

Azacitidine: 10 Years Later¹

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Azacitidine has been undergoing clinical trials for almost 20 years and is internationally considered to have a useful place in the treatment of acute nonlymphocytic leukemia. However, its role in the various combinations for induction, intensification, maintenance, or relapse regimens has not yet been clearly defined. This review outlines the last 10 years' clinical experience with the drug in acute nonlymphocytic leukemia, analyzes what critical information has yet to be obtained, and suggests what phase III trials may still be feasible to gather that information. [Cancer Treat Rep 71:737-746, 1987]

Azacitidine is a ring analog of the natural pyrimidine nucleoside cytidine synthesized in 1964. Clinical trials began in Europe in 1967 and in the United States in 1970. Preclinical information and results of early clinical trials were comprehensively discussed in a previous review (1). In that article the authors described a drug with "consistent antitumor activity in patients with acute myelogenous leukemia resistant to previous treatment" and "an overall response rate of 36% with 20% complete remissions... in... acute myelogenous leukemia... of adults and children." Furthermore, the median duration of remission with azacitidine was noted to be "encouragingly long." In summary, Von Hoff et al (1) suggested four potential directions for the development of azacitidine as an antileukemic agent, as follows: (a) to define the use of azacitidine in the first-line treatment of acute nonlymphocytic leukemia (ANLL); (b) to explore in a controlled manner the use of infusions of the drug versus other modes of administration; (c) to examine the use of sc administration, since this would be a more desirable route, especially in children; and (d) to define the role of azacitidine in consolidation, maintenance, and late intensification regimens.

The review also suggested two other areas of exploration: (a) to develop the early observations of the protective effect of azacitidine on mice exposed to lethal x-irradiation; and (b) to expand animal experiments using azacitidine with cytidine as an antidote, to improve the therapeutic index of the drug.

After a brief update on preclinical issues, this paper will examine the last 10 years' clinical development of azacitidine, summarize how these six recommendations have been pursued, and finally examine what still needs

to be done to establish the role of azacitidine in the treatment of ANLL.

MECHANISM OF ACTION

Several steps in cellular metabolism are affected by azacitidine and previous papers (1-5) have dealt with these in some detail. The accompanying paper on the biochemistry of azacitidine reviews these data at length (6) and only the main features are summarized here.

Azacitidine is transported into the cell and sequentially phosphorylated by the same mechanisms as for cytidine and uridine. 5-Azacitidine triphosphate is incorporated into RNA, disrupting the synthesis and processing of both nuclear and cytoplasmic species and leading to inhibition of protein synthesis. It is also incorporated to a lesser extent into DNA leading to inhibition of DNA synthesis.

The most interesting development at the biochemical level since previous reviews (1-3) has been the increasing use of azacitidine as a "laboratory tool" to examine the role played by DNA methylation upon gene expression and activation. After incorporation into DNA, azacitidine noncompetitively inhibits DNA methyltransferase, causing a block in cytosine methylation in newly replicated DNA (7-12). There is now a large body of evidence, which has been comprehensively reviewed (13-18), indicating that alterations in the pattern of cytosine methylation are causally related to gene expression and cell differentiation. This hypomethylation of DNA is thus probably the mechanism underlying the differentiating activity of azacitidine (19) and may be related to its tumorigenic effects (20-27).

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PHARMACOKINETICS

The initial study by Troetel et al (28) had shown that the plasma half-life of total radioactivity (parent drug plus metabolites) after iv administration was about 3½ hours. Subsequently, Israili et al (29) obtained similar results for total radioactivity. In addition, they identified at least two metabolites and/or decomposition products of azacitidine in plasma. They also showed that levels of the parent drug declined much more rapidly than those of total radioactivity so that in one patient levels of azacitidine were < 2% of total radioactivity after 30 minutes. Renal clearance of radioactivity varied from 74 to 210 ml/minute in the five patients in Israili's study and most of the administered radioactivity was excreted via the urine.

