CLINICAL TRIAL SUMMARY REPORT

Acronym: Viola

Title: A Phase I Trial of Combined Azacitidine and Lenalidomide Salvage Therapy in Patients who have relapsed after undergoing an Allogeneic Stem Cell Transplantation for Acute Myeloid Leukaemia (AML) or Myelodysplasia (MDS)

Sponsor: University of Birmingham

Sponsor Ref Number: RG_13-002

EudraCT Number: 2013-002118-11

REC Reference Number: 13/SC/0581

Details of Investigational Medicinal Product(s)

Azacitidine was supplied as a lyophylised powder in 100mg single-use vials. The IMP was packaged and labelled in accordance with local regulations and Good Manufacturing Practice (GMP), stating that the drug is for clinical trial use only.

Lenalidomide was supplied in 2.5, 5, 10, 15 and 25mg capsules for oral use. The IMP was packaged and labelled in accordance with local regulations and GMP, stating that the drug is for clinical trial use only.

Arms: This was a prospective, open label, single arm, phase I, multicentre, dose-finding study of lenalidomide in combination with azacitidine.

Analysis Stage: End of trial

Start Date: 05-Feb-2014

End of Trial: 07-Mar-2019

This report was prepared by the Chief Investigator and the Cancer Research UK Clinical Trials Unit (CRCTU) on behalf of the Sponsor.

Contact Details

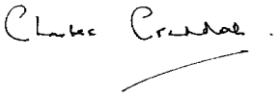
Cancer Research UK Clinical Trials Unit (CRCTU)
Institute of Cancer and Genomic Sciences
University of Birmingham
Edgbaston
Birmingham
B15 2TT

☎0121 3714365 🖷 0121 41 371 7874 ✉ Viola@trials.bham.ac.uk

SIGNATURE PAGE:

Signatory

Chief Investigator: Charles Craddock

Signature: 

Date: 30 / Apr / 2020

GENERAL INFORMATION

- **Trial Design**

This was a prospective, open label, phase I dose-finding, multicentre clinical trial to determine the maximum tolerated dose (MTD) of lenalidomide in combination with azacitidine therapy in patients who have relapsed after undergoing an allogeneic stem cell transplant using a sibling or unrelated donor for acute myeloid leukaemia (AML) or Myelodysplasia (MDS). The MTD was determined using a continual reassessment method (CRM) to allow a more accurate determination of the MTD.

Patients were recruited sequentially in planned cohort sizes of 3 with a maximum sample size of 30. AZA (75mg/m2) was administered by subcutaneous injection on a 5+2+2 schedule, commencing on day 1 of a 42 day cycle for up to 6 cycles. A pre-determined dose level of LEN was administered from days 10 to 30 followed by a 12-day rest period according to a modified one-stage, one parameter Bayesian CRM (Table 1). Tolerability and safety was assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0 criteria for all patients who commenced treatment. Response to trial therapy was assessed using modified Cheson criteria22. Patients achieving a major clinical response-defined as a CR, CR with incomplete blood count recovery (CRi) or partial response (PR)- within the first 6 cycles of treatment, were permitted to continue study treatment until loss of response. Non-responding patients discontinued trial therapy. A Dose Limiting Toxicity (DLT) was defined as the occurrence of grade 3 or 4 acute GVHD, recurrent grade 2 acute GVHD, any increase in GVHD grade within the first 2 cycles of therapy or grade 2 GVHD persisting for ≥ 42 days. New onset grade 3 or 4 non-hematological toxicity or death related to treatment were also considered to be DLTs and were formally evaluated up to the end of cycle 1 treatment (day 30).

Table 1: **Dose levels and prior and posterior probabilities of DLTs for each dose level with associated 90% credible intervals (based on the CRM dose-toxicity model)**

Combination Dose of LEN with 75mg/m^2 AZA	Prior DLT rate	Number of Evaluable Patients	Number of DLTs	Posterior DLT rate (90% credible interval)
Dose -2 (AZA Only)	0.03	0*	0*	0.001 (0, 0.012)
Dose -1 (2.5mg LEN)	0.07	0*	0*	0.004 (0, 0.035)
Dose 0 (5mg LEN)	0.12	3	0	0.013 (0.001, 0.068)
Dose 1 (10mg LEN)	0.20	3	0	0.037 (0.005, 0.131)
Dose 2 (15mg LEN)	0.30	2	0	0.085 (0.019, 0.218)
Dose 3 (25mg LEN)	0.40	13	2	0.153 (0.048, 0.314)
Dose 4 (35mg LEN)	0.52	0*	0*	0.263 (0.115, 0.437)

- **Scientific Background**

Allogeneic stem cell transplantation (allo-SCT) plays an increasingly important role in the management of acute myeloid leukemia (AML) and myelodysplasia (MDS) consequent upon the advent of reduced intensity conditioning (RIC) regimens and increased availability of alternative donors[1]. Although considerable progress has been made in reducing transplant toxicity, 30-80% of patients allografted for AML are still destined to relapse[2]. The outcome of patients with recurrent disease post-transplant remains extremely poor and fewer than 10% survive long term. Consequently, disease relapse is now the major cause of treatment failure in patients allografted for AML/MDS[3]. Although a second allograft and donor lymphocyte infusion (DLI) both have the capacity to deliver durable survival in patients with recurrent disease they are only effective in patients who achieve a morphological complete remission (CR) after salvage therapy[4,5]. Currently however salvage options for this patient are highly unsatisfactory. Intensive chemotherapy results in acquisition of a CR in a proportion of patients relapsing post-transplant but is toxic and often poorly tolerated resulting in lengthy hospital admissions[6]. As a consequence, most patients who relapse after an allograft are palliated and the development of effective salvage regimens represents a major unmet need.

