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# Short course infusional idarubicin plus intermittent cytarabine and etoposide for refractory hematologic malignancies: clinical and preliminary pharmacological results

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**ABSTRACT**

***Background and Objective.*** **Idarubicin (IDA) is relatively immune to the multidrug resistance P-gp mechanism that is frequently expressed in recurrent and refractory hematologic malignancies. Owing to rapid metabolism *in vivo*, a continuous infusion (CI) of IDA might prolong exposure time to the parent drug rather than its more P-gp susceptible alcohol metabolite. For this reason we developed a brief retreatment schedule incorporating CI IDA in order to obtain clinical as well as preliminary pharmacological data in patients with refractory leukemias and lymphomas.**

***Design and Methods.*** **Eligible patients had either advanced-stage acute myeloid or lymphoid leukemias (AML, ALL) or high-grade non-Hodgkin's lymphomas (NHL) which failed curative-intent frontline or salvage regimens in use at our institution during the study period (July-October 1992). CI IDA 5 mg/m²/d was employed together with intermittent (every 8 hours) intermediate-dose cytarabine (500 mg/m²) and etoposide (200 mg/m²); all drugs were given for 2-4 days. A preliminary pharmacokinetic evaluation of CI IDA was carried out in three patients, including a comparison with bolus delivery in one. The *in vitro* effects of CI-type vs bolus-type IDA delivery in terms of intracellular IDA accumulation and related pro-apoptotic activity were assessed in P-gp- and P-gp+ human leukemic CEM cells by means of cytofluorimetry (IDA fluorescence intensity = FI, annexin V expression), with and without the addition of P-gp inhibitor cyclosporin A (CsA).**

***Results.*** **Complete (2) or partial (4) responses were achieved in a total of 12 patients (17% and 33%, respectively), despite prior treatments with anthracyclines (100% of cases) and cytarabine-etoposide (33% of cases). Hematological toxicity caused the duration of treatment to be reduced from 4 days to 2 days after the first 4 patients. The procedural death rate was 42% (5/12), which was probably related in part to the sum of adverse prognostic characteristics: median patient age 55 years, two-thirds of cases having previously failed second/third-line regimens. The pharmacokinetic study showed an increased plasma AUC value with CI IDA in one patient (2.9-fold increase vs bolus delivery) due to the prolonged presence of low IDA plasma levels (10-20 ng/mL vs 50 ng/mL), as seen in two other cases as well. On the other hand, the *in vitro* study did not prove to be in favor of CI IDA because the FI threshold (>1500 units) associated with increased apoptosis of P-gp+ cells (>10%) was achieved only with bolus-type IDA exposure (50 ng/mL for 30') plus CsA.**

***Interpretation and conclusions.*** **This short regimen demonstrated activity against end-stage leukemias and lymphomas and might prove to be more effective and less toxic in younger patients and in those with less advanced disease. In view of the results from plasma pharmacokinetics and *in vitro* intracellular IDA accumulation and apoptosis assays in lymphoblastic CEM cells, CI IDA 5 mg/m²/day may not represent a better therapeutic option than a rapid bolus injection, particularly in P-gp+ neoplasms. If obtaining an adequate intracellular drug concentration is the primary treatment goal, a higher CI IDA dosage, the addition of a P-gp down-regulator such as CsA and others, and an *in vivo* study focusing on tumor samples from patients could all be helpful.**


*Key words: chemotherapy, idarubicin, multidrug resistance, hematologic malignancies, continuous infusion*

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Idarubicin (IDA) is a recently developed anthracycline whose metabolism, pharmacokinetics and vulnerability to the P-glycoprotein (P-gp)-mediated multidrug resistance mechanism differ sensibly from other similar compounds. IDA is rapidly and extensively metabolized by liver cells to idarubicinol (IDOL), which has a very prolonged plasma half-life of about 50-90 hours and is as cytotoxic as the parent drug.[1] The reduced P-gp vulnerability of IDA and the prolonged infusion-like IDOL-related cytotoxicity could explain, among other things, the higher therapeutic index of IDA reported in acute myelogenous leukemia trials[2,3] and the fact that IDA is being increasingly considered for the management of lymphoid malignancies.[4-6] However, because IDOL is less resistant than IDA to P-gp-mediated cell transport,[7] the

rapid decay of the plasma IDA concentration represents a potential therapeutic limitation in P-gp+ tumors.