From preclinical studies it is known that azacitidine is rapidly deaminated by cytidine deaminase which is

present in serum as well as in the liver, granulocytes, and gastrointestinal epithelium. This enzyme can be inhibited both in human leukemic cells in vitro and in the mouse in vivo by tetrahydrouridine (30,31). The relative role and clinical significance of deamination in the metabolism of azacitidine in humans as compared with other enzymatic degradation pathways cannot be determined until a specific and sensitive assay for azacitidine is developed.

RESULTS OF CLINICAL TRIALS

This paper will only review those trials of azacitidine conducted in ANLL of adults and children. Phase II trials in several solid tumors including colorectal, breast, lung, melanoma, head and neck, and renal carcinoma did not reveal adequate activity to justify further investigation in these tumor types. A summary of these trials is shown in table 1.

TABLE 1.—AZA in solid tumors (phase II trials only)*

Disease site (group)	Dose (mg/m ²)	Schedule	Tumor	No. of —		Ref No.
				Evaluable patients (No. entered)	Responses	
Advanced gastrointestinal cancer (Mayo)	750	Daily × 5 or daily × 10	Colon cancer	27	1 PR	32
			Pancreatic cancer	1	1 < PR	
			Gastric cancer	1		
Breast cancer (RPMI)	60 100	Daily × 10 then 2 ×/wk		27	2 PR	33
Broad phase II (COG)	60	Daily × 10†	Breast cancer	29	6 PR	34
			Lung cancer	24	1 PR	
			Large cell cancer			
			Intestinal cancer	26	0	
			Melanoma	12	0	
			Hodgkin's disease	6	1 PR	
			NHL	8	2 PR	
			Other cancer	59	9 PR	
Broad phase II (SWOG)	225	Daily × 5 every 3 wks	Colon cancer	15	0	35
			Renal cancer	17	0	
			Breast cancer	14	0	
			NSCLC	12	2 PR	
			SCLC	14	1 PR	
			Testicular cancer	4	2 PR	
			Melanoma	13		
			Other cancer	85		
Broad phase II (SEG)	150	2 ×/wk for 6 wks	Breast cancer	6 (8)	0	36
			Renal cancer	10 (12)	1 PR	
			Colon cancer	7 (9)	0	
			Melanoma	10 (10)	1 < PR	
			SCLC	2 (3)	0	
			NSCLC	26 (33)	1 PR; 1 < PR	
			H and N	14 (14)	0	
			Other cancer	16 (24)	0	
Broad phase II (Mt. Sinai)	30-120 25-200	24-hr CIV + Pyrarazofurin	Other cancer	6	0	37
Sarcoma	150-200	Daily × 5	Osteogenic sarcoma	7	0	38
			Ewing's sarcoma	7	0	

*AZA = azacitidine; Mayo = Mayo Clinic; RPMI = Roswell Park Memorial Institute; COG = Central Oncology Group; SEG = Southeastern Cancer Study Group; Mt. Sinai = Mt. Sinai Hospital; CIV = continuous iv infusion; NHL = non-Hodgkin's lymphoma; NSCLC = non-small cell lung cancer; SCLC = small cell lung cancer; H and N = head and neck cancer; PR = partial response; and < PR = objective response not meeting criteria for PR.

† = 1.6 mg/kg.

