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ORIGINAL ARTICLE

A limited sample model to predict area under the drug concentration curve for 17-(allylamino)-17-demethoxygeldanamycin and its active metabolite 17-(amino)-17-demethoxygeldanomycin

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Abstract
Purpose The Hsp90-directed anticancer agent 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) is currently undergoing phase I and phase II clinical investigation. Our goal was to develop a simple limited sampling model (LSM) for AUC of 17-AAG and its active metabolite, 17-(amino)-17-demethoxygeldanomycin (17-AG) using drug concentrations from a few time points.
Methods Pharmacokinetic data from 34 patients treated at 11 dose levels on a Mayo Clinic Cancer Center phase I clinical trial of 17-AAG was utilized. Blood samples were collected at 11 different time points, spanning 25 h. Graphical methods and correlations were used to assess functional forms and univariate relationships. Multivariate linear regression and bootstrap resampling were used to develop the LSM.
Results Using log-transformed data, the two and three time point 17-AAG LSMs are log-AUC (17-AAG) = $0.869 + 0.653*(C_{55min}) + 0.469*(C_{5h})$ and log-AUC (17-AAG) = $2.449 + 0.400*(C_{55min}) + 0.441*(C_{5h}) + 0.142*(C_{9h})$. The two and three time point LSMs for 17-AG are log-AUC (17-AG) = $3.590 + 0.747*(C_{5h}) + 0.169*(C_{17h})$, and log-AUC (17-AG) = $3.797 + 0.650*(C_{5h}) + 0.111*(C_{9h}) + 0.122*(C_{17h})$. Ninety-seven percent and 94% of the predicted log-AUC values were within 5% of the observed log-AUC for the two and three time point models for 17-AAG and 17-AG respectively.
Conclusions The precise calculation of AUC is cumbersome and expensive in terms of patient and clinical resources. The LSM developed using a multivariate regression approach is clinically and statistically meaningful. Prospective validation is underway.

Keywords AUC · Limited sampling · Pharmacokinetics · 17-AAG · 17-AG

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Introduction

HSP90 is a critical protein in cells that assists in the folding, trafficking and degradation of many other proteins critical in cancer cell growth and survival [7]. The Hsp90-directed anticancer agent 17-(allylamino)-17-demethoxygeldanamycin (17-AAG) is currently under phase I and phase II clinical investigation [2, 7, 11, 17]. While pharmacokinetics of 17-AAG and its active metabolite 17-aminogeldanamycin (17-AG) were characterized in the phase I single-agent trials, additional pharmacokinetic data is necessary to evaluate pharmacokinetic-pharmacodynamic relationships in those trials. Our goal was to develop a simple predictive model for AUC using drug concentrations

collected from a few time points in order to minimize patient burden and research resources, similar to previously published work in developing limited sampling models for other agents [16].

Materials and methods

Data

Pharmacokinetic data for model building was obtained from a Mayo Clinic Cancer Center phase I clinical trial involving 34 patients who received 15 to 431 (median 220) mg/m^2 17-AAG given by a 1-h intravenous infusion [8, 12]. Plasma specimens were collected during infusion and for 24 h after infusion (at 30, 55, 65, 75, and 90 min; and 2, 3, 5, 9, 17, and 25 h). Drug concentration values for missing data were imputed with a minimum value approach for 36 individual data points on 15 patients out of 748 possible data points (4.8%; Table 1). The AUC was calculated by standard non-compartmental analysis methods using WinNonLin software (Pharsight).

Statistical analysis

Basic summary statistics were used to inspect the distributions of 17-AAG and 17-AG AUCs, as well as each individual drug concentration. The association between normal scale and log AUCs for 17-AAG and 17-AG and the normal scale and log drug concentrations were examined via Pearson and Spearman correlation coefficients. Multivariate linear regression using stepwise regression and "all subsets" regression was used to select individual drug concentration time points that best predicted log AUC [9, 10]. For stepwise regression, an alpha of 0.05 for entry and removal to the model was used. All subsets regression ranked the best 2 and 3 variable LSMs in order of their R^2 values.

