Abstract

Background: Many agents in antineoplastic chemotherapy are highly schedule dependent. Therefore, variables such as total dose and also the area under the curve (AUC) that are schedule insensitive are generally insufficient to adequately represent treatment strength.

Purpose: To establish a descriptor of treatment strength that takes into account the differential contribution of plasma concentrations (C) and exposure times (T) towards the cytotoxic effect and to investigate whether such a pharmacodynamically weighted descriptor is better correlated to the clinical effect than conventional variables.

Patients and Methods: The paradigm “CN × T = constant” (for an isoeffect) incorporates a weighing factor N (concentration coefficient) into the conventional description of the AUC that quantitates the differential contribution of C and towards the cytotoxic effect. N was to be numerically derived from a multitude of in vitro isoeffect analyses of the major agents in acute myeloid leukemia (AML) therapy from patient samples (n = 57).

Results: For cytarabine, N was 0.45, numerically expressing the substantially higher relevance of T versus C for its cytotoxic effect. In a meta-analysis of 49 study arms involving >10,000 patients, neither total dose, dose intensity, nor AUC was correlated to the clinical effect. However, when AUC was pharmacodynamically weighted (N-weighed AUC, N-AUC = CN0.45 × T), this new descriptor was highly significantly correlated to the clinical effect (P < 0.001).

Conclusion: The N-AUC concept is able to characterize schedule-dependent agents and is the only descriptor of cytarabine treatment strength actually correlated to the clinical effect in AML.

Antineoplastic therapy comprising intensive induction and consolidation chemotherapy (plus allogeneic blood stem cell transplantation in selected patients) is nowadays able to cure ~35% of patients with acute myeloid leukemia (AML) in large randomized trials (1–3). Apart from improvements in supportive care, the reason for the gradually increasing cure rates have been attributed to “treatment intensification.” However, the term “intensification” is rather vague because diverse aspects of the treatment regimens, especially concerning cytarabine as the mainstay of antileukemic pharmacotherapy, were altered. For example, (a) doses (of cytarabine) were raised; (b) dose intensity was increased by early repetition of induction chemotherapy without waiting for intermittent normalization of peripheral blood counts (double induction); (c) maximization of active metabolite formation was induced by biochemical modulation; and (d) schedules were targeted to vulnerable phases of the leukemic population (e.g., splitting of the schedule as in the sequential high-dose cytosine arabinoside and mitoxantrone regimen; ref. 4).

Therefore, especially if this historically successful approach of treatment intensification is intended to be maximally exploited, treatment strength needs to be clearly defined before intensification of this variable is rational. Conventionally, dose is used as a measure of treatment strength. However, according to Field and Raaphorst, “…the concept of dose is far from simple…: The purpose of the dose is to provide a number which relates to a specific biological response. A knowledge of the dose, therefore, provides a means of predicting the biological response to a given treatment and hence also a means of communicating when one wishes to compare biological responses given in different places at different times. It follows that the principle requirements of a dose are that it relates to the biological response in a relevant manner, it should be a well defined physical quantity and there should be a proper means of intercomparison….” (5).

In the case of cytarabine, a combination of this drug with an anthracycline will achieve complete remissions rates, as a measure of early cytoreduction, of ca. 60% to 80%. However, the range of cytarabine doses applied in the various studies is enormous with daily doses ranging from 100 to 6,000 mg/m² (60-fold). Even lower doses of the drug (20 mg/m²/d), given...
on a palliative basis, may reach complete remission rates of 30% to 40% in previously untreated patients therefore giving a dose range of up to 300-fold in induction therapy. Quite obviously, cytarabine dose alone is an inappropriate measure of treatment strength, at least concerning early cyto reduction as expressed by the complete response (CR) rate, because highly different doses may be associated with similar CR rates. In fact, three randomized trials have shown the equal efficacy of standard dose (100-200 mg/m²/d) and high-dose (2,000-6,000 mg/m²/d) cytarabine schedules in induction chemotherapy (2, 6, 7).

The mode of application is also highly diverse with infusion times ranging from bolus applications to continuous 24 hours of infusions. Quite obviously, the mode of application (e.g., bolus versus continuous infusion) is of substantial relevance for this “S phase-specific” agent; that is, the antileukemic effect is highly schedule dependent.

Hence, cytarabine might serve as an example of a very useful drug in the treatment of AML, whose exact relationship to treatment outcome however cannot be easily described. The merely empirical relations between certain doses/schedules and antileukemic effect as well as the merely qualitative knowledge of highly schedule-dependent pharmacodynamics are not very useful for, for example, further attempts of treatment intensification. Therefore, a variable describing treatment strength is needed, which significantly correlates with treatment outcome. We hypothesized that several aspects apart from the absolute dose needed to be taken into account to obtain such a clinically relevant variable: (a) pharmacokinetics of cytarabine following the various modes of application [i.e., the resulting area under the curve (AUC) and also the shape of this AUC, plasma levels, and exposure times], (b) pharmacodynamics of the drug [i.e., especially the differential cytotoxic effects of cytarabine AUCs of identical absolute size but different shapes; e.g., high plasma levels for short exposure times versus low plasma levels for long exposure times]. For these considerations, the following steps were taken in the present study:

(a) In the first step, an analysis of published pharmacokinetic studies of cytarabine was done with the intention to develop an algorithm that allows the approximation of the resulting AUCs in the treatment arms of larger phase III studies in which no pharmacokinetic analyses had been done. This approximation was not only to give the absolute AUC of the respective induction cycle but also the average steady-state plasma concentration (C) of cytarabine and the exposure time (T).