TABLE 2.—AZA alone in ANLL*

Group or institution	Dose (mg/m ²)	Schedule	No. of—									Ref No.
			Evaluable patients			CRs			PRs			
			T	RI	Rf	T	RI	Rf	T	RI	Rf	
SWOG†	100	Every 8 hrs × 15	50	50		10	10		5	5		39
	750	Single iv										
	75	4-hr infusion every 6 hrs × 20										
SWOG†	250	Iv every 4 hrs × 3	101	101		8	8					40
	300	Daily CIV × 5										
	200	Daily CIV × 5										
	200	Daily CIV × 7										
	150	Daily CIV × 10										
MDA	150-600	Daily iv × 5	18	18		3			4			41
MSKCC	120-300	Daily iv × 5	6			0			1			42
BCRC	200-250	Daily iv × 5	18	14	4	5	3	2	1			43
WCG	60	Every 8 hrs × 15	6			0			2			44
	100	Every 8 hrs × 15	23			3			4			
	180	Daily iv × 5	5			1			0			
	300	Daily iv × 5	21			2			7			
SEG	150-200	Daily CIV × 5	45			11			4			45
University of Chicago	200	Daily iv × 5	15	9	6	5	3	2	2	2	0	46
MMC	150	Daily CIV × 5	10		10	3		3	3		3	47
ECOG	50-90	3-hr infusion every 8 hrs × 15	3	—	3	0	—	—	0	—	—	48
Subtotal			321	174	23	51	24	7				
CCSG	150-200	Daily iv × 5	14		14	5		5	1		1	49
Total			335		56							

*MDA = M. D. Anderson Hospital and Tumor Institute; MSKCC = Memorial Sloan-Kettering Cancer Center; BCRC = Baltimore Cancer Research Center; WCG = Western Cancer Study Group; MMC = Maine Medical Center; ECOG = Eastern Cooperative Oncology Group; CCSG = Children's Cancer Study Group; T = total No. of evaluable patients who were considered by the investigators to have had an adequate trial of azacitidine; RI = No. of patients relapsed after induction on standard therapy; Rf = No. of patients refractory to standard induction regimens; and CR = complete response.

†Breakdown by infusion schedule included all leukemia types, so only total for ANLL given here.

Azacitidine as a Single Agent in ANLL

Table 2 shows the azacitidine single-agent data from trials sponsored by the National Cancer Institute as of June 1985. Some of these studies included acute lymphocytic leukemia and blast crisis of chronic myelocytic leukemia, but the data presented in table 2 are only for ANLL. The single-agent data base shown in the earlier review (1) has now been expanded from a total of 200 patients to a total of 335. The overall complete response rate has fallen from 20% to 16.7%. As can be seen, the pediatric data base has not been expanded in single-agent use since 1976. Furthermore, the largest study (40) observed only eight complete responses among 101 evaluable patients who received an adequate trial of azacitidine.

Effect of Schedule on Activity of Azacitidine

Azacitidine was active on both daily and intermittent schedules against L1210 leukemia. While its predominant effects were in the S phase, its action was not

limited to the S phase (3). In early clinical trials three schedules were used: daily × 5; every 8 hours × 15; and daily continuous infusion × 5-7 days. Because of the instability of the drug most of the continuous-infusion studies actually employed a 3-hour infusion every 8 hours (40,45,47). [There has been conflicting advice in various publications about the stability of azacitidine in iv solutions. This issue has recently been reviewed (50) and a comparative analysis of decomposition rate in several infusion solutions carried out (51). The recommendation from these studies is that the reconstituted solution be further diluted within 30 minutes in lactated Ringer's injection, USP, which provides optimum pH for stability. Under these conditions there will be 90% of intact drug remaining after 2 hours and 80% after 6 hours. Therefore, the dilute solution should be infused over ≤ 2-3 hours.]

Table 3 summarizes the response rate in acute leukemia according to the three schedules used in the initial trials. Since data are pooled from all the trials in table 2, the analysis includes not only patients with ANLL

TABLE 3.—Effect of schedule on AZA response rate*

Dose (mg/m ²)	Schedule	No. of evaluable patients	Response rate (%)	
			CR	PR
150-500	Daily × 5	122	21	17
50-100	Every 8 hrs × 15	110	24	8
150-300	Daily CIV × 5-7	138	23	9

*Data are pooled from all the trials in table 2; therefore Nos. include not only patients with ANLL but also patients with acute lymphocytic leukemia and blast crisis of chronic myelocytic leukemia.

but also patients with acute lymphocytic leukemia and blast crisis of chronic myelocytic leukemia. Using this form of analysis to achieve large denominators, the complete response rate does not vary between any of these schedules. However, the question of schedule dependence has not been addressed in an appropriate prospectively randomized trial with statistically defined endpoints.

