



Combination romidepsin and azacitidine therapy is well tolerated and clinically active in adults with high-risk acute myeloid leukaemia ineligible for intensive chemotherapy

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Introduction

Treatment options for patients with acute myeloid leukaemia (AML) who are ineligible for intensive chemotherapy are limited and, as a consequence, patient outcomes remain poor.¹ A significant advance has been the use of epigenetic therapies in patients with AML and high risk myelodysplastic syndromes (MDS), and azacitidine remains the backbone of regimens involving novel agents, including venetoclax.^{2,3} However, in patients with relapsed/refractory AML, the

Summary

Azacitidine (AZA) is important in the management of patients with acute myeloid leukaemia (AML) who are ineligible for intensive chemotherapy. Romidepsin (ROM) is a histone deacetylase inhibitor which synergises with AZA *in vitro*. The ROMAZA trial established the maximum tolerated dose (MTD) of combined ROM/AZA therapy in patients with AML, as ROM 12 mg/m² on Days 8 and 15, with AZA 75 mg/m² administered for 7/28 day cycle. Nine of the 38 (23.7%) patients treated at the MTD were classified as responders by Cycle 6 (best response: complete remission [CR]/incomplete CR *n* = 7, partial response *n* = 2). Correlative next-generation sequencing studies demonstrated important insights into therapy resistance.

Keywords: acute myeloid leukaemia, relapsed, refractory, early phase, clinical trial, hypomethylating agent.

activity of azacitidine monotherapy is reduced⁴ and strategies which increase the activity of azacitidine are required. *In vitro* studies demonstrated evidence of synergistic anti-leukaemic activity between azacitidine and histone deacetylase inhibitors (HDACi).⁵

Emerging clinical data suggest that azacitidine used with the HDACi, romidepsin, may have significant anti-tumour activity and synergy.⁶ Romidepsin belongs to the cyclic peptide family⁷ and inhibits different pathways to previously investigated HDACi.⁸ Romidepsin has been shown to be

clinically potent and well-tolerated as monotherapy in patients with peripheral T-cell lymphoma,⁹ but has not been investigated in AML. Therefore, in this phase I/II ROMAZA trial, we examined the safety and efficacy of romidepsin and azacitidine in patients with newly diagnosed, relapsed or refractory AML who are ineligible for conventional chemotherapy.

Methods

Study conduct

Review boards of participating institutions approved the study protocol, which was conducted according to the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Conference on Harmonization (EudraACT No: 2011-005023-40 and ISCRTN:69211255).

Patients and investigations

Patients with newly diagnosed, relapsed or refractory AML as defined by the World Health Organisation classification and deemed ineligible for intensive chemotherapy on the grounds of age or co-morbidities, were eligible for trial inclusion. Patients with prior treatment with demethylating agents were ineligible.

Treatment. The maximum tolerated dose of romidepsin in combination with azacitidine was determined using an escalating/de-escalating 3 + 3 cohort design. Patients were recruited in planned cohort sizes of three up to a sample size of 18, with an additional 35 patients recruited to an expansion cohort. (Figure S1, Data S1). Patients initially received up to six cycles of combination therapy and continued if benefiting clinically.

Adverse event reporting. Tolerability and safety were assessed according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. A dose-limiting toxicity (DLT) was defined as a clinically significant grade 3 or 4 non-haematological toxicity, excluding nausea (manageable with anti-emetics) and fatigue (persisting for more than seven days), which occurred within the DLT monitoring period and was related to the trial medication.

Endpoints. The primary endpoint for the escalation cohort was to determine the maximum tolerated dose (MTD) of the romidepsin/azacitidine combination (Figure S3). The objective for the expansion cohort was to provide preliminary efficacy and safety data of the combination at the MTD with response defined as acquisition of complete remission (CR), incomplete CR (CRi) or partial response (PR) as the primary endpoint. This was assessed at the end of three and six cycles of treatment according to modified Cheson criteria.¹⁰ Secondary endpoints were tolerability and safety of the combination

and overall survival for patients treated at the MTD. Further details of statistical and mutational analysis using next-generation sequencing (NGS) are available in Data S1.

