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Frontline management of acute myeloid leukaemia eligible for intensive chemotherapy

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Abstract

The phrase ‘Panta Rhei - everything flows’ by the Greek philosopher Heraclitus, a purported reference to a constantly changing flowing river, or more philosophically, ‘continual transformation’, can be aptly extended to describe the evolution of treatment strategies for many human diseases. In disappointing contrast, the drug treatment for patients with non-promyelocytic, acute myeloid leukaemia (AML) has remained essentially unchanged for over 50 years, with improved outcomes over this period, largely, a consequence of incremental improvements in supportive care and the application of allogeneic stem cell transplantation. The anti-leukaemic effectiveness of single-agent daunorubicin (D) or cytarabine (Ara-C) was first recognised over half-a-century ago, and intensified leukaemic cell kill with these genotoxic drugs (DA) became the standard approach for treating newly diagnosed AML patients. At the time of writing, induction therapy combining these two pharmacological classes of drugs, followed by intensified consolidation or allogeneic stem cell transplantation remains the only proven strategy for curing AML. Here, through a review of the development of different anti-leukaemic drug combinations, we evaluate the effectiveness of various intensive chemotherapy platforms, and the evidence for using adjunctive or sequential therapy with newer, genotoxic or non-genotoxic agents.

Intensive chemotherapy in AML – a historical perspective

There is little evidence to support the recognition of AML as a unique cancer until the early 20th century [1,2]. Developments in the late 19th century that established the relevance of the bone marrow to blood cell production, and improvements in staining techniques enabled recognition of the myeloblast as a granulocyte precursor cell. The distinction between myeloblasts and lymphoblasts lead to the identification of sub-types of acute leukaemia by Reschad and Schilling-Torgau in 1913 that included AML and monocytic leukaemia [1,2]. Compared to chronic leukaemias, this delayed recognition of AML may, in part, have been due to a rapidly fatal clinical course, with limited diagnostic or therapeutic opportunities. Thus, descriptive reports on arsenic and x-ray treatment for 'leukaemia' at the turn of the 19th century include patients with likely chronic myeloid leukaemia (CML) or chronic lymphoid neoplasms, but not AML [3,4]. Following the identification of AML as a sub-type of acute leukaemia [1,2,5], early attempts to achieve disease control in these patients would have relied on similar approaches used to treat CML and acute lymphoblastic leukaemia (ALL), and included the use of urethane [6]. Notwithstanding limited scope for rigorous scientific scrutiny, an occasional, durable response following combination therapy with radiation, arsenic and thorium-X (RAT) has been suggested [7], but in most patients, survival remained unaltered despite a modest, transient reduction in disease burden.

Research into nitrogen mustards during the second World War benefitted patients with lymphoma more than leukaemia; an indirect consequence was the development of alkylating agents, subsequently incorporated into induction or maintenance regimens for AML [1,2]. Important discoveries crucial to the successful

pharmacotherapy of ALL followed and included folate antagonists, prednisone and 6-mercaptopurine (6-MP), with the effectiveness in AML then investigated [8,9]. In a series of 15 patients with acute leukaemia including 11 with AML, treatment with 6-MP (2.5mg/kg/day for 3 weeks) resulted in clearance of marrow leukaemic infiltrate and blood count recovery. This state of complete remission (CR) was maintained for 3 months and improved survival [9]. It would be easy to underplay the significance of these and other observations of the time, or the seemingly 'negative' results of older studies, but two principles that would form the cornerstone of future therapies, in particular intensive chemotherapy (IC) of AML, began to emerge. These included the recognition of 'remission' as a critical pre-requisite for improving survival, and the existence of unique differences between the drug susceptibility of ALL and AML blasts that necessitated a different pharmacological approach to improve the durability of responses.

By the early1960s, amidst the acknowledgment that extending the drug-therapy of ALL to AML, could at best achieve CR rates of 20% [10,11], pre-clinical and clinical studies of two drugs began, that would subsequently alter the treatment paradigm for AML. One of these, arabinosylcytosine or cytarabine, a cytidine analogue exhibited anti-leukaemic properties in murine models of the disease [12], with additive effects in combination with thioguanine [13]. In human studies, as a single agent, CR rates with cytarabine were not dissimilar to other forms of monotherapy of the time and approached 30% [14], but multi-agent therapy with methylglyoxal bis guanilhydrazole [15], 6-MP [16] and cytarbine increased CR rates to 40% [17]. In separate studies [18], a role for daunorubicin (daunomycin) in the therapy of AML was beginning to be recognised: starting with a dose of 2mg/kg/day

(60-80mg/m^2 as the maximum daily dose), titrated to changes in circulating and marrow blasts each week, pronounced leucopaenia was observed in most patients within 3-5 successive days of treatment. Remarkably, over half the patients achieved CR after 2-8 weeks, with marrow aplasia around day 8, a consistent feature. Treatment with methotrexate and 6-MP maintained remission and was interrupted at intervals for re-induction with daunorubicin and methyl-GAG. The average duration of response was 155 days, with some patients maintaining remission even after a year. Based on this study, a cumulative daunorubicin dose ceiling of 750mg/m^2 was suggested, but concerns around the drug's narrow therapeutic index [19] and acquired drug resistance with monotherapy meant that the subsequent focus was combination approaches incorporating daunorubicin at lower doses. By the late 1960s, cytarabine in combination with 6-MP, thioguanine or daunorubicin was considered standard remission-induction therapy in newly diagnosed AML patients, based more on the clinical impression of efficacy than statistical significance [20,21]. Studies relating changes in marrow cellularity to DNA synthesis in patients receiving 6-MP and 6-methylmercaptopurine highlighted the attainment of early post-treatment aplasia as a pre-requisite for CR and improved survival [22], thus establishing the importance of intensified induction therapy, despite its potential for toxicity.

