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The FLAM regimen: revisiting time sequential induction therapy for patients with poor-risk acute myeloid leukemia

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Although the last 30-40 years have seen a steady rise in long-term survival rates for younger patients with acute myeloid leukemia (AML), these improved outcomes are largely attributable to better supportive care strategies and the wider accessibility of allogeneic stem cell transplantation. Induction chemotherapy has changed very little in that time, with the trusted combination of anthracycline and cytarabine (usually the standard '7+3' regimen) remaining the mainstay, this despite many attempts to improve upon it by changing the anthracycline (e.g. using idarubicin instead of daunorubicin[1]), escalating the cytarabine dose, or adding a third drug (such as etoposide[2] or 6-thioguanine[3]). In recent years, intensified dosing of daunorubicin to 90 mg/m^2 for three days has been suggested as a new standard of care,[4] although recently published data have questioned the superiority of this regimen over 60 mg/m^2 dosing.[5] Unfortunately, recent improvements in overall survival (OS) described through the addition of the immunoconjugate gemtuzumab ozogamicin to induction therapy do not appear to extend to patients with adverse risk disease.[6] For patients with poor-risk disease features, including those with secondary AML (related to therapy or arising from an antecedent myeloid neoplasm) or adverse cytogenetics, the prognosis remains particularly bleak: with conventional chemotherapy, complete remission (CR) is achieved in fewer than 50% of cases (compared to 80% of patients with non-poor-risk disease), and long-term survival remains at around 10%.[7]

Several approaches have been adopted to improve responses to induction therapy that involve maneuvres designed to recruit leukemia cells synchronously into the cell cycle and thus render them potentially more sensitive to cell cycle-specific cytotoxic agents such as cytarabine. One study in younger adults with AML suggested that the cytotoxicity of induction chemotherapy could be enhanced in this way through the concurrent addition of granulocyte colony stimulating factor (G-CSF), although reported improvements in disease-free survival did not translate into an OS benefit.[8] 'Timed sequential therapy' (TST) refers to treatment strategies, arising originally from *in vitro* and animal models, in which a second course of chemotherapy including cell cycle-specific agents is given in the very close aftermath of first induction treatment to best exploit the synchronously-cycling proliferative state that appears to peak in residual leukemia cells approximately 6-10 days after initial exposure to chemotherapy. To date, the most encouraging results for TST have been in childhood AML where the Children's Oncology Group reported superiority of repetitive DCTER induction courses, the second being administered ten days after the first cycle, over standard timed induction therapy with improved 3-year event-free (42% *vs*. 27%) and disease-free (55% *vs*. 37%) survival rates.[9] Translating these results into adult AML has proved challenging, however, with a trade-off needing to be made between efficacy and toxicity. The same repetitive DCTER regimen proved too toxic for adults when given at an interval of 6-10 days, although the German Acute Leukaemia Group did demonstrate the feasibility of delivering a dose-dense induction regimen comprising two sequential cycles of S-HAM (high-dose cytarabine and mitoxantrone) over 11-12 days with an encouraging overall response rate of 83% and an acceptable toxicity profile, along with a relatively short duration of neutropenia.[10] Although attempts to develop optimal time sequential schedules now span almost four decades, TST has yet to be fully embraced into the mainstream of AML therapy.

The sequential FLAM regimen represents an alternative approach to delivering TST through exploiting the cell cycle-regulatory properties of flavopiridol. Flavopiridol is a semi-synthetic flavonoidal alkaloid, initially isolated from the bark of an Indian tree (*Dysoxylum binectariferum*) used in herbal medicine. It is a potent pan-cyclin-dependent kinase (CDK) inhibitor that functions through several distinct mechanisms, including the induction of apoptosis through blockage of cell cycle progression and release of E2F, the prevention of activation of RNA polymerase II which leads to a downregulation of expression of genes that promote leukemic cell proliferation, such as *Cyclin D1*, and the blockage of tumor-promoting pathways mediated by STAT3.[11] Flavopiridol entered clinical trials in the late 1990s and has been studied across a range of hematologic malignancies. Particular evidence of its single-agent potency came in chronic lymphocytic leukemia (CLL) where significant clinical responses, often accompanied by acute tumor lysis syndrome, were reported in patients with poor-risk (including *p53*-deleted) fludara-

bine-refractory disease.[12]

