Leukemia Research 81 (2019) 43–49

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Research paper

Sequential azacitidine and lenalidomide for patients with relapsed and refractory acute myeloid leukemia: Clinical results and predictive modeling using computational analysis

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ARTICLE INFO

Keywords:
Acute myeloid leukemia
Relapsed
Lenalidomide
Azacitidine
Computational modeling
Prediction

ABSTRACT

Background: Patients with relapsed and refractory (R/R) acute myeloid leukemia (AML) have limited treatment options. Genomically-defined personalized therapies are only applicable for a minority of patients. Therapies without identifiable targets can be effective but patient selection is challenging. The sequential combination of azacitidine with high-dose lenalidomide has shown activity; we aimed to determine the efficacy of this genomically-agnostic regimen in patients with R/R AML, with the intention of applying sophisticated methods to predict responders.

Methods: Thirty-seven R/R AML/myelodysplastic syndrome patients were enrolled in a phase 2 study of azacitidine with lenalidomide. The primary endpoint was complete remission (CR) and CR with incomplete blood count recovery (CRi) rate. A computational biological modeling (CBM) approach was applied retrospectively to predict outcomes based on the understood mechanisms of azacitidine and lenalidomide in the setting of each patients' disease.

Findings: Four of 37 patients (11%) had a CR/CRi; the study failed to meet the alternative hypothesis. Significant toxicity was observed in some cases, with three treatment-related deaths and a 30-day mortality rate of 14%. However, the CBM method predicted responses in 83% of evaluable patients, with a positive and negative predictive value of 80% and 89%, respectively.

Interpretation: Sequential azacitidine and high-dose lenalidomide is effective in a minority of R/R AML patients; it may be possible to predict responders at the time of diagnosis using a CBM approach. More efforts to predict responses in non-targeted therapies should be made, to spare toxicity in patients unlikely to respond and maximize treatments for those with limited options.

1. Introduction

Acute myeloid leukemia (AML) patients with relapsed or refractory (R/R) disease have few effective treatment options, limited ability to tolerate toxicity, and very poor outcomes, with a median overall survival measured in weeks [1–3]. Given their specificity and relative tolerability, targeted therapies are appealing for this population [4]; however, despite recent United States Food and Drug Administration approval of the targeted therapies enasidenib, ivosidenib and gilteritinib for *IDH2, IDH1* and *FLT3* positive AML, respectively, patients with these mutations constitute a minority of the R/R AML population. A multitude of interventions over many years of clinical research in R/R AML have resulted in few therapies deemed worthy of pursuit in later phase or registration studies. However, most studies report some patients who derive benefit; these therapies are typically not pursued because there is no efficient way to identify the patients most likely to

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https://doi.org/10.1016/j.leukres.2019.04.005
Received 3 January 2019; Received in revised form 4 April 2019; Accepted 8 April 2019
Available online 13 April 2019
0145-2126/

respond and thus spare needless toxicity to the majority of non-responders. We posited that with new computational modeling approaches, it may be possible to predict responders to regimens with low response rates, or even with therapies whose mechanisms of action are poorly understood. This approach could maximize treatment options for those patients in greatest need.

Lenalidomide (Revlimid, Celgene) is an immunomodulatory agent that is active in myeloid malignancies. In patients with del(5q) myelodysplastic syndrome (MDS), lenalidomide induces the ubiquitination of the haploinsufficient casein kinase 1A1, allowing for the selective eradication of disease cells [5]. Due to frequent and deep responses [6], lenalidomide is the first-line therapy for these patients [7]. While lenalidomide has activity as a single agent in R/R [8,9] and untreated [10] AML patients, the response rate is significantly lower than in del (5q) MDS; this is also true for AML patients with a del(5q) [11], suggesting the mechanism of action of lenalidomide in AML is different. In an attempt to improve responses, lenalidomide has been added to the DNA methyltransferase inhibitor azacitidine (Vidaza, Celgene) in sequential combination in newly diagnosed patients [12]; however, a randomized study did not show improvement with this regimen compared to azacitidine alone [13]. In the R/R setting, this combination resulted in an overall response rate (ORR) of 25% and a median overall survival (OS) of 9.8 months for responders [14]. Here we report the clinical results of a larger independent dataset of R/R AML patients who received the sequential combination of azacitidine and lenalidomide, with rigorous attempts to predict responders through the application of predictive analysis of patients' genomic profiles using computational biology modeling (CBM).

