HUMAN GENETIC VARIATION, POPULATION PHARMACOKINETIC – DYNAMIC MODELS, BAYESIAN FEEDBACK CONTROL, AND MAXIMALLY PRECISE INDIVIDUALIZED DRUG DOSAGE REGIMENS

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ABSTRACT

Variation in the behavior of drugs between people, and variation in drug behavior in a given patient over time, have both presented us with challenging problems in the optimal description of such behavior as well as challenges of how best to act on such information. New methods of genetic testing and measurement of variations in gene expression over time in a single patient present us with issues of 1) how best to use all such information in the overall process of planning drug dosage regimens for individual patients, especially if the drug is potentially toxic; 2) how to further refine our knowledge about the patient during of the course of therapy with the drug; and 3) how best to adjust the dosage regimen to the new information we obtain about him/her as a unique individual.

Human genetic variation, in the form of gene sequence or expression variability, provides us with important covariate information to help us further individualize our dosage regimen for a particular patient based on that information, just as does information about smoking status, age, gender, body weight, and renal function, for example. It helps us consider the patient as an individual rather than as a member of a larger group. Variation in gene expression over time (i.e., transcriptomic biomarkers) in an individual patient presents another problem, as it can cause significant differences in drug behavior over time. However, just as variation over time can occur in other covariates such as body weight and renal function, so can such changes in genetic expression over time be incorporated into models of drug behavior in individual patients, and used thoughtfully to optimize each patient's drug dosage regimen.

The overall structure of optimally precise Bayesian adaptive control is briefly reviewed in this paper, to define explicitly the context in which such human genetic/genomic information can be incorporated and used to optimize drug therapy for patients. Much of this has appeared elsewhere, but is reviewed briefly here for clarity in the present context of incorporating information about a patient's genetic makeup.

INTRODUCTION: ISSUES TO BE ADDRESSED.

When we make population pharmacokinetic / pharmacodynamic (PK/PD) models of the behavior of drugs in groups of people or patients, we often find that the model parameter distributions contain significant genetically determined subpopulations, for example, such as fast and slow metabolizers of drugs. In such cases, the customary assumed normal or Gaussian distributions of model parameter values are not present. Often the distribution is skewed, and a lognormal distribution instead may be used. The median may become a preferred estimator of a distribution which is not truly Gaussian, just as we often see it used as an index of personal income or the price of houses, for example.

This still may not be enough. Bimodal parameter distributions may need to be used, to better detect distinct subpopulations that display different drug behavior. Use of mixture models [1] to describe bimodal distributions has become increasingly popular to understand and describe such variable drug behavior.

Pharmacogenetics is the study of single gene variations in relation to variability in drug effects. This dates back to the 1950s when monogenic variations in drug metabolism first began to be discerned [2] *Pharmacogenomics* is a term introduced later, in the 1990s, based on the introduction of omics technologies such as genomics and proteomics [3]. Pharmacogenomics differs from pharmacogenetics in that it follows a broader multi-gene or genome-wide inquiry incorporating variation from the entire genome of a given person and its impact on variable drug effects. Importantly, one of the very first steps in translation of molecular genomic variation data into a form that can be useful in personalized medicine involves pharmacogenomic (or pharmacogenetic) *association* studies. Such association studies perform a correlational evaluation of genomic variation and drug effect variation in persons and populations. Unfortunately, it has been estimated that for effective translation of basic genomic discoveries to "real world" health outcomes in practice, only about 3% of the published human genomics studies presently focus beyond discovery-oriented applications [4]. Moreover, while building a foundation for personalized medicine, many of the pharmacogenomic association studies can only provide *approximate* guidance for individualized dosing regimens. Some have said, for example, that all that is needed is the genetic information, and then one knows the "right dose" to give. However, to be optimal, such information must be integrated into the techniques of maximally precise stochastic Bayesian adaptive control. What is needed is a *quantitative* approach, with models that can effectively identify genetic substructures in the variable human responses to medicines, and which can subsequently translate such raw data into specific and maximally precise dosing recommendations for each individual patient. In this regard, population pharmacokinetic and pharmacodynamic (PK/PD) models can be used effectively to address such quantitative needs on the critical path from the laboratory discovery of a human genetic variation to its meaningful use in the clinic or hospital to guide prescription decisions and optimal dosage regimens of potentially toxic drugs.

Despite their shared focus on understanding and describing the 'variability questions' in patient responses to medicines and other environmental exposures (e.g., toxins, bioactive nutrients, infectious agents, etc.), pharmacogenomics, pharmacogenetics and population modeling approaches (to be reviewed briefly in subsequent sections) have not intersected in a meaningful manner to date. This is timely for the field of population modeling. It is becoming increasingly clear that covariates previously used to increase the precision of dosing algorithms need to be reconsidered in light of the emerging human genomics variation data. For example, a recent study [5] found that race does not explain genetic heterogeneity in pharmacogenomic pathways, suggesting that most genetic

variation is determined by individual variation, and not simly by racial grouping, further indicating that a simple description of an apparent race is not adequate for individualized therapy [6].

The overall structure of optimally precise Bayesian adaptive control is reviewed briefly in this paper, to describe explicitly the context in which such human genetic/genomic information can be included and used to optimize drug therapy for patients. As the fields of genomics and population PK/PD modeling are rapidly coalescing, there is much to be learned from expertise available in both fields. Hence, the broader aim of this paper is to initiate a dialogue between these two highly complementary but hitherto separate communities and literatures among genomics, pharmaceutical sciences, modeling and simulation of drug effects, and practical clinical management of drug dosage regimens.

A BRIEF HISTORY OF PHARMACOMETRICS, POPULATION MODELING, AND BAYESIAN ADAPTIVE CONTROL: THEIR SIGNIFICANCE IN UNDERSTANDING AND CONTROL OF VARIABILITY IN DRUG BEHAVIOR

The science of pharmacometrics probably began with the work of Torsten Teorell, who in 1937 described the basic kinetic behavior of drugs in the body [7,8], upon which all else has been built. Various ways of fitting experimental data were also developed.

EARLY WAYS OF FITTING DATA

One of the most widespread and basic methods was linear regression upon the logarithms of the measured serum concentrations. This is because a logarithmic transformation converts an exponential decrease into a linear one, permitting the much more simple techniques of linear regression to be used, especially before computers became available.

Linear regression upon the logarithms of the measured concentrations was the cornerstone upon which the topic of “Basic Pharmacokinetics” was built. It was used extremely well by Sawchuk and Zaske [9] to describe the one-compartment apparent behavior of gentamicin in burn patients, to fit such a model to their data of measured serum concentrations, and to adjust gentamicin dosage to improve patient care.