Earlier studies by the Acute Leukemia Group B (renamed Cancer and Acute Leukemia Group B, CALBG in 1976) suggested the utility of two daily doses of daunorubicin with a 5 day schedule of cytarabine, administered as two 5 day courses [20]. Following a pilot study with a 7-day course of infusional cytarabine (100mg/$m^{2/}$day) combined with daily daunorubicin (45mg/m^2/day) for the first 3 days

of treatment ('DA 3 and 7'), a multicentre randomised investigation of 'DA 3 and 7', compared to the 5-day schedule (DA 2+5) was undertaken in 352 previously untreated AML patients, including 247 <60 years old [23]. The impact of the mode of cytarabine delivery on outcomes was also investigated: during induction, 24-hour continuous intravenous infusion of the drug (100mg/m^2/d) was compared with bolus injections (100mg/m^2) administered 12-hours apart, and in 4-monthly cycles of maintenance treatment that combined lower-doses of cytarabine (by subcutaneous or intravenous bolus route) with thioguanine, cyclophosphamide, lomustine or daunorubicin. The results were clear: a superior rate of CR and reduction in induction deaths with the 7-day induction schedules were observed in patients <60 years and older patients in whom, until then, the potential toxicity of intensifying induction therapy was believed to negate the anti-leukaemic benefits. The numerical difference in CR rates between cytarabine as an infusion (56%), or bolus (49%) may have been skewed by an unexplained 29% decrease in CR rates in the bolus arm during the latter part of the study, but did not meet statistical significance even after adjustment for age and time-of-entry to study. Whether overall survival (OS) is affected by infused or bolus cytarabine is difficult to ascertain since survival in responding patients was analysed in sub-groups categorised by the route of cytarabine administration in induction and maintenance, and included patients receiving DA 2+5. Nevertheless, by confirming the importance of CR to prolongation of survival, 'DA 3 and 7'-containing infusional or bolus cytarabine became an accepted standard for intensive induction therapy in many centres, to this day.

The British approach to induction IC had subtle differences: intravenous cytarabine (100mg/m^2) was administered as 12-hourly bolus doses for 10 days with

daunorubicin (50mg/m^2/d on days 1, 3 and 5) and oral 6-thioguanine (100mg/m^2 12-hourly, days 1–10), i.e. DAT 3+10 [24]. Although differences in CR rates were not statistically significant over the lower intensity schedule (DAT 1+5), time to CR was a median of 34 versus 46 days respectively and associated with superior survival at 5 years (25% versus 18%, $p<0.05$). Thus DA 3+10 (with thioguanine subsequently omitted due to non-availability, hepatotoxicity and questionable benefit) became an alternative standard to 'DA 3 and 7' against which investigative pharmacological approaches continue to be compared.

Over the last 30 years, strategies to optimise outcomes of IC have included changes to dosing intensity [25], conventional genotoxic drugs [26], drug-delivery systems [27] and the inclusion of small molecule drugs to target AML characterised by unique gene variants [28]. A meaningful comparison of results across studies presents challenges: fundamental differences exist in the design of induction and subsequent treatments, and dosing-schedules. For example, the administration of two courses of anthracycline-containing induction therapy is standard practice following DA 3+10 in some countries [29]; in others, this approach is reserved only for patients with detectable disease following one course [30]. German study groups tend to use a double-induction strategy in patients <60 years old, but to achieve early treatment intensification, the second cycle is administered at the pre-defined time-point of day 21 of cycle 1 [31]. In addition to this variation between IC protocols, as the biology of AML advances [32-37], the possibility that outcomes following same medicinal agent in contemporaneous studies may be confounded by an unrecognised heterogeneity in disease sub-types, also requires consideration.

Intensifying induction chemotherapy – looking beyond 'DA'

Optimising cytarabine

Over time, improvements in supportive care have enabled better management of chemotherapy-associated toxicity [38] facilitating the investigation of the effects of dose-intensification in induction. Initial studies compared the effects of increasing the duration or dose of cytarabine within the 'conventional' range of 100-200mg/m^2/day. Extending the duration of 'DA 3 and 7' to 10 days, with additional days of cytarabine (100mg/m^2/day) as an infusion did not impact on remission-rates or duration [39]. Likewise, response-rates, relapse and deaths in remission remained unaffected despite doubling the bolus cytarabine dose, from 100mg/m^2 to 200mg/m^2 in DAT 3+10 [40]. This lead to randomised comparisons of considerably higher doses of cytarabine in induction: conventional protocols containing 100-200mg/m^2/day for 7-10 days, and thus a cumulative dose of 0.7-1.4g/m^2 were compared against schedules containing cumulative doses of 5-24g/m^2 [41-45]. Confounding a comparison of these studies are differences in concomitant therapy, including daunorubicin (40-50mg/m^2/day for 3 days), idarubicin or mitoxantrone, with additional drugs given in some protocols [41,44,45]. Post-remission strategies between studies too differed, including the application of autologous or allogeneic stem cell transplantation, known to impact survival outcomes [46-48], and maintenance treatment. These confounders notwithstanding, the improvement in remission-duration or disease-free survival (DFS) with higher doses of cytarabine in induction regimens did not improve OS in most studies, including those investigating dose-dense regimens [31]. Exceptions include patients <46 years in the EORTC-

GIMEMA AML-12 study [44] that investigated the effects of increased cytarabine dosage (3g/m^2 every 12 hours by 3-hour infusion on days 1, 3, 5, and 7) and more recently, in a Chinese study with patients receiving relatively lower doses [100 mg/m^2/day days 1-4 followed by 1 g/m^2 every 12 hours, days 5–7 (cumulative dose of 6.4g), with 40mg/m^2 of daunorubicin and omacetaxine mepesuccinate [45]. In the EORTC-GIMEMA study [44], the survival benefit with higher dose cytarabine (42.5% versus 38.7%) was not statistically significant, except in patients aged 15-45 years. In the study from China [45] that included a second randomisation to different cytarabine doses (3g/m^2 vs 1.5g/m^2) in consolidation, a DFS (67% versus 54%, p=0.005) and OS (68% versus. 59%; p=0.014) benefit was observed with the higher dose of cytarabine in induction, even after censoring for transplantation. While the second randomisation did not affect outcomes, the survival benefit with the higher cytarabine dose in induction was restricted to patients who received 3g/m^2 in consolidation.

Since some protocols investigating higher-dose cytarabine in induction (3g/m^2) had de-escalated drug-doses [41,44] or avoided cytarabine [43] during consolidation, the sequential administration of higher-intensity cytarabine may achieve the depth of remission need for cure. This hypothesis was investigated through the randomisation of patients who had achieved remission following higher-dose cytarabine (at a cumulative dose of 24g/m^2), etoposide and daunorubicin (ICE) induction, to a further cycle of ICE or two courses of attenuated ICE that contained a lower, cumulative cytarabine dosage of 500 mg/m^2 [49]. Sequential intensified chemotherapy was more toxic without affecting relapse-free or OS. These results, supported by subsequent HOVON/SAKK [43] and AML-CG [50] studies indicate that

intensified cytarabine-induction is unlikely be advantageous to patients receiving second induction, or consolidation at the same cytarabine dose.