Recently both the DNA methyltransferase inhibitor azacitidine (AZA) and the immunomodulatory drug lenalidomide (LEN) have been shown to possess significant anti-leukemic activity in newly diagnosed and relapsed AML and benefit from a broadly favorable toxicity profile permitting administration as an outpatient[7,8]. In patients who relapse after allo-SCT AZA is well tolerated and active in a proportion of patients particularly those relapsing more than 12 months post-transplant with a low tumor burden[9]. However, the overall CR rate is only 15-20% and strategies with the ability to increase the activity of AZA monotherapy are required. Whilst low dose LEN demonstrates anti-leukemic activity in patient who relapse after allo-SCT it is associated with very high rates of severe, often life-threatening, graft-versus-host disease (GVHD) and its administration is generally viewed to be contra-indicated post-transplant[10,11]. In addition to its anti-leukemic activity AZA accelerates reconstitution of T regulatory cells post-transplant in murine models resulting in a reduced risk of severe GVHD[12]. These observations have been replicated in patients allografted for AML[13-15]. We therefore hypothesized that co-administration of AZA may deliver additive anti-leukemic activity whilst serving to ameliorate the risk of severe GVHD associated with LEN administration post-transplant.

A major factor limiting the expeditious examination of novel drug combinations in complex clinical settings, such as post-transplant relapse, has been the limitations of standard early phase trial designs conventionally used to establish the maximum tolerated dose (MTD)[16,17]. Emerging data has highlighted the superior performance of model-based designs, such as the continuous reassessment method (CRM), in correctly identifying the MTD by permitting more efficient patient allocation thereby enabling a more rapid progression to later phases of clinical trial assessment[18,19]. We therefore examined the tolerability and activity of combined LEN/AZA therapy in patients who had relapsed after allo-SCT for AML utilizing a novel Dose Transition Pathway (DTP) tool which provides a bespoke CRM design, integrating important clinical judgements in a revised statistical model[20].

- **Objectives**

Primary objective

To assess the MTD of lenalidomide when administered in combination with azacitidine.

Secondary objectives

To assess the tolerability and safety of lenalidomide in combination with azacitidine and to assess major response rate as defined by the European LeukemiaNet (ELN) criteria and the IWG response criteria and survival at 1 year.

- **Statistical Considerations**

The MTD was defined as the dose level with an estimated DLT rate closest to 20% (target) with its associated DLT rate and 90% credible interval. The DLT evaluable population included those patients who had received all 7 days of AZA and at least 17/21 days of LEN in Cycle 1. The Overall survival (OS) curves for responders and non-responders were generated using Kaplan Meier plot and compared using the log-rank test. For immune evaluation analysis, 2-tailed Mann-Whitney U test or t-test for unpaired and paired samples respectively, were performed. Statistical analyses were conducted using R version 3.4.2 and GraphPad Prism, USA. *p* values <0.05 were considered statistically significant.

- **Patient Safety**

Over and underactive thyroid function has been observed in patients receiving therapy with lenalidomide. Symptoms related to changes in thyroid function were monitored and thyroid function tests were performed where required. Clinical interventions were used to manage symptoms where appropriate.

Blurred vision is associated with lenalidomide. Adverse effects to vision were monitored throughout the trial adverse event reporting period.

Lenalidomide carries a risk of developing secondary primary malignancies. Patients were monitored for secondary malignancies throughout the trial and during the follow up period.

Nervous system disorders are very commonly associated with the use of lenalidomide. Nervous system disorders were monitored and managed by clinical interventions where appropriate.

Pain (e.g., muscle, joints, non-cardiac chest pain) is associated with lenalidomide and episodes of pain were monitored and managed by appropriate pain relief interventions.

SUBJECT DISPOSITION

- **Eligibility Criteria**

The VIOLA trial was delivered by the Bloodwise Trials Acceleration Program as a prospective, open label, phase I dose-finding, multicenter trial designed to determine the MTD of LEN in combination with AZA in patients who have relapsed after allo-SCT for AML or MDS. Patients with active acute or chronic GVHD or a history of grade 3 or 4 GVHD were excluded. Impaired renal or hepatic function (defined as total bilirubin ≥ 2.5 x upper limit of normal (ULN), aspartate aminotransferase or alanine aminotransferase ≥ 3.0 x ULN and estimated glomerular filtration rate ≤ 40mls/min) were exclusion criteria. Patients on immunosuppressive therapies at the time of relapse and those who had received anti-tumor therapies within 28 days before the start of protocol treatment were also excluded.

- **Recruitment**

31 patients who had relapsed after an allograft for AML or MDS were recruited between February 2014 and December 2016.

BASELINE CHARACTERISTICS

- **Age**

  16 years and over

- **Gender**

  13 female patients and 18 male patients were recruited

- **Study Specific Characteristics**

29 patients commenced trial therapy (AML n =24, MDS n=5) with a median follow up of 23 months. 25 patients had received a RIC allograft. 13 patients were transplanted using a matched sibling donor and 16 a matched unrelated donor. The median percentage blasts at commencement of treatment was 33% and the median time from transplant to relapse prior to the trial was 10.2 months. Patients received a median of 2 cycles of therapy on study (range 0-11).

Table 2: Baseline Patient Characteristics – Safety Population (n=29)

Characteristic		N (%)
Disease	AML MDS	24 (83) 5 (17)
Age (Years)	Mean (range)	54 (18-71)
Cytogenetic Risk Group	Favourable Risk Intermediate Risk Poor risk Not Known	3 (10) 18 (64) 7 (23) 1 (3)
Conditioning regimen	Reduced intensity Myeloablative	25 (86) 4 (14)
Donor	HLA identical sibling Unrelated donor	13 (45) 16(55)
Prior Acute GVHD	No Yes	22 (76) 7 (24)
% Blasts at Baseline	Mean (range) Not known	40 (7-90) 8 (28)
Relapse – time from transplant (Months)	Median (range) Not known	10 (1-39) 1 (3)

RESULTS

- **MTD assessment**:

21 patients were evaluable for MTD assessment. Sequential updates of the estimated dose-toxicity curves were determined using the CRM (from initial prior curve) after each cohort and the final model's estimated posterior probabilities of DLT for each dose level and their associated 90% credible intervals were calculated (Table 1). The MTD for LEN when administered with AZA was determined as 25 mg based on an estimated posterior probability of DLT of 15.3% (90% credible interval: 4.8% - 31.4%). 13/21 (62%) of evaluable patients were treated at the MTD.