(b) In the second step, the differential cytotoxic effects of cytarabine AUCs of identical absolute size but different shapes were to be formalized and subsequently quantified. This was done by use of the so-called concentration coefficient N, which weighs the relative importance of concentration versus exposure time in the equation:

\[ C^N \times T = \text{constant} \]

where \( C \) = plasma concentration of cytarabine and \( T \) = exposure time. If \( N = 1 \), then both concentration and exposure time are of equal importance for the cytotoxic effect, and one can, for example, be lowered by a factor of 2, if the other is raised by a factor of 2 for an unchanged effect level. If \( N > 1 \), then concentrations are more relevant than exposure time, and a raise of the concentration by a factor of 2 will require a >2-fold reduction in exposure time for an isoeffect and vice versa for \( N < 1 \). This relationship has successfully been used for the preclinical characterization of various antineoplastic agents (8–10).

In the present study, the value \( N \), primarily of cytarabine and also of the major other agents in AML therapy, was to be numerically derived from a multitude of (in vitro) isoeffect analyses in 57 AML patient samples. After obtaining the numerical value of \( N \) for cytarabine, the previously derived pharmacokinetic variable AUC was to be weighed by this “pharmacodynamic” descriptor in the form of \( C^N \times T = N\text{-AUC} \), in which \( N\text{-AUC} \) is defined as the concentration coefficient \( N \)-weighed AUC.

(c) In the third step, 28 large randomized phase III studies since 1990 with cytarabine-based induction chemotherapy involving >10,000 patients with newly diagnosed AML were identified. In these studies, the established pharmacologic characteristics of a given treatment arm (cytarabine dose and dose intensity) as well as the newly derived characteristics (cytarabine AUC and \( N\text{-AUC} \)) were analyzed for their relationship to treatment response to identify the variable that was best correlated with treatment effect and therefore realistically represents treatment strength.

(d) In the fourth step, simulations were made in how far alterations in clinically accessible variables, such as dose and infusion time, can be made to optimize this newly identified variable. Such increasing of a variable that is closely related to treatment response constitutes rational “treatment intensification.”

**Materials and Methods**

### Identification of the concentration coefficient \( N \)

**Patients.** Samples from consecutive adult (>18 years) patients with first diagnosis of AML were included into this study. Diagnosis was based on cell morphology according to the French-American-British criteria complemented by cytochemistry and immunophenotyping. A bone marrow infiltration of >70% and collection of a sufficient number of bone marrow cells (>10⁸ cells) during the initial diagnostic bone marrow aspiration was required. The conduct of the study was done in accordance to the declaration of Helsinki and was approved by the local ethics committee. All patients were informed of the investigational nature of the study and gave their informed consent.

**Materials.** Cytarabine was obtained from Cellpharm (Hannover, Germany); daunorubicine and idarubicine were from Pharmacia (Karlsruhe, Germany). Mitoxantrone and etoposide were purchased from Hexal (Holzkirchen, Germany). Topotecan was from GlaxoSmithKline (Munich, Germany); mofetamide was from AstraZeneca (Wedel, Germany). Cell culture medium, PBS, HEPES, streptomycin, L-glutamin, and FCS were from Life Technologies (Eggenstein, Germany). WST reagent was available from Roche (Grenzach, Germany). An OPTImax ELISA reader by Molecular Devices (Munich, Germany) was used.

**Sample processing.** Leukemic blasts were collected by bone marrow aspiration and subsequently subjected to Ficoll-Hypaque centrifugation. Prior studies have shown that following Ficoll-Hypaque centrifugation a purity of 90% to 100% of leukemic blasts could be obtained. Cells were diluted to a concentration of 1 x 10⁷ cells/ml in Iscove’s modified Dulbecco’s medium, which was supplemented with 20 mmol/L HEPES, 100 μg/ml streptomycin, 10 mmol/L L-glutamin, and 10% FCS. Cultures were kept at 37°C, 5% CO₂, and 95% humidity. Viability was checked by the trypan blue exclusion test, and only samples with a viability exceeding 90% were accepted for subsequent experiments.

**Cytotoxicity assay (WST).** Overall viability of cell samples was measured by the WST-1 assay. Briefly, this assay is based on the
which the concentration coefficient $N$ can be extracted (gradient = curve is transformed into a linear relationship (Fig. 1C), from exposure times were plotted on isoeffect curves. An example is for further incubation of 4 hours. The ELISA reader was set at a wavelength of 450 nm with a reference wavelength of 620 nm. Results following drug exposure were calculated as a percentage relative to untreated controls and plotted in semilogarithmic dose-effect curves. Using these data points, a curve was fitted (four-variable fit: $y = \frac{(A - D)}{[1 + \left(\frac{x}{C}\right)^2]} + D$; ref. 11), which was then used to intrapolate the LC$50$ (the hypothetical concentration that would reduce the viability of the cell population to 50% compared with the untreated control; Fig. 1A).