2. Methods

2.1. Patients

Eligible patients were adults aged 18 or older with R/R non-acute promyelocytic leukemia AML or MDS that was intermediate 2 or high risk by the International Prognostic Scoring System (IPSS) [15]. All patients had an Eastern Cooperative Oncology Group performance status of less than three, a white blood cell count less than 10,000/L at study entry (hydroxyurea was permissible to achieve this), creatinine less than 2.0 or creatinine clearance greater than 30 ml/min and total bilirubin less than two times the upper limit of normal. Prior allogeneic stem cell transplantation was allowable, but patients could not have relapsed prior to day 30 or have active acute graft versus host disease (GVHD) ≥ grade 2 or active extensive chronic GVHD. The study was approved by the institutional review board; all patients provided written informed consent prior to any procedures, and the study was performed in accordance with the Declaration of Helsinki. The complete protocol is available in the supplemental materials.

2.2. Study design and treatments

This was a single-institution, single arm phase 2 study (NCT01743859) of sequential azacitidine with lenalidomide. Patients received azacitidine 75 mg/m^2 intravenously or subcutaneously on days 1–7. On day 8 patients self-administered 50 mg lenalidomide daily through day 28. No treatment was administered on days 29–42; the 42-day period was considered a treatment cycle (Fig. 1). Patients had bone marrow biopsies performed for response assessments at the end of cycles 1, 3 and 6. Those who tolerated therapy and were deriving benefit could continue treatment cycles indefinitely. Response assessments were performed based on European LeukemiaNet guidelines [16]. The primary objective was to determine the efficacy as measured by the response rate (complete remission [CR] + CR with incomplete recovery of counts [CRi]); 37 patients were required to determine if the CR/CRi rate differed significantly from the null hypothesis of a 15% CR/CRi rate based on historical data, assuming an alternative hypothesis of a 30% CR/CRi rate. Secondary endpoints included the overall response rate (CR + CRi + partial remission [PR] + morphological leukemia free state [MLFS]), response duration, progression free survival, overall survival (OS), toxicity profile and the number of patients deemed eligible to proceed to an allogeneic stem cell transplantation or donor lymphocyte infusion if adequate disease control could be obtained.

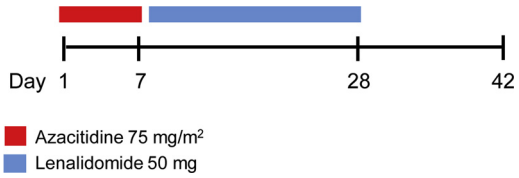

Fig. 1. Treatment cycle of the sequential azacitidine with lenalidomide regimen. Azacitidine was administered on days 1–7; lenalidomide was given on days 8-28. No therapy was given on days 29-42. A 42-day period constituted one cycle of therapy.

2.3. Next generation sequencing

Genomic DNA from whole bone marrow at AML diagnosis or relapse was assayed using the Rain Dance Thunder Bolts Myeloid Panel. Libraries were prepared and then purified using SPRISelect beads, index primers were added, and the resulting barcoded samples purified again with SPRISelect beads. Library quality and quantity were assessed on the Agilent TapeStation 2200 and run on an Illumina MiSeq sequencer. Variants at greater than or equal to 5% allelic frequency were reported. In many cases, a germline tissue source (buccal swabs or fingernails) was tested alongside the initial bone marrow sample to exclude germline mutations.