It is difficult to assess from pooled data whether toxicity is schedule-dependent. The Southwest Oncology Group (SWOG) study (40) tested several different schedules sequentially. Dose and schedule were altered de-

TABLE 4.—AZA: 2-drug combination regimens in remission induction of ANLL*

Group or institution	Regimen	Dose (mg/m ²)	Schedule	No. of—									Ref No.
				Evaluable patients			CRs			PRs			
				T	RI	Rf	T	RI	Rf	T	RI	Rf	
<i>Adult</i>													
BCRC	AZA MGBG	33 100	Every 8 hrs × 15 Daily CIV × 5	8	4	4	0			0			52
BCRC	AZA PF	150-250 7.5-30	Daily × 5 Daily × 1	16			2		2	1	1		53
SEG	AZA PF	150 25-150	Daily CIV × 5 Single iv	27			3			4			54
SEG	AZA TGdR	150 300	Daily CIV × 5 Daily × 5	81	81		16	16		5	5		55
CALGB	AZA Zorubicin	50 75	3-hr infusion every day × 5 Daily × 3	29	10	19	8	7	1				56
SEG	{ AZA AMSA	112-200 75-150	{ Daily CIV × 4 Daily × 4 }	27		27	3		3				44
	{ AZA AMSA	200 150	{ Daily CIV × 4 Daily × 4 }	53		53	7		7	5		5	57
ECOG	AZA AMSA	150 150	Daily × 5 Daily × 5	18	12	6	7	7	0				58
BCRC	AZA VP-16	50 75	Every 8 hrs × 15 Daily × 5	14	10	4	0			0			59
NWU	AZA AMSA	150 150	Daily × 5 Daily × 5	16	4	12	1	0	1				44
DFCC	AZA VP-16	300 200	Daily × 2 Daily × 3	13		13	0			0			44
<i>Pediatric</i>													
DFCC	AZA VP-16	300 200	Daily × 2 Daily × 3	14	0	14	3	0	3	2	0	2	44
St. Jude†	{ AZA VP-16	150 100	{ Daily × 2 Daily × 3 }	16		16	1		1				60
	{ AZA VP-16	300 200	{ Daily × 2 Daily × 3 }	14		14	6		6				61
St. Jude†	AZA VP-16	300 250	Daily × 2 Daily × 3	6		6	6		6				62
POG†	AZA VP-16	300 250	Daily × 2 Daily × 3	52	36	16	28	17	11	3	3	0	63
CCSG	AZA DNR	100 30	Daily × 5 Daily × 3	34		34	14		14	4		4	44

*CALGB = Cancer and Leukemia Group B; NWU = Northwestern University; DFCC = Dana-Farber Cancer Center; St. Jude = St. Jude Children's Hospital; POG = Pediatric Oncology Group; MGBG = mitoguazone; PF = pyrazofurin; TGdR = deoxythioguanosine; AMSA = amsacrine; VP-16 = etoposide; and DNR = daunorubicin.

†Ongoing.

pending on the toxicity in the preceding group of patients. They found that the proportion of severe nausea and vomiting was significantly less in patients receiving the low-dose infusion schedules (150-200 mg/m²/day in four divided doses) rather than the high once-weekly dose (750 mg/m² in three divided doses). In this trial there were several cases of coma. Upon review of each case it was concluded that some may be partially attributable to azacitidine. The incidence rate in the higher-dose schedules (300 and 750 mg/m²/day in divided doses) was 22%, whereas that on the 150-200-mg/m²/day regimens was 4.6%. Thus, the lower-dose infusion schedules appear to be associated with less gastrointestinal and neurological toxicity. These lower-dose schedules have been almost universally adopted for the more recent studies.