Optimising anthracyclines

Initial studies on daunorubicin investigated a dose schedule of 60-80mg/m^2/day, with a mean of 7 doses (range 2-17) [18] but concerns regarding toxicity resulted in a reduction to 45 mg/m^2/day for 3 consecutive, or alternate days in many protocols, including in the landmark 'DA 3 and 7' study [23]. More recently, 4 randomised controlled trials [51-56] have investigated whether a higher dose of daunorubicin (90mg/m^2) can optimise outcomes (Table 1). In a comparison of 90mg/m^2 with 45mg/m^2, a significant improvement in CR rates was observed following the higher dose, with a similar incidence of haematological toxicity or induction deaths, with improvement in survival outcomes in 3 studies [51-54]. However, in the UK NCRI AML17 trial [55,56] comparison of 60mg/m^2 with 90mg/m^2 in younger patients, a higher 60-day mortality was observed with daunorubicin dose-intensification, without an improvement in OS. Unlike the other studies, AML17 incorporated two cycles of anthracycline-containing induction and therefore, the cumulative daunorubicin dose in the 'lower-dose' cohort was 330mg/m^2, exceeding that in the high-dose, single-induction schedules (270mg/m^2) of the other trials. Nevertheless, and sub-group analysis notwithstanding, a survival benefit is evident with 90mg/m^2 of daunorubicin in patients with *FLT3* internal tandem duplications (ITD) in AML17 [56] commensurate with the ECOG study [53], that additionally reported a benefit in disease with *NPM1* and *DNMT3A* gene variants. Thus, daunorubicin can be used at

60-90mg/m^2 in induction, with a reduction in dose (45 mg/m^2), should re-treatment be required for persistent disease [30].

Idarubicin as a synthetic anthracycline analogue has been of interest as an alternative to daunorubicin [57] but a direct comparison of the two drugs across studies is challenging due to variation in dosage, concomitant and subsequent therapies. A meta-analysis of 1052 patients treated in 5 randomised trials from the 1980s suggests an advantage with idarubicin over daunorubicin in younger patients [58] but the applicability of the conclusions to modern practice is questionable. More recently, higher doses of daunorubicin have been compared with idarubicin: in the JALSG AML201 Study [59], an additional two days of daunorubicin, i.e. 50 mg/m^2/day for 5 days did not improve remission or survival outcomes over idarubicin (12 mg/m^2/day for 3 days). The ALFA-9801 study [60] randomised patients aged 50-70 years to daunorubicin at 80 mg/m^2 for 3 days or idarubicin, 12 mg/m^2 for 3 or 4 days, with a 7-day course of cytarabine. Idarubicin for 3 or 4 days produced superior remission rates (83% and 78% respectively) compared to daunorubicin (70%) but without a benefit to event-free survival or OS. Finally, a Korean study [61] of 3 consecutive days of idarubicin (12mg/m^2/day) and daunorubicin (90mg/m^2/day) too did not identify differences in remission or survival, except in patients with *FLT3*-ITD in whom daunorubicin appeared superior.

Studies of mitoxantrone have included its randomised comparison in combination with cytarabine and etoposide (MAE), against cytarabine, daunorubicin and etoposide (ADE) in the MRC AML12 trial that showed no differences response-rates or early mortality; while the relapse-risk was reduced with MAE (48% versus

57%; p=0.006), DFS survival and OS remained unaffected [40]. A similar lack of difference in OS at 5-years was observed by EORTC-GIMEMA investigators comparing outcomes following daunorubicin, idarubicin or mitoxantrone in induction and consolidation in patients <60 years [62]. Studies in older patients too have been inconclusive: randomised studies by EORTC-HOVON [63] and SAL [64] report improved response-rates with mitoxantrone compared to daunorubicin containing combination induction, but with many confounding variables, no benefit on survival outcomes has emerged.

Three drug combinations

Etoposide

Whether a 3-drug combination could lead to a superior and clinically meaningful anti-leukaemic effect was investigated by the Australian Leukemia Study Group in patients aged15 -70 years through the addition of etoposide to 'DA 3 and 7' [65]. Since recruitment started in 1984, data on cytogenetic risk groups was not routinely collected and variables within the two arms of the study were not evenly matched: the etoposide cohort contained a higher number of patients with APL, more patients with the FAB sub-type M1 received 'DA 3 and 7' and patients in this arm had a higher circulating leukaemic load. With these caveats, the addition of etoposide did not improve CR rates (59% vs 56%) but benefited remission-duration (18 months vs 12 months, p=0.01) without impacting OS. In patients <55 years, the improvement in remission-duration associated with improved OS suggesting the utility of etoposide in younger patients. The role of etoposide when added to DA (ADE) was evaluated

against DA and FLAG-Ida in the NCRI AML15 trial in patients under 60 years [66]. As in the Australian study, overall response-rates including CR and CR with incomplete count recovery (CRi) were similar in the randomised comparison of double-induction with ADE (86%) and DA (84%) although more patients achieved CR/CRi after the first course of ADE (70%) than DA (63%, p=0.002). In a separate comparison, overall response-rates after one course of FLAG-Ida (77%) were higher than ADE (67%, p<0.001), but similar following the second course (86% and 85% respectively). As with etoposide in the Australian study [65], adverse events affecting the gastrointestinal tract were more frequently reported with ADE, but survival outcomes were not different.

Purine analogues

Based on the *in vitro* potentiation of cytarabine cytotoxicity in blasts pre-treated with the purine nucleoside analogue fludarabine [67], and the clinical effectiveness of fludarabine-containing salvage regimens in relapsed or refractory AML [68,69], the MRC AML15 trial investigated fludarabine, cytarabine, granulocyte colony stimulating factor and idarubicin (FLAG-Ida) in induction for patients <60 years of age, compared to ADE [66]. Although a 10% improvement in CR/CRi was observed following one cycle of FLAG-Ida (77%), with the double-induction schedule, this difference was no longer evident after the second treatment-cycle with overall response-rates approaching 85% with both regimens. No differences in toxicity were evident after cycle 1 but haemopoietic recovery was delayed following the second cycle of FLAG-Ida, with more supportive care requirements. This excessive toxicity resulted in less than half the patients entering subsequent randomisation, with 54 patients having to

discontinue therapy entirely. Of interest, the survival of these patients receiving truncated therapy was similar to those treated with ADE or DA and a further 2 courses of HDAC. Others who completed the entirety of intended consolidation following double-induction with FLAG-Ida experienced superior survival (74% versus 54%, p<0.001) even after adjustment for age, white count, cytogenetics and secondary disease. For the entire cohort of FLAG-Ida recipients however, at a median follow-up 5.6 years, the reduction in relapse-risk (38% versus 55% p<0.001,) failed to translate into an OS benefit over standard treatment (44% versus 37%, p=0.2) due to excessive deaths in remission (17% versus 11%, p=0.02).