In order to lengthen the exposure to IDA in cases with refractory hematologic malignancy at high risk of expressing multidrug resistance mechanisms such as P-gp, we planned to give the drug as a continuous infusion (CI) together with intermittent etoposide and intermediate-dose cytarabine. In a complementary procedure, we performed a preliminary pharmacokinetic analysis of CI IDA plus an *in vitro* assessment of intracellular IDA accumulation and related pro-apoptotic effects on a P-gp+ leukemic cell line. The rational for this study was that CI or tightly-scheduled doses of anthracyclines, cytarabine, etoposide and other drugs have been reported to show varying degrees of effectiveness in advanced blood neoplasms[8-10] associated with clinical patterns of chemoresistance to several compounds, including anthracyclines and other P-gp substrates.[11]

## Patients and Methods

### *Patients*

Eligible patients had either advanced-stage acute myeloid or lymphoid leukemias (AML, ALL) or high-grade non-Hodgkin's lymphomas (NHL) which failed curative-intent front-line or salvage regimens in use at our institution during the study period (July-October, 1992). A patient was eligible for the study if his/her life expectancy was considered to be > 3 months, so as to permit evaluation of tumor response, if prior cumulative anthracycline dose and actual cardiopulmonary function did not contraindicate IDA treatment, and if serum creatinine was < 2 mg/dL and bilirubin < 3 mg/dL.

### *Treatment*

The short, 2-day idarubicin-cytarabine-etoposide (s-ICE) schedule is detailed in Figure 1. IDA was given as daily CI, and cytarabine and etoposide were alternated every 8 hours as 3-hour infusions. IDA dosage was intentionally low, i.e. 5 mg/m$^2$ daily, because of the uncertainty about the toxic side effects related to the CI schedule and because this dose had previously been shown to be effective in refractory acute leukemias.[12] All chemotherapy was given via two independent venous accesses, at least one of which was a central venous catheter for CI IDA. S-ICE was initially conceived as a 4-day regimen; however, the degree of myelotoxicity observed in the first four patients treated led to the shorter scheme presented in Figure 1. Concomitant medications included allopurinol and intravenous hyperhydration to prevent uric acid nephropathy. Subcutaneous granulocyte colony-stimulating factor (filgrastim, G-CSF) was prescribed from the end of IDA infusion until the neutrophil count exceeded $1.5\times10^9$/L following the neutropenic nadir. Responders were to receive two additional s-ICE courses and, whenever possible, very high-dose consolidation chemotherapy with autologous bone marrow or peripheral blood stem cell support.

### *Pharmacological studies*

*Pharmacokinetics.* This study was performed in three patients. Blood samples (5 mL) were collected in heparinized tubes at several times during and after the 48 hour IDA infusion or, in the case of bolus injection, at several times after delivery and up to 120 hours after the end of infusion. Plasma samples were obtained and frozen to –20°C until assayed. Plasma levels of unchanged IDA and its metabolite IDOL were determined after solvent



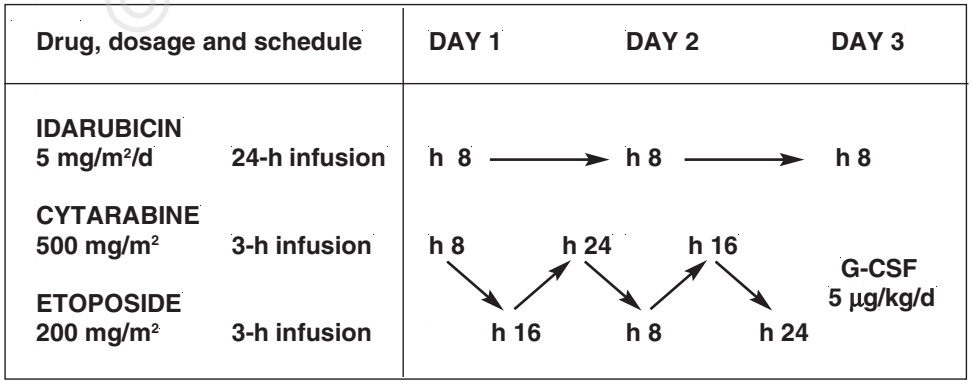


Figure 1. Short ICE regimen.

