Low-Dose Azacitidine, Pioglitazone and All-trans Retinoic Acid versus Standard-Dose Azacitidine in Patients ≥ 60 Years with Acute Myeloid Leukemia Refractory to Standard Induction Chemotherapy (AMLSG 26-16/AML-ViVA): Results of the Safety Run-In Phase I

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BACKGROUND

Patients (pts) with acute myeloid leukemia (AML) who are refractory to intensive frontline treatment have a dismal outcome. In case of ineligibility for allogeneic stem cell transplantation (HSCT), the median survival of chemo-refractory AML is about 2 months and less than 5% of these pts are alive after 1-year (retrospective analysis from the AMLSG database). To date, there is no universally accepted standard approach for the treatment of chemo-refractory AML in older pts., particularly in view of the fact that age is an independent adverse prognostic factor.

Several retrospective studies have assessed the role of hypomethylating agents in this patient group, but complete remission (CR) rates were disappointingly low (≤10%) when compared to first line treatment. The presented study represents a novel approach focusing on hematopoietic tissue reprogramming (i.e. anakoinosis).

Anakoinosis is a novel paradigm for cancer treatment based on therapeutic modulation of biological communications processes and aims at prioritizing alternative pathways for apoptosis induction, normalizing activity of dysregulated homeostatic pathways, at up-regulating non-mutated tumor suppressor genes, attenuation of stroma-mediated support for tumor growth, and at modulating cancer checkpoints.

METHODS

The initial dose-finding phase I of the study evaluated the combination of azacitidine (AZA) 75 mg/d s.c. for 7 days, repeated every 28-days, pioglitazone 45 mg/d p.o. continuously from day 1 and all-trans retinoic acid (ATRA). A modified 3+3 design has been used to establish the maximum-tolerated dose of ATRA. Patients have been enrolled at an ATRA dose of 45 mg/m²/d from day 1 to day 28 and 15 mg/m²/d continuously thereafter if no dose limiting toxicity (DLT) occurred until start of next cycle on day 29. The safety DLTs were defined as toxicities attributable to ATRA, expected or unexpected, except if these are likely associated with another cause.

Eligible patients had confirmed diagnosis of AML refractory to induction therapy and were not eligible for further intensive induction therapy or were not immediate candidates for allogeneic HSCT. The severity of adverse events was graded using the Common Terminology Criteria for Adverse Events (CTCAE) V. 4.03. The response to treatment was evaluated using standard criteria defined by the expert panel on behalf of the European LeukemiaNet and international working group (IWG) response.

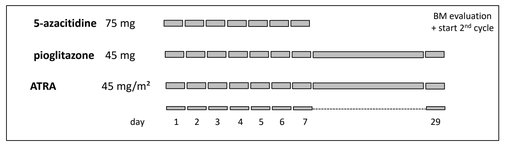

Figure 1: Treatment schedule

RESULTS

Ten pts were enrolled in the safety-run-in phase I (one pt withdrew informed consent on day 9 of cycle 1). Among all treated pts, the median age was 67 years (range, 62-76 years), and the majority of pts (70%) had an ECOG PS of 1 (see **Table 1**). Two pts had secondary AML; another two pts had therapy-related AML (t-AML). Eight pts had a complex karyotype.

Concerning safety, **hematological adverse events (AEs) were the most common toxicities observed**. Because pts with baseline cytopenias were included (leukopenia n=8; 80%; thrombocytopenia n=9; 90%), occurrences of many hematological AEs began before study drug initiation and were attributed to underlying hematologic disease. Common 3° /4° AEs included neutropenia (60%), anemia (50%), thrombocytopenia (40%), and infections (40%). 50% of pts experienced a serious AE; one 5° AE (gastric hemorrhage) occurred. **No DLTs were observed** (**Table 2).**

Five pts discontinued the study, with progressive disease (PD) or relapse being the most common reason for discontinuation.

Concerning efficacy, 3 pts (30%) achieved a CR and one pt a long-lasting stable disease (14 months). Morphologic review showed signs of differentiation of blasts in responding pts (see **Figure 2**), which has already been shown in *in-vitro* analysis (1). In line with this observation, one pts demonstrated resolution of fungal pneumonia during the study **(Figure 3)**.