- **Assessment of safety:**

20 treatment-related non-hematological toxicities occurred in >10% of patients at grade ≥ 3 at any time from cycle 1 through all cycles of treatment (Table 3). Three patients developed acute GVHD during therapy. One developed grade 2 lower gut GVHD after receiving 4 cycles of LEN at a dose of 15mg. Two patients receiving 25mg LEN developed GVHD – one with grade 2 acute skin GVHD in cycle 1 and one with grade 3 liver GVHD during cycle 6 of therapy. All patients who developed GVHD responded to steroid therapy and there were no GVHD related deaths. Two DLTs occurred in patients receiving 25 mg of LEN. One patient developed fatigue & rash (grade 3) the other experienced deranged liver function tests (grade 3). 22 patients died: 19 from resistant or progressive disease and three from infectious complications.

Table 3: Grade 3 or greater treatment-related non-hematological adverse events observed in > 10% of the trial population

Event	Grade	Overall Events (Patients)	Combination Dose of LEN with 75mg/ m^2 AZA			
			Dose 0 (5mg LEN) Events (Patients)	Dose 1 (10mg LEN) Events (Patients)	Dose 2 (15mg LEN) Events (Patients)	Dose 3 (25mg LEN) Events (Patients)
Febrile neutropenia	3 4	9 (6) 1 (1)	1 (1) 0 (0)	1 (1) 0 (0)	0 (0) 1 (1)	7 (4) 0 (0)
Infections and infestations	3	4 (3)	0 (0)	0 (0)	1 (1)	3 (2)
Sepsis	3 4	2 (2) 4 (3)	0 (0) 0 (0)	0 (0) 0 (0)	0 (0) 0 (0)	2 (2) 4 (3)

- **Clinical response and overall survival:**

7/15 (47%) patients who received at least three cycles of treatment achieved a major clinical response to LEN/AZA salvage (CR n=3, CRi n=3, PR n=1). There was no correlation between the administered dose of LEN and the response rate (Table 4). 5 patients who achieved a major clinical response subsequently received DLI (n=3) or a second allograft (n=2). Of the 3 patients who received DLI two patients are still alive 15 and 35 months after trial therapy commenced. One patient is still alive after a second transplant - 22 months after commencing trial therapy. The median OS in patients who responded to treatment was 27 months compared to 10 months in non-responders (p = 0.004).

Table 4: Best response to trial therapy in 15 evaluable patients

Best Response	Overall N (%)	Combination Dose of LEN with 75mg/ m^2 AZA			
		Dose 0 (5mg LEN) N (%)	Dose 1 (10mg LEN) N (%)	Dose 2 (15mg LEN) N (%)	Dose 3 (25mg LEN) N (%)
Complete Remission (CR) CR with Incomplete Recovery (CRi) Partial Remission (PR)	3 (20) 3 (20) 1 (6.7)	1 (33.3) 0 (0) 0 (0)	0 (0) 1 (50) 0 (0)	0 (0) 2 (100) 0 (0)	2 (25) 0 (0) 1 (12.5)
Resistant Disease (RD) Not Evaluable	7 (46.7) 1 (6.7)	1 (33.3) 1(33.3)	1 (50) 0 (0)	0 (0) 0 (0)	5 (62.5) 0 (0)

- **Immune evaluation:**

Exposure to both AZA and LEN can induce differences in T cell activation and function *in vitro* and we therefore sequentially characterized the number and phenotype of CD3+ T cells in patients before and after trial therapy. A significant reduction in the frequency of T cells was observed prior to commencement of trial therapy, compared to healthy controls ($p<0.0001$) (Figure 1a). T cells from trial patients at baseline expressed increased LAG3 and PD1 consistent with an exhaustion phenotype (Figure 1b and c). No CD4+FOXP3+ T cells were identified. T cells from trial patients demonstrated a significant reduction in release of Th1 cytokines IFN-γ and TNF-α following PMA-ionomycin stimulation ($p<0.0001$) compared with healthy controls (Figure 1d). Release of pro-inflammatory cytokines IL-2, IL-5, and IL-6 were similarly reduced. The 7 patients who demonstrated a clinical response (CR or PR) to combined AZA and LEN trial therapy were evaluated further. The CD3+ T cell frequency in the peripheral blood did not increase after 6 cycles of trial therapy (Figure 1e). No significant changes in the frequency of PD1/LAG3+ T cells (Figure 1f) or cytokine profile (Figure 1g) were identified following trial therapy. Taken together, these findings indicate that T cell function is impaired in patients who relapse post-allograft, consistent with an exhaustion phenotype, and that LEN/AZA treatment exerts anti-tumor activity independent of this pathway.