However, the method has major problems. It only fits data once a patient has reached a steady state on maintenance therapy, and it can only fit data that should be in a straight line during a single dose interval. Because of that, it was said that data should only be obtained after a steady state had been reached, and only after the last dose of drug had been fully distributed within the patient’s body. Probably for these reasons, although no rigorous explanation has ever been offered, to our knowledge, the custom grew in the 1970’s to wait for a steady state before starting to monitor a patient’s serum drug concentrations, and

never to obtain a sample until after distribution was complete within the body. Apparently because of that, one was told never to get a sample for serum digoxin measurements before 6 or 8 hours after an oral dose, and, again, always in a steady state. "Peak" aminoglycoside samples were supposed to be obtained ½ hour after the end of the intravenous infusion, and again, at the trough, the lowest concentration just before the next dose.

The method is limited to perceiving a 1 – compartment drug model, nothing more complex. No population model based on past experience can be used to augment the patent's data. If new data is obtained in a subsequent dose interval, the old data must be thrown away. The almost always incorrect assay error pattern of an constant assay coefficient of variation must be assumed. The method is now obsolete, though it is still taught in many pharmacy schools.

Weighted Nonlinear Least Squares Regression is a big improvement. One can now fit data accrued over all the doses the patient has received in the recent therapeutic history, without discarding past doses. One does not need to wait for a steady state any more, or for complete distribution after a dose. Data can be obtained at any time and be relevant. The method is not limited to 1 compartment models, and so can analyze models with significant structure to them. The correct assay weighting can be done, by the reciprocal of the assay variance at each measurement [10].

However, there still is no way to augment the patient data with data from previous experience with a population of patients receiving the drug. Further, the method needs at least one data point for each model parameter to be estimated.

Maximum Aposteriori Probability (MAP) Bayesian fitting is still better. It has all the above desirable features of weighted nonlinear least squares above. However, it also augments the patient data with that of the parameter values obtained from past experience with the drug in a population of patients studied previously. In addition, it can begin to fit with data as sparse as only a single measurement. Because of this, it is the best of the above 3 methods for fitting data from an individual patient. It was introduced into the PK community by Sheiner and Beal [11].

Population PK/PD modeling was then introduced into the PK community by Beal and Sheiner [12]. The method differs in approach in that it analyzes the entire data of all patients in a population at once. The only requirement is that the population data contain at least as many samples as there are parameters to estimate in the population model of the drug. An excellent review of population modeling in the past is [13]. This approach will be discussed in more detail further on.

VARIATIONS IN GENOTYPES AND GENE EXPRESSION: WHAT DO THEY MEAN FOR POPULATION MODELS AND PERSONALIZED MEDICINE?

Recent availability of human genetic variation data and new genomics technologies that make both gene sequence variation (genotypes) and gene expression data (that can vary over time) available now present us with issues on how best to use such information in the overall process of modeling drug behavior, planning the initial drug dosage regimen, (especially if the drug is potentially toxic), following up the initial regimen to further observe the patient and his/her responses to further refine our knowledge about the therapy during the administration of the drug, and how best to adjust the dosage regimen to the new information we obtain about him/her as a unique individual.

Already, a number of population pharmacokinetic and pharmacodynamic studies are emerging in the literature in which genetics/genomics data are incorporated to the analyses and modeling attempts [14-16]. Genetic testing is often proposed to help distinguish between various genetic subgroups. However, within each genetic subgroup there is still considerable diversity. Each such subgroup needs its own population PK/PD model to define the variability of drug behavior in each subgroup, and to link it to its class of genetic subgroups. We need a model that can use the covariate information of the genetic makeup of the population. This model should be linked to a method to develop a dosage regimen which is maximally precise for each potential newly discovered member of that population. Genetic testing can provide highly useful covariate information about patients, just as can information about smoking status, age, gender, body weight, and renal function can, for example.

A recent paper by Kaila et al [1] is most interesting in this regard. They developed a population model of Metoprolol and its relationship to the CYP2D6 phenotype. They used NONMEM, a parametric population modeling approach, and were quite successful in correctly classifying patients as poor versus extensive metabolizers. They used a bimodal mixture model to do this. There was some overlap between the two population subgroups, but the classification was generally quite successful. However, is such classification enough? Even within a classification, there is still considerable variability. Model parameter distributions all need to be quantified, not just classified, and a method is needed to develop an optimally precise dosage regimen for the overall population or for each specific subgroup to which a specific individual patient appears to belong.

The above authors made a useful step forward, but now what? The overall reason for their work was not stated or formulated in the paper. What is our real goal? Why do we model? Why do we do genetic testing and evaluate gene expression? What is the formal scientific structure of our approach, and what are our specific plans and tools to achieve our goals? Let us look at the main specific steps in this process.

At any stage of our knowledge of the behavior of a drug, we need tools which will best describe that behavior. The tool(s) should lead to a dosage regimen which will be most precise under the circumstances, based on the

information known at that time. It should hit our desired target goal(s) most precisely. Most current practice of modeling and simulation does not do this explicitly. Instead, it looks for various factors, descriptors, covariates, or genotypes or phenotypes such as CYP subtypes, for example, as in the paper by Kaila et al [1], but after making a model, there is little thought (a real cultural blind spot!) about how to proceed further. Monte Carlo simulation is done to predict variability in response. However, no specific target is usually defined. That is the problem and the blind spot.

Modeling and simulation approaches, currently dominated by the pharmaceutical industry, are not enough by themselves. The general process of "Bayesian forecasting" [11] needs to be re-examined. Such forecasting usually has no explicit target goal in mind, and no current tool in Bayesian forecasting has been developed to maximize the precision with which a dosage regimen will hit a specifically selected target goal.

Instead of Bayesian forecasting, we need maximally precise Bayesian adaptive control of a patient's PK/PD system to hit a target which our best clinical judgment leads us to believe will yield maximum benefit to a particular individual patient. Patients should be treated as individuals, not as members of a population. In addition, we may often learn that we should revise our target goal(s) based on what we observe about our patient's clinical sensitivity and response. Let us discuss the specific formal structure of this process.

POPULATION MODELS

Population models of pharmacokinetic (PK) and pharmacodynamic (PD) systems, physiologically based PK/PD models, and similar mechanistically based models are for the great part used these days to understand and describe the behavior of drugs and combinations of drugs in animals and in patients. These approaches have largely replaced the older approaches discussed earlier. They are quite well reviewed in [13]. Models are also made to find factors (descriptors, covariates, or genetic test results) associated with patient variability in such models. They are usually made to aid in the process of drug discovery and development, and to develop general guidelines for dosage regimens to be used in groups of patients.

