Leukemia Research 68 (2018) 22–28

Contents lists available at ScienceDirect

Leukemia Research

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

Research paper

AML refractory to primary induction with Ida-FLAG has a poor clinical outcome

Simon Kavanagh[a], Emily Heath[a], Rose Hurren[a], Marcela Gronda[a], Samir H. Barghout[a], Sanduni U. Liyanage[a], Thirushi P. Siriwardena[a], Jaime Claudio[a], Tong Zhang[b], Mahadeo Sukhai[b], Tracy L. Stockley[b,c,d], Suzanne Kamel-Reid[b,c,d,e], Amr Rostom[a], Andrzej Lutynski[a], Dina Khalaf[a], Anna Rydlewski[a], Steven M. Chan[a], Vikas Gupta[a], Dawn Maze[a], Hassan Sibai[a], Andre C. Schuh[a], Karen Yee[a], Mark D. Minden[a], Aaron D. Schimmer[a,*]

[a] Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada
[b] Advanced Molecular Diagnostic Laboratory, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada
[c] Clinical Laboratory Genetics, Laboratory Medicine Program, University Health Network, Toronto, Ontario, Canada
[d] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada
[e] Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada

ARTICLE INFO

Keywords:
AML
Chemotherapy
Molecular genetics
Chemosensitivity

ABSTRACT

We evaluated outcomes of 100 patients with high risk AML treated with Ida-FLAG induction as first-line therapy. 72 achieved remission with one cycle; 19 did not. High risk cytogenetics and *TP53* mutations were associated with failure to achieve remission. In those reaching remission, allogeneic bone marrow transplantation was associated with better relapse-free and overall survival. Those not achieving remission with induction therapy were extremely unlikely to reach remission with further therapy and had a dismal prognosis. Exploratory molecular analysis confirmed persistence of the dominant genetic mutations identified at diagnosis. Ex vivo chemosensitivity did not demonstrate significant differences between responders and non-responders. Thus, Ida-FLAG induction has a high chance of inducing remission in patients with high risk AML. Those achieving remission require allogeneic transplantation to achieve cure; those not achieving remission rarely respond to salvage chemotherapy and have a dismal outcome. Alternatives to conventional chemotherapy must be considered in this group.

1. Introduction

For over four decades, standard therapy for acute myeloid leukemia (AML) has been the combination of three days of an anthracycline and seven days of cytarabine ("3 + 7") [1]. This combination of agents produces complete remission (CR) in approximately 35% to 95% of patients, depending on cytogenetic risk group [2]. Those who do not achieve remission with this regimen can often achieve CR with an intensive reinduction regimen. For example, in patients who do not achieve CR with "3 + 7", reinduction with mitoxantrone, etoposide and high dose cytarabine (NOVE-HiDAC) produces an overall response rate of 53% [3]. Factors predicting response were absence of high risk cytogenetics, younger age and lower bone marrow blast percentage prior to reinduction. However, patients who do not reach CR with intensive reinduction have primary refractory disease and a very poor prognosis [4].

Given the importance of reaching CR in achieving long-term disease control, there has been significant interest in improving the initial chemotherapy regimen beyond '3 + 7' with a goal of increasing rates of remission, especially for patients with high risk disease. One approach has been the use of more intense induction regimens. An early, non-randomised, study of the intensive Ida-FLAG regimen in patients with de novo AML demonstrated CR in 82% of recipients [5]. Likewise, the MRC-AML15 trial treated younger patients with AML, including all cytogenetic risk groups, with DA (daunorubicin/cytarabine), ADE (daunorubicin/cytarabine with etoposide) and Ida-FLAG [6]. Ida-FLAG demonstrated significantly higher rates of CR or CR with incomplete hematological recovery (CRi) after the first cycle of chemotherapy than the other regimens. Patients receiving Ida-FLAG had lower rates of relapse compared to patient receiving DA or ADE, but they experienced an increased rate of deaths while in remission. As a result, overall survival did not differ between patients receiving Ida-FLAG, DA, or

* Corresponding author at: Princess Margaret Cancer Centre, Room 7−417, 610 University Ave, Toronto, ON, M5G 2M9, Canada.
E-mail address: aaron.schimmer@utoronto.ca (A.D. Schimmer).

https://doi.org/10.1016/j.leukres.2018.02.012
Received 19 January 2018; Received in revised form 15 February 2018; Accepted 16 February 2018
Available online 20 February 2018

ADE. However, given the higher rates of CR with Ida-FLAG, this regimen may be preferable for patients with high risk AML whose expected rates of CR with '3 + 7'are lower.

While patients who do not achieve CR with '3 + 7' can frequently be salvaged with higher-intensity reinduction regimens (e.g. 51% CR rate with NOVE-HiDAC [3]), rates of salvage appear lower [7] in those who receive initial therapy with HiDAC-based regimens. The outcomes of those not reaching CR despite initial induction with Ida-FLAG chemotherapy remain unknown. Here, we examined the clinical outcomes of patients with high risk AML who did not respond to first-line induction chemotherapy with Ida-FLAG. In addition, we conducted exploratory analysis of the effect of Ida-FLAG on the genetic subclones driving the disease and the ex vivo chemosensitivity of the leukemic cells from these patients.

2. Materials and methods

After receiving approval from the institution's Research Ethics Board (REB 16-5827), we retrospectively reviewed the outcome of all adult patients with AML who received Ida-FLAG as first-line induction therapy at the Princess Margaret Cancer Centre Cancer between February 2013 and January 2017. Patients were identified through the Leukemia Database in the Princess Margaret Cancer Centre Cancer Registry (REB 01-0573-C). Ida-FLAG was used as first induction for those who, in the opinion of the treating physician, were fit for induction chemotherapy and had high-risk AML. High-risk disease is defined by the presence of an unfavourable karyotype (by UKMRC criteria [2]), therapy-related AML or AML developing after a prior myeloid neoplasm. In some cases, Ida-FLAG was selected as induction therapy due to the clinical circumstances at presentation.

