin might have induced a better quality of response before allogeneic stem cell transplant. A similar finding emerged also in the phase 3 CPX-351 clinical trial (Lancet et al. 2018). These experiences indicate that a proper use of new drugs might increase the proportion of patients who are "pre-transplant MRD negative." On the other hand, the availability of new agents has also revitalized the role of maintenance therapy (Wei et al. 2019a), suggesting that pre-

emptive treatments are feasible even after allogeneic stem cell transplant (Platzbecker et al. 2019; Burchert et al. 2018). CC-486 (oral azacitidine) promises to be a strong candidate to investigate in clinical trials of post-transplant maintenance.

In conclusion, even though there is robust evidence of the negative prognostic role of "pre-transplant MRD positivity," we believe that it is not a valid justification to desist from a potentially curative approach such as allogeneic stem cell transplant. Such a habit appears even more convincing in an era of broad accessibility to new agents that might contribute to improving transplant outcomes. Also, the discovery of ever more sophisticated techniques promises to help to refine our therapeutic decisions in a way that they are tailored to the individual risk of recurrence. Controlled, clinical trials are needed to validate the value of these approaches, and patients should be encouraged to enter such trials.

18.6 MRD in the Era of Novel Therapies

MRD negativity is not yet an EMA/FDA accepted early surrogate outcome endpoint in AML but complete remission with MRD negativity (CR MRD−) is now included as a response criterion (Dohner et al. 2017) to categorize remissions that are ≥1–4 logs below the CR threshold (10^{-3} to 10^{-6}) as measured by standard MRD assessments (genetic markers by RT-qPCR or by MFC-MRD). In most published studies, CR MRD− frequencies are reported for composite mCR patients, that is, CR and CR with incomplete neutrophil or platelet recovery. Increasingly recent trials of newer AML therapies have reported rates of these deeper responses, either by standard MRD assessments or, in the case of IDH and FLT3 inhibitors, clearance of targeted mutations. In the absence of randomized studies, currently the only comparison for these data is from historical cohorts treated by chemotherapy.

Excluding gemtuzumab ozogamicin and midostaurin, novel treatments have been approved for (1) adults ≥75 years or unfit with newly diagnosed AML or (2) relapsed/refractory AML. In the setting of relapsed/refractory AML, a preliminary report suggests that about 60% of adults in remission following intermediate or high dose cytarabine salvage have a CR MRD− (10^{-3} to 10^{-4}, MFC-MRD) (Short et al. 2019). Regarding older adults in remission from standard treatments, previously published rates of CR MRD− (by MFC-MRD) ranged from 11% (Buccisano et al. 2015) to ~50% (Freeman et al. 2013) after intensive chemotherapy and 41% with HMA (hypomethylating agents) (Boddu et al. 2018). Table 18.1 shows the MRD data with frequencies of remission and CR MRD− reported so far for newer therapies. In some studies, especially for combination regimens, CR MRD− rates are certainly encouraging. However, the extent to which CR MRD− impacts on outcome compared to blast reduction below CR threshold of 5% remains uncertain. Factors that restrict determining this include the relatively small cohorts, modest, often short-lived outcome benefits and in some cases a selected MRD marker that may have lower prognostic value. Do less intensive regimens reduce the potential survival benefit of CR MRD− by limiting how much leukemia can be cleared below the MRD detection threshold? Interestingly, the prognostic advantage from CR MRD− (MFC) appears equivalent in adjusted analyses between intensive versus less intensive standard induction although more patients achieve negativity with the former (Hochman et al. 2019). It will be important to extend this evaluation to the newer combinations. A further consideration is that non-intensive novel drugs have different therapeutic activities from standard cytotoxics as they promote leukemic blast maturation; this could further alter the prognostic effect of MRD. Indeed, treatment benefit in AML may not always require leukemia clearance below 10^{-3} to 10^{-6} or even below the CR threshold as demonstrated in HMA trials (Santini and Ossenkoppele 2019; Yee et al. 2019). Moreover, any benefit from CR MRD− may be outweighed by greater treatment toxicity. A third of remission responses to HMA were CR MRD− (by MFC-MRD) (Boddu et al. 2018) (Table 18.1). Although relapse was reduced in these "best"

Table 18.1 New drugs and MRD: MRD information generated in studies of novel therapies