The general clinical need for planning, monitoring, and adjusting individualized doses of potentially dangerous and toxic drugs precisely is widely accepted, and gets a lot of lip service. However, in spite of this well – recognized need, specific techniques to achieve these goals have usually not been considered. General guidelines and intuitive adjustment of dosage have been the standard of such prescribing. The precise and badly needed clinical tools for optimal dosage individualization are described briefly below.

PARAMETRIC POPULATION MODELING APPROACHES.

Parametric modeling approaches assume that the model parameter distributions have a certain specific shape, such as normal, lognormal, multimodal, etc. Those distributions have formulas that describe their assumed shape. These formulas themselves have parameters such as means, medians, standard deviations, and correlations or covariances. They can also include the relative amplitude of the various means, for example, in a multimodal mixture distribution. It is the parameters in those formulas that define the assumed distribution which are estimated in parametric population modeling approaches, rather than the actual distribution itself. The normal distribution is completely characterized, for example, by means, standard deviations, and covariances or correlations. In the lognormal distribution, the logarithms of the parameter values are assumed to have a normal distribution. In a mixture model, two or more modes are assumed, and a weighting factor is estimated for each mode in the distribution.

In addition, both parametric and nonparametric (NP) models have clinical descriptors, covariates, and genetic test results with significant relationships to various model parameters, such as creatinine clearance for elimination or drug clearance, and body weight for volume of distribution, for example.

Strengths of parametric approaches:

1. They have been around a long time, and are very well-known and widely used by the modeling and statistical community. Indeed, they have been the standard of practice until recently.

2. They assume that the model parameter distributions are determined by a formula such as those which describe normal, lognormal, multimodal, or other distributions, as described above. These approaches are backed by many years of statistical experience.

3. Both population modeling methods can function with as few samples as 1 per subject, as long as the total number of samples is greater that the number of model parameters to be estimated. However, the richer the data, the better the results.

4. Statistical confidence limits can be computed for the various assumed parameter distributions. These are based on asymptotic theory - that is, when it is assumed that the number of subjects approaches infinity.

Weaknesses of parametric approaches:

1. The assumption that the model parameter distributions are normal, lognormal, or multimodal, for example, is frequently not borne out by data from

the genetically polymorphic populations of patients which exist. One may have to carry out many analyses of presumed multimodal distributions before one can conclude that one of them appears to describe what is in the raw data. This can be most tedious. Many metabolic processes are genetically determined, and can be quite polymorphic. This is true, for example, for subjects with various subtypes of CYP450 enzymes. Genetic testing helps us recognize such subpopulations, just as body weight and creatinine clearance also do. A population may well contain subgroups of rapid and slow metabolizers of drugs, for example, as described by Kaila et al [1].

2. Since current parametric approaches are usually maximum likelihood (ML) in type, constraints imposed by the assumed shapes of the parameter distributions guarantee only that the most likely point estimators of those distributions are found within the assumptions of their formulas.

3. The most widely used ML population modeling tool, NONMEM, uses approximate methods for calculating the likelihood, such as the first order (FO) and the first order, conditional estimation (FOCE) methods. These approximations can result in incorrect parameter values being found, because the approximate likelihood found is not the true one. Further, with approximate likelihood calculations, the highly desirable property of statistical consistency is lost. There is then no guarantee that studying more subjects in a population will really result in the parameter estimates, especially variances and correlations, becoming closer to the true values [17]. In fact, they can get worse [17].

4. Other parametric approaches such as S-ADAPT and MCPEM [18,19], do have exact likelihoods, and do have statistically consistent behavior. However, they still do not have the ability to develop maximally precise dosage regimens, as they are limited by their arbitrary assumptions about the shape of the model parameter distributions.

5. In addition, because the parameter distributions are usually assumed to be normal or lognormal, point estimates of the parameter values (means and medians, for example) are typically used to develop the dosage regimens to hit the desired therapeutic target goals. However, there is no way to estimate in advance, short of Monte Carlo simulation, the degree to which such a dosage regimen will fail to hit the target. The target is simply assumed to be hit exactly. Developing a maximally precise dosage regimen based on a skewed distribution or on a mixture model, especially of unknown proportions, can be an extremely daunting task using parametric population modeling approaches.

6. With parametric modeling approaches, it has also been customary to assume some general form of the error function due to the combination of assay error and environmental noise, and then to estimate its parameter values as well. Various forms of error functions are assumed and tested, and the one that gives the best fit is usually chosen. Users generally assume that assay error is a

negligible contributor to the overall noise. They have not sought to know how much of that noise is really due to the assay. The assay error has usually has been carefully determined, found to be “acceptable”, but then has been discarded in the further calculations. With such approaches, there is no way to know how much noise is actually due to the assay, and how much noise is due to the various environmental errors in the therapeutic or research environment such as:

1. errors in preparing and giving the various dosage amounts,
2. errors in recording the discrepancy between when the various doses were given versus when they were said to have been given,
3. errors in recording the discrepancy between when the various responses such as serum samples were obtained versus when they were said to have been obtained,
4. model mis-specification - the difference between the description by the model of reality and reality itself,
5. unrecognized changes in parameter values during the period of data analysis. These changes should be identified in a way that can be used clinically and not simply reported as interoccasional variability (see the IMM sequential Bayesian approach further below).

In summary. when the assay error has not been separated from the environmental error, one will never know how much noise in a population model is due to the assay, and how much is due to the other factors in the clinical environment. Parametric approaches are often used by those who are oriented to modeling for the pharmaceutical industry rather than to clinically relevant modeling oriented to optimal, individualized, personalized, patient care in clinical settings.

NONPARAMETRIC (NP) POPULATION MODELING APPROACHES.

In the NP approach, no assumptions are made about the shape of the parameter distributions [20-23]. The likelihood, and the distribution obtained, obtained is free from any constraints imposed by any assumed shape. Because of this, the resulting likelihood is usually greater when direct comparisons are made between the two approaches analyzing the same data [17]. The NP approach can better detect and describe the frequently unsuspected, often genetically determined, polymorphic subpopulations of patients present in any population. It does not simply classify them. It quantifies them – all the subpopulations and subgroups that are known, and all the ones that are not yet discovered. There is a lot we do not know yet about the DNA tree and our genetic makeup that has still not been discovered or classified by genetic testing. NP models do this already, without needing to ask what genetic classification a patient belongs to. The resulting parameter distributions are often provocative and stimulate vigorous discussions about what the characteristics might be of the

various subgroups found. This becomes a fertile field for genetic testing. What information characterizes the members of an unsuspected population subgroup?

STRENGTHS OF NONPARAMETRIC (NP) APPROACHES.

1. The calculated likelihood is exact. The most likely parameter distributions are therefore actually what are found. Statistical consistency is assured [20-23]. This means that as more patients or subjects are studied, the estimated parameter distribution really does approach the true distribution.