2.1. Treatment protocols

Ida-FLAG induction consisted of idarubicin (10 mg/m^2 IV days 1–3), fludarabine (30 mg/m^2 IV days 1–5), cytarabine (2 g/m^2 IV days 1–5) and granulocyte colony stimulating factor (G-CSF/filgrastim, 300 μg sc days 0–5). Patients who achieved CR and had an identified donor were recommended for allogeneic transplantation if they had suitable performance status and organ function. Two cycles of Ida-FLAG consolidation were recommended for patients not receiving allogeneic transplant.

The reinduction protocols for patients who do not achieve remission with Ida-FLAG were NOVE-HiDAC (mitoxantrone 10 mg/m^2 IV days 1–5, etoposide 100 mg/m^2 IV days 1–5, cytarabine 1.5 g/m^2 IV q12hourly days 6 and 7), a second course of Ida-FLAG, and high-dose cytarabine (1.5 g/m^2 IV days 1–4), mitoxantrone (10 mg/m^2 IV days 1–4), asparaginase (10,000 units/m^2 IM day 5), vinblastine (5 mg/m^2 IV day 5), ATRA (45 mg/m^2, divided, po continuously), valproate (750 mg po t.i.d. continuously) and imatinib (400 mg po daily continuously).

2.2. Response definitions

CR was defined as bone marrow blasts < 5%, absence of circulating blasts and blasts with Auer rods, absence of extramedullary disease and an absolute neutrophil count $\geq 1.0 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L [4]. CRi was defined as the above criteria except residual neutropenia ($< 1.0 \times 10^9$/L) or thrombocytopenia ($< 100 \times 10^9$/L) [4]. Those who did not achieve CR or CRi following a single cycle were considered to be refractory.

2.3. Molecular profiling to identify genetic mutations

In February 2015, our institution began performing routine DNA sequencing of leukemic cells obtained from consenting patients at the time of presentation. Peripheral blood and bone marrow samples were collected at diagnosis and after failure of Ida-FLAG and mononuclear cells were cryopreserved in dimethyl sulfoxide (DMSO). DNA was extracted using phenol/chloroform methods or an automated DNA extraction method (MagAttract DNA Blood Midi M48 Kit; BioRobot M48 workstation; Qiagen, Hilden, Germany) from 350 μL of each sample. Next Generation Sequencing (NGS) was performed using the TruSight Myeloid Sequencing Panel (TMSP; Illumina, San Diego, CA) on the Illumina MiSeq benchtop sequencer as previously described [8]. The TMSP examines fifty-four genes with amplicon-based library preparation and (2 × 250 bp) paired-end sequencing using 50 ng of input DNA. Sequence data were analyzed by the NextGENe (v.2.3.1, SoftGenetics) and MiSeq Reporter (MSR, Illumina) v2.4.60 software packages. Data files from each sample were uploaded into Cartagenia Bench NGS v4.2 (Agilent, Santa Clara, CA) for subsequent filtering to prioritize for reporting those variants that passed all MSR quality criteria including depth of coverage of at least 100× and a variant allele fraction (VAF) threshold of > 5% (> 2% for well-documented hotspots) [8]. Variants with a global population frequency > 1% according to population databases (1000 Genomes [9], NHLBI Exome Sequencing Project [10], Exome Aggregation Consortium [11]) and/or present in the AMDL internal database of recurring variants were excluded. Variants selected for downstream analysis included exonic frameshift and nonsense mutations, previously reported intronic splice site variants, missense variants and in-frame insertions/deletions.

2.4. Ex vivo chemosensitivity assays

Samples of peripheral blood were obtained from consenting patients at diagnosis. AML cells were separated by Ficoll density centrifugation and cryopreserved in DMSO. These cells were thawed, resuspended, centrifuged and resuspended in media (Myelocult H5100 from StemCell Technologies, supplemented with 1% pencillin/streptomycin, stem cell factor 100 ng/mL, interleukin (IL)-7 20 ng/mL, IL-3 10 ng/mL, IL-6 20 ng/mL, FLT3-ligand 10 ng/mL, G-CSF 20 ng/mL and granulocyte-macrophage colony stimulating factor, GM-CSF, 20 ng/mL) and maintained at 37 °C. 5×10^4 cells were treated with doubling concentrations of cytarabine (15.625–4000 nM) in phosphate buffered saline (PBS), fludarabine (31.25–8000 nM) in DMSO, or idarubicin (6.25–1600 nM) in DMSO. Blanks (media only) and negative controls (cells with appropriate solvent only) were used; three technical replicates were examined for each chemotherapy agent. Cell growth and viability was measured 72 h after treatment by CellTiter-Fluor (Promega, Madison, WI).

2.5. Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics (version 24, IBM, USA). Baseline demographic and disease related characteristics were expressed by mean (standard deviation) or median (range) for continuous variables and ratios (in percentages) for categorical variables. The differences in baseline characteristics, AML subgroups, and cytogenetic and molecular risk stratifications between those who did or did not receive a third induction were evaluated by either χ^2 or Fisher exact test for categorical variables and Wilcoxon-Mann-Whitney test for continuous variables. The Kaplan-Meier method was used to estimate survival functions and the Mantel-Cox log-rank test was used to compare the survival curves. Two sided tests were used for all statistical analyses. P values < 0.05 were considered statistically significant. IC50 values for the ex vivo chemosensitivity testing were calculated using GraphPad Prism (version 7.03, GraphPad Software, USA).