Mathematical considerations. A loss of viability of 50% of leukemic cells was chosen as the most appropriate isoeffect level for further analysis. These different LC$50$ values for the various exposure times were plotted on isoeffect curves. An example is given in Fig. 1B. Following a double logarithmic transformation the curve is transformed into a linear relationship (Fig. 1C), from which the concentration coefficient $N$ can be extracted (gradient = $-N$; ref. 8):

$$
C^N \cdot T = \text{const}
$$

$$
C^N \neq 0 \quad \Rightarrow \quad T = \frac{\text{const}}{C^N}
$$

$$
T, \frac{\text{dose}}{C^N} > 0 \quad \Rightarrow \quad \log(T) = \log\left(\frac{\text{const}}{C^N}\right)
$$

$$
\log(a^2) = \log(a) - \log(b) \quad \text{for} \ a, b > 0
$$

$$
\log(s^a) = n \cdot \log(s) \quad \text{for} \ a > 0
$$

$$
\log(T) = \log(\text{const}) - \log(C^N)
$$

$$
\log(N) = n \cdot \log(s)
$$

$$
\Rightarrow \quad y = -N \cdot x + k
$$

Analysis of dose-response correlations

To evaluate correlations of pharmacologic variables to clinical response, a retrospective analysis of published randomized clinical trials using cytarabine in primary AML was done. To be eligible for analysis, studies had to be published not before 1990 and had to have a minimum size of 100 patients. This was done (a) to minimize differences in supportive care, which has improved substantially because those years and (b) to minimize small size effects. In addition, studies were restricted to unselected patient groups, whereas studies only including high-risk patients (relapse, old age, and unfavorable karyotype) were excluded. Furthermore, minimal demands were made regarding pharmacologic information in the studies (i.e., total dose per application, infusion time for each application, and number of applications were required).

As clinical variables of the antileukemic efficacy of induction chemotherapy early blast clearance (EBC; after first induction course) and the rate of CR after induction chemotherapy were chosen as pragmatic readouts. Whereas CR following induction therapy is well defined, EBC was also assessed because several studies used double induction either regularly or in those patients not reaching a bone marrow with <5% blasts in the first control bone marrow aspiration. This aspiration was most commonly done between days 16 and 28. Therefore, EBC as used in this analysis comprises those patients achieving a CR after one cycle (no double induction) plus those that achieve an empty bone marrow with no increased blast count in the first aspiration after the first cycle during double induction. It is therefore the earliest assessment of antileukemic efficacy of the first induction cycle. In contrast, the eventual CR rate represents the antileukemic effect of up to two cycles of treatment (i.e., those patients that either by study design or by the fact that they still had residual blasts in their marrow were given two cycles: double induction).

Correlations between these variables and the following pharmacokinetic factors of cytarabine were assessed: total applied dose, dose intensity, AUC, and N-AUC. Dose intensity in this analysis was defined as total dose divided by treatment days. Average AUCs for a treatment regimen were calculated as $C \times T$ (average plasma steady-state levels of cytarabine [as approximated by the procedure given below] $\times$ exposure time [approximated by the infusion time]). The N-AUC was calculated by weighting the $C \times T$ term (also known from the pharmacokinetic approximation procedure below) by the exponent $N$, the concentration coefficient, in the following way:

$$
N \cdot \text{AUC} = C^N \cdot T
$$

$P < 0.05$ was considered statistically significant. Statistical analyses were done with the PC Statistics program for Windows software.

Evaluation of pharmacokinetics

Pharmacokinetically useful variables, such as the AUC, are usually only determined in small phase I and/or II studies. Large phase III studies almost never provide these data due to logistic problems of doing pharmacokinetic analyses in a large group of patients. Nevertheless, the AUC is often superior to dose as a descriptor of treatment strength (12).

An attempt was therefore made in this study to develop an algorithm that is able to predict from very general descriptors, such as total dose and infusion time, both the steady-state plasma concentration ($C$) and exposure time ($T$) of an individual cytarabine application. For this attempt, a retrospective analysis of published and own data on cytarabine pharmacokinetics was done, and the development of such an algorithm was attempted.

Results

Identification of concentration coefficient $N$

A total number of 57 patient samples was analyzed. All patient samples were primary AML blasts from the initial diagnostic bone marrow aspiration. Following Ficoll-Hypaque centrifugation, all samples had >90% blasts. Depending on the available blast count, up to seven agents were tested in the WST assay. For each agent, the following number of samples were
available for analysis: cytarabine, \( n = 56 \); etoposide, \( n = 57 \); mafosphamide, \( n = 33 \); daunorubicine, \( n = 25 \); mitoxantrone, \( n = 24 \); idarubicine, \( n = 23 \); topotecan, \( n = 23 \). Incubation durations of 24 to 96 hours were used for each agent with the drug concentrations as stated above. \( \text{LC}_{50} \) values were obtained for the various exposure times. Following logarithmic transformation of the exposure time and the respective \( \text{LC}_{50} \) value, a linear relation was fitted through these data points for each patient. From these linear relationships, the numerical value of \( N \) could be read off via determination of the gradient as described in Mathematical Considerations (gradient = \(-N\)). The results are given in Table 1. Both cytarabine and topotecan had a concentration coefficient \( N \ll 1 \) (absolute value, 0.45 in both cases), indicating a higher relevance of exposure time compared with drug concentration for the cytotoxic effect of these drugs. The other extreme was mafosphamide, which showed a value for \( N \) of 3.71. Although this pragmatic compound (water soluble and no hepatic toxification required) is not used in the treatment of AML, it was tested as a representative of the oxazaphophorine class of alkylating agents, of which for example, cyclophosphamide is used in conditioning before both autologous or allogeneic hematopoietic stem cell transplantation. \( N \gg 1 \) indicates that for this agent, high plasma concentrations are much more relevant for the antileukemic effect than exposure time. Intermediate values were found for the anthracyclins and the anthracendione (e.g., 0.78 for daunorubicine), where \( N \) was comparatively close to unity. In these cases, both drug concentration as well as exposure time are of similar relevance for the cytotoxic effect.