	AML status	Median age	Number of patients monitored by MRD	MRD marker	% CR[a] (overall cohort)	% MRD− (% of patients in remission)	% CR[a] MRD− (% of overall cohort)	? Improved outcome in MRD- patients		Comment
								Relapse	Overall survival	
Treatment										
HMA alone										
Decitabine (Boddu et al. 2018) Gaudecitabine Azacitidine Off-trial	New diagnosis	76 yrs	116	Flow cytometry	59%	41% (by 3 m post remission time-point)	24%	Yes (2 yr. CIR 48% vs 86% for other CR patients)	No	HIgh non- relapse mortality in MRD- group
Venetoclax										
Venetoclax with HMA (DiNardo et al. 2019c)	Treatment naive	74 yrs	145	Flow cytometry	67%	29%	19%	Trend for median duration of CR (not reached vs 11.3 months)	Uncertain (median OS not reached for CR)	Highest [a]CR % In *NPM1* and *IDH* mutated
Venetoclax with LDARAC (Wei et al. 2018, 2019b)	Treatment naïve or prior HMA	74 yrs	82	Flow cytometry	54%	32%	17%	Not reported	Uncertain (median OS not reached for [a]CR MRD−)	Highest [a]CR % In *NPM1* and *IDH* mutated
Venetoclax with Azacytidine (Winters et al. 2019) Off-trial	Treatment naïve or prior HMA	72 yrs (off trial)	14 (responders, off-trial)	Custom *ddPCR* assays based on diagnostic mutations	63%	29%	18%	NA	NA	No relapses in MRD- responders
Venetoclax with CLAD/LDARAC alternating with Aza (Kadia et al. 2019)	Newly diagnosed	69 yrs	24	Flow cytometry	92%	83%	76%	NA	NA	

(continued)

Table 18.1 (continued)

	AML status	Median age	Number of patients monitored by MRD	MRD marker	% CR[a] (overall cohort)	% MRD− (% of patients in remission)	% CR[a] MRD− (% of overall cohort)	? Improved outcome in MRD- patients		Comment
								Relapse	Overall survival	
Venetoclax with HMA or LDARAC (Tiong et al. 2019) Off-trial	NPM1 mutated with molecular relapse or persistence	61 yrs	10	*mNPM1*RT–qPCR	Not applicable	80%	Not applicable	NA	NA	
DiNardo Blood 2020										
IDH inhibitors										
Ivosidenib										
Ivosidenib monotherapy (Roboz et al. 2020)	New diagnosis	76.5 yrs	30	*IDH1 R132* mutations by ddPCR	42%	64%	27%	NA	NA	RTK mutations enriched for non-responders
Ivosidenib with standard chemotherapy (Stein et al. 2018)	New diagnosis	63 yrs	31	*IDH1* mutations by ddPCR Flow cytometry In some	78%	41% by mutation clearance 89% by flow cytometry (8 of 9 patients)	32% by mutation clearance	NA	NA	
Ivosidenib monotherapy (DiNardo et al. 2018)	Relapsed refractory	67 yrs	73	*IDH1* mutations by ddPCR	30%	21%	6%	Trend for median duration of CR (11.1 vs 6.5 months)	Trend for median OS <u>But</u> MRD+ not restricted to CR	

Enasidenib										
Enasidenib with standard chemotherapy (Stein et al. 2018)	New diagnosis	63 yrs	60	*IDH2* mutations by ddPCR Flow cytometry In some	69%	30% by mutation clearance 58% by flow cytometry (7 of 12 patients)	21%	Not reported	Not reported	
Enasidenib monotherapy (Stein et al. 2019)	Relapsed refractory	68 yrs	101	*IDH2* mutations by ddPCR	29%	28.6%	8%	Not known	Yes for median OS When MRD+ not restricted to CR	No survival difference for CR MRD− vs CR MRD+ *NRAS* and *Flt3* mutations enriched for non-responders
Flt3 inhibitors										
+/− FLT3 inhibitor with standard induction followed by CR1 allogeneic transplant (Levis et al. 2020)	New diagnosis *FLT3*-ITD mutated and *NPM1*-mutated	59 yrs	17 (8 had FLT3 inhibitor)	*FLT3*-ITD mutations by custom PCR-NGS assay plus CE-PCR	Not applicable	7 of 8 Flt3 inhibitor patients *Flt3*-ITD VAF <0.01%	Not applicable	NA	NA	Numbers very small but MRD significantly lower in Flt3 inhibitor vs chemo alone
Gilteritinib (Levis et al. 2018) CHRYSALIS trial	Relapsed/ refractory	61 yrs	80	*FLT3*-ITD mutations by custom PCR-NGS assay	55%	45% VAF $\leq 10^{-2}$ 30% VAF $\leq 10^{-4}$	25% VAF $\leq 10^{-2}$ 16.5% VAF $\leq 10^{-4}$	NA	Prolonged median survival But not restricted to CR	Median survival similar for MRD $\leq 10^{-2}$ To $\leq 10^{-4}$