The comparative performance characteristics used for model assessment were standard R^2, root mean square error (RMSE) as a measure of precision, and variance inflation factors (VIF) as a measure of collinearity. Because of the high level of correlation between AUC and the individual time point drug concentrations, we also investigated the percentage of patients whose predicted AUC was within ranges of 5 and 10% of the observed AUC value (for both

Table 1 Missing concentration values by dose level

Dose level (mg/m^2) (no. of patients)	Missing values for time since start of treatment (h)	17-AAG		17-AG	
		Missing data points *N* (%)	Imputed value (actual scale)	Missing data points *N* (%)	Imputed value (actual scale)
15 (1)	9	1 (100)	0.31	1 (100)	0.27
	17	1 (100)	0.13	1 (100)	0.16
	25	1 (100)	0.103	1 (100)	0.12
21 (1)	9	1 (100)	0.31	NA	NA
	17	1 (100)	0.13	NA	NA
	25	1 (100)	0.103	NA	NA
29 (1)	9	1 (100)	0.31	NA	NA
	17	1 (100)	0.13	NA	NA
	25	1 (100)	0.103	NA	NA
41 (1)	9	1 (100)	0.31	1 (100)	0.27
	17	1 (100)	0.13	1 (100)	0.16
	25	1 (100)	0.103	1 (100)	0.12
57 (2)	25	1 (50)	0.103	NA	NA
80 (2)	17	1 (50)	0.13	NA	NA
	25	1 (50)	0.103	NA	NA
112 (2)	25	1 (50)	0.103	NA	NA
157 (4)	17	1 (25)	0.13	NA	NA
	25	2 (50)	0.103	NA	NA
220 (7)	17	2 (29)	0.13	1 (14)	0.16
	25	5 (71)	0.103	2 (29)	0.12
308 (10)	25	1 (10)	0.103	NA	NA
431 (3)	NA	NA	NA	NA	NA

normal and log scale). The stability of our model was further assessed by performing bootstrap resampling [6]. A total of 1,000 simulated data sets of size 34 were drawn with replacement (with "patient" as the unit of replacement) from the original complete sample; stepwise and best subsets regression were then run on each of the 1,000 simulated data sets; and the number of times each time point was selected in the final model for each simulated data set was counted. Analyses were performed using S-PLUS 6.2 and SAS Version 9.1.3 computer software.

Results

The phase I trial results have been reported previously [8, 12]. The median age of the 34 patients enrolled onto this trial was 61.5 years (range 38–77) with 59% male ($N = 20$). There were a variety of tumor types in this patient population, the most frequent of which were GI tumors (24/34) (Table 2).

For 17-AAG, 15 patients (44%) had an entry of zero at the 25-h time point, eight patients (24%) had an entry of zero at the 17-h time point, and four patients (12%) had an entry of zero at the 9-h time point. For 17-AG, four patients (12%) had an entry of zero at the 25-h time point, three patients (9%) had an entry of zero at the 17-h time point, and two patients (6%) had an entry of zero at the 9-h time point. These entries correspond to responses that are so small as to be non-detectable. For our purposes, in order to be able to log-transform our data without excluding these patients, we assigned a small number in the place of each non-detectable value. For each of these time-points, this number corresponded to a value 100 times as small as the minimum non-zero concentration for each of these three measurements. For instance, the smallest non-zero measurement of 17-AG at the 9-h time point was 27, so a value of 0.27 was substituted for any patients with non-detectable values recorded at this time point (see also Table 1). Several other imputation techniques such as replacing non-detectable values with predictions from regression models were investigated and found to have similar results. Modeling was performed on the imputed data set of 34 patients. There were no noticeable differences between the complete and imputed data sets, thus only models using the imputed data set are reported. Modeling was also performed on both untransformed and logarithmically transformed data for 17-AAG and 17-AG. The logarithmically transformed data provided decidedly superior results, and as such only these results are reported.