In general, variation coefficients for \( N \) were high (e.g., 3.16 for cytarabine), most likely reflecting the fact that the samples used ranged from AML with favorable cytogenetics to those with complex aberrations (i.e., very unfavorable cytogenetics) and from clinical responders to complete nonresponders. The variability therefore reflects the known biological heterogeneity of this disease (or set of diseases).

### Evaluation of pharmacokinetics

A retrospective analysis of published articles and personal communication concerning cytarabine pharmacokinetics was done (13–18). From these studies, pharmacokinetic data based on a total of 166 patients were available (Supplementary Data). The doses investigated in these studies varied from 10 to 3,000 mg/m\(^2\), and the infusion times from 0.5 to 24 hours, therefore covering the whole clinical range of cytarabine applications. Cytarabine quickly reaches a steady state after the start of a continuous infusion and rapidly disappears after the end of the infusion with a \( t_{1/2} \) of 6 to 7 minutes (data not shown; ref. 15). The shape of the resulting concentration-time curve (defining the AUC) therefore actually resembles the rectangular features as “required” by the paradigm \( C^N \times T = \text{constant} \). Therefore, infusion time was used as an approximation of the exposure time.

|        | Ara-C | TOP | DNR | IDA | MIT | VP16 | MAF |
|--------|-------|-----|-----|-----|-----|------|-----|
| Median | 0.45  | 0.45| 0.71| 0.78| 0.72| 0.78 | 3.71|
| Mean   | 0.66  | 0.73| 1.13| 1.06| 1.12| 1.79 | 5.46|
| VC     | 3.16  | 1.07| 0.99| 3.11| 1.06| 5.40 | 3.77|
| \( n \) | 56    | 23  | 25  | 31  | 23  | 24   | 57  |

Abbreviations: Ara-C, cytarabine; TOP, topotecan; DNR, daunorubicine, IDA, idarubicine; MIT, mitoxantrone; VP16, etoposide; MAF, mafosphamide; VC, variation coefficient (SD/mean).

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Fig. 1. A, semilogarithmic dose-effect curve with four-parameter-fitted curve to determine the \( \text{LC}_{50} \). B, schematic isoeffect plot of \( \text{LC}_{50} \) of cytarabine in AML blasts. C, isoeffect plot after double logarithmic transformation. The gradient can be used to calculate the concentration coefficient \( N \).
When dose rate (i.e., total dose per unit of time) was related to the resulting, measured, plasma concentrations, a relation as shown in Fig. 2 was derived. There was a highly significant linear relation between these two variables ($P < 0.0001$, $r = 0.9581$) with a gradient of 13.41. Evidently, AUC was also highly linear to dose and was not influenced by the infusion time (data not shown). Therefore, dose and infusion time could be used to approximate the resulting plasma concentration via the following equation:

\[
\text{Plasma concentration in ng/ml} = 13.41 \times (\text{dose/application in mg/m}^2 / \text{infusion time in h})
\]

With approximations of drug concentration ($C$) and exposure time ($T$) now available, the AUC ($C \times T$) could be calculated for all treatment arms of the phase III studies used for the analysis of regimen descriptor/response relationships. We then defined the so-called N-AUC as a descriptor weighted by the concentration-coefficient $N$ as described below:

\[
\text{AUC} = C \cdot T
\]

\[
\Rightarrow N\text{-AUC} = C^N \cdot T
\]

In the case of cytarabine, for which the numerical value of $N$ has been explicitly derived, the equation reads thus:

\[
\Rightarrow N\text{-AUC}_{\text{ara-C}} = C^{0.45} \cdot T
\]

Because both $C$ as well as $T$ can be approximated via the aforementioned definition ($T$) or equation ($C$), a summation formula can be described which reads thus:

\[
N\text{-AUC}_{\text{ara-C}} = \sum \frac{\text{dose per application in mg/m}^2}{\text{infusion duration in h}} \times 13.41 \times 0.45 \times \text{infusion time in h}
\]

Via this summation formula, the N-AUC value for every cytarabine regimen can be calculated from variables conventionally well known for a treatment regimen: dose per application, infusion time, and number of applications. The respective variables of cytarabine application (number of applications per cycle, dose/m$^2$ per application, and infusion time) is analyzed studies with concomitant cytotoxic treatment and the respective variables of ara-C application (number of applications per cycle, dose/m$^2$ per application, and infusion time) is given in the Supplementary Data.

**Concomitant cytotoxic therapy.** The focus of this study was on cytarabine antileukemic efficacy, with a wide range in total cytarabine dose (dose range: 43-fold on a per cycle basis, 300-fold on a per day basis). However, all study arms, except for the low-dose ara-C arm of the study by Tilly et al. (39), had a cytotoxic combination partner in the induction regimen. Therefore, to attribute potential differences in clinical response to differences in descriptors of cytarabine treatment strength (dose, dose intensity, AUC, or N-AUC) of the various study arms, the comedication had to be accounted for. The first of two questions that had to be answered was therefore:

1. In study arms in which identical co-medications were applied (e.g., daunorubicine), were there substantial differences in (daunorubicine) dose that might account for any potential difference in the observed clinical results? The largest subgroups of study arms with identical comedication were those receiving daunorubicine ($n = 36$) or idarubicine ($n = 8$). The total applied dose between these arms differed only very slightly with a dose range of 1.33 for daunorubicine and 1.11 for idarubicine (Supplementary Data). For mitoxantrone ($n = 2$ study arms), identical doses were used in both study arms (dose range, 1.00). Aclarubicine and amsacrine were applied only in a single arm, respectively (dose range, 1.00). In

![Fig. 2. Correlation of dose/infusion time with measured steady-state concentrations.](image-url)
the two study arms of the single study using zorubicine, a 2-fold difference was used (dose range, 2.00). In conclusion, except for zorubicine, only very discrete variations in dose (dose range, 1.00-1.33) were observed between the various treatment arms, which has to be put into perspective in view of the substantially larger variations in cytarabine dose. Although a formal exclusion of variations in comedication dose as the underlying reason for differences in clinical results cannot retrospectively be done, this likelihood is low.