2.4. Computational biology modeling method

The computational biology modelling (CBM) system used in this study is a validated network of signalling, metabolic, epigenetic and transcriptional regulatory pathways underlying cancer physiology [17–20], created by manually curating and aggregating published experimental data representing the functionality of genes, proteins and their interactions mathematically [17]. Prospective and retrospective validations have been performed [18–21], and it was recently utilized to predict responses to a novel therapy [22]. The CBM coverage includes pathway networks underlying many cancer phenotypes including growth factor, cytokine and chemokine signalling pathways, transcriptional, post-transcriptional, translational and post-translational regulation, epigenetic regulation, cell cycle machinery, oxidative and ER stress, protein homeostasis, DNA repair pathways, apoptotic cascade and TP53 signalling, metabolic pathways, angiogenic and immune-suppressive pathways, and others. The CBM includes about 112 central pathways, over 75,000 reactions, and 3300 cancer specific-genes including comprehensive coverage of the kinome, transcriptome, proteome and metabolome. This extensively integrated network that makes up the CBM can be used to predict a patient's response to a single drug or a combination of drugs. Both prospective and retrospective validations have been shown in studies of glioblastoma multiform, multiple myeloma, myeloproliferative neoplasms, early T-cell precursor acute lymphoblastic leukemia, non-small-cell lung carcinoma, AML and MDS [18–21,31].

To create the profiles, baseline cytogenetic and mutational profiles of the patients were obtained, and genomic aberrations were interpreted for phenotypic implications (i.e., gain of function versus loss of function). The cytogenetic segments related to deletions, gains, translocations or derivatives were interpreted as amplifications and deletions of the genes residing in those segments. Genes found on the loci of affected regions of the chromosome were extracted from the human reference genome at ENSEMBL, and the complete list of genes was

matched with the CBM to identify those represented in the model. All genes that have coverage in the model (Supplemental Table 1) are included in the input file that is used to create the patient's cancer avatar. Genes reported to have a gain in copy number due to chromosomal amplifications are interpreted as being over-expressed at the gene expression level, while those genes in the deleted segments are considered a loss of copy number and are interpreted as having a knock-down in the model (Supplemental Table 1 and 2). For mutation signatures, the gene variants with known functional impact and therapeutic implication were searched in the public domain and recorded in a mutation library. Mutational signatures were processed through an internal variant calling workflow that utilizes DbNFSP, a database that uses multiple prediction algorithms including SIFT, FATHMM, Mutation Assessor, LRT, Mutation Taster, PROVEAN, MetaSVM, and others, to determine if the gene mutation would have a functional impact on the protein, classified as either deleterious or non-deleterious based on a concordance of more than five algorithms [23–27]. A deleterious mutation in an oncogene was assumed to be a gain of function mutation at the protein activity level, or a loss of function if present in a tumor suppressor gene. Frameshift and missense mutations were assumed to cause a loss of protein function, except in those cases where there is experimental evidence that the mutation causes a gain of function.

The input file was then overlaid on the control model (non-leukemic baseline) by indicating the mutations, amplifications, deletions and translocations, and the profile was simulated to create a dynamic disease state. Protein network maps were created for each patient profile based on their input data and disease specific biomarkers that were unique to each profile.

A digital drug model of azacitidine and lenalidomide was created by programming its mechanism of action (azacitidine modelled as an inhibitor of DNA methyltransferases and lenalidomide modelled to induce the cereblon-mediated degradation of casein kinase and Ikaros [5,28]) and the resultant effects on specific pathways and biomarkers determined from published literature. The digital drug model of azacitidine, lenalidomide and its combination was applied to each patient's disease via computer simulation. The efficacy of azacitidine, lenalidomide and its combination for each patient was measured as a function of disease inhibition score (DIS) – the degree to which crucial cancer signaling pathways and phenotypes were repressed. DIS is a composite of the percentage impact on proliferation and viability index with the drug in reference to the untreated disease network. The proliferation index is an average function of the active CDK-cyclin complexes that define cell cycle checkpoints and is determined by calculating contributions in the biomarkers CDK4-CCND1, CDK2-CCNE, CDK2-CCNA and CDK1-CCNB1. A viability index based on survival and apoptosis markers was also generated for each patient. The biomarkers constituting the survival index include AKT1, BCL2, MCL1, BIRC5, BIRC2 and XIAP, while the apoptosis index comprises the pro-apoptotic markers of caspases, Puma and cleaved PARP. Viability of a cell is calculated as a ratio of survival index/apoptosis index, and the weightage of each biomarker is adjusted to achieve a maximum correlation with experimental trends of the endpoint from peer-reviewed studies. For each case a DIS was calculated using the formula:

$$\text{Disease inhibition score} = \text{Proliferation} + \text{Viability} = \text{Proliferation} + \text{Survival/Apoptosis}$$