The effects of cladribine as a third drug with DA during induction (and consolidation) therapy in younger patients has been investigated by the Polish Adult Leukaemia Group (PALG) [70,71]. With cladribine added to a double-induction schedule consisting of 'DA 3 and 7' (60mg/m^2 daunorubicin and 200mg/m^2 cytarabine administered as an infusion) CR rates were equivalent to 'DA 3 and 7', but a greater proportion of patients achieved CR after one cycle of cladribine-containing treatment (64% versus 47%, p=0.0009). The difference in CR rates did not translate into improvements in OS at 3-years, although leukaemia-free survival improved (44 versus 28%, p= 0.05) in patients >40 years of age [70]. A follow-up study compared the addition of fludarabine or cladribine to 'DA 3 and 7' [71]. Here, superior remission rates with cladribine (67% vs 56%, p=0.01) associated with better survival (45% vs 33% p=0.02) at a median of 2.8 years, benefitting patients with poor-risk features: those older than 50 years, with high white counts or adverse karyotype. Although no obvious imbalance in patient demographics or disease characteristics was evident between the study cohorts, outcomes with DA were

lower than in comparable studies [55,56]. The addition of fludarabine (25mg/m^2 daily for 5 days) to DA suggested an OS benefit to patients with an adverse karyotype, with no other difference in outcomes.

Clofarabine (2-chloro-2′-fluoro-deoxy-9-β-darabinofuranosyladenine), a second-generation purine nucleoside analogue has been investigated as a 'third drug' in induction. As with fludarabine in AML15 [66], the addition of clofarabine (10mg/m^2 daily for 5 days) to cytarabine (200mg/m^2 infusion) and idarubicin (cycle 1) and amsacrine (cycle 2) in a HOVON-SAKK study [72] increased the speed of remission and reduced relapse-risk in adults <65 years old, without improving survival outcomes, except in patients with intermediate-risk disease [73]. Greater toxicity was observed in recipients of clofarabine. Clofarabine-containing IC and FLAG-Ida have only been directly compared as second induction in younger patients with high-risk disease following induction cycle one; here, the relapse-free survival and OS favoured FLAG-Ida over clofarabine and daunorubicin [74]. In patients >60 years old, the combination of daunorubicin and clofarabine (20mg/^{2} daily for 5 days) as induction chemotherapy delayed haemopoietic recovery but did not improve remission rates or survival over daunorubicin and cytarabine [75].

Gemtuzumab ozogamicin (GO, GO)

The frequent expression of CD33 on AML blasts and rapid internalisation of antibodies targeting this antigen, provided novel opportunities for therapeutic antibody-cytotoxic drug conjugates. Mylotarg/gemtuzumab ozogamicin (GO), a

humanized anti-CD33 monoclonal IgG4 antibody conjugate with calicheamicin, an antibiotic that cleaves DNA at specific sequences [76] produced response-rates of 30% as monotherapy in relapsed AML [77] and received accelerated approval for managing disease-relapse in patients unsuitable for IC. In the registration study, 9 mg/m^2 of GO was administered intravenously, two weeks apart, based on >75% of target sites being saturated at this dose [76,77]. In the post-marketing study commitment, the effects of adding GO to IC were investigated with a single dose of 6mg/m^2 in 'DA 3 and 7' (45mg/m^2 of daunorubicin) [78]. The control arm consisted of 'DA 3 and 7' (60 mg/m^2 of daunorubicin). Significant differences in toxic death during induction treatment in the experimental arm (17/296 vs 4/300 with DA, p=0.0062) were observed due to haemorrhage and pulmonary events, leading to pre-mature termination of the study. No difference in survival outcomes was evident between the treatment-arms; in subset analysis relapse-free survival in patients with favourable cytogenetics was improved. Concerns regarding the toxicity of GO at doses ≥6mg/m^2 were heightened further by Grade 3 or 4 hyperbilirubinemia (29%) reported in the final analysis [79] of the licensing study. This, combined with the rapidity of CD33 re-expression [80], investigators began to study lower, or fractionated doses of GO in combination with IC [81]. The UK NCRI AML15 study in patients <60 years of age randomised patients to 3mg/m^2 of GO in induction (DA, ADE or FLAG-Ida) and/or consolidation. GO did not impact on remission-rates or 30-day mortality, but supportive care requirements in recipients of GO were greater despite the equivalence in times to haemopoietic recovery [82]. There was a significant reduction to the relapse-risk, but a survival advantage only emerged when the follow-up was extended to 8 years [83]. When risk groups were analysed, an OS benefit was evident in patients with favourable cytogenetics and to a lesser extent, in

intermediate-risk disease. In a separate NCRI trial, AML16 [84], a single dose of GO (3mg/m^2), combined with DA 3+10 or daunorubicin-clofarabine based induction therapy for patients >60 years improved relapse-free survival (21% versus 16% at 3 years, p=0.04), as well as OS at 2 (35% versus 29%) and 3 years (25% versus 20%, p=0.05). This improvement in outcomes was not replicated in an EORTC-GIMEMA study investigating the sequential administration of a single-dose of GO (3mg/m^2) followed by intensive induction with mitoxantrone, etoposide and cytarabine in patients over 61 years of age, and excessive toxicity observed in older patients [85]. In the context of the ALFA-0701-based DA induction schedule however, fractionated doses of GO (3mg/m^2 with a maximum dose of 5mg given on days 1, 4 and 7) positively affected event-free survival (13.6 vs. 8.5 months, p=0.006) in patients between 50-70 years (who received an additional single dose during consolidation cycles), despite the absence of an OS benefit (HR: 0.81, p=0.16) [86,87]. The survival benefit in favourable and intermediate-risk AML including *NPM1* and *FLT3* mutated disease in sub-analysis, but not adverse cytogenetic risk AML, was confirmed in a meta-analysis of 5 frontline trials in which GO was combined with induction IC [83]. However, no improvement in event-free survival was evident in *NPM1* mutant disease with a single dose of GO (3mg/m^2) added to induction (and consolidation) therapy in a German study of sufficient statistical power that included older patients [88]. Here, the reduction in cumulative incidence of relapse was negated by a higher induction-death rate with GO that disproportionately affected older patients (20.4% vs 4%), possibly due to toxicity with idarubicin, cytarabine, etoposide-containing chemotherapy and all-trans retinoic acid (ATRA) in patients >70 years old. The reduction in relapse in recipients of GO associated with a greater

suppression of *NPM1* mutant transcript levels suggesting a potent, deeper anti-leukaemic effect [89].

The importance of the dose of GO (6mg/m^2 vs 3mg/m^2) during induction with DA in younger patients was seen in the NCRI AML17 trial [90]. Near-doubling of mortality with the higher dose was observed at 30 days (7% versus 3%, p=0.02), and 60 days (9% versus 5% p=0.01), with more veno-occlusive disease (5.6% versus 0.5%, p<0.0001) but unchanged response-rates or longer-term survival outcomes [30].