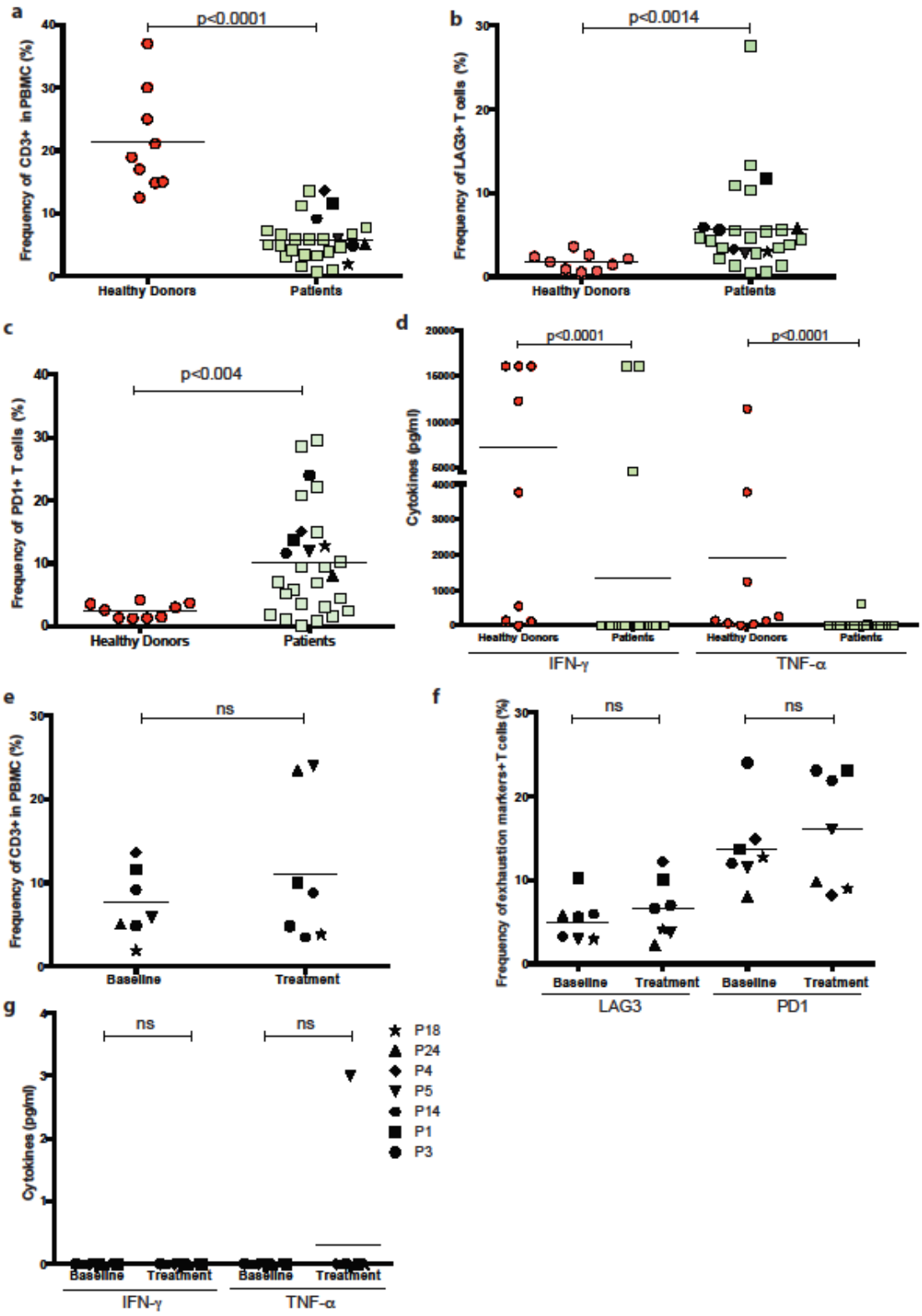


Figure 1 Cell numbers and cytokine expression in healthy controls and trial patients a) The frequency of CD3+ T cells in PBMCs from healthy donors and trial patients b) Expression of T-cell surface marker LAG3 in healthy donors and trial patients c) Expression of T-cell surface marker PD1 in healthy donors and trial patients d) Expression of Th1 cytokines IFN-γ and TNF-α following PMA ionomycin stimulation healthy donors and trial patients e) Frequency of CD3+ T- cells before and after commencement of trial therapy in patients demonstrating a clinical response to trial therapy f) Functional status of T-cells in patients who demonstrated a clinical response to trial therapy as determined by LAG3 and PD1 expression g) Expression of Th1 cytokines IFN-γ and TNF-α following PMA ionomycin stimulation in patients achieving a clinical response to trial therapy.

ADVERSE EVENTS

o Adverse events

All adverse events (AEs) were to be reported from the date of commencement of protocol-defined treatment until 28 days after the last treatment. Serious AEs (SAEs) that were possibly related to Azacitidine or Lenalidomide were reported irrespective of how long after IMP administration.

The collection and reporting of AEs were in accordance with the Medicines for Human Use Clinical Trials Regulations 2004 and its subsequent amendments. The Investigator should assess the seriousness and causality (relatedness) of all AEs experienced by the patient with reference to the Investigator Brochure.

Table 5 presents the same adverse event data

Category	Event	Grade	Overall Events (Patients)
Blood and lymphatic system disorders	Anemia	Any	80 (12)
Blood and lymphatic system disorders	Blood and lymphatic system disorders - Other, specify	Any	16 (5)
Blood and lymphatic system disorders	Febrile neutropenia	Any	36 (19)
Blood and lymphatic system disorders	Lymph node pain	Any	1 (1)
Cardiac disorders	Cardiac disorders - Other, specify	Any	1 (1)
Cardiac disorders	Palpitations	Any	2 (2)
Cardiac disorders	Sinus tachycardia	Any	16 (3)
Ear and labyrinth disorders	Ear and labyrinth disorders - Other, specify	Any	1 (1)
Ear and labyrinth disorders	Ear pain	Any	1 (1)
Eye disorders	Blurred vision	Any	3 (3)
Eye disorders	Dry eye	Any	1 (1)
Eye disorders	Eye disorders - Other, specify	Any	4 (3)
Gastrointestinal disorders	Abdominal pain	Any	14 (10)
Gastrointestinal disorders	Anal hemorrhage	Any	1 (1)
Gastrointestinal disorders	Anal pain	Any	1 (1)
Gastrointestinal disorders	Cheilitis	Any	1 (1)
Gastrointestinal disorders	Colitis	Any	1 (1)
Gastrointestinal disorders	Colonic perforation	Any	1 (1)
Gastrointestinal disorders	Constipation	Any	38 (17)
Gastrointestinal disorders	Diarrhea	Any	32 (18)
Gastrointestinal disorders	Dry mouth	Any	5 (5)
Gastrointestinal disorders	Dyspepsia	Any	3 (2)
Gastrointestinal disorders	Gastroesophageal reflux disease	Any	1 (1)
Gastrointestinal disorders	Gastrointestinal disorders - Other, specify	Any	23 (10)
Gastrointestinal disorders	Gastrointestinal pain	Any	3 (2)
Gastrointestinal disorders	Gingival pain	Any	1 (1)
Gastrointestinal disorders	Hemorrhoidal hemorrhage	Any	1 (1)
Gastrointestinal disorders	Hemorrhoids	Any	2 (2)
Gastrointestinal disorders	Mucositis oral	Any	11 (5)
Gastrointestinal disorders	Nausea	Any	40 (17)
Gastrointestinal disorders	Rectal hemorrhage	Any	1 (1)
Gastrointestinal disorders	Toothache	Any	2 (1)
Gastrointestinal disorders	Vomiting	Any	28 (17)