In patients with complete remission molecular-genetic aberrations at diagnosis persisted in bone marrow (data not shown). This observation indicates that a main effect of a pro-anakoinotic therapy is the capacity to evolve novel biologic hallmarks for attenuating tumor growth, particularly differentiation.

Differentiation induction of leukemic blasts to neutrophil-like cells may include the gain of function, namely phagocytic activity as shown in vitro and in vivo: Thus, resolution of severe fungal pneumonia in case No 4 could be related to clinically efficacious differentiation effects (**Figure 3**).

Term	Grade							
	Any		3		4		Total/Gr. 3+4	
	N	%	N	%	N	%	N	%
Anemia	9	90%	5	50%	0	0%	5	50%
Thrombocytopenia	7	70%	1	10%	3	30%	4	40%
Infection	6	60%	4	40%	1	0%	4	40%
Leukopenia	6	60%	4	40%	2	20%	5	50%
Neutropenia	6	60%	2	20%	5	50%	6	60%
Arthralgia	5	50%	2	20%	0	0%	2	20%
Bleeding	4	40%	0	0%	0	0%	0	0%
Cough	4	40%	0	0%	0	0%	0	0%
Edema	4	40%	0	0%	0	0%	0	0%
Dizziness	3	30%	0	0%	0	0%	0	0%
Dyspnea	3	30%	0	0%	0	0%	0	0%
Fever	3	30%	2	20%	0	0%	2	20%
Hypokalemia	3	30%	1	10%	0	0%	1	10%
Skin tissue disorders	3	30%	0	0%	0	0%	0	0%
Decrease of appetite	2	20%	0	0%	0	0%	0	0%
Diarrhea	2	20%	0	0%	0	0%	0	0%
Insomnia	2	20%	1	10%	0	0%	1	10%
Acute renal failure	1	10%	0	0%	0	0%	0	0%
Allergic reaction	1	10%	0	0%	0	0%	0	0%
Atrial Flutter	1	10%	0	0%	0	0%	0	0%
Bone pain	1	10%	0	0%	0	0%	0	0%
Chills	1	10%	0	0%	0	0%	0	0%
Constipation	1	10%	1	10%	0	0%	1	10%
Depression	1	10%	0	0%	0	0%	0	0%
Fatique	1	10%	0	0%	0	0%	0	0%
Fibrinogen decreased	1	10%	0	0%	0	0%	0	0%
Headache	1	10%	0	0%	0	0%	0	0%
Hyperkalemia	1	10%	0	0%	0	0%	0	0%
Hyperthyroidism	1	10%	0	0%	0	0%	0	0%
Hypothyroidism	1	10%	0	0%	0	0%	0	0%
Injection site reaction	1	10%	0	0%	0	0%	0	0%
Iron overload	1	10%	1	10%	0	0%	1	10%
Nausea	1	10%	0	0%	0	0%	0	0%
Neck pain	1	10%	0	0%	0	0%	0	0%
Thrombolic event	1	10%	1	10%	0	0%	1	10%
Wound complication	1	10%	1	10%	0	0%	1	10%

Table 2: Adverse Events (AE)

Patient	Sex	Age	ECOG	AML type	Cytogenetics	Mutations	Pretreatment 1/2	Treatment cycles	Best response	Reason for discontinuation
1	male	68	1	de novo	complex	none (*)	Thio/Cyt/Dauno	1	NA	IC withdrawal C1D9
2	male	60	2	sec.	complex	none (*)	Cyt/Dauno, Mito/Cyt	4	SD	PD
3	male	75	1	de novo	complex	ASXL1, TP53	Cyt/Dauno	10	CR	relapse
4	female	65	0	t-AML	complex	TP53, NF1	Cyt/Dauno	2	CR	allo SCT
5	male	66	0	t-AML	46 XY, del (9) (q13q22) [3/10]	ASXL1, IDH2, SF3B1, U2AF1	Cyt/Dauno	2	CR	allo SCT
6	male	76	1	de novo	complex	none (*)	Cyt/Dauno	2	SD	PD
7	male	62	1	de novo	complex	TP53	Cyt/Dauno	14	SD	PD
8	male	68	1	de novo	complex	none (*)	Cyt/Dauno	3	SD	PD
9	female	76	1	de novo	46,XX del(11)(q23)[23]	none (*)	Cyt/Dauno	2	SD	death
10	female	65	1	sec.	complex	none (*)	Thio/Cyt/Dauno, Mito/Cyt	1	NA	death