LDARAC low dose cytarabine, *CLAD* cladaribine, *NA* not assessable, m*NPM1* mutated *NPM1*, *ddPCR* droplet digital PCR, *NGS* next generation sequencing, *CE* capillary electrophoresis

[a]CR, composite complete remission i.e. CR with incomplete (CRi) or platelet recovery (CRp) and may in some studies include partial haematological recovery (CRh)

responders, this did not translate to a survival benefit due to a higher number of non-relapse deaths. However, when older patients were treated with a combination of HMA (decitabine) and vosaroxin (quinolone derivative, topoisomerase II inhibitor), MRD-negative status was associated with improved median overall survival (34.0 versus 8.3 months for other responders) (Daver et al. 2017). Currently investigated HMA plus novel agent combinations may be able to achieve deep remissions without concomitant increased toxicity. Encouragingly in the context of observed MRD-negative responses in phase 1/2 studies of IDH inhibitors and Venetoclax (as monotherapy or in HMA combinations) (Table 18.1, also (DiNardo et al. 2019a, b)), adverse events appear infrequent.

IDH Inhibitors: Mutations in either IDH1 or IDH2 can collectively be detected by NGS panels in up to 20% of AML patients by current technology (Bullinger et al. 2017). This prevalence increases in older AML cohorts (~25%) (Prassek et al. 2018) and in AML with normal cytogenetics (up to 30%) including NPM1 mutated AML (~30%) (Bullinger et al. 2017; Ferret et al. 2018; Ok et al. 2019). In retrospective studies, 45–60% of newly diagnosed IDH mutated AML patients attaining CR after standard chemotherapy cleared their *IDH* mutations (detection limit <0.2% VAF by standard dd PCR assay (Ferret et al. 2018) or <1% VAF by NGS (Ok et al. 2019)) and this was associated with reduced early relapse (Ferret et al. 2018; Ok et al. 2019). Some IDH inhibitor studies have monitored *IDH* mutations by a more sensitive dd PCR assay, (depth up to 10^{-4}) to combine a read-out of on-target efficacy with MRD. On-target molecular remissions are observed in 20–28% of relapsed /refractory *IDH* mutated patients achieving CR or CR with partial hematological recovery from IDH inhibitor monotherapy (Stein et al. 2019; DiNardo et al. 2018). Higher percentages have been reported in early data from phase 1 /2 IDH inhibitor studies (including azacitidine combinations) of newly diagnosed AML (DiNardo et al. 2019a; Roboz et al. 2020; Stein et al. 2018). While such deep *IDH* molecular remissions may be an indicator for response duration (with Ivosidenib (DiNardo et al. 2018)), improvements in survival compared to mutation positive CR/CRh patients have not yet been reported (Stein et al. 2019; DiNardo et al. 2018; Roboz et al. 2020). Furthermore, response and survival were comparable between patients with *IDH2*-R140 or *IDH2*-R172 mutations, but only the former had a major reduction in mutation VAF (Stein et al. 2019). Ongoing differentiation, clonal hematopoiesis, or later mutation loss from clonal evolution may all contribute to reducing the prognostic significance of detectable *IDH* mutations. Established assays (e.g., RT-qPCR of *NPM1* mutations or MFC-MRD) are clinically recommended to assess AML MRD (Schuurhuis et al. 2018). Combining them with *IDH* mutation analysis currently represents the optimal monitoring strategy for assessing the efficacy of IDH inhibitors in trials.