General disorders and administration site conditions	Edema limbs	Any	7 (6)
General disorders and administration site conditions	Facial pain	Any	2 (2)
General disorders and administration site conditions	Fatigue	Any	24 (17)
General disorders and administration site conditions	Fever	Any	24 (10)
General disorders and administration site conditions	Flu like symptoms	Any	4 (4)
General disorders and administration site conditions	General disorders and administration site conditions - Other, specify	Any	16 (10)
General disorders and administration site conditions	Hypothermia	Any	4 (1)
General disorders and administration site conditions	Infusion related reaction	Any	1 (1)
General disorders and administration site conditions	Injection site reaction	Any	12 (11)
General disorders and administration site conditions	Non-cardiac chest pain	Any	2 (2)
General disorders and administration site conditions	Pain	Any	4 (3)
Hepatobiliary disorders	Cholecystitis	Any	1 (1)
Hepatobiliary disorders	Hepatobiliary disorders - Other, specify	Any	2 (2)
Infections and infestations	Anorectal infection	Any	1 (1)
Infections and infestations	Catheter related infection	Any	1 (1)
Infections and infestations	Conjunctivitis infective	Any	1 (1)
Infections and infestations	Gum infection	Any	1 (1)
Infections and infestations	Infections and infestations - Other, specify	Any	22 (11)
Infections and infestations	Lip infection	Any	6 (5)
Infections and infestations	Lung infection	Any	5 (5)
Infections and infestations	Mucosal infection	Any	1 (1)
Infections and infestations	Papulopustular rash	Any	1 (1)
Infections and infestations	Rhinitis infective	Any	1 (1)
Infections and infestations	Sepsis	Any	9 (6)
Infections and infestations	Sinusitis	Any	1 (1)
Infections and infestations	Skin infection	Any	2 (2)
Infections and infestations	Upper respiratory infection	Any	3 (3)
Infections and infestations	Urinary tract infection	Any	3 (2)
Injury, poisoning and procedural complications	Bruising	Any	11 (6)
Injury, poisoning and procedural complications	Fall	Any	3 (2)

Injury, poisoning and procedural complications	Injury, poisoning and procedural complications - Other, specify	Any	1 (1)
Investigations	Activated partial thromboplastin time prolonged	Any	3 (1)
Investigations	Alanine aminotransferase increased	Any	5 (4)
Investigations	Alkaline phosphatase increased	Any	7 (3)
Investigations	Aspartate aminotransferase increased	Any	1 (1)
Investigations	Blood bilirubin increased	Any	2 (2)
Investigations	Creatinine increased	Any	1 (1)
Investigations	Electrocardiogram QT corrected interval prolonged	Any	1 (1)
Investigations	Hemoglobin increased	Any	1 (1)
Investigations	INR increased	Any	4 (1)
Investigations	Investigations - Other, specify	Any	101 (6)
Investigations	Lymphocyte count decreased	Any	16 (4)
Investigations	Lymphocyte count increased	Any	1 (1)
Investigations	Neutrophil count decreased	Any	44 (8)
Investigations	Platelet count decreased	Any	59 (12)
Investigations	Weight loss	Any	4 (4)
Investigations	White blood cell decreased	Any	14 (5)
Metabolism and nutrition disorders	Anorexia	Any	8 (5)
Metabolism and nutrition disorders	Hypercalcemia	Any	2 (1)
Metabolism and nutrition disorders	Hyperglycemia	Any	2 (1)
Metabolism and nutrition disorders	Hyperkalemia	Any	6 (3)
Metabolism and nutrition disorders	Hypermagnesemia	Any	5 (1)
Metabolism and nutrition disorders	Hypernatremia	Any	1 (1)
Metabolism and nutrition disorders	Hypoalbuminemia	Any	8 (3)
Metabolism and nutrition disorders	Hypocalcemia	Any	7 (2)
Metabolism and nutrition disorders	Hypoglycemia	Any	2 (1)
Metabolism and nutrition disorders	Hypokalemia	Any	7 (4)
Metabolism and nutrition disorders	Hypomagnesemia	Any	6 (3)
Metabolism and nutrition disorders	Hyponatremia	Any	3 (2)
Metabolism and nutrition disorders	Hypophosphatemia	Any	2 (2)
Metabolism and nutrition disorders	Metabolism and nutrition disorders - Other, specify	Any	2 (2)
Musculoskeletal and connective tissue disorders	Arthralgia	Any	3 (2)
Musculoskeletal and connective tissue disorders	Back pain	Any	12 (6)
Musculoskeletal and connective tissue disorders	Bone pain	Any	1 (1)
Musculoskeletal and connective tissue disorders	Muscle weakness lower limb	Any	1 (1)
Musculoskeletal and connective tissue disorders	Musculoskeletal and connective tissue disorder - Other, specify	Any	9 (5)
Musculoskeletal and connective tissue disorders	Myalgia	Any	8 (5)
Musculoskeletal and connective tissue disorders	Myositis	Any	1 (1)
Musculoskeletal and connective tissue disorders	Neck pain	Any	1 (1)
Musculoskeletal and connective tissue disorders	Pain in extremity	Any	6 (4)