2. If an "ideal" population model were desired, it would be most convenient if one could somehow observe each subject's parameter values directly and exactly. The parameter distribution obtained would be a discrete collection of the exact parameter values found in each subject. No population model could ever do better. The usual statistical summaries would degrade the richness of the actual often genetically polymorphic distribution found. However, even with this "ideal" model, it is interesting that one cannot make any statements about what the parameter distributions would be if the study were to be repeated many times. No statistical confidence limits can be determined even in this ideal case.

Of course, such "ideal and exact" models are clearly impossible to make. One cannot observe parameter values directly. Instead, one must give a dosage regimen (with dosage and timing errors) to each subject, measure responses (with assay, timing, and the other errors described earlier), and estimate the distribution of the model parameter values in the population.

3. The nonparametric (NP) modeling approach is the closest thing to the above ideal. The theorems of Lindsay [23], Mallet [20], and Caratheodory [24] prove that it is not necessary to evaluate infinite families of continuous distributions such as the parametric ones discussed above. Instead, out of all the infinity of distributions one might consider, the most likely one "can be found" in a particular discrete distribution containing discrete support points [20-24]. As shown in Figure 1, these discrete distributions do not have any equations determining their shape, as do the parametrically described ones. NP distributions are free of any such constraints. They simply are what they are.

4. Do the two methods (parametric and NP) get similar results? They do, if the distribution specified in the parametric approach happens to be the true distribution. For example, if the underlying distribution is truly Gaussian, as the number of subjects goes to infinity, parametric parameter estimates of the distribution converge to the true parametric distribution. However, if the true distributions are not Gaussian, for example, the results obtained are not certain. On the other hand, the Kiefer – Wolfowitz theorem [25] proves that for NP discrete distributions, as the number of subjects goes to infinity, the NP distribution converges to the true distribution, whatever that underlying distribution turns out to be.

The NP parameter distribution is therefore made up of a discrete collection of unconnected estimated points rather than any assumed continuous distribution. Each estimated point (support point of the distribution) consists of a point estimate of each model parameter value, plus an estimate of the probability of that set of specific parameter values. The overall shape of these discrete parameter distributions is determined only by the raw data itself, and by the stated assay and the estimated environmental errors.

The NP approaches [17,20-22] have exact likelihoods, and therefore have statistical consistency. They are also uniquely well suited to the design of maximally precise dosage regimens (see further below).

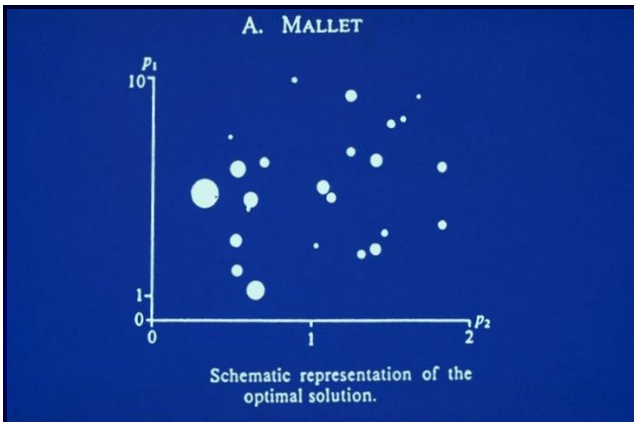

Figure 1. An example of an NP population model made by Mallet. Horizontal axis, a parameter such as the rate constant for elimination. Vertical axis, a parameter such as apparent volume of distribution. Note the clearly non-Gaussian shape of the horizontal parameter, with the central gap. This may well represent two unsuspected subpopulations such as fast and slow metabolizers of a drug. The size of each point reflects its estimated probability (reproduced by permission).

The NP approach, first developed by Mallet [20], now exists in several versions by various authors [20-22]. The data of the patient population is resolved into discrete points (support points), up to one for each subject studied in the population. More uncertainty results in fewer support points. Less uncertainty leads to more support points, up to one for each patient studied. Such a model, by Mallet [20], is shown in Figure 1.

A Weakness of the NP approach:

While one can calculate intervals that contain a specified proportion or percent of estimated parameter values (percentiles of the estimated distribution), one cannot calculate rigorous confidence limits of NP model parameter distributions. Note that this is also true of the unattainable ideal model consisting of exact directly observed parameter values, described above. However,

confidence limits for NP distributions can now be obtained, in theory, by bootstrap procedures [26].

PROPER DESIGN OF TDM POLICIES AND PROTOCOLS.

Many workers have had the impression that “sparse sampling” designs based on “routine” protocols of therapeutic drug monitoring, are suitable for population modeling, as such software can function with as few samples as one per subject. Many authors have published population models based only on trough samples obtained at the steady state. They usually conclude that a 1 compartment model is “determined to be best”. The models describe clearance, which is the only parameter that can be estimated under those very restricted and suboptimal circumstances.

One can never avoid the issues of proper experimental design [27]. A two - parameter model still needs at least two samples (not just the one of major interest) to capture well the information about both volume and clearance or elimination rate constant. Population models with real structure cannot be made from noninformative sampling strategies as “steady state, trough only”. One is led to conclude that many current policies of “routine” TDM are often wasteful, and need to be revised, so that the cost of doing TDM is not wasted, so that patient care can be improved by more informed and precise TDM models, and so that maximally precise dosage regimens can be developed, both from and for, the patients we care for.

Finally, if one starts with a rich data set and gets acceptable results, it is interesting then to withhold data from the analysis and to observe the results again, as the data points get progressively fewer and fewer. The worst results are obtained when only a single trough sample is used in the steady state [28]. Even in such data poor cases, NP modeling approaches have been shown to be very attractive [29].

DESCRIBE ASSAY ERROR BY THE RECIPROCAL OF ITS VARIANCE, NOT BY ITS PERCENT COEFFICIENT OF VARIATION (CV%).

Determining the Assay Error Polynomial.

One should first determine the error pattern of the assay quite specifically, by determining several representative assay measurements in at least quintuplicate, and to find the standard deviation (SD) of each of these points [10,30]. An example of this is shown graphically in Figure 2, above.