Azacitidine in Two-Drug Combinations in Relapsed or Refractory ANLL

Table 4 shows an analysis of the two-drug combination regimens separated into refractory and relapsed patients and into adult and pediatric populations. The total number of evaluable patients with ANLL who have received azacitidine in combination with one other drug as second-line or later therapy is now > 360. Two observations are immediately apparent. First, the patient denominator on each regimen (with the possible exception of azacitidine + deoxythioguanosine) is inadequate to demonstrate superiority of any one combination over another. Second, the denominators are again inadequate to show superiority of any combination over the single-agent results. Even after extracting the data

for the most frequently used combination in adults (azacitidine and amsacrine) from the three phase II trials, there are still only 15 complete responses and five partial responses among 87 patients. In pediatrics, the combination of azacitidine with etoposide was the most frequently tested. An overall complete response rate of 50% using these two drugs was seen in the phase II evaluation and complete responses were observed in both relapsed and refractory patients. None of these two-drug combinations has been brought into front-line therapy.

Azacitidine: Multidrug Combinations

Table 5 presents these data subdivided by adult and pediatric trials. Of particular note in the pediatric trials is the D-ZAPO (daunorubicin, azacitidine, cytarabine, prednisone, and vincristine) combination (65,66) which is the only azacitidine combination brought into front-line therapy in any leukemia trial, either adult or pediatric. The early promise of this combination suggested in the 1976 review (12 complete remissions among 24 patients) has been substantiated by the much greater patient denominator now on this trial (117 complete remissions among 163 patients). However, the superiority of D-ZAPO over regimens which do not include azacitidine remains to be substantiated in a randomized trial.

The MAZE (amsacrine, azacitidine, and etoposide) combination (67,68) has been tested in very few patients to date; the numbers are inadequate to substantiate whether the difference between the Medical Research Council (MRC) trial (seven complete responses among

TABLE 5.—AZA: multidrug combinations in remission induction of ANLL*

Group or institution	Prior treatment	Regimen	Dose (mg/m ²)	Schedule	No. of—			Ref No.
					Evaluable patients	CRs	PRs	
<i>Pediatric</i>								
SWOG	Rel and Ref	AZA	150	8-hr infusion daily × 4	56	8	7	64
		VCR	1.5	Single dose				
		Pred	60	Daily × 4				
CCSG†	No	DNR	30	Daily × 3	163	117		65, 66
		AZA	50	Every 12 hrs × 8				
		Ara-C	25	Every 8 hrs × 12				
		Pred	40	Daily × 4				
		VCR	1.5	Daily × 1				
<i>Adult</i>								
BCRC	Rel and Ref	AZA	50	Every 8 hrs × 15	15	2	1	59
		VP-16	50	Daily × 5				
		VBL	6	Days 1 and 6				
MRC	1	AZA	100	Daily × 6	10	7		67
		AMSA	100	Daily × 6				
		VP-16	100	Daily × 6				
SWOG	2 (1-5)	AZA	100	Daily CIV × 5	17	3 (15 marrow hypoplasia)		68
		AMSA	60	Daily × 5				
		VP-16	30	Daily × 5				

*VCR = vincristine; Pred = prednisone; ara-C = cytarabine; VBL = vinblastine; Rel = relapsed; and Ref = refractory.

†Ongoing.

ten patients) and the SWOG trial (three complete responses among 27 patients) is genuine. The difference in response rate may be due to selection of a lower dose of amsacrine and etoposide in the SWOG trial. Patient selection could also be a factor, since all patients in the MRC trial were in first relapse, whereas the median number of previous treatment regimens in the SWOG trial was two.