Results

Baseline characteristics

Forty-eight patients were recruited from October 2013 to October 2017 (Figure S1). Thirteen patients were recruited to the dose-finding cohorts, which included four replacements for those who did not complete the DLT monitoring period. A subsequent 35 patients were recruited to the expansion cohort of patients treated at the MTD (cohort 2) (Figure S2). Baseline patient characteristics are listed in Table I, 36/48 (75%) had relapsed/refractory disease.

Treatment administration

Patients received a median of 2.5 cycles of treatment across the whole trial. Twenty out of 38 patients treated at the MTD discontinued their treatment before the third cycle of treatment and were not assessed for their response. Only 4/20 discontinued treatment due to treatment toxicity.

MTD assessment

In the absence of DLTs in the first cohort (10 mg/m² romidepsin on Day 8 and 15, and 75 mg/m² azacitidine on Day 1–9 (in a 5-2-2 combination)) and second cohort (12 mg/m² romidepsin on Day 8 and 15, and 75 mg/m² azacitidine on Day 1–9) (Figure S1), a third cohort of four patients was opened at 12 mg/m² romidepsin on Day 8, 15 and 22 and 75 mg/m² azacitidine on Day 1–9. A total of four patients were recruited as one patient was unevaluable. All patients experienced at least one serious adverse event (SAE). Whilst there were no reported DLTs at this dose level, a proportion of patients suffered from nausea, vomiting and associated weight loss. As a result, the dose was de-escalated to that of Cohort 2 (12 mg/m² romidepsin on Day 8 and 15, and 75 mg/m² azacitidine on Day 1–9 (in a 5-2-2 combination)) which was used as the MTD.

Safety and tolerability

The combination therapy was well tolerated: five non-haematological treatment-related adverse events of Grade 3 or higher affecting at least 10% of all trial patients were reported in the study across the 38 patients treated at the MTD (Tables SI and SII).

Response and survival

Using an intention-to-treat (ITT) approach, nine of the 38 (23.7%) patients treated at the MTD were classified as

Table I. Patient characteristics across the whole trial.

Characteristic	Cohort 1 <i>n</i> = 6	Cohort 2 <i>n</i> = 38	Cohort 3 <i>n</i> = 4	Overall <i>n</i> = 48
Disease, <i>n</i> (%)				
Primary	5 (83)	19 (50)	1 (25)	25 (52)
Secondary	1 (17)	19 (50)	3 (75)	23 (48)
Age (years), median (range)	54 (18–67)	69 (31–84)	52 (46–63)	68 (18–84)
Sex, <i>n</i> (%)				
Male	3 (50)	22 (58)	2 (50)	27 (56)
Female	3 (50)	16 (42)	2 (50)	21 (44)
Cytogenetics, <i>n</i> (%)				
Favourable risk	0 (0)	1 (2)	0 (0)	1 (2)
Intermediate risk	2 (33)	22 (58)	0 (0)	24 (50)
Poor risk	3 (50)	12 (32)	4 (100)	19 (40)
Not known/failed	1 (17)	3 (8)	0 (0)	4 (8)
Disease status, <i>n</i> (%)				
Untreated	0 (0)	11 (29)	1 (25)	12 (25)
Relapse	6 (100)	22 (58)	3 (75)	31 (65)
Primary refractory disease	0 (0)	5 (13)	0 (0)	5 (10)
<i>FLT3-ITD</i> , <i>n</i> (%)				
Present	1 (17)	6 (16)	1 (25)	8 (17)
Absent	2 (33)	27 (71)	2 (50)	31 (64)
Unknown	3 (50)	5 (13)	1 (25)	9 (19)
<i>NPM1</i> mutation, <i>n</i> (%)				
Present	0 (0)	4 (11)	0 (0)	4 (8)
Absent	3 (50)	29 (76)	3 (75)	35 (73)
Unknown	3 (50)	5 (13)	1 (25)	9 (19)
ECOG status, <i>n</i> (%)				
0	6 (100)	22 (58)	3 (75)	31 (65)
1	0 (0)	12 (32)	0 (0)	12 (25)
2	0 (0)	4 (10)	1 (25)	5 (10)
Prior allogeneic stem cell transplant, <i>n</i> (%)	4 (67)	10 (26)	3 (75)	17 (35)