FLT3 Inhibitors: There is a paucity of MRD data in published trials of FLT3 tyrosine kinase inhibitors. On-target molecular monitoring is available at low sensitivity (10^{-2} VAF) by the established clinical test of capillary electrophoresis (CE) *FLT3* ITD detection. A more sensitive (up to 10^{-4} VAF) combination PCR NGS assay (propriety) demonstrated a 16% CR MRD− frequency in 80 relapsed/refractory AML adults who received gilteritinib monotherapy (CHRYSALIS phase 1 /2 study) (Levis et al. 2018). CR MRD− patients had a significantly longer median survival compared to those in an MRD-positive remission. However, lower levels of MRD ($\leq 10^{-3}$ VAF, detected in 25% of total cohort) did not impact on median survival (Levis et al. 2018), suggesting that in this setting an MRD threshold of 10^{-3} is most predictive. This or a similar assay has also been applied to remission samples of 17 newly diagnosed *FLT3* 1TD */NPM1* mutated adults (Levis et al. 2020) (Table 18.1) and in the ongoing CTN 1506 (gilteritinib post-transplant maintenance) and Quantum-First (quizartinib in newly diagnosed AML) trials. Other NGS-based platforms linked with differing bioinformatics strategies can also monitor *FLT3* ITD mutations to the same ITD VAF depth in research settings (Thol et al. 2018; Hourigan et al. 2020; Blatte et al. 2019; Kim et al. 2018). The above higher sensitivity assays

could be validated for routine clinical practice in the next couple of years. However, as late subclonal leukemic mutations, *FLT3* ITD mutations may be unreliable MRD markers (clinical false negatives) from instability / VAFs below MRD detection limits (Freeman and Hourigan 2019); this is particularly likely beyond early response and when monitoring FLT3 inhibitors as maintenance therapy. Therefore, independently of on-target *FLT3*-ITD mutation monitoring, clinically validated MRD assays (presently MFC if no RT qPCR target such as *NPM1* mutations) continue to be recommended for MRD assessment (Schuurhuis et al. 2018).

Venetoclax: Composite CR/CRi frequencies for the BCL2 inhibitor venetoclax in combination with either low dose AraC or azacitidine are high, ranging between 54 and 67% for elderly adults unfit for intensive chemotherapy (DiNardo et al. 2019c; Winters et al. 2019; Wei et al. 2019b). When measured in the overall cohorts, MRD levels were below 10^{-3} in up to a third of the remissions (Table 18.1) and duration of response may be prolonged in these patients (DiNardo et al. 2019c; Winters et al. 2019; Wei et al. 2018) although data are preliminary. There are early but encouraging indications that MRD-negative remissions to the depth of the sensitive *NPM1* mutant RT-qPCR assay are frequent and prolonged in *NPM1* mutated patients (Tiong et al. 2019; DiNardo et al. 2020) (discussed further below). Notably, MRD detection of *IDH2* mutations appears to be a poor predictor of relapse-free survival in venetoclax treated *IDH2* mutated elderly adults. Most tested patients had detectable persistent *IDH2* mutations by ddPCR despite high rates of durable clinical remissions (at least 24 months in one study) (Winters et al. 2019; DiNardo et al. 2020).

Glasdegib: Although MRD results are not yet available for glasdegib studies, CR MRD− (by centrally assessed MFC-MRD) is included as a secondary endpoint in the Phase 3 BRIGHT AML1019 trials of glasdegib with standard chemotherapy or azacitidine.

Immunotherapies: Immunotherapies are an active area of early phase studies in AML. As well as checkpoint inhibitors there are immune constructs targeting myeloid surface proteins (CD33, CD123, CLL-1) (Assi et al. 2018). CD33 positivity is a requirement for the approved use of gemtuzumab. Flow cytometric diagnostic screening for AML markers targeted by new constructs is likely to evolve into "on-target" flow cytometric MRD monitoring to assess response efficacy and evaluate target loss on residual leukemic blasts. Relevant to this is identifying and monitoring targets on immunophenotypic blast populations that are most likely to be reservoirs of relapse as enriched in leukemic stem cells (LSC) or relapse initiating cells (Haubner et al. 2019; Zeijlemaker et al. 2019). CD34+CD38− is the most tractable immunophenotype for flow cytometric monitoring of candidate LSC / relapse initiating populations. High frequencies of CD34+CD38− blasts in diagnostic samples are indicators of poor prognosis (Zeijlemaker et al. 2019; Khan et al. 2015), consistent with this immunophenotype as a baseline biomarker for resistant leukemic cells. An initial screen for immunotherapeutic targets on CD34+CD38− and other blast populations could be simplified by a single "LSC" tube that combines multiple aberrant "LSC" markers (Zeijlemaker et al. 2016).