extraction using an HPLC method and a fluorescence detector.[13] The lower limit of quantitation was 2 ng/mL for both compounds. The half-lives of IDA and IDOL were obtained by regression analysis of the log-concentration vs. time data in the elimination phase of the drugs. The AUCs (areas under the curve) were calculated according to the trapezoidal rule from time 0 up to the last experimental point plus the extrapolated portion of the curve to infinity.

*In vitro study.* This study was conducted to assess the intracellular IDA accumulation and related antiproliferative effects (apoptosis) of bolus and CI IDA, respectively. P-gp$^-$ CEM and P-gp+ CEM-VBL human T-lymphoblastic cell lines were grown in humidified 5% $CO_2$ at 37°C in cell culture medium supplemented with 10% fetal calf serum glutamine, penicillin and streptomycin. IDA was purchased from Pharmacia-Farmitalia Group (Milan, Italy). Working solutions were prepared by dilution in cell culture medium. Experiments mimicking the *in vivo* pharmacokinetics of bolus and CI IDA were carried out according to the drug concentrations reached in patient plasma. CEM cells were exposed to IDA 50 ng/mL for 30' every 24 hours twice (reproducing bolus) or to IDA 10-20 ng/mL for 48 hours (reproducing infusion), respectively, with and without the P-gp blocking agent cyclosporin A (CsA, Sandoz, Bern, Switzerland). CsA was diluted in absolute ethanol and cell medium to a cellular concentration of ethanol < 0.1%, added 90' before IDA, and maintained at a final concentration of 1.5 μg/mL, which is both clinically achievable and able to inhibit P-gp function.[14] IDA-related apoptosis was detected by assessing the expression of phosphatidylserine on the outer leaflet of the cell membrane, using fluorescein isothiocyanate (FITC)-labelled annexin V (Bender MedSystems distributed by Prodotti Gianni, Milan, Italy) as previously described,[15] and according to the manufacturer's technical recommendations. FITC-annexin V expression and intracellular IDA incorporation were determined as described[15,16] on a FACScan flow cytometer (Becton Dickinson, San José, CA, USA) equipped with an argon excitation laser light (488 nm wavelength) and 530/30 nm (FL1) and 585/42 nm (FL2) band pass filters. Cellular fluorescence of $1\times10^6$ cells/mL was determined using FL1 (FITC-annexin V) and FL2 (IDA) filters with the appropriate negative controls and fluorescence compensation. Results of intracellular IDA accumulation were expressed as fluorescence index (FI = % fluorescent cells × mean cellular fluorescence intensity).

### Definitions

For acute leukemia patients, the definition of complete remission (CR) required the reduction of blast cells to < 5% (< 1% obviously leukemic by morphologic criteria and immunophenotype) in the bone marrow, with restoration of normal trilineage hemopoiesis, and an untransfused hemoglobin level >10 g/dL, platelets $>100\times10^9$/L and neutrophils $>1.5\times10^9$/L. For NHL patients, the definition of CR required the normalization of previously abnormal clinical findings. A partial response (PR) consisted of a greater than 50% reduction in tumor load, and no response (NR) was anything less than PR. Toxicity criteria were those defined by the *World Health Organization* (WHO). Grade 3 and 4 toxicities only were reported. Comparisons were made by means of Student's t-test.

## Results

### Patients

Patients and their disease status at the time of s-ICE rescue are detailed in Table 1. In no case were tumor cells tested for the expression of P-gp. Four patients received the 4-day schedule as originally intended, while the next eight followed the amended 2-day schedule. Median patient age was >50 years and virtually all cases manifested clinical multidrug resistance, since they had previously failed either first-line (25% of cases) or salvage (75%) therapies that included anthracyclines (100% of cases), cytarabine (33%), and etoposide (33%).