Modulators of chemotherapy

Optimisation of induction treatment has also been investigated through the inclusion of drugs with differentiation-inducing or cytotoxicity-potentiating capabilities. The use of ATRA [91,92] with chemotherapy failed to improve outcomes in randomised studies [93-95], except in sub-analysis of patients with *NPM1*^{mut} disease [94] or low *MN1* expression [96]. These results are however not supported by other studies [97] or meta-analysis [98]. Pharmacological inhibition of the membrane transporter P-glycoprotein (P-gp) to reduce chemotherapy efflux from leukaemic cells [99,100] using valspodar (PSC-833) has been attempted, but the toxicity was problematic and disease control remains unchanged [101-103]. The use of G-CSF either as a priming agent [104] or to ameliorate the toxicity of chemotherapy has also yielded disappointing results: the benefits restricted to DFS survival [106] have not been reproducible [107], and in the post-treatment phase, infection-related complications

remain unchanged despite faster neutrophil recovery and reduced hospitalisation [108]. A meta-analysis of 19 trials including colony-stimulating factor therapy too supports the limited utility of G-CSF in unselected patients receiving IC [109].

Post-remission strategies in AML – consolidation and maintenance

Consolidation-intensity – a determinant of outcomes

The re-emergence of AML in patients achieving CR with IC had underlined the need for additional treatment to eliminate persisting leukaemic cells [22, 105], now called measurable residual disease. Based on studies of the kinetics of leukaemic cell reduction and proliferation in patients achieving remission with 6-MP and 6-methylmercaptopurine in the 1960s, an additional year-and-a-half of therapy of similar intensity had been suggested for disease eradication [22]. Post-remission strategies between 1960 and 1980 generally consisted of combinations of various drugs and doses, including those used in remission-induction, in cyclical rotation and with variable treatment-intensity [20,21]. Toxicity and supportive care requirements were the surrogates that defined the ceiling of treatment-intensification, with consolidation courses arbitrarily accepted as more intensive than maintenance treatment. The benefits of post-remission therapies administered as ‘consolidation’ or ‘maintenance’, or both, in sequence, on the prolongation of remission and survival however remained uncertain.

In 1980, in an ECOG study [110], 146 AML patients (including APL) achieving remission after DAT 3+5 (daunorubicin 60mg/m^2/day, infusional cytarabine 200mg/m^2/day) were randomised to two-years of maintenance therapy with cytosine

arabinoside and 6-thioguanine, or two courses of DAT (daunorubicin 45mg/m^2/day x 2 days, single bolus dose of 100mg/m^2 of cytarabine) as consolidation, followed by the same maintenance schedule. Sequential consolidation and maintenance therapy was associated with greater, non-fatal, haemopoietic toxicity and a non-significant improvement in 2-year DFS (28% vs 14%), but not OS. Thus, while consolidation therapy had the potential to improve disease control, survival was unlikely to improve through mere repetition of previous drugs at attenuated doses. The possibility of acquired cytarabine resistance with conventional-dose (100-200mg/m^2/d) schedules [111-113] provided further impetus for the investigation into alternative, intensified consolidation strategies.

Studies in relapsed acute leukaemia, including AML suggested the feasibility of administering single doses of cytarabine at up to 7.5g/m^2 at weekly or 4-weekly intervals [114]. Subsequently, trials on dose and duration were undertaken [115]: beginning with 3g/m^2 of cytarabine (termed high-dose cytarabine, HDAC) administered 12 hourly for 2, 4, 6 or 12 days, followed by 50% increments in drug-dose. Thus, 3 g/m^2 12 hourly for 6 days was identified as the maximum tolerated treatment, but concerns regarding neurotoxicty in subsequent CALGB studies [116] lead to the schedule being revised to 6 doses of 3g/m^2/dose administered over 3 hours, 12 hours apart on alternate days, and a cumulative dose of 18g/m^2. The dose-finding studies further identified 400mg/m^2/day dose for 5 days as the maximum tolerated cytarabine 24-hour infusional schedule [116]. These doses were then compared against conventional schedules (100mg/m^2/day as a continuous infusion for 5 days) as post-remission consolidation in a randomised study (n=693), with eligibility not restricted by age [117]. Four cycles of consolidation at the 3 different doses (3g/m^2, 400mg/m^2 or 100mg/m^2) were followed by 4 cycles of

daunorubicin (day 1) and subcutaneous cytarabine (100mg/m^2/day twice daily) for 5 days in all patients. Tolerance of the 3g/m^2 by older patients was poor with frequent treatment discontinuation, but DFS at 4-years, 39% (3g/m^2), 25% (400mg/ m^2) and 21% (100mg/ m^2) favoured the highest dose, even after adjusting for age (p=0.003). The OS was 46%, 35% and 31% respectively (p=0.04), with patients <60 years experiencing particular benefit (52%, 40% and 35%, p=0.02). These results supported the use of HDAC as consolidation strategy, particularly in patients receiving conventional-dose cytarabine during induction.

However, since the effects of graded increments in cytarabine dose (i.e. between 400mg/m^2 and 3g/m^2) were not investigated, the necessity of 3g/m^2 as the standard-defining dose of cytarabine in consolidation has been questioned [25,118]. In addition, whether survival could be improved using multi-agent rather than single agent consolidation, and the optimal number of consolidation courses required clarification.

Identifying the standard for intensive consolidation

High-dose cytarabine – studies defining the 'optimal' dose in consolidation

Retrospective sub-analysis of the CALBG study [117] suggested that the survival advantage with HDAC was limited to patients with core-binding factor (CBF) AML (n=57) and AML with *RAS* mutations (n=34) [119]. A benefit to younger patients with favourable cytogenetics (n=218) has also been suggested in a Japanese study

comparing 2g/m^2 of cytarabine for 5 days with multi-agent chemotherapy p=0.050) [120]. In the entire patient cohort however, survival outcomes following the administration of 3 courses of higher-dose cytarabine did not differ from 4 courses of multi-agent chemotherapy.

Results from a German SAL trial also questioned the use of HDAC in consolidation: in this study [121], cytarabine administered 12 hourly for 6 days at 1g/m^2 (at a cumulative dose of 12g/m^2) or 3g/m^2 (36g/m^2 cumulatively), both with mitoxantrone showed no differences in survival outcomes, including in CBF AML. It would be important to highlight that patients in the SAL study received double-induction therapy including 1g/m^2 of cytarabine (12 hourly for 5 days, i.e. a cumulative dose of 10g/m^2) in cycle 2 and therefore, the exposure to cytarabine by the end of consolidation cycle one was 22.8g/m^2 cumulatively in the 'lower' dose cohort, compared to 19.4g/m^2 in the CALBG study [117]. In addition, the cumulative amount of cytarabine per 'high-dose' consolidation cycle (36g/m^2) in the SAL study was double that in the CALGB study (18g/m^2). The permissibility of risk-adapted approaches including autologous and allogeneic stem cell transplantation, and sub-optimal compliance with protocol-directed therapy further compromised evaluation of the SAL study.