2.5. Statistics

OS was calculated from the date of study entry to the date of death from any cause. Patients who were living on September 25th, 2018 were censored for OS, and median OS was calculated on the same date. No patients were lost to follow up. Univariate logistic regression analyses were used to assess the effects of each covariate on the response status. For each model the outcome was response status. Two-sided p-values were calculated, and a significance threshold of $p = 0.05$ was used for all tests. Analysis was carried out in R v 3.5.1.

For CBM predictive results, a DIS ≥ 30% was classified as sensitive (responder), and a DIS < 30% was classified as resistant (non-responder) for single drug. For the combination, a DIS ≥ 40% was classified as sensitive (responder), and a DIS < 40% was classified as resistant (non-responder). The thresholds for CBM response prediction have been validated previously in independent datasets [18,22,29–31]. Post-hoc analysis of virtual responders and non-responders were performed to determine all unique cytogenetic and genomic identifiers between the two groups (clinical responders vs non-responders).

To measure the predictive capability of CBM, correlation between actual response and predicted response was assessed by using a confusion matrix. Further, the CBM performance was evaluated by Area Under Receiver Operating Characteristic (AUROC). The confusion matrix calculations included the following definitions:

$$\text{PPV (Positive predictive value)} = TP/(TP + FP)$$

$$\text{NPV (Negative predictive value)} = TN/(TN + FN)$$

$$\text{SPECIFICITY} = TN/(TN + FP)$$

$$\text{SENSITIVITY} = TP/(TP + FN)$$

$$\text{ACCURACY} = (TP + TN)/(TP + TN + FN + FP)$$

TP – true positive – clinical responder and predicted as a responder

TN – true negative – clinical non-responder and predicted as a non-responder

FP – false positive – clinical non-responder but predicted as a responder

FN – false negative – clinical responder but predicted as a non-responder

The statistical analyses also involved the calculation of effective sample size for testing the CBM predictive power to demonstrate its reliability/robustness (for level of significance = 5%).

In high risk MDS or AML, azacitidine with lenalidomide has not demonstrated an overall response rate greater than 41% [12]. Therefore, statistical analysis was performed to determine the effective sample size [32] required to demonstrate the reliability/robustness of the CBM prediction, with an accuracy of 83.3% and statistical power of 90%. The calculated sample size was N = 23 for an effect size of 42%. Because the calculated effective sample size was smaller than actual evaluated patients (N = 24) the number of predictions made by CBM are sufficient to demonstrate its predictive power (Supplemental Table 3).

3. Results

3.1. Patients

Thirty-eight patients were consented and screened and 37 enrolled. The median age was 73 years (range 18–87), and nine (26%) had relapsed after an allogeneic hematopoietic stem cell transplantation. Thirty-four had AML; 21 (57%) had relapsed and 16 (43%) had refractory disease. Three had MDS, all of whom had prior therapy with a hypomethylating agent. Table 1 includes baseline data for all enrolled patients.

3.2. Toxicity

There were 631 adverse events (AEs) recorded. Eighty were considered serious AEs, 37 of which were deemed treatment-related. There were 11 grade 5 events, 3 of which were deemed treatment related (renal failure, infection and hemorrhagic stroke). All related AEs and their incidences are listed in Table 2. During the first cycle, two patients required lenalidomide dose reductions, and 11 patients had

Table 1
Baseline characteristics of enrolled patients.