### Response

An objective response was noted in six cases: 2 CR (case 5 with AML after one 2-day course, case 3 with NHL after one 4-day course) and 4 PR (NHL cases #1, 9, 10, 11). Five patients died early of treatment-related complications, three of whom were not yet evaluable for response and two in PR (cases #10 and 11). The early mortality rate was slightly higher with the 4-day schedule: 2/4 or 50% vs 3/8 or 37.5%. Four out of the 5 early deaths occurred in patients receiving s-ICE as third or fourth-line regimen. The overall response rate was 66% (6/9) among evaluable patients, 57% (4/7) in patients surviving s-ICE retreatment, and 50% (6/12) overall. Three (50%) and five (83%) of the six responders had already been exposed to cytarabine and etoposide, respectively.

### Survival

Duration of survival was 7 and 19 months for two CR patients, respectively, and 4-17 months for PR patients surviving the induction course. Survival was shorter in non-responders (range 1-5 months). Two CR patients (cases 3 and 5) received additional s-ICE courses with a mean intercycle time of 42 and 30 days, respectively, and were both consolidated with high-dose therapy supported by an autologous hematopoietic stem cell transplant. Thereafter, one suffered early progression of NHL and the other with AML died of late heart failure after 18+ months of CR.[17] One PR patient (case #9

**Table 1. Patients and outcome.**

| Pt. no. | Age/sex | Diagnosis* | State of disease° | Prior treatments# | No. of total ICE courses | Outcome@ (Survival) |
|---|---|---|---|---|---|---|
| **4-day s-ICE** | | | | | | |
| 1 | 66/M | NHL, IVm | 3rd RE | CHOP, DHAP, VACOP-B | 1 | PR (4) |
| 2 | 66/F | ALL | 1st RE | IVAP | 1 | ED (1) |
| 3 | 52/F | NHL, IVm | 2nd RE | VACOP-B, DHAP | 2 | CR (7) |
| 4 | 47/F | NHL, IVm | 3rd RE | CHOP, DHAP, MACOP-B | 1 | ED (1) |
| **2-day s-ICE** | | | | | | |
| 5 | 5/F | AML | 2nd RE | DAT, ABMT | 3 | CR (19) |
| 6 | 58/M | CML-LT | 1st RE | IVAP | 1 | NR (2) |
| 7 | 2/F | AUL | 1st RE | VDP | 2 | NR (5) |
| 8 | 59/F | CLL-IB | 2nd RE, PRO | Chl, CHOP | 1 | NR (5) |
| 9 | 59/M | NHL | 2nd RE | CHOP, P-VEBEC | 3 | PR (17) |
| 10 | 46/M | NHL, IVm | 3rd RE/PRO | CVP, CHOP | 1 | PR/ED (1) |
| 11 | 48/M | NHL, IV | 2nd RE | VACOP-B, MACOP-B | 1 | PR/ED (1) |
| 12 | 62/M | NHL, IVm | 3rd RE | CVP, CHOP, DHAP | 1 | ED (1) |

**IVm, stage IV with bone marrow involvement; CML-LT, chronic myelogenous leukemia in acute lymphoblastic transformation; CLL-IB, chronic lymphocytic leukemia-immunoblastic transformation; °RE, recurrence; PRO, progressive disease; #CHOP, cyclophosphamide-doxorubicin-vincristine-prednisone; DHAP, dexamethasone-high-dose cytarabine-cisplatinum; IVAP, idarubicin-vincristine-L-asparaginase-prednisone; VACOP-B/MACOP-B, etoposide/methotrexate-doxorubicin-cyclophosphamide-vincristine-prednisone-bleomycin; ABMT, autologous bone marrow transplantation; VDP, vincristine-daunorubicin-prednisone; Chl, chlorambucil; P-VEBEC, prednisone-vincristine-epirubicin-bleomycin-etoposide-cyclophosphamide; CVP, cyclophosphamide-vincristine-prednisone; @To first s-ICE course: ED, early death; survival expressed in months.*



with NHL) received two more s-ICE courses with a mean intercycle interval of 34 days, without any improvement in response. Case #7 with AUL did not respond to a second course given after 24 days.