Nervous system disorders	Dizziness	Any	9 (4)
Nervous system disorders	Dysgeusia	Any	1 (1)
Nervous system disorders	Headache	Any	8 (8)
Nervous system disorders	Lethargy	Any	5 (4)
Nervous system disorders	Nervous system disorders - Other, specify	Any	4 (4)
Nervous system disorders	Peripheral sensory neuropathy	Any	2 (2)
Nervous system disorders	Somnolence	Any	1 (1)
Nervous system disorders	Syncope	Any	1 (1)
Psychiatric disorders	Confusion	Any	1 (1)
Psychiatric disorders	Insomnia	Any	2 (2)
Psychiatric disorders	Psychiatric disorders - Other, specify	Any	2 (2)
Renal and urinary disorders	Acute kidney injury	Any	2 (2)
Renal and urinary disorders	Cystitis noninfective	Any	2 (2)
Renal and urinary disorders	Renal and urinary disorders - Other, specify	Any	3 (3)
Renal and urinary disorders	Urinary frequency	Any	3 (2)
Renal and urinary disorders	Urinary incontinence	Any	1 (1)
Reproductive system and breast disorders	Menorrhagia	Any	2 (1)
Reproductive system and breast disorders	Reproductive system and breast disorders - Other, specify	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Cough	Any	15 (13)
Respiratory, thoracic and mediastinal disorders	Dyspnea	Any	11 (7)
Respiratory, thoracic and mediastinal disorders	Epistaxis	Any	15 (5)
Respiratory, thoracic and mediastinal disorders	Hiccups	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Hypoxia	Any	2 (2)
Respiratory, thoracic and mediastinal disorders	Pleuritic pain	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Pneumothorax	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Productive cough	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Respiratory, thoracic and mediastinal disorders - Other, specify	Any	6 (5)

Respiratory, thoracic and mediastinal disorders	Cough	Any	15 (13)
Respiratory, thoracic and mediastinal disorders	Dyspnea	Any	11 (7)
Respiratory, thoracic and mediastinal disorders	Epistaxis	Any	15 (5)
Respiratory, thoracic and mediastinal disorders	Hiccups	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Hypoxia	Any	2 (2)
Respiratory, thoracic and mediastinal disorders	Pleuritic pain	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Pneumothorax	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Productive cough	Any	1 (1)
Respiratory, thoracic and mediastinal disorders	Respiratory, thoracic and mediastinal disorders - Other, specify	Any	6 (5)
Respiratory, thoracic and mediastinal disorders	Sore throat	Any	3 (3)
Skin and subcutaneous tissue disorders	Alopecia	Any	1 (1)
Skin and subcutaneous tissue disorders	Dry skin	Any	6 (5)
Skin and subcutaneous tissue disorders	Erythema multiforme	Any	2 (2)
Skin and subcutaneous tissue disorders	Pruritus	Any	15 (11)
Skin and subcutaneous tissue disorders	Purpura	Any	2 (1)
Skin and subcutaneous tissue disorders	Rash acneiform	Any	1 (1)
Skin and subcutaneous tissue disorders	Rash maculo-papular	Any	22 (12)
Skin and subcutaneous tissue disorders	Skin and subcutaneous tissue disorders - Other, specify	Any	27 (15)
Skin and subcutaneous tissue disorders	Skin hyperpigmentation	Any	1 (1)
Skin and subcutaneous tissue disorders	Urticaria	Any	1 (1)
Vascular disorders	Flushing	Any	1 (1)
Vascular disorders	Hypertension	Any	1 (1)
Vascular disorders	Hypotension	Any	9 (5)
Vascular disorders	Phlebitis	Any	1 (1)
Vascular disorders	Thromboembolic event	Any	4 (2)
Vascular disorders	Vascular disorders - Other, specify	Any	1 (1)
	Total	All	1119 (29)

- **Serious Adverse Events**

Table 6 presents the details for the serious adverse events that occurred.

Category	Event	Patients	Occurences	Related Occurences	Deaths	Related Deaths
Blood and lymphatic system disorders	Anemia	2	2	0	1	0
Blood and lymphatic system disorders	Blood and lymphatic system disorders - Other, specify	1	1	1	0	0
Blood and lymphatic system disorders	Febrile neutropenia	15	27	9	5	1
Blood and lymphatic system disorders	Hemolysis	1	1	0	0	0
Gastrointestinal disorders	Colonic perforation	1	1	0	1	0
Gastrointestinal disorders	Diarrhea	2	2	0	0	0
Gastrointestinal disorders	Rectal hemorrhage	1	1	0	0	0
Gastrointestinal disorders	Vomiting	1	1	1	0	0
General disorders and administration site conditions	Edema face	1	1	0	0	0
General disorders and administration site conditions	Fever	9	19	3	1	0
General disorders and administration site conditions	Flu like symptoms	1	1	0	0	0
General disorders and administration site conditions	General disorders and administration site conditions - Other, specify	2	2	0	0	0
General disorders and administration site conditions	Infusion related reaction	1	1	0	0	0
Infections and infestations	Anorectal infection	1	1	0	0	0
Infections and infestations	Infections and infestations - Other, specify	3	3	0	0	0
Infections and infestations	Lung infection	5	6	1	2	0
Infections and infestations	Sepsis	3	3	2	1	1
Infections and infestations	Skin infection	1	3	1	0	0
Infections and infestations	Soft tissue infection	1	1	1	0	0
Infections and infestations	Tooth infection	1	3	3	0	0
Infections and infestations	Upper respiratory infection	1	1	1	0	0
Infections and infestations	Urinary tract infection	1	1	0	0	0
Investigations	Platelet count decreased	2	2	0	0	0
Metabolism and nutrition disorders	Hypokalemia	1	1	0	0	0
Musculoskeletal and connective tissue disorders	Back pain	1	1	0	0	0
Nervous system disorders	Dizziness	1	1	0	0	0
Nervous system disorders	Syncope	1	1	0	0	0
Psychiatric disorders	Confusion	1	1	0	1	0
Reproductive system and breast disorders	Menorrhagia	1	1	1	0	0
Respiratory, thoracic and mediastinal disorders	Cough	1	1	0	0	0
Respiratory, thoracic and mediastinal disorders	Epistaxis	1	1	1	0	0
Respiratory, thoracic and mediastinal disorders	Hypoxia	1	1	0	0	0
Respiratory, thoracic and mediastinal disorders	Pleuritic pain	1	1	0	0	0
Skin and subcutaneous tissue disorders	Skin and subcutaneous tissue disorders - Other, specify	1	1	1	0	0