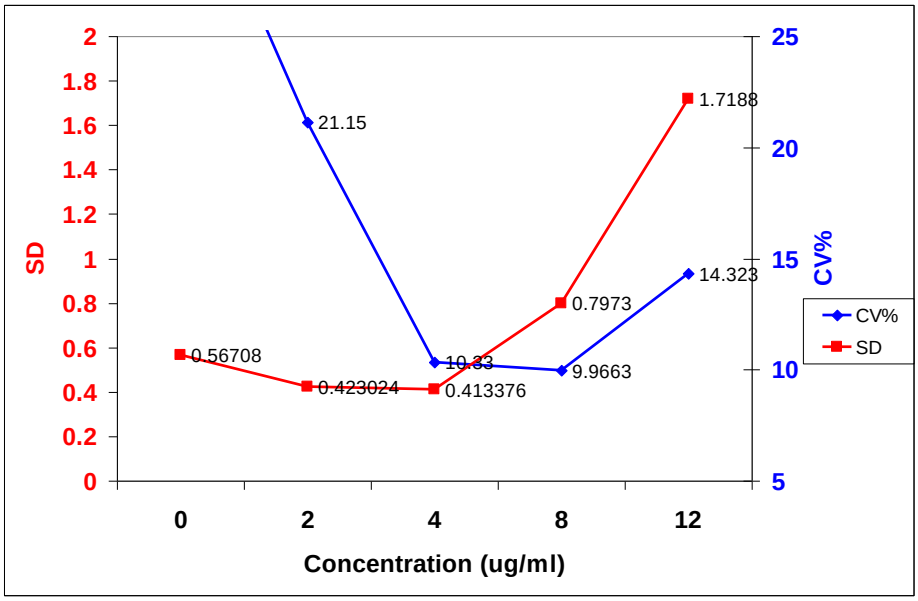

Figure 2. Relationship between measured concentration (horizontal scale), CV% (right hand scale) and Assay SD (left hand scale). CV% (diamond symbols) increases as shown at low values. On the other hand, the assay SD is always finite at any value, all the way down to and including the blank. Because of this, there is no need to censor any data at all. The measurement and the SD, done in this way, enhance the sensitivity of any assay all the way down to and including the blank, with a well documented statistical measure of credibility.

One can measure, in at least quintuplicate (and the more the better – some say at least 10), a blank sample, a low one, an intermediate one, a high one, and a very high one. For each collection of replicate samples, one can then fit the relationship between the mean serum concentration (or any other measured response) and the SD (not the percent coefficient of variation as is currently done) with which it has been measured, with a polynomial of up to third order if needed, so that one can then compute the 1/variance associated with any single sample that goes through the laboratory assay system [10,30]. One can then express the relationship as a polynomial,

$$SD = A_0 + A_1C + A_2C^2 + A_3C^3 \qquad (1)$$

where SD is the assay SD, A_0 through A_3 are the coefficients of the polynomial, C is the measured concentration, C^2 is the concentration squared, and C^3 is the concentration cubed. A representative plot of such a relationship, using a second order polynomial to describe the error pattern of an assay of gentamicin, is shown in Figure 2. Such a procedure permits giving correct weight in the fitting process to each measured serum concentration.

Determining the Remaining Environmental Noise.

In addition, a parameter which we have called lambda, a further measure of all the additional environmental sources of intra-individual noise, can also be computed by software for population PK modeling. We use it in our population modeling software as a noise term in addition to the assay error polynomial described above. The nominal value of lambda is zero, indicating that there is no other source of variability than the assay error pattern itself. Lambda is therefore greater than zero. It summarizes the various environmental errors such as those described earlier above Lambda is thus an overall measure of all the other sources of intraindividual variability besides the assay error.

In this way, one can know just how much of the overall noise is due to the assay, and how much is due to the remaining environmental noise. If lambda is small, it suggests that the sum of the environmental sources of noise is small. If it is large, it suggests that the overall environmental noise is large, and that the protocol of the clinical study might be re-examined to tighten it up.

EVALUATING CHANGING RENAL FUNCTION IN ACUTE CLINICAL SETTINGS.

When you are caring for acutely ill and unstable patients, often in ICU settings, it is imperative to be able to track changing renal function accurately and precisely. However, most methods for estimating creatinine clearance have been based only upon a single serum creatinine specimen. It often takes several days to a week for serum creatinine to stabilize after a change in renal function. Methods that use only a single serum creatinine cannot track rapid changes in renal function,

Our laboratory developed a method using a pair of serum creatinine samples instead [31,32]. It takes into account the rate of rise or fall of serum creatinine between the two samples. It calculates the creatinine clearance that would make serum creatinine rise or fall from a first to a second value over a stated time in a patient of a stated age, gender, height, and weight. It predicted creatinine clearance in postsurgical renal transplant patents, starting with the day of surgery, with a precision not different from that found with the standard 24 hour urine gold standard [31,32].. This has been a great practical help in TDM and in individualizing clinical drug therapy. Other similar methods have been described since our original article [33-35].

THE CULTURAL BLIND SPOT: OPTIMAL CONTROL OF PK/PD SYSTEMS.

We need to formulate specific strategies, especially for the management of therapy with potentially dangerous drugs, as they need the most precise and thoughtful management with respect to both safety and toxicity.

SELECT A SPECIFIC THERAPEUTIC TARGET GOAL, NOT A WINDOW.

Our overall goal is to develop the most precise dosage regimen to hit a selected therapeutic target goal, such as a desired serum concentration profile, most precisely. At first, we know nothing about the patient as an individual unless, perhaps, we have had occasion to measure covariates such as age, gender, body weight, creatinine clearance, smoking status, or genetic test results before planning the initial dosage regimen.

Problems with "Therapeutic Ranges" Figure 3 shows the usual means by which "therapeutic ranges" appear to have been obtained. It is usually done by eyeball, and nothing more rigorous. It is stated that first, there is a "significant" incidence of therapeutic effects with increasing serum drug concentrations. This is supposed to define the beginning of the therapeutic range. Later on the incidence of toxic effects also becomes "significant", and the "toxic range" has been entered. However, this procedure does not consider the need to develop a gentle dosage regimen for a sensitive patient who needs only a gentle touch, or a more aggressive one for a patient who may be less sensitive, or who may have a bioavailability problem, and who needs the dosage "pushed".

In addition, the risks and benefits in choosing a "therapeutic range" or window are quite complex to analyze. One could, for example, easily develop a multiple model (MM) dosage regimen (see below) to maximize the probability of having the patient's serum concentration being within some chosen window. This sounds good at first, but one must also weigh the benefit and the probability of a desirable response against the risks, and their probabilities, of being outside that window, either below or above it.

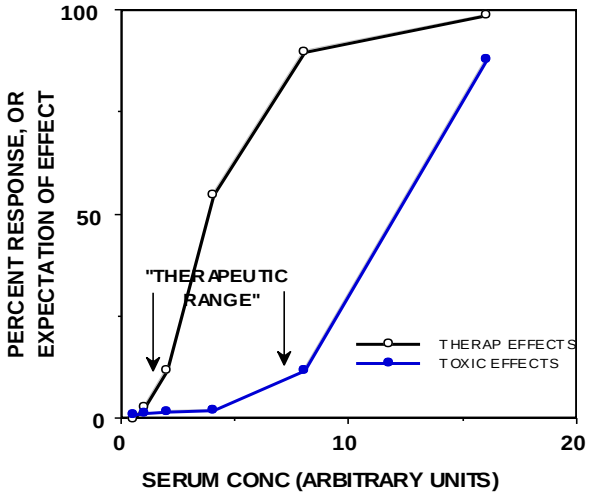

Figure 3. General relationships usually found between serum drug concentrations and the incidence of therapeutic and toxic effects. The eye is drawn to the bends in the curves, and the therapeutic range is classified in relation to these bends. This qualitative procedure of classification discards the important quantitative relationship of the incidence of toxic effects versus serum concentration.