### *Toxicity*

Only toxicity related to the first s-ICE course is reported. Myelosuppression was severe. With 4-day s-ICE absolute neutropenia < $0.5\times10^9$/L lasted 10-16 days (median 13 days), and 2 of 4 patients succumbed to infectious complications. With 2-day s-ICE the absolute neutropenic period was shorter: 4-18 days (median 9 days). Thrombocytopenia < $20\times10^9$/L lasted 0-28 days (median 6) (days from last platelet transfusion). Infectious complications developed in 6 patients (50%) and were fatal in 5, including two PR cases. Infections consisted of bacterial or fungal pneumonia (n=3) and/or septicemia (n=4). Severe nonhematologic and noninfectious toxicity consisted of reversible kidney failure (n=1) and gastrointestinal illness (n=2).

### *Pharmacokinetics*

A pharmacokinetic study was performed in three patients (#5, 9, and 11), including a comparison with IDA 5 mg/m²/d given by rapid intravenous bolus (30') for 2 consecutive days (patient #5 during second s-ICE course). In this latter case, after the two short (30') daily injections (course 2) IDA very quickly reached its peak plasma concentration (50 ng/mL) and then disappeared very rapidly; it remained detectable in the plasma at very low levels (> 2 ng/mL) for a total of 48 hours, reproducing the usual pharmacokinetic pattern of bolus IDA delivery.[1,13] IDOL plasma levels persisted longer than those of the parent drug, being detectable at 144 hours and peaking at 35 ng/mL by the end of day 2. In the same patient after CI IDA (course 1), the drug was found to be present in the plasma for a total of 70 hours, whereas its metabolite was detectable for 168 hours. However, in contrast with bolus injection, lower peak plasma levels were achieved for both IDA (10-20 ng/mL) and IDOL (20-30 ng/mL) at the end of infusion, and were relatively stable for the following 48 (IDA) and 72 (IDOL) hours. Results were similar in patients #9 and #11, who received the drug only as a continuous two-day infusion: IDA was continuously detectable for 70 hours, IDOL plasma levels persisted up to 120-144 hours, and peak plasma levels were reached at the end of daily infusions (10-20 ng/mL for both IDA and IDOL). Table 2 reports the terminal half-lives and AUC values of IDA and IDOL in the 3 patients investigated, showing a 2.9-fold greater exposure to IDA with the infusional schedule in patient #5. Since IDOL is reported to

**Table 2. Pharmacokinetic parameters of IDA and IDOL.**

| Pt. no. | Infusion schedule | Terminal half-life (hr) IDA | IDOL | AUC (ng/mL h) IDA | IDOL | IDA+IDOL |
|---|---|---|---|---|---|---|
| 5 | 30' day 1 | 8.5 | NE | 200 | NE | – |
| | 30' day 2 | 8.8 | 62.0 | 220 | 2140 | 2550 |
| | 48 h | 10.0 | 53.0 | 710 | 1940 | 2650 |
| 9 | 48 h | 13.2 | 31.0 | 695 | 1354 | 2049 |
| 11 | 48 h | 13.0 | 41.5 | 615 | 1571 | 2186 |

*NE, not evaluable.*

be as cytotoxic as IDA and is released *in vivo*, we also show the sum of the AUC values for the parent drug and its metabolite, which better reflects the total pharmacological effect.