ECOG, Eastern Cooperative Oncology Group.

responders by Cycle 6 (best response: CR/CRi *n* = 7, PR *n* = 2) (Figure S4) (a pre-specified success criteria by A'herns design required 10/38 responders). Of the 11 patients who were previously untreated, four (36.4%) had a response. In the 27 patients with relapsed/refractory disease there were five responses (18.5%). Of the 29 non-responders, 20 discontinued treatment prior to end of Cycle 3 and consequently did not undergo response assessment.

At the date of data cut-off (21st May 2020), one patient in Cohort 2 remained on treatment and there were 45/48 deaths. The median overall survival of the 38 patients treated at the MTD was 6.21 months (95% confidence interval [CI]: 3.71, 8.87), whilst the median overall survival for the 9/38 patients who responded from Cohort 2 was 15.3 months (95% CI: 4.3, 29.1).

Mutational analysis by NGS at baseline and sequentially post-treatment

We obtained diagnostic mutational status across 97 genes in 35 patients using NGS. The median number of variants per patient was six (Figure S5). The most common mutations

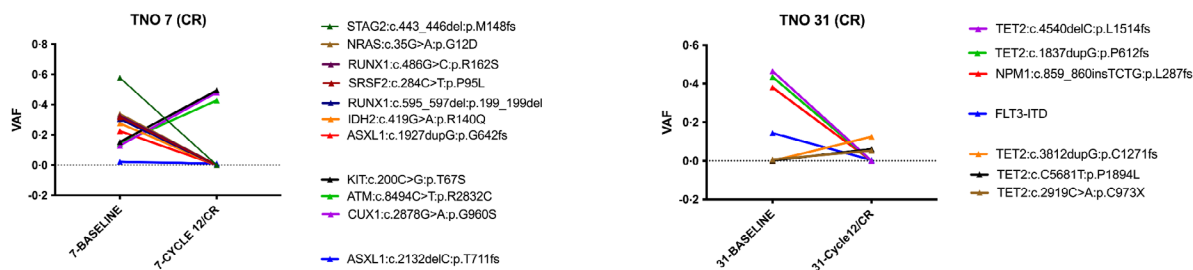
were in *RUNX1* and *FLT3*, reflecting predominance of adverse risk genetic mutations,^{11,12} consistent with our population disease history.

Serial clonal structures in responding and non-responding patients

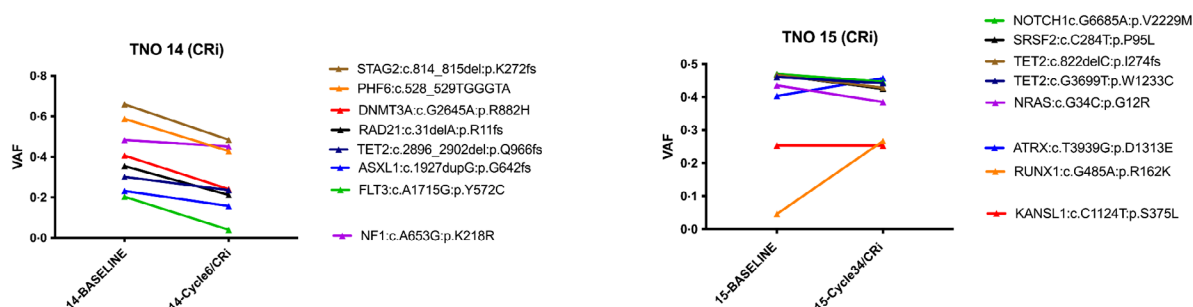
We next examined the pattern of clonal mutational architecture at different sequential time points in response to azacitidine and romidepsin, and related this to their clinical responses. In two patients achieving a CR (Fig 1A), the dominant clone present at commencement of therapy was suppressed, suggesting clonal selection in responding patients. For example, in TNO 7 mutations, pre-treatment include *STAG2*, *NRAS*, *RUNX1* and *SRSE2*. However, NGS analysis of the sample after Cycle 12, at a time when the patient remained in CR, a subdominant clone – present at diagnosis – consisting of *ATM/KIT/CUX1* mutations becomes the dominant clone.