Azacitidine: Consolidation and Maintenance Regimens

Table 6 lists the trials in which azacitidine has been incorporated into front-line consolidation and maintenance regimens. Most of these trials are ongoing. None of these trials was designed to address the role of azacitidine as an individual agent. The VAPA protocol (71,72) used early and late intensification and sequencing of different chemotherapy combinations (which included azacitidine) after remission induction with a standard combination of cytarabine, daunorubicin, vincristine, and prednisone. The aim of the intensive sequential regimen was to achieve adequate reduction of residual leukemic cells and prevent the emergence of drug-resistant cells. When last reported, with a median follow-up of 43 months (72), there was a complete remission rate of 70% and a 3-year actuarial probability remission duration of 56% for patients < 18 years of age and of 45% for patients 18–50 years of age. The D-ZAPO protocol (65,66) brought azacitidine into front-line remission induction regimens in pediatric ANLL. It also continued its use in both arms of a randomized maintenance program, comparing a more intensive schedule with a less intensive schedule (daily rather than intermittent 6-thioguanine and cyclophosphamide added to the monthly pulses of cytarabine and azacitidine and earlier administration of intrathecal methotrexate). The more intensive regimen showed a significantly improved duration of marrow remission, although survival was similar for both schedules. The results of the SEG study using azacitidine in two of the three arms of a randomized consolidation study in adult ANLL are so far only available in abstract form (69,70). In this study there was no advantage to adding beta-deoxythioguanosine to the azacitidine arm in terms of percentages of patients remaining in complete remission.

DISCUSSION

The development of azacitidine over the last 10 years has been disappointing in its lack of systematic progression. Of the four goals delineated in the earlier review for azacitidine in ANLL, only one has been partially achieved, namely, the demonstration that there is no major difference in response rate between the three schedules of administration. However, even this conclusion is based on pooled data from trials in all forms of leukemia. The other three goals have not been achieved: (a) except for one series of pediatric trials, azacitidine has not been brought into first-line treatment; (b) the route of administration has not been explored; and (c)

the role of azacitidine in consolidation, maintenance, and intensification regimens has not been defined.

Over 400 patients have been entered in two-drug combination trials, but no attempt has been made to demonstrate in a rigorous manner the superiority of any one combination over another.

Promising preclinical leads such as the use of cytidine as a rescue agent (73) and enhancement of *in vitro* cytostatic activity by thymidine and deoxycytidine (74) have not been pursued systematically. The radioprotective effect of azacitidine noted in mice (75) has not been investigated further except for one study of combined teratogenic effects on the mouse central nervous system (76) and one study of chromosomal aberrations in the broad bean (77). Azacitidine was not demonstrated to be protective in either of these studies. However, the original study of Vesely et al (75) undoubtedly needs replication and extension since it has significant clinical implications if confirmed.

In summary, the development of azacitidine, since its promising biological activity was first described > 20 years ago (78), has not established a definitive role in either front-line or salvage therapy of ANLL. The consequence is that azacitidine has not become generally available to physicians as a marketed drug for the treatment of leukemia.

These problems in the development of azacitidine exemplify the difficulties inherent in the development of an antileukemic agent which has shown "promising" activity in phase II trials. As seen from tables 1 and 3, considerable expenditure of patients and investigators' resources can easily occur without conclusive results being obtained.

We would like to propose a sequence of priorities for antileukemic drug development so as to achieve optimal use of all clinical and economic resources. (a) Pharmacokinetic parameters (particularly clearance and elimination half-life) need to be established as early as technically possible in the drug's development so that optimal schedules for phase II and III trials can be developed. In the case of azacitidine the development of a specific and sensitive assay for azacitidine had special problems due both to its chemical instability in aqueous solution and susceptibility to enzyme degradation in serum (4). Progress towards a useful assay in biological fluids was made 10 years ago (79) but this has not been developed further.

(b) The aim of single-agent trials of an investigational antileukemic drug in relapsed or refractory patients is to differentiate an active agent from an inactive one and to determine within reasonable confidence limits whether the response rate warrants further investigation of the drug in this disease. Since these trials provide little information about the true therapeutic efficacy of the drug, it is important that the number of patients be kept to the minimum necessary to provide a reliable basis for decision making. The standard approach has been to enter enough patients to establish a response rate for the agent in relapsed or refractory patients. The

TABLE 6.—AZA: use in front-line intensification regimens after remission induction*

