Azacitidine: 10 Years Later[1]

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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-Azacytidine 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 — Evaluable patients (No. entered)	No. of — Responses	Ref No.
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 + Pyrazofurin	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— Evaluable patients T	Rl	Rf	CRs T	Rl	Rf	PRs T	Rl	Rf	Ref No.
SWOG†	100 750 75	Every 8 hrs × 15 Single iv 4-hr infusion every 6 hrs × 20	50	50		10	10		5	5		39
SWOG†	250 300 200 200 150	Iv every 4 hrs × 3 Daily CIV × 5 Daily CIV × 5 Daily CIV × 7 Daily CIV × 10	101	101		8	8					40
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	60 100 180 300	Every 8 hrs × 15 Every 8 hrs × 15 Daily iv × 5 Daily iv × 5	6 23 5 21			0 3 1 2			2 4 0 7			44
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; Rl = 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 $\leq$ 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— Evaluable patients T	Rl	Rf	CRs T	Rl	Rf	PRs T	Rl	Rf	Ref No.
				Adult									
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
				Pediatric									
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/m^2/day in four divided doses) rather than the high once-weekly dose (750 mg/m^2 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/m^2/day in divided doses) was 22%, whereas that on the 150–200-mg/m^2/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— Evaluable patients	CRs	PRs	Ref No.
				Pediatric				
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				
				Adult				
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.

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