2. The second question that needed to be addressed is in how far the fact that different agents (however, all in the anthracycline/anthracendione family) were used in different study arms might account for differences in the observed treatment results. In other words, were equieffective doses of the various anthracyclines/anthracendione used in the various study arms?

To address this question, a list of all major randomized comparisons of various anthracyclines/anthracendione is given in the Supplementary data (19, 26, 33–35, 38, 40, 41). These data indicate that for the following total doses/m² per cycle of these agents, approximate equieffectiveness regarding CR rate can be assumed:

Daunorub. 150 ≃ Idarub. 36 – 40 ≃ Mitox.36

(≃ Amsacrine600 ≃ Aclarub.225)

Therefore, the median dose applied in the various study arms, especially of those agents that were most frequently applied accounting for the comedication of 46 of the total 50 study arms, was very close or even identical to those total doses for which antileukemic equieffectiveness had been shown (median total dose/m² per cycle: daunorubicine, 150 mg; idarubicine, 39.5 mg; mitoxantrone, 36 mg).

In conclusion, there is no evidence that the cytotoxic comedication is a likely reason for potential differences in the clinical outcome of the various study arms in the presence of the substantially larger variations in cytarabine dose.

**Calculation of the various descriptors of cytarabine treatment strength.** From the aforementioned studies, the following data on cytarabine application during induction chemotherapy could be extracted: dose in mg/m² per application, infusion time in hours of the individual application, number of application, and duration (number of days) of the induction cycle. From these data, the following descriptors of cytarabine treatment strength could be calculated: total dose (in mg/m² per cycle) and dose intensity (in mg/m²/d of the induction cycle). Evidently, definition of the dose intensity requires the definition of a time period and is commonly used only for the description of repeated cycles of (identical) therapies. In the case of our study, which used as a readout early treatment response, such as EBC and CR rate, the duration of the (first) induction cycle was chosen to account for substantially different time periods, over which the total cytarabine dose was applied with a range from 3 to 21 days. The AUC was calculated by using the aforementioned linear relationship between dose/infusion time as described above (AUCtotal in ng/mL h = dose per application in mg/m² × 13.41 × infusion time in hours per application × number of applications). The N-AUC was similarly calculated as described above with the numerical value of N = 0.45 as empirically derived from the experimental data on 57 AML samples. For facilitation of calculation of both the AUC as well as the N-AUC, a short computer program was written (ZIP file in the Supplementary Data). A summary of the various descriptors of treatment strength is given in the Supplementary Data with a median total dose of 1,400 mg/m² (range, 580-45,360), median dose intensity of 100 mg/m²/d (range, 20-6,000), median AIC of 18,774 ng/mL h (range, 7,772-447,358), and median N-AIC of 1,386 ng/mL h (range, 525-2,402).

**Correlation of different pharmacologic variables with clinical response.** Linear correlations were done among the four descriptors of treatment strength and EBC and CR rate. The results as described below did not change when done with or without the aforementioned study by Tilly et al.

In the first analysis, only the first induction cycle was used for the correlations. Under these circumstances, neither total dose (Fig. 3), dose intensity, nor AUC showed any meaningful relation to early blast clearance with P = 0.49, 0.50, and 0.58, respectively. However, N-AUC showed a highly significant correlation to EBC with P = 0.0009. Regarding CR rate, a similar picture emerged with no meaningful relation to total dose, dose intensity, or AUC. In contrast, N-AUC again showed a significant correlation also to CR rate (P = 0.04). However, the correlation to CR rate was worse than for EBC, which was most likely due to the fact that a considerable number of studies applied not only a single course of induction chemotherapy but used regular double induction or applied another cycle of therapy if an inadequate blast clearance was observed after the first induction cycle. We therefore extracted the average number of applied cycles during induction chemotherapy of a respective study arm (range, 1-2) and used this variable to multiply total dose, AUC, and N-AUC to obtain the average treatment strength of the whole induction treatment of a respective study arm. Dose intensity evidently did not change according to our definition, because total dose/treatment days was not altered when applying a repeated cycle of the same treatment. The comparison of the respective variables after adjustment to actually received cycles with CR rate again yielded no significant correlation among total dose, dose intensity, and AUC but a highly significant correlation with N-AUC (P < 0.0499).
When another analysis was done in which the data points were weighed according to the number of patients included into the respective study arm, a virtually unchanged and also highly statistically significant correlation was observed ($P < 0.001$). The regression curve for this correlation was $y = 0.0218x + 30$ ($r = 0.5608$). An overview over all correlation coefficient and $P$ values are given in Table 2. In addition, the Spearman rank test was used to show monotony without the assumption of a linear correlation for the relation between the N-AUC and EBC and CR rate after one cycle and CR rate after complete induction treatment ($P < 0.001$, $P < 0.05$, $P < 0.01$, respectively).