Characteristic	Median (range) or N (%)
Age, years	73 (18-87)
AML	34
AML not otherwise specified	15 (44%)
AML from antecedent disease	15 (44%) (14 MDS, 1 essential thrombocythemia)
Treatment related AML	3 (8%)
AML with inv(16)	1 (3%)
MDS	3
IPSS Intermediate 2	2
IPSS High	1
SWOG Cytogenetics Category	
Favorable	1 (3%)
Intermediate	9 (26%)
Unfavorable	22 (65%)
Unknown	2 (6%)
Complex cytogenetics	14 (38%)
Monosomal karyotype	12 (32%)
European Leukemia Net Prognostic Group	
Favorable	1 (3%)
Intermediate-1	4 (12%)
Intermediate-2	9 (26%)
Adverse	20 (59%)
Prior therapies	2 (1-5)
Prior hypomethylating agent	23 (62%)
Relapsed	21 (62%)
Duration of last remission (months)	6 (2-36)
Refractory	16 (47%)
Prior allogeneic stem cell transplantation	9 (24%)
Baseline aspirate blasts (%)	33 (6-95)
Required hydroxyurea to enroll	9 (24%)

AML = acute myeloid leukemia.
MDS = myelodysplastic syndrome.
IPSS = international prognostic scoring system.
SWOG = Southwest oncology group.

lenalidomide dose interruptions for infection (N = 4), disease progression (N = 2), rash (N = 1), neuropathy (N = 1), renal insufficiency (N = 1), non-compliance (N = 1) or gastrointestinal toxicity (N = 1).

3.3. Responses

Median follow up time as of the censor date of September 25, 2018 was 4.4 years. The median number of cycles completed was 1 (range 0–5). Nine patients did not complete the first cycle; all nine died of disease progression. The complete remission rate was 11% (CR = 1, CRi = 3), and the overall response rate was 49% (CR = 1, CRi = 3, MLFS = 14). Six additional patients had blast reductions but did not meet criteria for response. The median response duration for all responders was 125 days (23–308) and for patients who achieved a CR/CRi was 216 days (140–308). The median number of cycles to achieve best response was 1 (1–3). Sixteen patients were identified pre-treatment as potential candidates for a first or second allogeneic stem cell transplantation or donor lymphocyte infusion, and 4 (25%) were able to achieve this endpoint. Univariate analysis of potential response predictors did not reveal any to be significant (Table 3a); this was true both when considering MLFS a response and a non-response (Supplemental Table 4).

3.4. Survival

One patient remains alive, 3.8 years after enrollment. Of the 36 who died, 33 died of disease progression and three died of treatment related mortality (acute renal failure, infection and hemorrhagic stroke). Thirty-day mortality was 14% and 60-day mortality was 24%. Median progression-free survival was 112 days for all patients; 216 days for responders (defined as CR + CRi) and 104 days for non-responders. Median OS was 166 days for all patients; 340 days for responders (defined as CR + CRi) and 145 days for non-responders (Fig. 2). Supplemental Fig. 1 shows no differences in outcomes when response is defined as a CR + CRi + MLFS.

Table 2
Related adverse events.

Adverse Event	Any grade	Grade 3	Grade 4	Grade 5
Fatigue, malaise or generalized muscle weakness	36	13		
Thrombocytopenia	25	8	8	
Leukeopenia	23	6	10	
Injection site reaction	19			
Constipation	17	1		
Neutropenia	16	5	11	
Anemia	16	11		
Nausea	15	2		
Diarrhea	15	2		
Pruritus	14	1		
Emesis	9			
Rash	6	1		
Anorexia	6			
Febrile neutropenia	6	2	4	
Increased creatinine	4	1		
Dysgeusia	3			
Peripheral sensory neuropathy	3			
AST increased	3	1		
ALT increased	3			
Purpura or bruising	3			
Acute kidney injury	2	1		1
Edema of limbs	2			
Body odor	1			
Pulmonary hypertension	1	1		
Hyperbilirubinemia	1			
Arthralgia	1			
Myalgia	1			
Periorbital edema	1			
Sinus bradycardia	1			
Flushing	1			
Hemorrhagic stroke	1			1
Malaise	1			
Abdominal pain	1			
Hypotension	1	1		
Back pain	1			
Syncope	1			
Headache	1	1		
Chills	1			
Hyponatremia	1	1		
Hypomagnesemia	1	1		
Lung infection	1			1

AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Table 3a
Univariate predictors for complete remission or complete remission with incomplete recovery of blood counts.