Table 2 Patient and trial characteristics

Characteristics	No. of patients
17-AAG Dose (mg/m^2)	
15	1
21	1
29	1
41	1
57	2
80	2
112	2
157	4
220	7
308	10
431	3
Tumor site	
GI	24
Melanoma	2
Lung	3
Stomach	1
Thyroid	1
Gyn	2
Unknown	1
Age (in years) median (range)	61.5 (38–77)
Gender	
Male	20
Female	12

Scatterplots showed that 17-AAG and 17-AG did not appear linear over the dose range studied in this trial (Fig. 1a, b). Therefore we were unable to perform dose-normalization. However, dose was included as a possible predictor in the multivariate regression model to account for differences in a patient's AUC due to the amount of 17-AAG received. Due to the issue of non-detectable concentrations at the later time points, it is difficult to reliably estimate the tail of the curve. As Schoemaker et al. [14] point out, the time–concentration curves for the lower dose levels have a tendency to level off without providing information to estimate the tails. This needs further exploration (given the work of Banerji et al. [1]; and Ramanathan et al. [13]) and it is likely that this apparent non-linearity in the curve could be attributed to the non-detectable concentrations at the later time points in the lower dose levels.

Individual drug concentration time points were correlated with AUC for 17-AAG and 17-AG. The drug concentration time points with the highest correlation with AUC for 17-AAG were between 75 min and 3 h after beginning infusion, with both Spearman and Pearson correlations at least 0.98 (Table 3). The AUC versus drug concentration time point correlation values for 17-AG were lower than those of 17-AAG. Later time points between 3 and 9 h after the start of treatment had higher correlation values for 17-AG (Table 3).

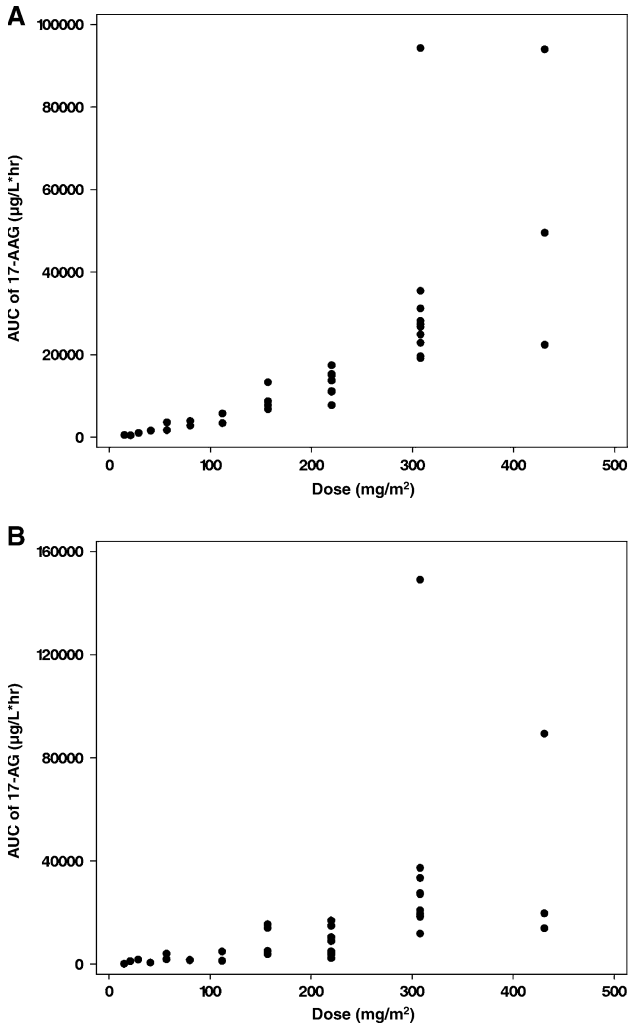

Fig. 1 **a** Area under the drug concentration curve of 17-AAG (µg/L*hr) by dose (mg/m^2). **b** Area under the drug concentration curve of 17-AG (µg/L*hr) by dose (mg/m^2)

Stepwise and best subsets regressions were performed after log-transforming all the individual time point drug concentrations, dose, and the respective AUCs of 17-AAG and 17-AG. The natural log transformation of the data was done to satisfy the assumption of normality of error distribution, which is required for applying the linear regression methodology. Dose was not selected as a significant predictor in the two and three time point LSMs for 17-AAG and 17-AG. The two and three time points selected using stepwise and best subsets regression for 17-AAG were similar with 55 min and 5 h after start of treatment and 55 min, 5 and 9 h after start of treatment (Table 4). Stepwise and best subsets regression for 17-AG yielded similar two and three time point models of 5 and 17 h after start of treatment and 5, 9 and 17 h after start of treatment (Table 4).