Molecular Determinants of Response: Potential molecular determinants of benefit and response durability have been explored for several novel regimens, following the paradigm of CBF AML with gemtuzumab ozogamicin (GO). For example, mutations in receptor tyrosine kinase pathway genes such as *NRAS* may be associated with primary and adaptive resistance to IDH inhibitors (Amatangelo et al. 2017; Stein et al. 2019; DiNardo et al. 2018) and venetoclax (DiNardo et al. 2020) while mutations in *IDH2* and *NPM1* appear to be molecular determinants of more durable remissions from venetoclax (DiNardo et al. 2020). In the case of GO, however, activating signaling mutations such as *NRAS* correlated with improved event-free survival in the 2017 ELN good/favorable risk subgroups, including for NPM1 mutated patients (Fournier et al. 2020). Although signaling mutations are linked to resistance to IDH inhibitors and venetoclax, the observed higher CD33 levels

on blasts with these mutations (Fournier et al. 2020) may be a mechanism for improved sensitivity to GO. *TP53* mutations confer resistance across different therapies including CPX-351 (Goldberg et al. 2018) and venetoclax (DiNardo et al. 2020). Even when *TP53* mutated patients enter remission after CPX-351, CR MRD− frequency may be lower (Goldberg et al. 2018) but this needs confirmation in ongoing randomized trials with integrated MRD (such as NCRI AML18 and AML19).

To use these newer agents to their full potential, response profiles need further investigation by superimposing MRD data to mutation screens in sufficiently large cohorts. This should uncover which genetic subgroups are most treatment sensitive, whether clinical activity correlates with deeper responses and the best combination of MRD assays and genetic subgroups for MRD status to provide an early indicator of outcome endpoints. Concerning the latter, there is a strong rationale for MRD in NPM1 mutated AML to assess and direct newer therapies.

18.7 Combining MRD with Molecular Determinants for Outcome Prediction: *NPM1* Mutated AML

NPM1 mutations are AML-specific (as causative driver mutations) and in >90% of cases remain stable in the relapse initiating clone (Ivey et al. 2016; Cocciardi et al. 2019; Hollein et al. 2018b). Treatment responses in *NPM1* mutated patients can be measured to a depth of 1×10^{-6} by RT-qPCR of *NPM1* mutant transcripts (Schuurhuis et al. 2018). MRD status by this very sensitive assay is highly prognostic in *NPM1* mutated AML after induction with standard chemotherapy as well as at later time points in younger adults (Freeman and Hourigan 2019; Schuurhuis et al. 2018). Durable responses and MRD negativity have been observed not only after standard chemotherapy but also in older and relapsed /refractory *NPM1* mutated patients following novel therapies (Tiong et al. 2019; Levis et al. 2020; DiNardo et al. 2020). From the present combination of best-standard AML MRD assay and leukemia response profile in *NPM1* mutated AML, it is plausible that MRD is most likely to be a predictive measure of treatment efficacy for newer treatments in this AML subtype as compared to others. MRD data from gemtuzumab (GO) trials support this. There is a survival benefit from the addition of gemtuzumab (GO) to standard chemotherapy induction despite no concomitant increase in response (Hills et al. 2014). Specifically for *NPM1* mutated patients, there were significantly fewer relapses with GO compared to standard induction for patients achieving a remission in the AMLSG 09-09 trial (Schlenk et al. 2020).

Response depth from gemtuzumab has been compared to standard treatment arm in two trials of older patients (NCRI AML16 (Freeman et al. 2013) and ALFA-0701 (Lambert et al. 2014)) by frequencies of CR and CR MRD− (below 10^{-3} to 10^{-4}, measured by MFC-MRD in NCRI AML16 and by WT1 RT-qPCR in ALFA-0701). No significant differences between the treatment arms were observed although MRD was prognostic for survival in the overall cohorts. Notably however, a post-hoc analysis of the *NPM1* mutated subgroup in the ALFA-0701 trial, showed that improved survival from GO did correlate with CR MRD− frequency by *NPM1* mutant RT-qPCR (CR MRD−, 39% in GO arm versus 7% in control, $p = 0.006$) (Lambert et al. 2014).