In contrast, recurrent myeloid mutations tended to persist, at similar VAFs, in patients who achieved a CRi (*n* = 3,

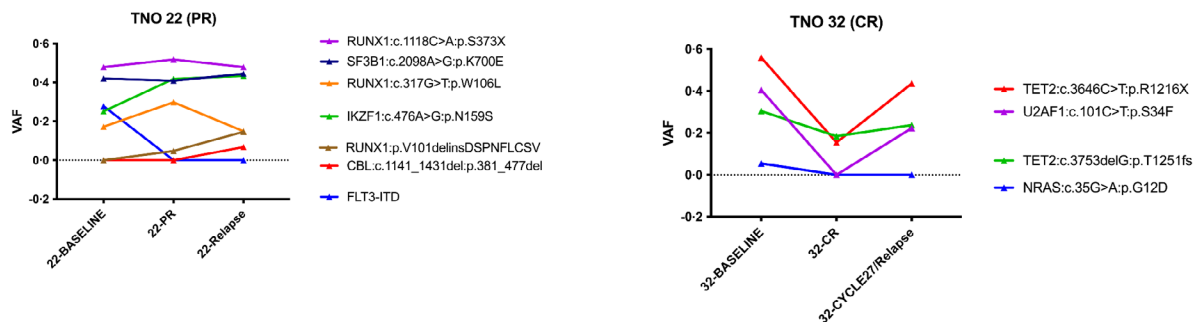
(A)
Mutation analysis at baseline and at CR



(B)
Mutation analysis at baseline and at CRi



(C)
Mutation analysis at baseline, response and at relapse



(D)
Mutation analysis at baseline, and at resistant disease

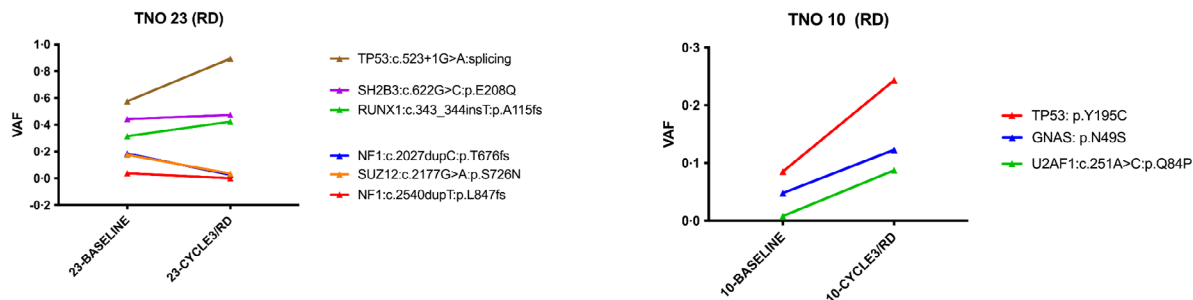


Fig 1. Mutational analysis of patients at baseline and in sequential samples post-azacitidine and -romidepsin treatment. Mutational analysis at baseline and sequential treatment samples for patients with (A) complete remission (CR); (B) with an incomplete CR (CRi), and (C) patients who achieve a response but subsequently relapse, and (D) patients with resistant disease. [Colour figure can be viewed at wileyonlinelibrary.com]

Fig 1B; Figure S6B). This could be consistent with the persistence of pre-leukaemic or leukaemic clones. If these clones are leukaemic then it would suggest that the mechanism of action of romidepsin and azacitidine is to promote differentiation.