For 17-AAG, the two time point LSM (55 min and 5 h after starting treatment) and the three time point LSM (55 min, 5 and 9 h after starting treatment) were equally effective, predicting 97% (33/34) of the log-AUC values within 5% of the observed log-AUC values (Fig. 2a). Table 4 provides the regression model diagnostic results for the two and three time point LSMs for 17-AAG. The variance inflation factors for the time points selected in both models were below ten, and the R^2 and adjusted R^2 values greater than 97% indicate that the chosen time points explain nearly all the variability in the data. Results for 17-AG were different from those for 17-AAG, both in terms of the time points as well as the model based predictions. The two time point and three time point LSM predicted 94% (32/34) of the log-AUC values within 5% of the actual log-AUC for the metabolite 17-AG (Fig. 2b). The regression model diagnostics for the two and three time point LSM models for 17-AG are also presented in Table 4. These were similar to those found in the 17-AAG models with all VIF values <10 and R^2 and adjusted R^2 values >96%.

Table 3 Pearson and Spearman correlations for log AUC versus log plasma concentrations for 17-AAG and 17-AG

Time since start of treatment	17-AAG AUC		17-AG AUC	
	Spearman	Pearson	Spearman	Pearson
30 min	0.93	0.94	0.79	0.86
55 min	0.94	0.97	0.85	0.88
65 min	0.96	0.97	0.83	0.86
75 min	0.98	0.99	0.87	0.91
90 min	0.99	0.99	0.92	0.92
2 h	0.99	0.98	0.91	0.93
3 h	0.99	0.98	0.95	0.95
5 h	0.97	0.96	0.95	0.96
9 h	0.95	0.92	0.97	0.93
17 h	0.83	0.74	0.92	0.80
25 h	0.66	0.64	0.87	0.72

Bootstrap analysis

To assess the stability of the models chosen above, we bootstrapped 1,000 samples of size 34 stratified by dose level with replacement from the original data set and applied the same model-building techniques to the data sets (best subsets regression procedure carried to a maximum of two and three variables on each of the 1,000 created samples). For 17-AAG, the sample time points selected most frequently in the two-variable best subsets procedure were 90 min, and 5 h, which appeared respectively in 41 and 40% of the 1,000 models (Fig. 3a). The three most frequently selected sample time points in the three-variable best subsets procedure were 55 min, 5 and 9 h, which appeared respectively in 46, 82, and 54% of the 1,000 models

Table 4 Two and three time point regression models for 17-AAG and 17-AG log AUC

	17-AAG		17-AG	
	Two time point	Three time point	Two time point	Three time point
Time points	EST (SE)/VIF	EST (SE)/VIF	EST (SE)/VIF	EST (SE)/VIF
Intercept	0.869 (0.32)/0	2.449 (0.31)/0	3.590 (0.21)/0	3.797 (0.23)/0
55 min	0.653 (0.08)/5.09	0.400 (0.06)/8.00	–	–
5 h	0.469 (0.07)/5.09	0.441 (0.04)/5.14	0.747 (0.04)/1.84	0.650 (0.06)/4.70
9 h	–	0.142 (0.02)/4.64	–	0.111 (0.06)/8.25
17 h	–	–	0.169 (0.03)/1.84	0.122 (0.04)/3.39
R^2	0.975	0.990	0.968	0.971
Adjusted R^2	0.973	0.989	0.966	0.968
RMSE	0.215	0.136	0.262	0.251
log AUC within n% (number (%) of patients)				
Within 5%	33/34 (97)	33/34 (97)	32/34 (94)	32/34 (94)
Within 10%	34/34 (100)	34/34 (100)	34/34 (100)	34/34 (100)
Within 15%	34/34 (100)	34/34 (100)	34/34 (100)	34/34 (100)

LSM Limited sample model, *EST* regression coefficient, *SE* regression coefficient standard error, *VIF* variance inflation factor, *RMSE* root mean square error, *AUC* area under the drug concentration curve