**Simulations.** To show the potential effect of the N-AUC approach on schedule optimization, we did a simulation of $N$-oriented optimization of the high-dose cytosine arabinoside and mitoxantrone regimen (high-dose ara-C plus mitoxantrone). In this regimen, which is presently evaluated in the first-line therapy of the German AML Cooperative Group studies (1), high-dose cytarabine is applied in a dose of 3,000 mg/m$^2$ over 3 hours every 12 hours on days 1 to 3. The (idealized) rectangular plasma concentration-time curve features plasma concentrations of 13,410 ng/mL and an exposure time (infusion time) of 3 hours. The resulting AUC of 3 days of therapy is 216,000 ng/mL h. If nothing else is altered but the infusion time (3-6 hours), the plasma concentration drops to 50% (i.e., 6,705 ng/mL) and the AUC is evidently unchanged (216,000 ng/mL). However, when the N-AUC for these two schedules is calculated, a substantial difference is observed (1,296 ng/mL when the N-AUC for these two schedules is calculated, a...)

Table 2. Correlation of the various descriptors of treatment strength with EBC or CR rate

| Descriptor        | EBC after one cycle | CR rate after one cycle | CR rate after complete induction (1-2 cycles) |
|-------------------|---------------------|-------------------------|-----------------------------------------------|
| Total dose        | $r = 0.1056$, $P = 0.4949$ | $r = 0.0047$, $P = 0.9740$ | $r = 0.1852$, $P = 0.2027$ |
| Dose intensity    | $r = 0.1049$, $P = 0.4880$ | $r = 0.0026$, $P = 0.9857$ | $r = 0.2780$, $P = 0.1532$ |
| AUC               | $r = 0.0857$, $P = 0.5804$ | $r = -0.0167$, $P = 0.9085$ | $r = 0.1963$, $P = 0.0176$ |
| N-AUC             | $r = 0.4843$, $P = 0.0009$ | $r = 0.2825$, $P = 0.0469$ | $r = 0.5608$, $P < 0.0001$ |

Thus, the N-AUC approach can be used to estimate the effect on early treatment response by changes in dose or scheduling.

**Discussion**

Conventional descriptors of treatment strength, such as dose or dose intensity, are of questionable use for cytarabine-based regimens in AML. What is well known to hematologic common wisdom is also systematically shown by the present study, which shows in a large group of patients that in fact, total dose alone or dose intensity are not correlated in any meaningful way to early treatment response, such as early blast clearance or CR rate. The well-known schedule-dependent cytotoxicity of cytarabine is the most likely reason for this lack of correlation, making dose alone without the consideration of the mode of application an insufficient descriptor; that is, it does not fulfill the principle requirement of the definition by Field and Raaphorst, “...that it relates to the biological response in a relevant manner...” (5). In fact, this phenomenon of schedule dependency, in most cases due to a relative S-phase specificity, with consecutive problems in determining treatment strength via dose constitutes more the rule than the exception in the treatment of acute leukemias (e.g., topotecan and methotrexate). Hence, any attempt at rational treatment intensification is hampered by the lack of both qualitative knowledge about what variable to increase as well as quantitative knowledge about relations between a delta in this descriptor and the resulting delta in treatment response.

The approach of the present study was to go beyond merely qualitative findings (“a given dose of cytarabine is more effective if given over a prolonged period of time than over a short infusion”) and to first formalize the relative contribution of drug concentration versus exposure time and then to quantitate these differential effects. This was done by using a previously used paradigm that relates $C$ and $T$ for isoeffects by the use of a weighting factor, the so-called concentration coefficient $N$ in the form of $C^N \times T = $ constant (8–10). By explicitly deriving the numerical value of $N$ for an agent used against a certain cell population, this approach has been successfully used in the past for the advanced pharmacodynamic description of antitumor agents in preclinical development, such as crisnatol (8). To our knowledge, our study is the first to use such an approach in a clinical setting. In contrast to prior studies, which have used $N$ primarily as a qualitative descriptor of the differential relevance of $C$ and $T$ for a given drug, the present study developed the concept of the N-AUC. This approach uses $N$ to weigh the various AUCs, or to be more exact, the steady-state concentration used in the term $C \times T = $ AUC. By this procedure, the differential...
contribution of C versus T for an agent, such as cytarabine, is quantitatively taken into account; that is, the schedule specificity of the antileukemic effect following AUCs of identical absolute value but of different shape can thus be represented. In fact, in contrast to conventional descriptors of treatment strength, N-AUC, as a pharmacokinetically and pharmacodynamically weighted variable, showed a highly significant linear relation to treatment response.

The absolute value of $N = 0.45$ was derived from in vitro experiment and represents the median of 56 analyzed patient samples. Hence, this variable was independently derived and was not a derivative of the clinical studies later on analyzed. However, this in vitro value of $N$ was also confirmed by independently searching for the best linear fit for the $C_n^N \times T/CR$ rate relation of the treatment arm. For this approach, increasing values for $N$ spanning a range of 0.20 to 2.00 in increments of 0.05 were imposed onto the known $C$ and $T$ values of the various study arms. As shown in Fig. 5, an identical value for $N = 0.45$ could be obtained with this different analytic approach and a completely independent data set. [Especially, the latter approach might also be useful for pharmacodynamic modeling of other agents (in other diseases), for which a large amount of historical data of the kind requested in this study is available.] This indicates that the presented paradigm and the empirically derived numerical value of $N$ together represent a useful descriptor of average cytarabine pharmacodynamics concerning early cyto-reduction.