NPM1 mutations are prevalent in older as well as younger adults (Prassek et al. 2018; Buccisano et al. 2018) and were present in about 20% of the elderly adults enrolled in the venetoclax phase 2 trials. Venetoclax in combination with HMA or low dose cytarabine has striking efficacy by remission rates (~90% (DiNardo et al. 2019c, 2020)) in NPM1 mutated older adults ineligible for intensive chemotherapy. This responsiveness correlates with a favorable 2 years survival of over 70%, albeit in a small number of patients so far. This overall survival rate has not previously been achieved in historical cohorts of NPM1 mutated older adults treated with either HMA (Prata et al. 2018) or intensive chemotherapy including with GO (Fournier et al. 2020; Burnett

et al. 2012), even for those in a CR MRD− by flow cytometry (Freeman et al. 2013). Is there any evidence that these encouraging outcomes are associated with increased and sustained MRD clearance? *NPM1* mutant MRD monitoring data are limited for venetoclax regimens. However, durable MRD negativity by *NPM1* mutant RT-qPCR from venetoclax combinations has been reported as common in the few patients tested (Tiong et al. 2019; DiNardo et al. 2020). These include patients treated for *NPM1* mutant molecular persistence or relapse (Tiong et al. 2019). Thus, *NPM1* mutant MRD is promising as a surrogate for clinical benefit from venetoclax but also may enable selection of patients with molecular progression following chemotherapy for pre-emptive venetoclax treatments.

A significant proportion of patients with actionable mutations will also have the highly sensitive MRD marker from RT-qPCR of *NPM1* mutant transcripts. Due to the association between *NPM1* and *IDH1* or *IDH2*R140 mutations, up to 45% of younger and 10–20% of older AML patients with IDH mutations (excluding *IDH2*R172) are *NPM1* mutated (Bullinger et al. 2017; Prassek et al. 2018).

NPM1 mutations are also frequent in FLT3 mutated patients, as evident from relapsed/refractory as well as younger newly diagnosed FLT3 mutated trial cohorts; 47% of adults recruited to the ADMIRAL trial (Perl et al. 2019) (gilteritinib versus chemotherapy for relapsed/refractory AML) had co-mutated *NPM1* and 57% in the RATIFY trial (Stone et al. 2017) (midostaurin added to chemotherapy in younger untreated AML). Survival benefits from midostaurin and gilteritinib are independent of *NPM1* genotype risk/*FLT3* ITD allelic ratio (AR) risk groups (Perl et al. 2019; Döhner et al. 2020). However, it is unclear whether CR1 allogeneic transplantation should be deployed for 2017 ELN favorable (*NPM1* mutated / *FLT3*-ITD low AR) and intermediate risk patients whether or not they receive frontline midostaurin (Döhner et al. 2020) or in the future a second generation FLT3 inhibitor. *NPM1* MRD has the potential to both inform the early efficacy of FLT3 inhibitors in these risk groups and guide further transplant decisions. Recent evidence points to *FLT3* ITD mutated patients with pre-transplant MRD positivity having a very poor outcome after allogeneic transplantation (Hourigan et al. 2020; Dillon et al. 2020). Whether available peri-transplant strategies could alter this remains to be determined. It is anticipated that ongoing trials such as those testing post-transplant maintenance with integrated MRD assays (gilteritinib, BMT CTN 1506; MRD directed azacitidine, RELAZA2 (Platzbecker et al. 2019); oral azacitidine/CC-486, AMADEUS) should contribute important data to help address this critical question.

These initial results from MRD testing in trials of emerging therapies are preliminary due to tested cohort sizes. They are, however, already generating information about the relative utility and limitations of certain markers and assays as MRD read-outs. For instance, the promising CR MRD− responses observed in NPM1 mutated AML with venetoclax and FLT3 inhibitors suggests that the higher sensitivity of RT-qPCR MRD will be advantageous in this subtype to assess and direct treatment. On the other hand, MRD detection of persisting IDH mutations in CR, even in the setting of IDH inhibitors, does not appear to preclude a survival benefit. We would encourage the future incorporation of sequential MRD into studies to aid the selection and timing of further interventions by, for example, accruing data on the kinetics of MRD re-emergence in those patients relapsing after a CR MRD−.