Group or institution	Protocol No.	Ref No.	Induction regimens	Intensive consolidation/maintenance/postremission-induction regimen†
<i>Adult</i>				
SEG		69, 70	Ara-C and DNR	AZA (× 3) or AZA and TGdR (× 3) or 6TG, ara-C, and DNR
ECOG	EST P-B477	44	Ara-C and DNR	AZA, then DNR, then ara-C (× 2)
SEG	AML 81-312‡		Ara-C and DNR	Ara-C and DNR or Ara-C and DNR, then AMSA, then AZA, 6TG, and DNR or AZA, 6TG, and DNR, then allogeneic BMT
DFCC	D81-018-590		Ara-C and DNR	DNR, ara-C, 6TG, and i.t. ara-C (× 4), then DNR, AZA, and i.t. ara-C (× 4), then ara-C, 6TG, and i.t. ara-C (× 4)
Baylor	D81-044-077‡		Ara-C, DNR, and 6TG	DNR + ara-C, then VP-16 + AZA
Hahnemann Medical College	D82-061-724‡		Ara-C, DNR, and 6TG	AMSA, AZA, and ara-C or Ara-C + AMSA, then AMSA + AZA
Stanford	D82-051-605‡		Ara-C, DNR, and 6TG	VP-16, AMSA, and AZA
<i>Adult and pediatric</i>				
Weinstein et al		71, 72	VAPA twice	DNR and ara-C (× 4), then DNR and AZA (× 4), then VCR, MePred, MP, and MTX (× 4), then ara-C (× 4)
<i>Pediatric</i>				
CCSG		73	D-ZAPO	6TG, AZA, ara-C, and VCR or 6TG, AZA, ara-C, VCR, and immunotherapy
CCSG		74	D-ZAPO	6TG, ara-C, and VCR or 6TG, AZA, ara-C, VCR, and CPM
CCSG	CCG-251		Ara-C and CNR	6TG, ara-C, and i.t. MTX (or allogeneic BMT), then 6TG, VCR, ara-C, AZA, and CPM (repeated continuous) or DNR and ara-C, then Pred and VCR, then AZA and DNR, then BCNU and CPM, then 6TG and ara-C
CCSG‡			Ara-C and DNR or Ara-C, DNR, VP-16, 6TG, and DXR	Ara-C and Asp, then 6TG, VCR, ara-C, and CPM, then VP-16, DNR, ara-C, AZA, and CPM, then VP-16, DNR, ara-C, DXR, and 6TG
POG	8101‡		Ara-C and DNR or Ara-C, VCR, and Dex§	6TG, ara-C, and AZA, then ara-C and 6TG, then VCR, ara-C, CPM, and Pred or DNR and ara-C, then ara-C and 6TG, then 6TG, ara-C, and AZA, then VCR, ara-C, CPM, and Pred
DFCC	T84-0245‡		HD ara-C, DNR, and i.t. ara-C, then ara-C, DNR, and i.t. ara-C	VP-16, AZA, and i.t. ara-C (× 3), then ara-C and DNR
Hawaii Cancer Center	D81-011-693‡		Ara-C, DNR, VCR, and Pred	DNR and ara-C, then DNR and AZA, then VCR, MePred, MP, and MTX, then ara-C
MRC	T83-1410		DNR, ara-C, and 6TG	DNR, ara-C, 6TG, then CPM, VCR, and ara-C. or AMSA, AZA, and VP-16, then ara-C and 6TG every 4 wks, then CPM, VCR, and ara-C every 4 wks

*6TG = 6-thioguanosine; BMT = bone marrow transplant; i.t. = intrathecal; MePred = methylprednisone; MO = mercaptopurine; MTX = methotrexate; CPM = cyclophosphamide; BCNU = carmustine; DXR = doxorubicin; Asp = asparaginase; Dex = dexamethasone; and HD = high dose.

†Consolidation has not been separated from maintenance since in many protocols different working definitions are used, and in recent practice these amount to intensive postremission-induction regimens of comparable durations.

‡Ongoing study or final report not yet published.