Still, there are several limitations to our study. Quite evidently, this newly developed global variable $N$ averages over the whole treatment arm and can only be used for the approximate treatment strength of a whole treatment arm. No analyses of individual patients with individual $N$ values, used on individually measured pharmacokinetic data, were done. Also at present, this approach is limited to the phase of early cyto-reduction, both the in vitro analyses for $N$ as well as its derivation from fitted $C_n^N \times T/CR$ rate relations by definition define $N$ as a function of cyto-reductive activity (over a period of days in the in vitro assays and over a period of a few weeks for the fitted $C_n^N \times T/CR$ rate relations). Although N-AUC, as shown by the data, evidently is a predictor of early cyto-reduction and CR rate, it is yet unclear in how far the presented approach will be useful also for prediction of long-term results.

Especially, relapse rates will be primarily determined by regrowth induced by surviving leukemic stem cells, whose kinetic characteristics are most likely not represented by both methods for deriving $N$. However, similar analyses using stem cell assay as a readout might be used in the future for deriving $N$ for this quo-ad long-term disease-free survival, most relevant leukemic subpopulation.

Another potential problem is the nonconsideration of the combination chemotherapy in our analyses. However, in contrast to cytarabine that was used in doses covering a range of 60- to 300-fold, there were comparatively minute variations in dose in the other agents (daunorubicine, 1.33-fold; idarubicine, 1.11-fold, etc., across all study arms). In addition, although different anthracyclins or related compounds were used, their average applied dose can be considered equieffective from comparative studies or from individual phase II data. Together, this indicates that the antileukemic medication was rather constant, and that the largest variations by far were in the cytarabine dose and in its modes of application ranging from 0.5-hour infusions to 24-hour continuous infusions. An interesting aspect of the observed regression curve ($y = 0.0218x + 30$) is its $y$-intercept (30% CR rate), because this most likely reflects the average contribution of the non-cytarabine component of the applied regimen to the CR rate. Another relevant point is the fact that the $N$ values obtained for the anthracyclines or anthracendiones are comparatively close to unity (e.g., $n = 0.78$ for daunorubicine). This indicates that for these agents, both $C$ and $T$ are of similar relevance for the antileukemic effect; that is, the mode of application will only have a comparatively small effect on cell kill, in contrast to agents, such as cytarabine or topotecan with $N << 1$ (both 0.45). In fact, for these agents with a value for $N$ close to unity, dose might therefore serve in fact as a realistic descriptor of treatment strength.

Furthermore, it might be questioned why the well-elucidated mechanisms of action of cytarabine are not taken into account. Quite obviously, there is ample mechanistic evidence for the rather S phase-specific action of cytarabine, and even the asymptotic antileukemic effect at increasing dosages might be mechanistically explained by the saturation of the rate-limiting anabolizing enzyme in the toxification of the drug to its triphosphate (ara-CTP), deoxycytidine kinase, at ca. 2.5 (−5) µg/mL (43). These mechanistic findings, apart from their explanatory value for cytarabine pharmacology, are even of potential clinical use, such as by providing the basis for biochemical modulation strategies to shift deoxycytidine kinase saturation to higher levels thereby maximizing the production of the cytotoxic metabolite ara-CTP (44). There is even some evidence that variables, such as intracellular ara-CTP levels and its respective half-life, might have prognostic value thus representing a proxy of treatment strength (45, 46). Compared with the presented approach of characterizing cytarabine exposure merely by concentration and exposure time, these more advanced variables (ara-CTP levels, amount of DNA double-strand breaks, and variables of impending apoptosis) offer the advantage of being “closer” to the cytotoxic effect. However, the principal disadvantage of such variables is (a) that their effect on cell kill in relation to exposure time is not yet quantitatively known and would require considerable analytic effort, and (b) that at present, no algorithm exists that would make approximation of these variables from a priori
known characteristics of a treatment regimen possible, which means that, for example, ara-CTP levels and their kinetics would have to be actually measured in patients of one particular treatment arm, whereas in the presented concept, ara-C plasma concentration and exposure times as the determinants of cell kill can be approximated by a straightforward algorithm done on a priori known characteristics (dose/application, infusion time per application, and total number of application/cycle). Hence, although pharmacologic variables of cytarabine more elaborate than plasma concentration or exposure time offer theoretical advantages, they are at present of no use for the pragmatic estimation of treatment strength in the clinical setting as was the aim of the present study.

These considerations stress an important point. That is, the concept of the concentration coefficient $N$ and its product, the N-AUC, is obviously helpful for the quantitative description of (cytarabine) pharmacodynamics. In fact, the numerical values for $N$ in AML therapy could be derived not just for cytarabine but also for the other main agents in AML therapy. However, $N$ alone as a pharmacodynamic descriptor is not sufficient for the approximation of treatment strength. Basic pharmacokinetic data (i.e., plasma concentrations and exposure time) of the applied regimen are also required. This requirement might in fact be limiting. In the case of cytarabine, several advantageous circumstances made the formulation of an algorithm for the approximation of cytarabine pharmacokinetics possible:

(a) The real life plasma concentration curves of cytarabine infusions actually resemble the idealized rectangular form of our paradigm. Steady-state concentrations ("static drug concentrations") are reached quickly and, following the end of the infusion, ara-C disappears from the plasma very rapidly with a $t_{1/2}$ of 6 to 7 minutes and a $t_{1/2a}$ of 2 to 3 hours.

(b) Due to this rectangular shape, infusion times are in fact a good approximation for the exposure time (at steady-state plasma concentrations).