Risks associated with the patient being below the window are usually associated with inadequate therapeutic effect. Those associated with being above the window are usually associated with toxicity. Each outcome - subtherapeutic, therapeutic, or toxic - has not only its associated probability but also its own positive or negative quantitative utility function of goodness or badness. Optimizing such a complex set of probabilities and utilities is poorly amenable to rigorous clinical decision analysis, especially at the bedside.

Choose specific target goal(s) based on each patient's need and sensitivity.

A more intuitive and individualized approach is one in which the clinician evaluates the magnitude of each patient's individual clinical need for the drug in question, and selects an estimated risk of toxicity which is felt on clinical grounds to be justified by that patient's need. In this approach, there is no illusory window of neutrality about a target, as in a "therapeutic range". Things are not the same at the bottom as at the top of the range. Based on the relationship between serum concentration and incidence of toxicity shown in Figure 3, for example, one selects a specific target serum concentration goal to be achieved for a specific patient. One does not want the patient to run any greater risk of toxicity than is justified by the patient's clinical need for the drug. Within that constraint, however, one wants to give the patient as much drug as possible, to get the maximum benefit. This approach provides the rationale for selecting a specific target serum concentration goal, rather than some wider window, and then to attempt to achieve that target goal with the greatest possible precision, just as if one were shooting at any other target.

In this approach, the risks of being just slightly above the desired target goal are only minimally different from those associated with being just slightly below it, in the sense of an infinitesimal difference in calculus. Because of this, it appears easier, and also more intuitive for a clinician, to choose a specific target goal rather than a window, and then to attempt to achieve it or hit it with the greatest precision (least error) possible.

THE STAGES OF LEARNING ABOUT A PATIENT.

1. We need to select a specific target goal (serum concentration profile, for example), not just a therapeutic window in which most patients do well. We need to use our clinical judgment to consider each patient's individual need for the drug, perhaps the probable sensitivity of the patient's infecting organism, and we need to set a specific target goal suited to his/her specific needs, and to the risk of toxicity it appears appropriate to accept in order to obtain the hoped - for benefits.

2. Next, we need to hit that desired target most precisely, to get the efficacy we think we want, and not to incur any greater risk of toxicity than we think is acceptable.

3. We begin with our NP population model, as we have no other information to guide us than what we know about a past population of patients like the one in front of us. We develop the dosage regimen to hit our target most precisely, using multiple model MM) dosage design, as described below.

4. Then we will monitor the response of the patient, both clinically and by obtaining measures of response such as serum concentrations and effect measurements, weighting them by 1/variance, as described above and fitting the patient's data, to make a model of the patient as an individual. We then know our patient more precisely as an individual, as we have supplemented data from a population of similar patients with data from this individual patient. This process takes into account all the covariate and genetic information we already may know about the patient, as well as all that we have not discovered yet, plus all the drug-drug interactions we know of, plus all those we have not discovered yet as well.

5. We then use this individual model of the patient (the Bayesian posterior model) to plan the next dosage regimen, once again with maximum precision. This process is then repeated as many times as needed. The process is described briefly below.

MULTIPLE MODEL (MM) DOSAGE DESIGN.

MM dosage design develops dosage regimens which hit desired therapeutic target goals for an individual patient with maximum precision, based on all data available up to the present. It uses NP population models, not parametric ones, as those do not work here. The multiple support points in the NP population model, each one with its own parameter values, provide multiple predictions of future serum concentrations and other responses from any candidate dosage regimen. Each prediction is weighted by the probability of the support point giving that particular prediction (see Figures 1 and 4). When one selects a specific target goal at a target time, one can use the multiple predictions and their probabilities, to calculate the weighted squared error of the failure of any dosage regimen to hit the specific selected target goal. It is then also easy to find the regimen which specifically minimizes that predicted error.

This MM method, using NP population models, provides, for the first time, dosage regimens which hit desired targets with maximum precision (minimum expected weighted squared error). In this way, one can develop a maximally precise initial dosage regimen of a drug [36-39]. One can see visual plots of the diversity of the multiple predictions provided by the NP population model and that particular maximally precise MM dosage regimen. The MM approach to Bayesian

adaptive control has been widely used in the aerospace industry for flight control and spacecraft guidance systems.

Especially when model parameter distributions are not Gaussian, or when they are multimodal, as in [1], use of MM dosage design provides, for the first time, dosage regimens for any genetically polymorphic population, known or not yet discovered, which will hit the target(s) with minimum expected weighted squared error. It considers all subpopulations in the overall distribution, their relative prevalence and probabilities, and uses the entire distribution as the means by which the maximally precise regimen is determined, not just some point estimator (mean or median, for example) obtained from it. When distributions are not Gaussian, the use of mean parameter values to determine the dosage regimen can be quite dangerous, as, for example, when the mean is located at the 73rd percentile of that distribution, for example, as has been found. Furthermore, unless the entire distribution is computed, one may never know these things [17].