Measure	Effect Estimate with 95% CI	p-value
Age	1.0 (0.937, 1.072)	0.939
AML subtype (secondary vs AML NOS)	1.1 (0.13, 9.339)	0.93
Number of prior therapies	1.4 (0.589, 3.324)	0.447
Relapsed vs refractory	0.6 (0.072, 5.034)	0.638
Prior transplantation	1.0 (0.087, 11.525)	1
Prior hypomethylator	2.5 (0.23, 28.02)	0.447
SWOG cytogenetics score	0.58 (0.067, 5.11)	0.636
Complex karyotype	2.0 (0.236, 16.93)	0.525

AML = acute myeloid leukemia.
CI = confidence interval.
NOS = not otherwise specified.
SWOG = Southwest Oncology Group.

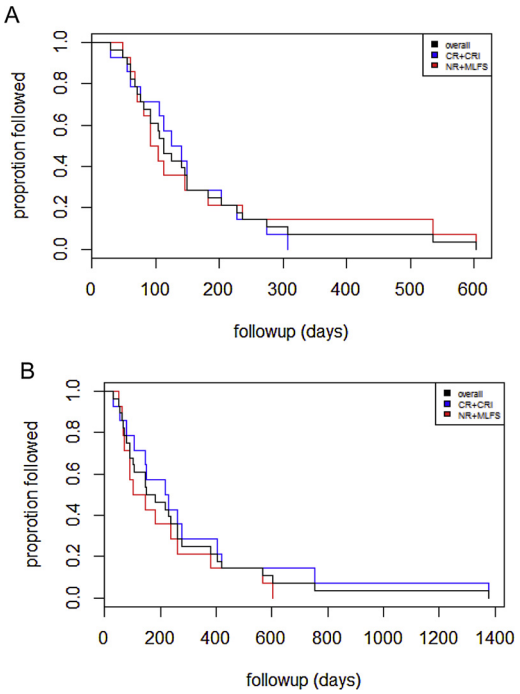

Fig. 2. Progression-free survival (A) and overall survival (B) for all patients (black), responders (blue) and non-responders (red). Responders defined as CR + CRi; non-responders defined as MLFS or no response. No differences were noted between the groups (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.5. Computational biology modeling

Utilizing digital drug simulations to connect the proposed mechanism of action with the effects of azacitidine and lenalidomide on cell signaling networks, we sought to predict patient responses to this regimen. Models were generated from 32 of the 37 patients in the study; of these, 24 patients were clinically evaluable for response. The confusion matrix shows that the CBM method accurately predicted responses in 20/24 (83%) of evaluable patients; the positive predictive value was 80%, the negative predictive value 89%, the sensitivity 73% and the specificity 92% (Supplemental Table 5). Patients with a CR/CRi were accurately predicted in 3/4 (75%) cases, those with a CR/CRi/MLFS were accurately predicted in 12/13 (92%) of cases, and patients without a response were predicted in 8/11 cases (73%) (Table 3b). Supplemental Table 2 shows the accuracy of predicting whether a patient would be likely to respond to azacitidine or lenalidomide as single agents, as well as to the combination of therapie.

The CBM predictive performance was evaluated using a receiver operating characteristics curve which utilized the DIS, an output from CBM. The AUROC was 0.83, which highlights the satisfactory performance of CBM predictive capability (Fig. 3a).

Table 3b
Computational biology method prediction accuracy across responder and non-responder categories.

Category	Sample Size	CBM Prediction Accuracy
Total Evaluable Patients	24	83.33%
CR/CRi	4	75.00%
CR/CRi/MLFS	13	92.31%
No response	11	72.72%

CR = complete remission.
CRi = complete remission with incomplete count recovery.
MLFS = morphologic leukemia free state.
CBM = computational biology modeling.