(c) Sufficient pharmacokinetic data exist because several studies have been done in the past on various schedules of the drug ranging from low-dose applications over so-called standard doses to high-dose regimens. The resulting plasma levels for a dose of cytarabine, given over a unit of time, are highly linear to dose, the mathematical description of which made the approximation of steady-state plasma concentrations from applied dose and infusion time possible.

Only the combination of these favorable features of cytarabine made it possible to approximate $C$ and $T$ (and therefore also the AUC) for the various treatment arms. Only on these pharmacokinetic characteristics could the pharmacodynamic descriptor $N$ be used. To our knowledge, there are hardly any similar algorithms for other cytotoxic agents. An exception is etopoide, for which a similar approach for the approximation of pharmacokinetic characteristics from basic characteristics, such as dose and infusion time, has been published (47, 48).

In contrast to the conventional descriptors of therapy strength, the N-AUC/CR rate relation shows a linear relationship over the (limited) span of the available data. We are aware of the fact that over the whole range of theoretically possible applications of a drug, a linear relation to response is unlikely. In fact, a Hill model is one of the most commonly used pharmacodynamic models used for such descriptions (in principle, these curves have a similar aspect to that shown in Fig. 1A). However, unlike in the preclinical setting where even minute doses of a drug can be given (which will then be responsible for the minute effect in the initial flat part of the curve) and also exceedingly high doses can be used [which will result in very high but hardly changing (i.e., asymptotic) response rates], the situation is very different in the clinical setting. Both "too-low" as well as "too-high" doses will have been eliminated in the early phase I to II trials due to lack of clinical efficacy or due to unacceptable toxicity. That is, the clinical situation, especially in the "late" phase III studies as covered in this article, centers around the middle (steep) part of the Hill curve, which can be and in fact is commonly approximated by a linear relation. In fact, defining this linear part of the Hill curve is clinically very worthwhile, because this defines the interval, where modest alterations in treatment strength (dose, N-AUC, etc.) can be expected to result in an appreciable clinical benefit (i.e., a delta in response).

Another relevant aspect of the observed linear relationship is its rather low gradient. This phenomenon might be explained by the following. Consider a very steep dose-response relationship with a certain dose $X$ given to the study population with a resulting response rate $Y$, this situation implies a very homogenous study population with regard to treatment response, because a small increase in dose ($AX$) would result in a substantial increase in the number of responders ($\Delta Y$). On the other hand, a rather shallow dose-response relationship as in the N-AUC/CR rate relation implies a very heterogeneous study population with regard to their chemosensitivity. This interpretation is in good accordance with the biological evidence that describes "AML" as a very heterogeneous group of multiple entities with substantial differences in their respective underlying genetic alterations, biological features, and prognosis (49). Therefore, it might be postulated that AML subgroups with a purported high sensitivity to cytarabine treatment, such as those patients with an inversion (16) or translocation (8;21), would show a steeper gradient in their N-AUC/CR rate relationship (50, 51). On the other hand, cytogenetic subgroups with a particularly low chemosensitivity, such as those AML with complex cytogenetic aberrations (52), would be expected to show an exceptionally low gradient, indicating a negligible probability of increasing response rate by N-AUC escalation. If these hypotheses can be confirmed by ongoing analyses in our group, these findings might specify those AML subgroups in which further treatment intensification (via cytarabine N-AUC escalation) might be promising.

The advantage of the N-AUC concept is that it is intuitive from a pharmacologic point of view by relating concentration and exposure time, and that both variables are accessible for the clinician via dose and infusion time. Our simulation of a $N$-based modification of the high-dose cytosine arabinoside and mitoxantrone regimen shows this feature. By prolonging the infusion time to 6 hours per application (with unchanged dose and total AUC), a substantial delta in the N-AUC can be achieved, which, according to our regression curve, would
translate into an expected increase in CR rate of 13%. In this way, an evidence-based estimate of the expected change in response rate can be obtained and used in, for example, formulating the null hypothesis of new phase III studies. However, two points need to be mentioned: (a) Evidently, the regression curve is only backed by data ranging from ca. 500 to 2400 ng/ml - h, and extrapolation beyond these limits is therefore merely speculative. (b) Although escalation of cytarabine N-AUC concept might be rationalized in terms of toxicity, this might of course be offset by an increase in toxicity. For example, increased gastrointestinal toxicity has been limiting during prior attempts at delivering high-dose cytarabine regimens as a continuous infusion (53–55). Similar quantitative analyses for the relation of descriptors of therapy strength and toxicities are therefore merely speculative. (b) Although escalation of treatment protocols large improvements in clinical results will be achievable. However, in the long-term quest for increasing overall survival rates, the approach of optimizing current pharmacotherapy might contribute some valuable percentage points and thus complement other efforts of improving antileukemic therapy. In particular, the N-AUC concept might be useful, apart from cytarabine as shown in this study, for the description of all those agents with N << 1 (such as topotecan) or N >> 1 (such as mafosfamide as a representative of the class of alkylating agents). In these agents, optimization of C and T relations are much more worth the effort than in those agents with N values close to unity (such as the anticyclines). Moreover, characterization of new agents via the concentration coefficient N may facilitate the schedule design for early clinical studies and reduce the amount of empiricism in schedule optimization. Despite its imperfection, the N-AUC concept is a pharmacologically oriented variable and constitutes, at present, the only evidence-based descriptor of cytarabine based treatment strength and is therefore superior to other potential descriptors, such as total dose, dose intensity, or AUC.

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Modeling the Pharmacodynamics of Highly Schedule-Dependent Agents: Exemplified by Cytarabine-Based Regimens in Acute Myeloid Leukemia

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