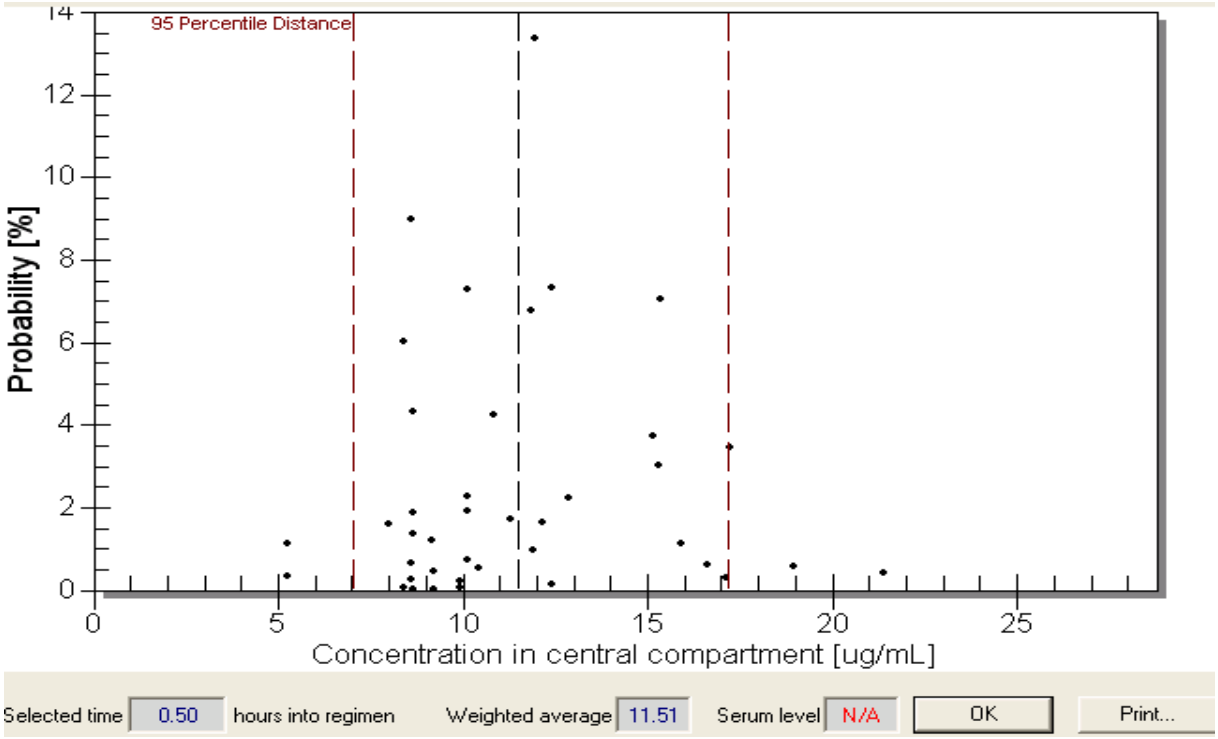

Figure 4. Time slice of the multiple predictions at ½ h into the regimen, the time of the peak serum concentration. Horizontal = the multiple predicted serum concentrations. Vertical = probability of each prediction. Middle dashed vertical line = predicted weighted average concentration of 11.51 ug/mL (target = 12). Outer dashed lines = 95 percentile distributions (roughly 7 to 17 ug/mL) of the predictions.

There are 76 support points in the population PK model for Gentamicin in the MM-USCPACK clinical software [36-39]. These 76 points generate 76 different predictions of future serum concentrations from any candidate dosage

regimen. In this respect, each MM dosage regimen is an optimized clinical trial and simulation. Results of a once daily regimen, developed for a stated individual patient, hit the target goals of a peak concentration of 12 ug/ml and a trough of 0.5 ug/ml, most precisely, given the constraints of q 8 h, q12 h, or q 24 h dosing intervals, as shown in Figure 4. It shows those predictions for the peak concentrations at the end of the 30 minute infusion of the first dose (½ hr into the regimen). The predicted weighted average concentration is 11.5 ug/ml, close to the target of 12 ug/ml. However, the great diversity of the predictions based only on the population model is also easily seen. The 95 percentile distances range from a low of 7.0 to a high of 17.2 ug/ml. The patient clearly needs TDM.

MONITORING THE PATIENT BOTH CLINICALLY AND BY MAKING INDIVIDUALIZED MODELS BASED ON DATA OBTAINED BY THERAPEUTIC DRUG MONITORING (TDM).

MM Bayesian posterior joint distributions can be used, based on data acquired during thoughtfully designed TDM [27], to model drug behavior in that individual patient and to adjust dosage regimens as patient physiology (or the desired therapeutic target) may change over the course of therapy. As serum concentrations and other responses are obtained, the population model is now presented with and supplemented by this new individual patient data. In contrast to the maximum aposteriori probability (MAP) parametric Bayesian estimator [11], the MM Bayesian process computes the posterior probability of each population support point given the new data from that individual patient. Those population model support points (combinations of model parameter values) that fit and predict the individual patient's new data well have much greater probability. Those that fit it poorly have much less. Usually, a few or perhaps only a single point remain, while the probabilities of the other population support points become negligible.

One can see graphically just how much one has learned by the TDM one has done, by the usually much narrower distribution of the estimated serum concentrations. After this, the MM Bayesian posterior joint distribution can be used to develop the next dosage regimen to hit most precisely the specific selected target goal, which may or may not be changed, based on clinical judgment. Comparing the patient's clinical behavior with plots of the patient's past serum concentrations and the estimates of them from the patient's posterior individual model is the most useful way we know to evaluate a patient's clinical sensitivity to a drug.

A representative patient undergoing Gentamicin therapy received first intuitive dosage adjustment and then Bayesian reconstruction and analysis of his clinical situation. He initially received 80 mg of Gentamicin roughly q 8 h (all times were known exactly). His first serum results were 3.8 ug/mL peak and 1.8 trough. Because of this, his dosage was intuitively raised to 100 mg q 8 h. The 3rd dose was given intramuscularly, late, as shown, as the IV had infiltrated. A serum sample in the middle of that dose interval was 5.2 ug/mL. A subsequent peak

was 9.1 ug/mL, but the trough, almost 15 h after that third dose of 100 mg, was very high (4.1 ug/mL). Because of this, his dosage was reduced intuitively to 80 mg once again, as shown. At this point the patient's data were analyzed by the MM Bayesian clinical software. Note that the patient's serum creatinine rose rapidly, within two days, from an initial 1.2 mg/dL, to 1.5, and then to 2.1 mg/dL. Using the method to estimate changing creatinine clearance described earlier, it was 56 ml/min/1.73 M^2 initially. It then fell to 41 as the SCr began rose from 1.2 to 1.5 mg/dL, and declined further to 27 as the SCr rose further from 1.5 to 2.1 mg/dL. Clinically, the therapeutic situation was not at all clear. It was not possible to judge clinically, at that time, what the proper dosage regimen should be.

At that point, the MM Bayesian analysis was used to make an individualized PK model for this patient, fitted both to his serum concentration data and his changing CCr over time. The results are shown in Figure 6. A good fit (somewhat hard to see in the figure) was obtained. The combination of the estimation of changing CCr and the pharmacokinetic model with the elimination rate constant linked to that changing CCr and the central compartment volume of distribution linked to changing body weight as covariates, was able to track the behavior of the drug in this patient quite well, as shown in the plots.