18.8 NGS-Based MRD Detection: Advances and Challenges

MFC-based MRD detection has been the standard for MRD assessment in AML patients for many years and is applicable to the majority of patients (Schuurhuis et al. 2018; Hourigan et al. 2017). In contrast, the use of molecular enumeration of MRD has been limited to specific recurrent molecular aberrations, such as the core binding factor fusion transcripts *RUNX1/RUNX1T1* and *CBFB-MYH11* and mutant *NPM1* (Schuurhuis et al. 2018; Hourigan et al. 2017). NGS now

enables detection of all mutations, including hotspot as well as patient-specific mutations, in AML at diagnosis and in CR after chemotherapy (Levine and Valk 2019). In fact, it has recently been shown that molecular MRD detection by applying NGS is potentially applicable to virtually every newly diagnosed AML patient because of the frequent prevalence (>90%) of multiple (on average 3) molecular aberrations among patients with AML (Levine and Valk 2019). However, MRD detection based on NGS faces several challenges before it can be reliably introduced in clinical practice.

Sensitive detection of all mutant (minor) cell populations at diagnosis and during the course of disease is a prerequisite for NGS-based MRD detection in routine analyses. Sequencing artifacts are introduced during DNA isolation, library prep and the actual NGS-procedure (0.1–1%), which makes sensitive detection of all possible mutations at low level (<0.01%) a challenge (Salk et al. 2018). The rate of sequencing artifacts can be reduced biochemically, for example, by using proof-reading polymerases, or computationally; however, these corrections are only modest and cannot entirely resolve the introduction of artifacts. Alternative strategies should be explored. For instance, error corrected NGS approaches using unique molecular barcodes have been shown to increase the specificity of low-frequency mutation detection (Salk et al. 2018). Recently, several studies addressed NGS-based MRD detection in relatively large AML cohorts from clinical trials, all demonstrating that NGS-based MRD carries profound prognostic impact for patients with AML (Jongen-Lavrencic et al. 2018; Thol et al. 2018; Hourigan et al. 2020; Klco et al. 2015; Hirsch et al. 2017; Getta et al. 2017; Morita et al. 2018; Press et al. 2019). In these studies, persisting mutations in CR were measured with gene panels (Jongen-Lavrencic et al. 2018; Hirsch et al. 2017), capture-based deep sequencing (Klco et al. 2015; Hirsch et al. 2017; Salk et al. 2018; Guenot et al. 2019), or targeted sequencing (Thol et al. 2018; Hourigan et al. 2020). Only in the latter two studies NGS-based MRD detection included error-correction using unique molecular identifiers, indicating that the other NGS-based MRD studies may not have been optimal. Another successful approach to correct for noise is the usage of site-specific error models (Jongen-Lavrencic et al. 2018). In these models the distribution of variants is determined in a reference set without mutations, for example, remission samples. MRD is subsequently defined by those mutations, that is, those present at diagnosis, which are statistically significantly different in CR to the distribution of the same variants in the reference set. A major drawback of such models is the requirement of a series of reference samples. In a routine setting MRD measurement in a single sample without the dependence of a large reference is obviously the preferred method. Nevertheless, since molecular MRD in CR has consistent prognostic value in AML (Jongen-Lavrencic et al. 2018; Thol et al. 2018; Hourigan et al. 2020; Klco et al. 2015; Hirsch et al. 2017; Getta et al. 2017; Morita et al. 2018; Press et al. 2019) technological improvements should be accomplished to increase both sensitivity and specificity of NGS-based MRD detection.