Whether an 'intermediate' dose of cytarabine was as effective as HDAC from the CALGB study was investigated in the prospective randomised MRC AML15 trial in younger patients [66]. Here, the results showed no survival difference regardless of whether 3g/m^2 or 1.5g/m^2 of cytarabine was used; a trend towards a reduction in

relapse-risk was observed with the higher dose, with greater supportive care requirements. The 'intermediate' dose of cytarabine is therefore an attractive option, particularly for older patients [30].

Defining the optimal drug combination

Given the risks of resistance with repeated cytarabine exposure [113], the advantages of multi-agent non cross-resistant post-remission therapy has been of interest. The MRC AML9 trial between 1984-1990 recruited patients aged between 1-79 years to compare two cycles of cyclophosphamide, vincristine, prednisolone and 5 days of conventional-dose cytarabine, with amsacrine, azacytidine (substituted subsequently with 200mg/m^2 intravenous cytarabine days 1–5) and etoposide (MAZE/MACE), also two cycles [24]. The relapse-risk at 5 years was reduced with MAZE (66% *v* 74%, p=0.030) but supportive care requirements and toxic deaths were higher, with no OS benefit (37% *v* 31%). In a CALGB study involving older patients, adding mitoxantrone to cytarabine administered by intravenous infusion resulted in greater toxicity than single-agent cytarabine [122]. The dose of cytarabine (500mg/m^2 versus 100 mg/m^2), number of cycles (2 versus 4) and inter-cycle interval (60 days and one month) differed between the combination and monotherapy arms, and no difference in OS was evident with the more intensive approach.

With the emergence of HDAC (3g/m^2) as a 'standard of care', this regimen formed the comparator against which multi-agent consolidation was studied. In the CALGB 9222 study, patients (15-59 years, including APL) in remission after

conventional-dose cytarabine-containing induction were randomised to 3 cycles of HDAC, or in sequence, HDAC, followed by cyclophosphamide with etoposide, and finally, mitoxantrone and diaziquone [123]. Multi-agent consolidation was associated with greater non-haematological toxicity but did not confer a benefit on survival outcomes, overall or in any cytogenetic sub-group. A similar lack of benefit and greater toxicity including delayed haematological recovery was observed in the MRC AML15 study [65] that randomised 1,445 adults <60 years old to cytarabine (3g/m^2 or 1.5g/m^2) or MACE (containing 200mg/m^2/day infusional cytarabine for 5 days), followed by mitoxantrone with cytarabine (1g/m^2 given as a 2 hour infusion twice daily for 3 days). In sub-analysis of patients with adverse karyotype (n=54) however, a survival benefit with multi-agent consolidation (39% *v* 0%, p=0.0004, with p=0.003 for interaction) emerged, despite higher levels of toxicity. The AML15 trial also investigated whether the randomised addition of GO as a single 3mg/m^2dose in consolidation (course 3) improved relapse-risk or survival, but no difference in outcomes was observed [82]. This negative result remains the only randomised study of GO in conjunction with chemotherapy in consolidation, despite its approval for use in this setting. More recently, a French phase 2 randomised trial has compared the effects of consolidation with HDAC against clofarabine and 'intermediate-dose' cytarabine (1g/m^2/day for 5 days) (CLARA) on relapse-free survival in younger patients with intermediate or poor-risk cytogenetics (n=223) [124]. Originally intending to exclusively recruit patients without stem cell donors, the subsequent availability of donors and transplantation confounded interpretation of the results; nevertheless, combination therapy improved 2-year relapse-free survival (53.3% versus 31% p=0.043), even after adjustment for stem cell transplantation. However CLARA was associated with more adverse events and despite the absence

of toxicity-related deaths, conferred no OS benefit, particularly in allograft recipients. The exclusion from the trial, of patients with favourable-risk cytogenetics, who would normally not receive an allograft in first CR, meant that the relapse-risk following CLARA could not be investigated in this sub-group of patients.

Defining the optimal number of consolidation courses

While intermediate or HDAC is now an established consolidation therapy particularly in younger patients, the optimal number of courses remains less well-defined. An alternative approach has been to include autologous stem cell transplantation; usually undertaken after a single consolidation course, this strategy is probably as effective as repetitive courses of consolidation, at least in patients with no adverse-risk cytogenetics [46]. In sequential NCRI trials, AML12 [40], AML15 [66] and AML17 [125] in patients <60 years old, the number of consolidation courses for optimal outcomes following double-induction therapy was investigated. In AML12, of a total of 992 patients completing MACE as first consolidation course, those randomised to a total of 2 consolidation courses went on to receive a higher-dose cytarabine-containing regimen (MidAc). In patients receiving 5 courses, conventional-dose cytarabine with idarubicin and etoposide, followed by MidAc were administered as cycles 4 and 5 respectively. The results demonstrated no difference in relapse-free or OS with the additional course of consolidation; of concern, survival in patients older than 40 years was adversely affected. The absence of benefit with a 5th cycle, consisting of single agent cytarabine (1.5g/m^2) was confirmed in the follow-up MRC AML15 study, further suggested capping intensive consolidation

chemotherapy to a maximum of 2 cycles in younger patients, a decision that CALGB had reached [118], even prior to the availability of data from these studies.

With risk-stratification of AML, it became important to determine whether consolidation treatment could be de-escalated further in disease-sub-types, without impacting on relapse. In the NCRI AML17 trial [125], patients <60 years in remission after two cycles of induction, and classed as having 'favourable'- or 'intermediate'-risk disease (n=1017) based on a weighted scoring-system [126,127], were randomised to either 1 or 2 courses of consolidation predominantly with HDAC ($3gm/m^2$) although a minority received multi-agent chemotherapy. In the entire cohort, the relapse-free survival at 5-years favoured the use of two consolidation cycles (43% versus 36%, p=0.030). Furthermore, a trend towards improved OS (63% versus 56%, p=0.090) was apparent in analysis confined to those receiving HDAC consolidation. These results have to be interpreted on the basis that all patients in this study had received two cycles of induction chemotherapy prior to randomisation but suggest that at least when HDAC consolidation is used, two cycles are to be recommended in those not being considered for allogeneic transplantation.

Consolidation therapy in older patients

Identifying the optimal strategy to consolidate remission in older patients (>60-70 years old) has proven more challenging than in younger patients due to poor tolerance of treatment-intensification [24, 117]. In addition, whether the frequent presence of poor-risk features including adverse cytogenetics and secondary AML in

older patients can be overcome through repeated administration of less, or more intensive post-remission strategies requires clarification.