In patients who responded and subsequently have a documented relapse (Fig 1C), complex patterns of clonal evolution were seen. In TNO22, a *FLT3*-ITD clone decreased in size when the patient was in PR and remained low at relapse. In contrast, cells with *RUNX1/CBL* mutations were selected for at PR and the VAF of these mutations increased at relapse – consistent with resistance to azacitidine and romidepsin. In contrast, in patient TNO32 the VAF of mutations in *TET2*, *U2AF1* and *NRAS* were reduced at CR, only to increase at relapse. This is consistent with leukaemic cells showing initial therapy sensitivity followed by therapy resistance, possibly through epigenetic mechanisms. Finally, we observed that in patients with resistant disease ($n = 5$, Fig 1D; Figure S6A) mutant clones persist or expand.

Discussion

Responses in the relapsed/refractory setting are challenging to obtain and this study exemplifies the difficulty in treating this cohort of less fit patients with rapid kinetic disease.¹³ Of particular note, 17 (35%) had previously received an allogeneic stem cell transplant.

The tolerability of the ROM/AZA regimen is notable because previous experiences of HDACi were associated with increased toxicity. Romidepsin belongs to a different class of HDACi, as previously investigated in AML,^{7,14} and we demonstrate that this combination can be safely delivered in the outpatient setting.

In order to obtain insights into the mechanism of this novel combination we monitored the clonal architecture of the disease through the serial NGS detection of mutations. The prevalence of *TP53* mutations appear to reflect the heavily pre-treated nature of this cohort of patients, and in patients who subsequently have progressive disease, these mutant clones expand, in keeping with previous results of patients treated with decitabine/panobinostat.¹⁵

In summary, this study established a MTD for combined ROM/AZA therapy that is safe and clinically active within adults with relapsed AML. Further studies will be required to compare the clinical activity of ROM/AZA directly to a comparator treatment arm.

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Author contributions

JL, MM, RB, AJ, PV and CC analysed the data. LH, RB, AJ and SF were involved in the management of the trial. ET, JL, AP, MD, PV and CC recruited patients to the study. CC designed the trial. All authors had access to the primary data and contributed to manuscript development. All authors assumed responsibility for executing the study according to the protocol and statistical analysis plan, completeness and integrity of the data, and the decision to submit the manuscript.

Disclosures

PV is on the advisory boards for BMS, Daiichi Sankyo, Astellas, AbbVie, Pfizer, Jazz; speaker bureau for AbbVie, Novartis, Jazz, Daiichi Sankyo, BMS, the Takeda scientific advisory board for Auron, Oxford Biomedica and receives research support from CD47 Inc, BMS, Novartis, GSK. CC has received honoraria from Celgene, Daichi-Sankyo, Novartis and Pfizer as well as research funding from Celgene. JL has received travel funding from Novartis and Daichi-Sankyo, and honoraria from Pfizer, Janssen and Amgen. JP has received travel funding and honoraria from Daichi-Sankyo and Jazz.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Supplementary Methods.

Table SI. Treatment-related adverse events.

Table SII. Treatment-related serious adverse events.

Fig S1. Flow diagram summarising trial.

Fig S2. Overview of patients treated at the maximum tolerated dose.

Fig S3. Schema to determine MTD.

Fig S4. Swimmer plot of response for cohort treated at MTD.

Fig S5. Mutation profile at baseline for 35 patients.

Fig S6. Mutational analysis at baseline and samples subsequent to treatment.

Table SIII. Sequencing coverage per gene.

Table SIV. Sequencing coverage of each patient sample.

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