### *In vitro study*

These tests were conducted on P-gp$^-$ and P-gp$^+$ leukemic CEM cells to assess whether CI of IDA could increase the intracellular drug concentration (FI) and its cytotoxic effect (apoptosis) with respect to bolus delivery, as suggested by the increased AUC levels. IDA concentrations and cell exposure times for these tests were chosen to mimic the pharmacokinetic study on the patients, i.e. 50 ng/mL for 30' on two consecutive days or 10-20 ng/mL for 48 hours in the bolus and CI experiments, respectively. The CI-type experiment with IDA 10 ng/mL was closer to clinical conditions, since plasma IDA concentrations were < 15 ng/mL in 35/42 (83%) total determinations performed over 72 hours in the three patients. The activity of the P-gp inhibitor CsA was also tested. The results are reported and compared in Figure 2. In both P-gp$^-$ and P-gp$^+$ cells, FI was consistently higher following bolus delivery, and the difference was statistically significant in all tests except that with CI IDA 20 ng/mL plus CsA (Figure 2a). In a similar fashion, marked pro-apoptotic changes (arbitrarily >10%) were induced by bolus IDA, but only when the FI threshold was >1500-2000 units (Figure 2b). The experiments with the P-gp downregulator CsA showed that only bolus IDA, associated with a FI > 1500, was able to induce an apoptotic cell rate > 10% in P-gp$^+$ cells.

## Discussion

A 50% response rate, 17% complete and 33% partial, was achieved with a brief three-drug combination including CI IDA plus intermittent cytarabine-etoposide (s-ICE) in a group of subjects with mostly end-stage hematological malignancies. This result and the high incidence of treatment-related deaths, 42% overall, could be anticipated in a series in which the median patient age exceeded 50 years (25% were > 60 years) and in which two-thirds of the cases had previously failed one or two different



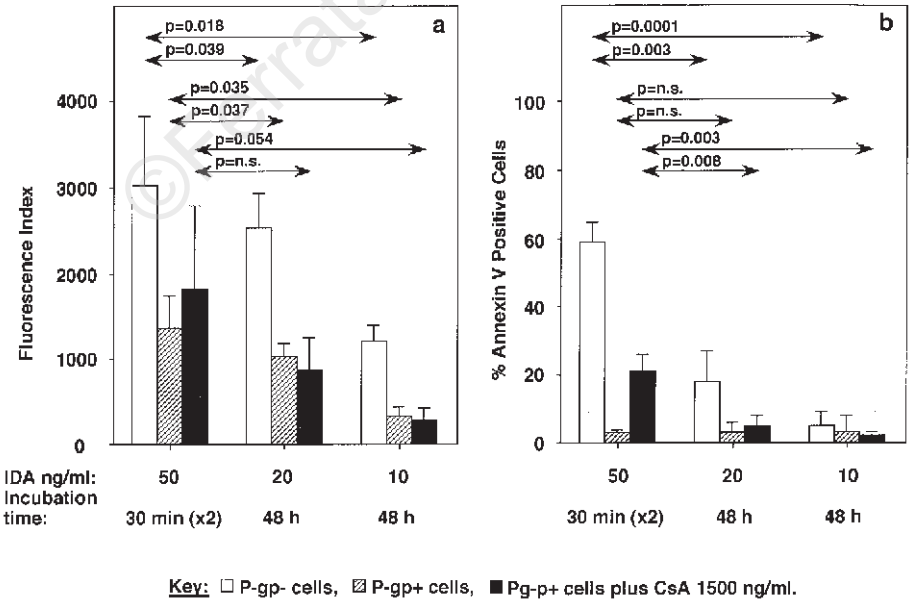


**Figure 2. Intracellular IDA accumulation (FI) (a), and related pro-apoptotic effect (binding to annexin V after subtraction of spontaneous activity) (b). Reported figures are means±SD from three experiments. P values from comparison with bolus experiments (Student's t-test).**

multidrug salvage regimens. It is possible that younger patients treated early on during the course of their illness might respond better and suffer less regimen-related toxicity.

With these cautionary notes in mind, the principle of CI drug delivery as an optimal way of administering some drugs has been raised by other recent trials. At John Hopkins University, newly diagnosed adult AML patients treated with two brief sequential courses including CI high-dose cytarabine fared unusually well for this disease,[18] as did adult ALL patients with standard-risk features treated at M.D. Anderson Cancer Center with the infusional VAD (vincristine-adriamycin-dexamethasone) and hyper-CVAD (cyclophosphamide-VAD) regimens.[19,20] The pharmacology and clinical effects of prolonged infusion high-dose cytarabine have been described.[8,21] An intermittent cytarabine schedule was shown to enhance Ara-CTP retention by leukemic blast cells but not by normal hemopoietic cells.[22] Interestingly, in a rat model gastrointestinal toxicity from cytarabine was reduced without compromising antineoplastic efficacy by adopting a 9-hour interval between infusions.[23] CI high-dose etoposide proved to be both safe and clinically effective,[9,10,24] even in patients already exposed to this same and other antineoplastic agents. These observations led us to developed the intermittent intermediate-dose cytarabine and etoposide backbone to which CI IDA was added.