In the MRC AML11 trial [128], older patients achieving remission after two courses of DAT (2+7 and 2+5) were randomised to a further cycle of (DAT 2+7), i.e., a total of 3 cycles, or two cycles of COAP containing cyclophosphamide, vincristine, conventional dose subcutaneous cytarabine and prednisolone) with an intervening cycle of DAT 2+7, i.e. a total of 6 treatment cycles. Disease control and OS were not improved with additional treatment. Subsequent studies failed to demonstrate any advantages with 4 cycles of multi-agent chemotherapy compared to 3 (AML14) [103], or indeed 3 cycles over 2 (AML16) [26]. As mentioned previously [122], treating older patients with two cycles of intensified multi-agent consolidation with higher doses of cytarabine compared to 4 cycles of cytarabine monotherapy at conventional (100mg/m^2) confers no survival advantage. Whether doses of 1g/m^2 of cytarabine will be more effective than standard doses, or no consolidation therapy in older patients is unclear.

Maintenance therapy

The intensity of therapies aiming to ‘maintain’ remission is operationally less than with consolidation regimens. Traditionally, maintenance genotoxic treatments were scheduled to commence at the time of marrow regeneration in-between courses of intense regimens, or as ‘stand-alone’, long-term treatment following the completion of intensive therapies but fell out of favour due to no definitive improvement in OS [reviewed in 129]. An interest in maintenance therapies has been re-invigorated

recently with new approaches including modulators of immune function, hypomethylating agents or as will be described subsequently, kinase inhibitors.

One of the earlier approaches to maintenance following IC was immunotherapy in CR with subcutaneous injections of irradiated autologous blasts and BCG injections [130,131]. Although statistically underpowered, these studies promoted enthusiasm for immunomodulatory drugs such as interferon or interleukin-2. Interferon as maintenance therapy [132,133] however failed to improve disease control or survival. In contrast, low-dose IL-2 plus histamine dihydrochloride improved DFS [134] and received regulatory approval, but uptake remains limited. Additional agents of promise include the androgen norethandrelone [135]. Given concomitantly with IC and with 6-MP and methrotrexate maintenance, 5-year OS in older patients improved with norethandrelone (26.3% versus 17.2% respectively). The mechanism of action is unclear, and the limited availability of the drug may preclude wide-spread use. Recently, the oral formulation of the hypomethylating agent azacitidine (CC-486), administered as maintenance therapy following IC in patients ≥55 years of age has demonstrated an OS improvement (24.7 months compared to 14.8 months with placebo; $p<0.001$), with an acceptable safety profile [136].

The success of CC-486 is not unexpected: the NCRI AML16 trial in patients >60 years had investigated 12 months of subcutaneous azacitidine (75mg/m^2 daily as 5-day cycles at intervals of 6 weeks) following IC [26]. Although no improvement in OS was evident for the entire cohort, azacitidine associated with improved 5-year

survival in two patient sub-groups: 1. those in whom measurable disease was undetectable by flow-cytometry following intensive induction [137] and 2. patients who had been randomised to just 2 courses of chemotherapy [26]. Patients receiving 3 courses had no benefit from azacitidine maintenance. The HOVON97 trial too has reported improved DFS (64% versus 42% at 12 months) with subcutaneous azacitidine (50mg/m^2 for 5 days) as 1-year of maintenance therapy following IC in older patients [138]. The differences in pharmacokinetic and pharmacodynamic properties between CC-486 and parenteral azacytidine [139] may confer greater potency to the oral drug which translates into a survival benefit.

Risk-adapting intensive therapy in AML

Although the existence of AML sub-types was recognised over 100 years ago [1,2,5], the heterogeneity in post-treatment responses amongst disease sub-groups was described in the 1960s [140,141]. Advances in conventional karyotyping provided in part, a biological rationale for the variation in outcomes [32-34], with the identification of the clinical efficacy of retinoic acid in APL with t(15;17) [91] highlighting the need to adapt therapeutic strategies to AML sub-type. With increasing genetic variants now recognised in AML, disease re-classification continues to evolve alongside efforts to identify drugs against 'actionable' targets [35-37]. Therapeutic decisions can thus be adapted to biological information at diagnosis [28]. Another strategy for risk-stratifying AML relies on measuring treatment-responses at sensitive levels in patients in CR: unique gene or protein-expression for detecting 'measurable residual disease' (MRD) at pre-defined time-points during treatment can serve as a surrogate

for longer-term responses [142-144]. Thus, in patients predicted to have a higher relapse-risk based on MRD, treatment-intensification could enable a 'real-time' risk-adapted approach. There however remain challenges to the standardisation of techniques in characterising and quantifying MRD; these, combined with differences in treatment protocols, limit the generalisation of results across studies [142-144].

Intensive combinations and 'actionable' genetic sub-types of AML

Examples of adapting intensive strategies to improve outcomes in sub-types of AML include the use of GO and HDAC in patients with CBF-AML described previously. In these patients, the quantification of disease-transcripts following therapy can be used to predict the relapse-risk [145,146] and molecular stratification through *KIT* or *FLT3* analysis [147-150] can inform decisions regarding integrating tyrosine kinase inhibition with IC [150-152].

The utility of identifying 'actionable' AML sub-types has been confirmed by superior survival outcomes observed in patients with AML with *FLT3* variants (ITD or tyrosine kinase domain mutations) treated with midostaurin, a small molecule multi-kinase inhibitor administered in sequence with 'DA 3 and 7' and HDAC [28]. Midostaurin-containing treatment resulted in a 22% reduction in death (hazard ratio for death, 0.78; one-sided p = 0.009) compared to placebo, in patients <60 years old, regardless of the mutant to wild-type *FLT3* ratio. The statistical significance for difference in survival was lost when patients were censored for allogeneic stem cell transplantation (4-year OS 63.7% versus 55.7% with placebo), nevertheless

midostaurin has been approved for use in all age-groups in conjunction with anthracycline and cytarabine-containing chemotherapy, and as maintenance treatment.

More recently, drugs with the ability to attenuate signalling pathways critical to leukaemia cell survival have been shown to result in clinically and statistically meaningful improvements in survival, when combined with non-IC or hypomethylating agents. The newer drugs include inhibitors of the pro-survival protein Bcl-2 (venetoclax) [153] and oncometabolite-generating mutant IDH1 (ivosidenib) [154] and IDH2 (evasidenib) [155] proteins, and appear to benefit distinct genetic AML sub-types. A logical extension of these data is to investigate outcomes after combining these drugs with IC. An early-phase investigation of the dosing schedule of venetoclax with cytarabine and idarubicin containing IC (CAVEAT) in older patients has highlighted the potential for haemopoietic toxicity, particularly affecting the platelet count [156]. Overall response rates were 72%, and 97% in *de novo* AML indicating potential anti-leukaemic benefits of administering venetoclax around IC. Likewise, the use of ivosidenib or evasidenib with IC in younger patients with mutant *IDH1* or *IDH2* AML was associated with manageable toxicity, with no excess non-haematological adverse events [157]. Thus, the incorporation of small molecule drugs [156-158] with IC could be a promising risk-adapted curative strategy in AML.