§Arm was closed once lesser efficacy was established.

small numbers in such trials (as exemplified for azacitidine in table 2) mean that the confidence limits for the estimated response rate are very broad. For example, if three responses are observed among 25 patients, the bounds of the 95% confidence interval are 3% and 30%. In other words, a conclusion as to whether the drug is active or inactive usually cannot be reached from such a result in a phase II trial. A more appropriate design to achieve the aim of the trial would be a Fleming two-stage design (80). This design obliges the investigator before starting the trial to specify what response rate would be of sufficient interest to take the drug into combination phase II and possibly phase III studies as part of a front-line combination regimen. The design also allows for early termination should the observed response rate be below a predetermined minimum level of interest.

(c) In leukemia the new agent will need to be tested as part of a combination as soon as its single-agent activity has indicated that further development of the drug is warranted. At this stage a randomized pilot phase III trial using a sequential design may be appropriate (81-84). The aims of such a design are to determine the level of activity of a combination; to minimize the number of patients entered if early results indicate that the test combination is unlikely to produce a clinically meaningful improvement over a standard combination; and to avoid overinterpretation of unrandomized phase II studies in highly selected patients.

(d) Establishing the specific contribution of the drug to front-line combination regimen is extremely difficult in a disease like leukemia, with multiple effective agents of comparable activity being used in several different combination regimens for induction and salvage. It is important to establish whether the new agent is merely equivalent to existing agents or constitutes a significant medical advance. The clearest definition of a drug's contribution is obtained from a randomized trial in which the new agent is the only variable between the two arms. There are two possible experimental arms. In one arm, the new drug substitutes for a standard drug of similar mechanism of action and level of activity. In the second arm, the new agent is added to the standard induction regimen. The control arm in each case would be a standard induction regimen. This approach assumes that there will be small differences in effectiveness between arms; any design should include early stopping rules in the event that one arm is substantially superior.

Azacitidine has been brought into the D-ZAPO front-line combination with a promising remission rate (72%) in pediatric ANLL (65,66). However, without a randomized comparison of this regimen versus the same group of drugs minus azacitidine (ie, daunorubicin, cytarabine, prednisone, and vincristine) and no additional differences between the two arms in terms of intensification or maintenance regimens, it is impossible to say whether azacitidine materially adds to the complete remission rate or to survival.

The risk of not proceeding in a sequential fashion is that new drugs of poorly defined activity will add toxicity without additional efficacy or may compromise the efficacy of standard regimens because of reductions in dose intensity. These and other issues involved in the development of combination chemotherapy are explored in some detail by Wittes and Goldin (85).

(e) Similarly, the place of individual drugs in consolidation and maintenance regimens can only be reliably established by a stepwise progression comparing a standard current intensification regimen to one incorporating the new agent without compromising dose intensity of the standard regimen.

With respect to azacitidine, it is no longer feasible to perform this sequence of trials. However, the following trials are proposed as ways in which a conclusion could be reached as to whether the drug is a safe and effective component of standard leukemia therapy. (a) Use of azacitidine in front-line therapy in addition to a standard induction combination should be compared with the standard induction combination alone. The success of the D-ZAPO regimen (65,66) indicates that this could be done without compromising the total dose of the standard induction agents to a major extent. This dose of cytarabine in these trials was 25 mg/m² every 8 hours for 12 days, but that of the other agents was the same as in induction therapy without azacitidine. (b) A trial should be conducted using a combination such as MAZE which has appeared promising in noncomparative trials and evaluating the role of azacitidine by doing a randomized trial of the two-drug versus three-drug combination in first relapse. It is encouraging that this concept is being piloted for toxicity by the Pediatric Oncology Group.

The essential feature of these trials is the randomized addition design which would enable a conclusion to be reached about the role of azacitidine without compromising progress in the treatment of leukemia.

Another approach would be a randomized phase III trial to compare an alternating non-cross-resistant regimen of daunorubicin, cytarabine, and thioguanine (DAT), then MAZE with repeated cycles of DAT alone. This approach would not clarify the role of azacitidine itself. However, it would establish the role of a promising combination in front-line therapy. It would also address the more general question of the value of intensive non-cross-resistant regimens alternating in front-line therapy.

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