As regards anthracyclines, continuous infusions were partially successful in patients with refractory leukemias and lymphomas,[7,25,26] even though a recent review on adriamycin was unable to document the superiority of CI over bolus delivery.[27]

Indeed, the main feature of the current short ICE regimen was the use of CI IDA. To the best of our knowledge this is an unprecedented attempt, based on the peculiar pharmacological properties of this drug. IDA was considered because it had not been previously employed in most of these cases, but especially because it is relatively resistant to the P-gp-mediated cellular drug efflux mechanism[6,28] that is often overexpressed by recurrent and refractory hematological tumors.[11] Moreover, since IDA was reported to be more cytotoxic than IDOL against P-gp$^+$ human leukemic cell lines but has a relatively short half-life,[1,6] we chose the CI modality in order to prolong the exposure of potentially P-gp$^+$ cells to the parent drug rather than the less active metabolite. In fact, when the pharmacokinetics of bolus and CI IDA was compared in the same patient, plasma AUC values were higher with CI due to its longer detectability in patient plasma, albeit at a rather low concentration. Another substantial difference from the usually sharp decline of the IDA peak plasma level observed following bolus injection[1,13] was the stable plasma concentration during infusion and for up to 48 hours afterward. These data were confirmed by the pharmacokinetic study of CI IDA in two other cases. These preliminary results suggest that prolonged exposure to IDA *in vivo*, desirable in patients with P-gp$^+$ tumors,[6,28] can be achieved using a CI, although peak plasma levels are likely to be lower than those achieved with a rapid bolus.

Given these observations and the unknown P-gp status of the patients, we set up an *in vitro* study to determine cellular incorporation and pro-apoptotic effects from IDA concentrations mimicking CI and bolus delivery, respectively. At variance with the plasma pharmacokinetic study, the results reproducing CI IDA indicated a lower cellular uptake and lower induction of apoptosis in both P-gp$^-$ and P-gp$^+$ cells, and with or without the addition of the P-gp blocker CsA. Knowing that intracellular IDA concentration is usually many times higher than the concurrent plasma concentration,[1] these results suggested that higher drug levels (bolus) rather than longer exposure time (CI) were associated with increased IDA cellular uptake by CEM cells and higher apoptosis rates. It is worth mentioning that a similar conclusion on the fundamental antiproliferative role of intracellular drug concentrations rather than plasma AUC values was reached in a study comparing bolus and CI doxorubicin in chronic lymphocytic leukemia.[29]

Even though the very limited number of patients studied prevents drawing any firm conclusion and the in vitro study in CEM cells may not be fully representative of the *in vivo* situation, our clinical and preliminary pharmacological experience does not suggest that CI IDA at 5 mg/m$^2$/d would be very effective in refractory blood malignancies at risk of expressing the P-gp multidrug resistance mechanism. It remains to be seen whether higher IDA dosages can be administered safely as CI, with P-gp inhibitors in P-gp$^+$ states, in order to obtain the desired plasma concentrations and intracellular drug accumulation rates.

### Contributions and Acknowledgments

*R. Bassan was the principal investigator responsible for the conception of the study, its design, data handling, and the writing of the paper. B. Chiodini performed* in vitro *studies (mdr1+ cells, apoptosis). M. Zucchetti carried out pharmacokinetic studies. T. Lerede collected and handled data. P.E. Cornelli and S. Cortelazzo followed the patients enrolled in this trial. T. Barbui contributed to the study design and writing of the paper. The order in which the names appear is that of decreasing contribution, with the last name (T. Barbui) indicating the principal clinician involved and senior author.*

### Disclosures

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

***Manuscript processing***

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