The post hoc analysis identified some genomic features associated with response in this cohort. CBM identified *WT1*rs16754 (N = 7) and SETBP1 (N = 2) mutations as predictors for response to azacitidine and lenalidomide in this retrospective cohort (Supplemental Table 6). A representative case of a CBM-derived protein network map of a patient with a mutation in *WT1* and a trisomy 8 is shown (Fig. 3b).The *WT1*rs16754 was predicted to result in increased *WT1* expression, which transactivates *DNMT3A* contributing to increased CpG methylation. Trisomy 8 was predicted to result in higher expression of *KAT6A* and *MYC*. *KAT6A* transcribes *EZH2* which recruits DNA methyltransferases required for CpG methylation. *MYC* transcribes *IKZF1* which is a target of lenalidomide [28]. In a digital drug simulation, azacitidine, which inhibits CpG methylation, and lenalidomide, which inhibits *IKZF1*, were predicted be effective because of activation of these targets; this patient did experience a CR (Fig. 3b). Genomic profiles for each patient, along with their true and predicted response status, is included in Supplemental Table 2 and Supplemental Table 6.

4. Discussion

AML is clinically and genetically heterogeneous, especially in the relapsed setting. For that reason, it is unlikely that a universal therapy will be effective for a majority of R/R AML patients. This has led to increased interest in the development of genomically-driven targeted therapies with the hopes that treatment can be personalized based on mutational status. However, there are dozens of genes recurrently mutated in AML, and most patients have several mutations; years of drug development have delivered targeted therapies for *IDH1*, *IDH2* and *FLT3*, with few others in the pipeline. Even if a majority of patients had a druggable mutation, this strategy is not a panacea. The CR/CRi rate for relapsed *IDH*-positive AML patients with enasididenb, the *IDH2* inhibitor, and ivosidenib, the *IDH1* inhibitor, is around 30% [33,34]. Viewed more globally, the 30% CR/CRi rate is only relevant to the 20% of AML patients with an IDH mutation, meaning that if there were 100 R/R AML patients each year, six would achieve a CR/CRi from an IDH inhibitor. Therefore, in the current study of a genomically-unselected R/R population, we must try to identify ways to retrospectively understand the 11% of patients who responded, and then try to apply this knowledge prospectively for future patients with similar features who may be predicted to respond.

In the current study we demonstrate in a limited cohort of patients that response prediction can occur with a high level of accuracy using a CBM approach that accounts for gene mutations, chromosomal abnormalities and the understood mechanism of the treatment. Applied retrospectively, this method accurately predicted responses in 20/24 evaluable patients, including 3/4 with a CR/CRi and 12/13 with a CR/CRi/MLFS. There are limitations to this technique, particularly when considering how to apply it prospectively in real time to patients in need of treatment; a clinical trial is planned to directly test the feasibility of this approach (NCT03446638). In addition, not every intracellular pathway is software coded; the system is representative, but the pathways coded were curated to those that are known to drive AML cell proliferation, AML cell survival, drug mechanism of action and drug resistance. Another limitation is that the computational technology relies on genomic profiling, in this study next-generation sequencing and cytogenetics, which can take 7–28 days for results, limiting its prospective use. In this study the required sample size for the CBM model to have an accuracy of 83.3% and power of 90% was 23, which was exceeded by the actual evaluated patients (N = 24); however, sample size can in some cases limit this approach. The genomic heterogeneity of AML can be a limitation to attempting to predict responders to a novel therapy, particularly with a small sample size. The advantage of CBM is that response prediction is based on the individual patients' genomics and their predicted interaction with the understood mechanism of the treatment, but more experience with larger number of patients will be useful. Future iterations of the model that