Table 1: Patient Characteristics: sec. = secondary, Thio=Thioguanin, Cyt=Cytarabin, Dauno=Daunorubicin, Mito=Mitoxantron, NA=not analyzed, SD= stable disease, CR=complete remission, PD=progressive disease, *analyzed in standard panel: MLLT3/MLL, CEBPA, PML/RARA, RUNX1, FLT3-ITD, FLT3-TKD, NPM1

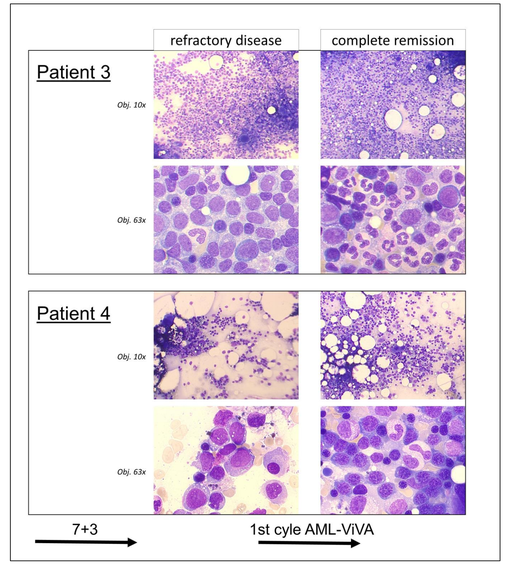

Figure 2: Cytomorphology of bone marrow smears of patient No. 3 and 4. After ‚7+3' both patients showed a significant bone marrow infiltration with leukemic blasts which were completely abolished after one cycle of AML-ViVA. The presence of neutrophils at complete remission may be due to differentiation of leukemic blasts (especially because some neutrophils show dysplasia like hypogranulation).

AML-VIVA treatment may establish **a long-term stable co-existence** of a low rate of leukemic blasts besides clinically predominant normal hematopoiesis (pt. No 7).

Allogeneic transplantation following induction of CR with the AML-VIVA protocol is followed by continuous CR for 12+ and 8+ months. Pt. No 7 is still alive with rescue therapy after disease progression.

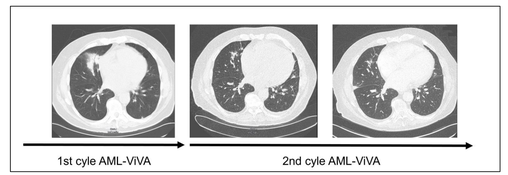

Figure 3: Resolution of fungal pneumonia in patient No. 4.

CONCLUSIONS

- In summary, the low-intensity, biomodulatory regimen of low-dose AZA, pioglitazone and ATRA demonstrated **a tolerable safety profile** and **encouraging signals for efficacy** in pts with AML refractory to standard induction chemotherapy warranting further investigation.
- Further, the study - in context with published data (1-6) - exemplarily shows that AML may be considered as a non-leukemia-cell-autonomous disease. Anakoinosis represents a third therapy column resetting pathologic homeostatic processes besides the classic targeted therapies tackling either AML blasts or adjacent stroma cells (5).
- Anakoinosis may establish AML differentiation on a more general basis (1,2,4) in comparison to currently available differentiation inducing targeted therapies or might be supplementary administered to targeted or low-dose cytotoxic therapy.

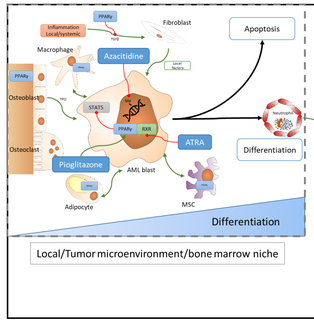

Figure 4: Graphical abstract depicting the effects of AML-ViVA treatment which leads besides apoptosis also to differentiation of leukemic blasts.

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Funded by
Celgene and Anticancer Fund, Belgium;
ClinicalTrials.gov Identifier: NCT02942758

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