Intensive drug-delivery platforms for secondary AML

Secondary AML evolving from an antecedent myelodysplastic syndrome or myeloproliferative neoplasm or occurring after previous genotoxic therapy (t-AML) is poorly responsive to conventional IC [73,140,141]. In a minority of t-AML patients with CBF lesions, disease control and survival following conventional intensive treatment is comparable to *de novo* CBF AML; in others, the outlook remains dismal [30]. Recently, CPX-351 (VYXEOS), a liposomal encapsulation of cytarabine and daunorubicin (in a 5:1 synergistic molar ratio) was compared to 'DA 3 and 7' in patients aged 60-75 years with secondary or t-AML [27]. CPX-351 improved remission rates (47.7% versus 33.3%; two-tailed p=0.016), early mortality and OS (9.56 versus 5.95 months, one-tailed p=0.003), with no excess non-haematological toxicity despite delayed haemopoietic recovery. Estimated OS at 1- and 2- years (41.5% and 31.1% with CPX-351 versus 27.6% and 12.3% with DA 3 and 7 respectively) favoured CPX-351, and the statistical difference in survival is maintained at 5-years. A greater proportion of patients treated with CPX-351 was able to receive allogeneic stem cell transplantation, with exploratory analysis suggesting a post-transplant survival benefit in these patients. Whether CPX-351 will confer superior outcomes in other AML sub-types in older patients compared to GO-containing intensive induction therapy is currently being investigated.

Measurable residual disease (MRD)-adapted therapy – genetic strategies

The use of MRD measurement to identify patients at higher risk of relapse despite morphological remission potentially enables the risk-adaptation of subsequent

treatments [142-144]. The utility of MRD monitoring in CBF-AML has been mentioned previously, but this disease sub-type constitutes a relatively small proportion of AML cases. Defining a reliable molecular genetic marker for MRD detection and its standardised measurement can be difficult but in younger patients with AML characterised by the nucleophosmin1 (*NPM1*) mutation [159], the persistence of *NPM1*-mutated transcripts in blood after the second cycle of anthracycline-containing induction chemotherapy associates with a higher relapse-risk (82% versus 30%, p<0.001) and lower survival (24% versus 75% p<0.001) at 3-years, even after adjustment for concomitant genetic drivers of prognostic significance [160]. Thus, based on MRD analysis, majority of younger patients with *NPM1*-mutant disease are likely to be cured with standard HDAC consolidation, without the need for treatment intensification and allogeneic stem cell transplantation.

Measurable residual disease (MRD)-adapted therapy – multi-parametric flow-cytometry (MFC)-based strategies

The frequency and fidelity of genetic markers to reliably inform relapse-risk is currently restricted to a small proportion of patients, but the ability to identify a unique leukaemia-associated immunophentype (LAIP) in almost all AML patients, provides an alternative strategy for MRD detection [142-144]. In patients <60-years old with AML and wild-type *NPM1*, detection of MRD by MFC after the second cycle of induction therapy confers a higher relapse-risk (HR 1.88, p<0.001) and lower survival (HR 1.77, p<0.001) [161]. In patients >60 years, the detection of MRD by MFC after

the first cycle of induction therapy is predictive of a 12% higher relapse-risk and 16% difference in OS at 3 years [162]. In both patient groups, MRD measurements provide opportunities to select patients for early intervention with novel therapies or allogeneic stem cell transplantation.

Too early to draft the obituary for IC?

The identification of prognostic and predictive biomarkers in AML, and newer anti-leukaemic therapies has renewed optimism for prolonging survival and cure in patients. The early attainment of CR is critical for better outcomes, and for an overwhelming majority of patients, IC currently represents the best chance of achieving rapid CR. The success of tyrosine kinase inhibition in CML [163], or arsenic trioxide-ATRA combinations in APL [164] has led to enthusiasm for therapeutic strategies not reliant on IC to cure AML. The greater repertoire of cellular and molecular drivers in AML relative to CML or APL, however appears to confer context-dependent redundancy or subtlety that facilitates disease-escape, to explain the absence of durable responses to monotherapy with current small molecule drugs against 'actionable' targets in AML. Thus, while it may become possible to cure subsets of AML, for example, patients with *NPM1* mutant disease using non-intensive non-genotoxic therapy [165,166], analogous to the current therapy of low-risk APL [164], for the foreseeable future, a backbone of IC will remain the mainstay of cure for most AML patients.

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Table 1. Summary of randomised clinical trials investigating the effects of daunorubicin-intensification in induction therapy. The daunorubicin (**D**) dose in the experimental arm of each study was 90mg/m^2, but the cytarabine (**A**) doses and administration schedules for both drugs ('inf.' Indicating continuous intravenous infusion over 24 hours) between the experimental and control arms (shown here) were identical.

Study	Median age (range)	n	Median follow-up (months)	End-point(s)	Drugs	CR	EFS	OS	Sub-group benefit
HOVON-SAKK-AMLSG [51]	67 (60-83)	813	40	EFS	**D**45 mg/m^2 d1-3 **A**200 mg/m^2 inf. d1-7	64% vs 54% (p = 0.002)	2y 20% and 17% (p = 0.12)	-	age (60-65y) favourable risk CG
ECOG E1900 [52,53]	48 (17-60)	657	80.1	OS	**D**45 mg/m^2 d1-3 **A**100 mg/m^2 inf. d1-7	70.6% vs 57.3% (p<0.001)	-	4y 39% vs 41% (p = 0.001)	age (<50y), all CG risk - groups, *NPM1*^{mut}, *DNMT3A*^{mut}, *FLT3* ITD
Cooperative Study Group A for Hematology [54]	43 (15-60)	383	52.6	CR, OS, EFS and RFS	**D**45 mg/m^2 inf. d1-3 **A**200 mg/m^2 inf. d1-7	82.5% vs 72% (p = 0.014)	5y 46.8% vs 34.6% (p = 0.03) RFS unchanged	5y 40.8% vs 28.4% (p = 0.03)	intermediate-risk CG
NCRI AML17 [55,56]	53 (16-72)	1206	28	OS	**D**60 mg/m^2 d1,3,5 **A**100 mg/m^2/12 h d1-10	73% vs 75% (p = 0.6)	-	2y 59% vs 60 (p = 0.16)	*FLT3*-ITD

OS: overall survival; EFS: event-free survival; CR: complete remission rate; RFS: relapse-free survival; CG: cytogenetic, PS: Karnofsky performance score