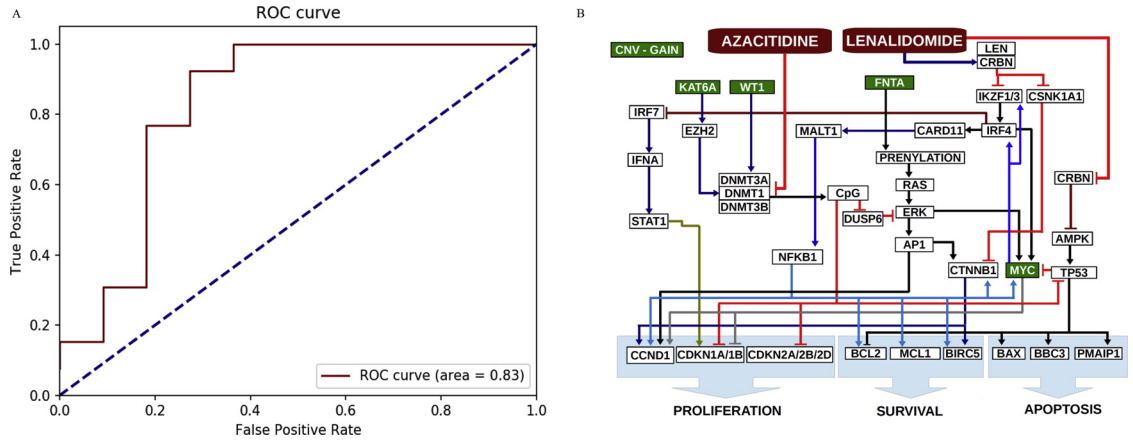

Fig. 3. (a) Statistical analysis of computational biology modeling prediction. The receiver operator curve utilized the disease inhibition score to generate the area under thereceiver operating characteristics, which equaled 0.83. (b) Computational biological modeling (CBM) identifies the molecular rationale for a clinically responsive patient to azacitidine and lenalidomide. CBM correctly predicted the combination of azacitidine and lenalidomide to be responsive in this patient. The responsiveness to azacitidine may have been the result of KAT6A and WT1 amplification due to trisomy 8 and the WT1 rs16754 polymorphism, respectively. Trisomy 8 also resulted in upregulation of MYC which transctivates IKZF1/3, a primary target of lenalidomide.

incorporate non-genomic features such as age and blast percentage may enhance its predictive abilities as well.

In conclusion, we cannot afford to continue to discard therapies that are effective in a minority of patients. When response predictors are genomically defined, it is easier to select patients felt to be appropriate for a given targeted therapy; however, only a minority of patients have genomically-driven targeted therapies available. In the current example, a computational model was applied retrospectively and successfully predicted responders and non-responders using an algorithm that accounts for gene mutations, chromosomal abnormalities and the understood mechanism of a particular therapy. This study of sequential azacitidine and lenalidomide for R/R AML patients had a low response rate, similar to that of other studies reported for this regimen [14]; however, a minority of patients did derive benefit, and efforts should be made to apply this knowledge to other similar patients who would be expected to respond. More efforts to understand predictors for responses to regimens in which only a minority respond could improve overall outcomes for R/R AML patients.

Author contributions

BS, AW, NM, QW, TA, SV, JKS, LD, CRC, AH, DA and CTJ analyzed and interpreted the data. JAG, AF, GH, DS and CS provided study materials or patients and analyzed and interpreted the data. DAP drafted the first draft of the manuscript, analyzed and interpreted the data and provided study materials or patients. All authors participated in writing and gave final approval for publication.

Declaration of interests

DAP has served as an advisory board member for Celgene, Agios, Pfizer, Abbvie, Astellas, Gilead and Diachi and has received research funding from Celgene. TA, SV, NKS, LD and CRC are or have been employed by or received research funding from Cellworks.

Funding

Celgene.

Acknowledgements

BS, AW, NM, QW, TA, SV, JKS, LD, CRC, AH, DA and CTJ analyzed and interpreted the data. JAG, AF, GH, DS and CS provided study materials or patients and analyzed and interpreted the data. DAP drafted the first draft of the manuscript, analyzed and interpreted the data and provided study materials or patients. All authors participated in writing and gave final approval for publication.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.04.005.

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