MORE INFORMATION

- Substantial Amendments

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version

Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment
1	28-Mar-2014	3.0	Substantial	Patient inclusion criteria amended to include patients with relapsed MDS; SCT prior conditioning and donor

				clarified; IWG response criteria to be used for MDS
2	23-Oct-2014	4.0	Substantial	Clarification of exclusion criteria and schedule of events post cycle 6. Timeline for DLT evaluation changed
3	17-Nov-2015	N/A	Substantial	Change of PI at a participating site
4	05-Apr-2016	5.0	Substantial	The last dose level of Lenalidomide amended from 50mg to 35mg

CONCLUSIONS&DISSCUSSION

The demonstration that high doses of LEN, in combination with AZA, exerts significant anti-leukemic activity with modest toxicity in patients relapsing after allo-SCT identifies a potentially important new salvage strategy in patients who relapse post-transplant. Intensive chemotherapy is often ineffective in this setting is often ineffective and associated with substantial toxicity and attendant mortality. Novel salvage therapies in this important patient population are clearly required. Whilst the targeted therapies sorafenib and AG221 demonstrate significant clinical activity in patients with relapsed disease post-allograft their use is restricted to specific biological sub-types of AML[23,24]. AZA is well tolerated post-allograft but has only modest clinical activity. Our data, for the first time, demonstrate that LEN can be safely administered post allograft, in combination with AZA, and appears to be associated with a higher CR/PR rate than AZA monotherapy[9]. Whilst clearly demanding confirmation in a larger patient population our results identify LEN/AZA combination therapy as a potentially novel salvage therapy in post-transplant relapse. The observed clinical activity may be consequent upon the additive anti-leukemic activity of combination therapy of LEN and AZA or alternatively represent pharmacological manipulation of the graft-versus-leukemia (GVL) effect by one or both agents. AZA has previously been shown to up-regulate tumor antigen expression on AML blasts and can also induce a CD8+ T cell response post allograft whilst LEN has direct T cell activation properties[13].

A striking observation in this study was the tolerability of LEN after allo-SCT. Previous studies using LEN as maintenance or pre-emptive therapy in the first three months post-transplant reported excessive rates of severe, often life-threatening GVHD, after LEN administration-even at modest doses of 5-10 mg. In contrast, in this study patients with overt relapsed disease tolerated doses of up to 25 mg LEN, in conjunction with AZA, with acceptable rates of GVHD. Why then might this study have yielded results different from previous reports in which LEN was administered post allograft? It may be relevant that the majority of patients treated with LEN maintenance in previous studies were recipients of T replete allografts whereas patients in this series received either alemtuzumab or ATG as GVHD prophylaxis. It is also possible that the later timing of LEN administration in this study, 6 months versus 60 days in maintenance studies, may have contributed to the markedly lower incidence of GVHD. Alternatively, the co-administration of AZA may augment T regulatory cell expansion which may decrease the risk of GVHD as observed in mouse models[12]. It will therefore be of interest to explore whether AZA co-administration reduces the risk of GVHD when LEN is administered early post-transplant.

In order to obtain the MTD in such a complex patient population we adopted a bespoke CRM design that integrates important clinical judgements. Implementing an early stopping criterion that permitted clinical judgement in this population was also of value to manage excessive DLTs seen at the starting and lower doses in addition to having favourable statistical properties. This is enabled, via utilization of a novel investigator oriented tool, the DTP which maps out dose decisions in advance. This is the first trial that has implemented a tailored CRM coupled with DTP, translating a complex dose-finding design to simple decision making for trialists. Benefits of using this innovative design were seen not only at the design stage, but also during the running of the trial with the ease of visualisation of dose pathways – simplifying the statistical "black-box" of complex designs. Additionally, assessment of the MTD in this very high risk patient population was accelerated by the adoption of such flexible design which coped effectively with the challenges of unexpected dosing error and cohort size variation due to early patient drop out and not having to necessarily replace all inevaluable patients. These have collectively led to substantial savings in time and resources. Such unforeseen occurrences would have been difficult for a conventional 3+3 design.

T cells are a major arm of the anti-cancer immune response yet controversy remains over their functional state in AML[25,26.] In a smaller cohort, T cell exhaustion in AML patients may predict relapse post allogeneic stem cell transplant[27]. Correspondingly, we have shown that the T cells in post-transplant patients have an exhaustion phenotype manifested by increased PD1 and LAG3 expression, in combination with reduced IFN TNF, IL-2, IL-5, and IL-6 secretion. We show that combined AZA/LEN therapy does not reverse the T cell phenotype and T cell status does not correlate with response to these agents post allo-SCT. The findings

extend our previous results that AML creates an immunosuppressive microenvironment to T cells[28]. Alternative approaches to reverse T cell exhaustion could be an adjunct to enhance post-transplant immune-surveillance for AML patients.

Our data establish a potentially important role for a LEN/AZA combination as salvage therapy in patients with relapsed AML post allograft and support a randomized comparison of this novel regimen with intensive chemotherapy in this area of major unmet need. Alternatively, combined LEN/AZA therapy could be administered either as maintenance therapy to reduce the risk of relapse post-transplant[15] or pre-emptively in patients with mixed T cell chimerism or evidence of measurable residual disease.

The study has been published in the Journal of Clinical Oncology:
Combination Lenalidomide and Azacitidine: A Novel Salvage Therapy in Patients Who Relapse After Allogeneic Stem-Cell Transplantation for Acute Myeloid Leukemia Charles Craddock, MD1,2; Daniel Slade, MSc2 ; Carmela De Santo, PhD2 ; Rachel Wheat, MSc2 ; Paul Ferguson, MD3 ; Andrea Hodgkinson, PhD2 ; Kristian Brock, MSc2 ; Jamie Cavenagh, MD4 ; Wendy Ingram, MD5 ; Mike Dennis, MD6 ; Ram Malladi, MD1 ; Shamyla Siddique, MPhil2 ; Francis Mussai, MD2 ; and Christina Yap, PhD; 17- Jan-2019, Vol 37, Issue 7.