In AML, *in vitro* studies showed that blasts surviving initial flavopiridol-induced cytotoxicity enter synchronous cell cycling; an increased proportion are noted to be in S phase after 2-3 days, a situation that persists for a further 3-4 days during which time synergistic cell killing can be demonstrated when S phase-specific agents such as cytarabine are added in a time-sequential manner.[13] These observations provided the impetus for the 'FLAM' regimen, in which flavopiridol is administered by rapid infusion for three days for the dual purpose of initial cytoreduction and enhancing the cell-cycle progression of the remaining leukemia cells, followed three days later by cytarabine and mitoxantrone. The Johns Hopkins group have conducted a series of single center studies of FLAM induction, initially establishing a flavopiridol MTD of 50 mg/m^2 in a phase I study in which clinical responses were associated with downregulation of targets including RNA polymerase II and cyclin D1.[14] In a subsequent phase II study in 62 patients with poor-risk, mainly relapsed/refractory AML, a CR rate of 75% in a setting of acceptable, reversible toxicity was seen in patients with newly-diagnosed secondary disease or first relapse.[15] These encouraging observations in secondary AML prompted a further phase II study of FLAM, this time restricted to patients with newly-diagnosed AML; a 67% CR rate was reported in a group of 45 patients with significant poor-risk disease features, including a high median age (61 years), secondary AML (37 patients), and adverse cytogenetics (24 patients).[16]

In this issue of *Haematologica*, Zeidner and colleagues report results of the first multi-center, randomized trial of the FLAM regimen in newly-diagnosed AML.[17] In this study, 165 patients at 10 centers were randomized on a 2:1 basis between sequential induction with FLAM and conventional '7+3'. This was undoubtedly a 'poor-risk' group of patients; cases with core binding factor fusions were excluded, the median age was 60 years, and many patients had adverse cytogenetics (57%) or secondary AML (47%). The primary end point for the study was achievement of remission following induction therapy, and the headline difference in CR/CRi rate between 70% of FLAM patients and only 46% of '7+3' patients is impressive. A confounding factor in the straightforward interpretation of the results is that patients allocated to the conventional therapy arm were permitted to receive an additional optional '5+2' course of daunorubicin and cytarabine in the setting of persistent bone marrow disease activity at day 14, a practice that continues to divide the opinions of those physicians who manage AML.[18] Twenty-four (44%) patients in the '7+3' arm had residual leukemia at day 14 and 10 of these patients subsequently went on to achieve CR (interestingly, these included 4 of 11 patients who were not actually given the additional '5+2'). Taking these maneuvres into account, the overall response rate in the control arm was actually 57%; not statistically inferior to FLAM. No differences in OS or event-free survival were noted between the arms, although the study was not powered to detect such differences and there was considerable variation in post-induction treatment strategies.

Despite these caveats, the use of 'sequential induction' with FLAM clearly holds considerable promise, particularly for patients with adverse risk AML; a greater number of secondary AML patients (60% *vs*. 35%) achieved CR with FLAM than with '7+3' in the Zeidner study and, cumulatively, 66% (105 of 158) of secondary AML patients treated across four phase II studies have now achieved CR following FLAM induction. In the challenging setting of secondary AML, these remission rates are superior to those recently reported in a phase III study of amonafide/cytarabine combination therapy,[7] and are comparable with the promising liposomal cytarabine/daunorubicin formulation CPX-351,[19] currently the subject of an ongoing phase III study in secondary AML. Given that several previous AML induction regimens, such as FLAG-Ida in the MRC AML15 study,[20] have been associated with increasing remission rates without significantly impacting on survival, we should avoid over-interpreting these encouraging results. There is, however, clear justification for the larger scale phase III testing of FLAM induction in newly-diagnosed adverse risk AML, employing rigorous trial design, including uniformity of approach to the 'day 14 question', standardized post-induction treatment, and a survival-based primary end point. As one of the so-called '1st generation' of CDK inhibitors, flavopiridol has a broad CDK-inhibitory profile and a relatively wide range of targets; should phase III testing of FLAM yield positive results, there will be a strong rationale for further studies to explore the incorporation of a new generation of more highly targeted cell-cycle inhibitory agents into time sequential AML regimens.[21]

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