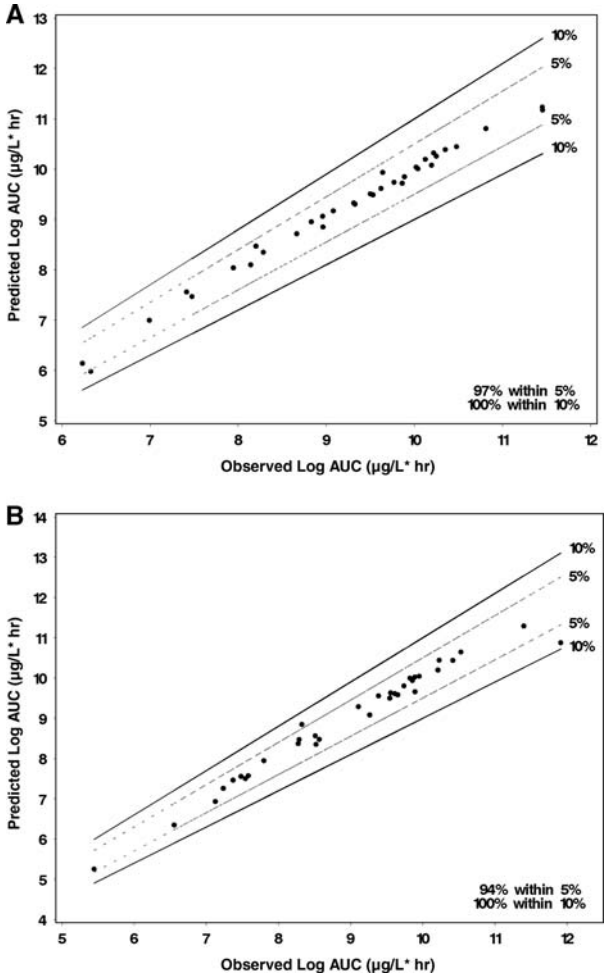

Fig. 2 **a** Observed versus predicted log AUC values for 17-AAG. **b** observed versus predicted log AUC values for 17-AG

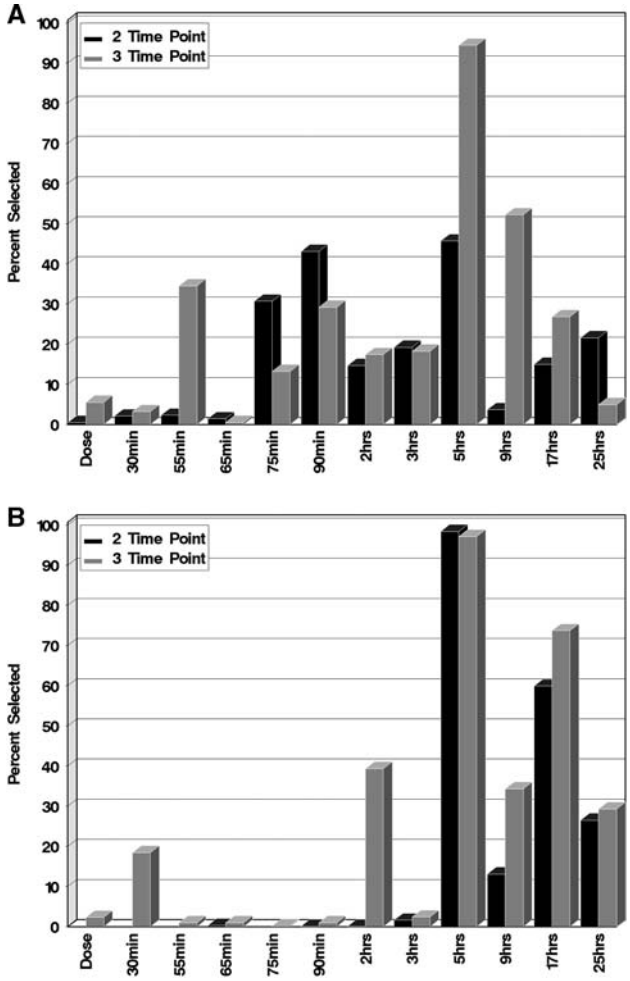

Fig. 3 **a** Results of bootstrap analysis for 17-AAG. **b** Results of bootstrap analysis for 17-AG

(Fig. 3a). For 17-AG, the 5 and 17 h sample time points were selected respectively in 91 and 59%, of the 1,000 models in the two variable best subsets procedure. The most frequently selected time points in the three-variable best subsets procedure were 5, 9 and 17 h which appeared respectively in 94, 39, and 74% of the 1,000 models (Fig. 3b).