REFERENCES

1. Cornelissen JJ, Gratwohl A, Schlenk RF, et al: The European LeukemiaNet AML Working Party consensus statement on allogeneic HSCT for patients with AML in remission: an integrated-risk adapted approach. Nat Rev Clin Oncol 9:579-90, 2012
2. Craddock C, Versluis J, Labopin M, et al: Distinct factors determine the kinetics of disease relapse in adults transplanted for acute myeloid leukaemia. J Intern Med, 2017
3. Schmid C, Labopin M, Nagler A, et al: Treatment, risk factors, and outcome of adults with relapsed AML after reduced intensity conditioning for allogeneic stem cell transplantation. Blood 119:1599-606, 2012
4. Schmid C, Labopin M, Nagler A, et al: Donor lymphocyte infusion in the treatment of first hematological relapse after allogeneic stem-cell transplantation in adults with acute myeloid leukemia: a retrospective risk factors analysis and comparison with other strategies by the EBMT Acute Leukemia Working Party. J Clin Oncol 25:4938-45, 2007
5. Christopeit M, Kuss O, Finke J, et al: Second allograft for hematologic relapse of acute leukemia after first allogeneic stem-cell transplantation from related and unrelated donors: the role of donor change. J Clin Oncol 31:3259-71, 2013
6. Motabi IH, Ghobadi A, Liu J, et al: Chemotherapy versus Hypomethylating Agents for the Treatment of Relapsed Acute Myeloid Leukemia and Myelodysplastic Syndrome after Allogeneic Stem Cell Transplant. Biol Blood Marrow Transplant 22:1324-9, 2016
7. Dombret H, Seymour JF, Butrym A, et al: International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. Blood 126:291-9, 2015
8. Fehniger TA, Uy GL, Trinkaus K, et al: A phase 2 study of high-dose lenalidomide as initial therapy for older patients with acute myeloid leukemia. Blood 117:1828-33, 2011
9. Craddock C, Labopin M, Robin M, et al: Clinical activity of azacitidine in patients who relapse after allogeneic stem cell transplantation for acute myeloid leukemia. Haematologica 101:879-83, 2016
10. Sockel K, Bornhaeuser M, Mischak-Weissinger E, et al: Lenalidomide maintenance after allogeneic HSCT seems to trigger acute graft-versus-host disease in patients with high-risk myelodysplastic syndromes or acute myeloid leukemia and del(5q): results of the LENAMAINT trial. Haematologica 97:e34-5, 2012
11. Kneppers E, van der Holt B, Kersten MJ, et al: Lenalidomide maintenance after nonmyeloablative allogeneic stem cell transplantation in multiple myeloma is not feasible: results of the HOVON 76 Trial. Blood 118:2413-9, 2011
12. Choi J, Ritchey J, Prior JL, et al: In vivo administration of hypomethylating agents mitigate graft-versus-host disease without sacrificing graft-versus-leukemia. Blood 116:129-39, 2010
13. Goodyear OC, Dennis M, Jilani NY, et al: Azacitidine augments expansion of regulatory T cells after allogeneic stem cell transplantation in patients with acute myeloid leukemia (AML). Blood 119:3361-9, 2012
14. Schroeder T, Frobel J, Cadeddu RP, et al: Salvage therapy with azacitidine increases regulatory T cells in peripheral blood of patients with AML or MDS and early relapse after allogeneic blood stem cell transplantation. Leukemia, 2013
15. Craddock C, Jilani N, Siddique S, et al: Tolerability and Clinical Activity of Post-Transplantation Azacitidine in Patients Allografted for Acute Myeloid Leukemia Treated on the RICAZA Trial. Biol Blood Marrow Transplant 22:385-90, 2016
16. Rogatko A, Schoeneck D, Jonas W, et al: Translation of innovative designs into phase I trials. J Clin Oncol 25:4982-6, 2007
17. Nie L, Rubin EH, Mehrotra N, et al: Rendering the 3 + 3 Design to Rest: More Efficient Approaches to Oncology Dose-Finding Trials in the Era of Targeted Therapy. Clin Cancer Res 22:2623-9, 2016
18. O'Quigley J, Pepe M, Fisher L: Continual reassessment method: a practical design for phase 1 clinical trials in cancer. Biometrics 46:33-48, 1990
19. Iasonos A, Wilton AS, Riedel ER, et al: A comprehensive comparison of the continual reassessment method to the standard 3 + 3 dose escalation scheme in Phase I dose-finding studies. Clin Trials 5:465-77, 2008

20. Yap C, Billingham LJ, Cheung YK, et al: Dose Transition Pathways: The Missing Link Between Complex Dose-Finding Designs and Simple Decision-Making. Clin Cancer Res 23:7440-7447, 2017

21. Goodman SN, Zahurak ML, Piantadosi S: Some practical improvements in the continual reassessment method for phase I studies. Stat Med 14:1149-61, 1995

22. Cheson BD, Bennett JM, Kopecky KJ, et al: Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 21:4642-9, 2003

23. Stein EM, DiNardo CD, Pollyea DA, et al: Enasidenib in mutant IDH2 relapsed or refractory acute myeloid leukemia. Blood 130:722-731, 2017

24. Sharma M, Ravandi F, Bayraktar UD, et al: Treatment of FLT3-ITD-positive acute myeloid leukemia relapsing after allogeneic stem cell transplantation with sorafenib. Biol Blood Marrow Transplant 17:1874-7, 2011

25. Kong Y, Zhu L, Schell TD, et al: T-Cell Immunoglobulin and ITIM Domain (TIGIT) Associates with CD8+ T-Cell Exhaustion and Poor Clinical Outcome in AML Patients. Clin Cancer Res 22:3057-66, 2016

26. Schnorfeil FM, Lichtenegger FS, Emmerig K, et al: T cells are functionally not impaired in AML: increased PD-1 expression is only seen at time of relapse and correlates with a shift towards the memory T cell compartment. J Hematol Oncol 8:93, 2015

27. Kong Y, Zhang J, Claxton DF, et al: PD-1(hi)TIM-3(+) T cells associate with and predict leukemia relapse in AML patients post allogeneic stem cell transplantation. Blood Cancer J 5:e330, 2015

28. Mussai F, De Santo C, Abu-Dayyeh I, et al: Acute myeloid leukemia creates an arginase-dependent immunosuppressive microenvironment. Blood 122:749-58, 2013