The recent NGS-based MRD studies in larger AML cohorts (Jongen-Lavrencic et al. 2018; Thol et al. 2018; Hourigan et al. 2020; Morita et al. 2018) revealed that gene mutations persisting in CR that are well-known to be associated with clonal hematopoiesis of indeterminate potential (CHIP) (Genovese et al. 2014; Jaiswal et al. 2014), such as mutations in *DNMT3A*, *TET2,* and *ASXL1* (*DTA*), do not impact on risk of relapse. After high dose chemotherapy, these AML patients are in a state of clonal hematopoiesis (CH), where AML-specific mutations occurring late in leukemogenesis are eradicated and CHIP-related mutations persist. However, the definition of true molecular MRD by the non-*DTA* mutations is not yet optimal. Besides acquired mutations in *DTA*, other well-known pathogenic mutations such as those in *TP53, PPM1D, JAK2, CBL, SRSF2,* and *SF3B1* have also been associated with CHIP in healthy individuals, however, at lower frequencies (Genovese et al. 2014; Jaiswal et al. 2014). Since these mutations appear at lower frequencies in newly diagnosed AML, it will require sufficiently large AML cohort to

determine if and to what extent persisting mutations other than *DTA* represent either true residual leukemia or CH with and without increased risk of relapse, respectively. The association of the persisting mutations to relapse risk may relate to type of mutation(s) but also the time and order of mutation acquisition, the allelic burden and/or number of mutations. For instance, later events such as mutations in the RAS pathway-related mutations *FLT3, RAS, KRAS, PTPN11,* and *KIT* as well as *NPM1* are generally cleared by high dose chemotherapy and persistence of these mutations, representing the frank leukemia, has been shown to be clearly associated with a higher risk of relapse (Jongen-Lavrencic et al. 2018; Thol et al. 2018; Hourigan et al. 2020; Klco et al. 2015; Hirsch et al. 2017; Getta et al. 2017; Morita et al. 2018; Press et al. 2019). AML patients with *TP53* mutations at presentation either fail to reach a CR or can relapse quickly after induction therapy, irrespective of their molecular MRD status from data in the HOVON study (Jongen-Lavrencic et al. 2018) (personal communication, Peter Valk). Thus, certain subtypes of AML may whereas others may not benefit from NGS-based MRD testing. Altogether, the definition of true residual leukemia needs to be refined in the coming years with a focus on the persistence of AML-specific mutations with a clear association to an increased risk of relapse.

Today, only a few studies compared NGS- to MFC MRD detection in AML (Jongen-Lavrencic et al. 2018; Ok et al. 2019; Getta et al. 2017). A concordance of 70% in MRD detection between both technologies existed, where those AML patients with detectable MRD by both MFC and NGS having the highest risk of developing a relapse (Jongen-Lavrencic et al. 2018; Ok et al. 2019; Getta et al. 2017). Interestingly, however, those AML cases with MRD detected by NGS or MFC were also associated with an inferior prognosis (Jongen-Lavrencic et al. 2018; Ok et al. 2019; Getta et al. 2017). Improvement of the sensitivity as well as specificity of our NGS-based MRD assays and our understanding of the biology of CH after high dose chemotherapy will enable us to better understand the discordant cases and determine whether we require both technologies or not.

Thus, NGS-based MRD detection focusing on certain (combinations of) mutations persisting in CR carries profound prognostic value for AML patients. The major limitations of the NGS-based MRD detection methodology relate to limited sensitivity and specificity of the assay and the inability to correctly discriminate between residual leukemia and CH. Improvements should be made in all these areas before NGS-based MRD detection can successfully be implemented in routine practice. Initial studies of NGS-based MRD detection were focused on the time of CR after intensive chemotherapy; however, AML patients with a high risk of relapse can also be recognized by NGS-based MRD detection post-allogeneic transplant (Kim et al. 2018; Thol et al. 2019). In addition, NGS-based MRD data of AML patients receiving alternative treatment schedules, including the novel therapies, exist but are limited. It is therefore essential to collect this type of data during the course of disease in the current clinical trials. The ultimate goal will be to dynamically monitor all AML-specific mutations during the course of disease by NGS to adequately follow therapy responses in AML and guide treatment.

18.9 Conclusions

The feasibility of MRD risk-directed and preemptive strategies has been demonstrated and its utility will be informed further by reporting of key studies in 2020/2021. Experience of MRD testing to identify deep responders with novel regimens is also building and combined with genetic subtyping should provide further insights into how best to target therapies and evaluate their clinical benefit. High-quality NGS-based MRD assays could contribute to this but more data, in different treatment settings, are required to clarify the prognostic value of MRD levels of mutations that are associated with CH as well as leukemia.

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