Discussion

17-AAG and 17-AG pharmacokinetic data can be modeled with excellent accuracy using a limited sampling model approach. The best three time point LSM to predict the log AUC for 17-AAG includes the drug concentration values measured at 55 min, 5 and 9 h after start of treatment. The LSM to predict the log AUC for 17-AG includes the drug concentration values measured at 5, 9, and 17 h after start of treatment.

Potential limitations to the analysis included non-detectable values that occurred with greater frequency at lower doses, use of log-normal data distribution, use of a bootstrap approach with data obtained with an accelerated titration dose escalation design and log AUC transformation for evaluation of predicted versus observed values. Non-detectable values were assigned a small number in order to facilitate log-transformation on the entire dataset. Because a large number of the patients had non-detectable values at the 9-h concentration and beyond, the form of the model may be greatly distorted by the imputation of arbitrary small numbers. As a result, this method can lead to models that fit the data much more poorly, as well as complications in the modeling of random effects and other dependencies [10]. Based on the approach (M1) suggested by Beal [3], we refit our model using only data from the higher dose levels which had two advantages: data from three patients instead of just one patient, and detectable concentrations at the later time points. Our results were exactly the same as using the complete data in that the same time points were chosen to be optimal. As Beal [3] suggests in their article, all approaches to account for non-detectable concentrations would be biased, and caution needs to be used in interpreting the final results. We recognize that the two approaches used in this manuscript to address the non-detectable concentrations can impact the variance of the parameter estimation, but given that using only the data from the higher dose levels resulted in the selection of the same time points, we believe that our approach is indeed robust. To minimize impact, we also tested several different imputation methods and found that the LSMs selected were similar to those presented here. In addition, much pharmacokinetic data also follows distributions other than lognormal, such as a gamma distribution, or if extreme individuals are present, a log-Cauchy distribution [10]. The graphical analysis for the AUC and concentration values provided enough evidence that the lognormal distribution seemed plausible. While the bootstrap validation approach confirmed the multivariate results, it was less applicable due to the accelerated titration design used for this trial (only one patient treated at the first four dose levels). Finally, in predicting the percentage of patients whose AUC was predicted within a certain range, we compared the observed AUC with the predicted AUC on an anti-log (i.e., linear) scale. This comparison was not as favorable as on the log scale, however formal analyses on PK data are generally non-parametric and therefore modeling on the log transformation would not affect the statistics used such as Spearman's rho [5].

Alternative methods besides the regression approach described here for log AUC can be used to model this type of pharmacokinetic data. It would be reasonable to consider a random effects model with covariates, which operates under the assumption that a given individual has fixed values of all parameters for the duration of the trial [10]. By including an autoregressive process in such a random effects model, we could also adjust for the fact that each subject has an individual curve (that is, that residuals from one time-point to the next are dependent on each other) [10]. An exponential error model may be used to account for intersubject variability, while a compound covariance error model may account for intrasubject variability [15]. Nonlinear mixed effect modeling techniques can also be used to generate a population pharmacokinetic model [4, 15]. Finally, optimal sampling theory used in conjunction with a Bayesian algorithm may also be a valuable tool in modeling pharmacokinetic data, as it is more flexible with regard to sample collection time and design variables. The Bayesian approach also allows for the actual time to be used, rather than the assumption that the sample was taken at the specified time [9].

LSMs such as the two and three time point models are valuable for the study of 17-AAG and 17-AG pharmacokinetics in future clinical trials. The approach described here using a multivariate regression best subsets and stepwise model selection methods provided clinically and statistically meaningful results. The use of this LSM will facilitate further analysis of relationships between pharmacokinetics and pharmacodynamic parameters in phase II testing where larger sample sizes and more homogeneous populations and dosing are included to determine whether PK parameters can predict effects on target proteins. This LSM strategy is currently under evaluation prospectively in a Phase I trial of 17-AAG with Cytarabine. Finally, if 17-AAG is deemed to be clinically active, the LSM developed here can be explored as a predictor of therapeutic benefit and clinical toxicity.

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