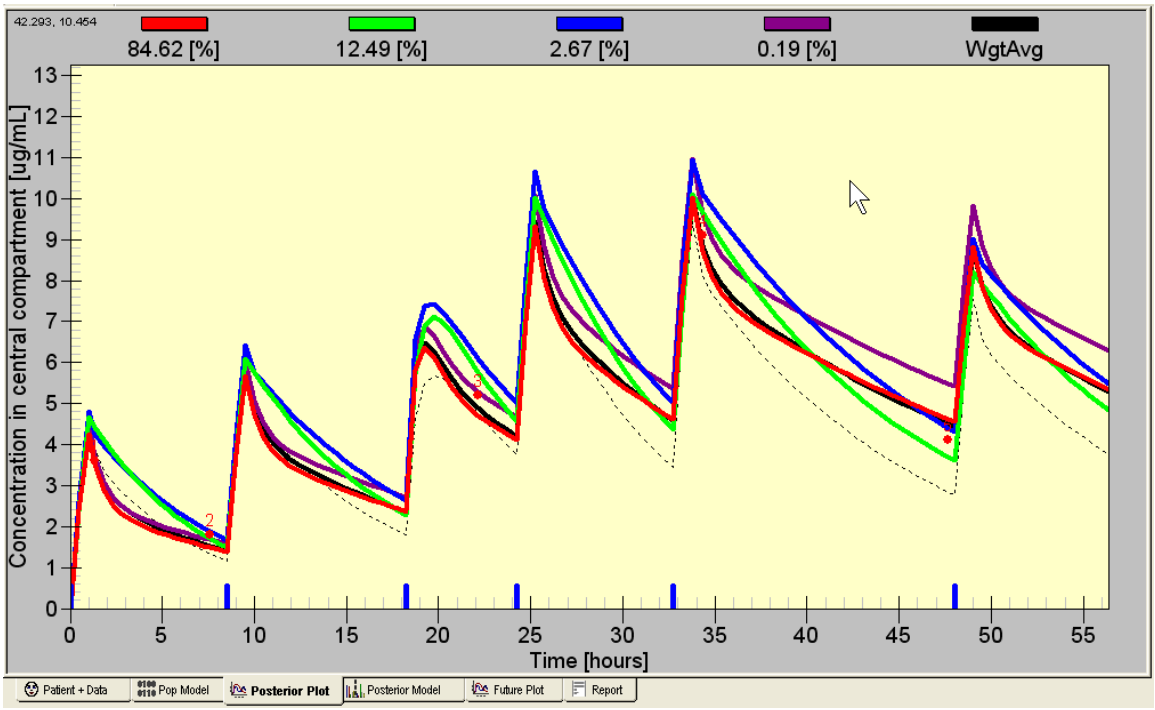

Figure 5. Plot of the trajectories of estimated serum concentrations produced by MM Bayesian analysis of the above patient's data. Instead of 76 estimations as shown in the population PK model in Figure 4, only 5 support points survived the Bayesian analysis. Note their very much increased probabilities, up to 84%, (top horizontal of the figure) compared to those of the population model, where the highest probability of the various support points was 13%. The five measured serum concentrations are shown by the round dots and their nearby numbers.

Figure 6 below shows the scatter of the estimates of the serum concentrations at the time of the first predicted peak serum concentration in the new regimen. The figure shows the much narrower range of estimates around that serum level compared to the range based only on the population model, as shown in Figure 4 earlier. This provides visual information of the more precise knowledge of the behavior of the drug in the individual patient at that time, compared to knowledge based only on the population model. We can see visually just what TDM has done for us, in terms of the new information and the more precise knowledge acquired about our patient. The more precise individualized model now provides a useful way to predict serum levels resulting from subsequent dosage regimens, and to develop a maximally precise dosage regimen to hit the selected target goals, for example, a 12 ug/ml peak and a 0.5 ug/ml trough.

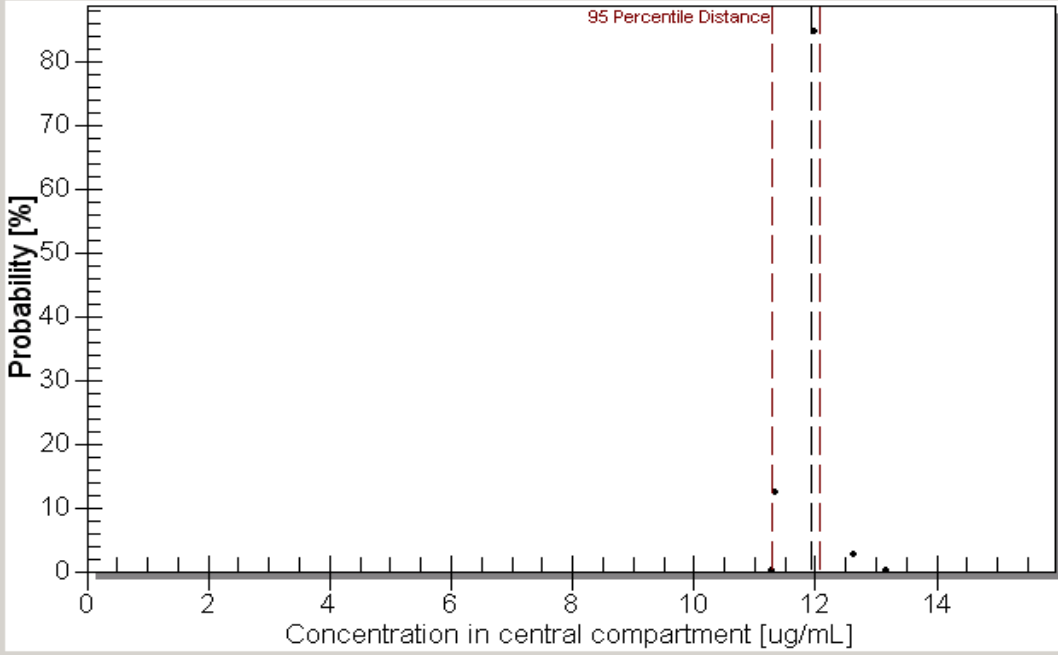

Figure 6. Profile of the multiple predictions at the time of the first peak achieved on the new regimen. Horizontal = the multiple predicted serum concentrations. Vertical = probability of each prediction. Middle dashed vertical line = predicted weighted average concentration of 11.93 ug/mL (target = 12). Outer dashed lines = 95 percentile distributions (roughly 11.3 to 12.2 ug/mL) of the predictions. Note the much narrower bandwidth of the 95% distance of predictions around the predicted peak serum concentration of 11.93 ug/mL, Compare this figure with Figure 4 above.

PATIENTS WITH CHANGING PARAMETER VALUES: INTERACTING MM (IMM) SEQUENTIAL BAYESIAN ANALYSIS.

A particular problem can be presented by patients who are very unstable, and whose parameter values (volumes of distribution, rate constants, etc.) may

change rapidly without any change in any known covariate or descriptor such as body weight or creatinine clearance. This can also be the case when variations in gene expression over time alter the behavior of a drug, or when a child, who may receive therapy for years, grows up and changes over time.

All other current Bayesian methods obtain either the parameter values or distributions which best fit all the data. Parameter values are assumed to be fixed and unchanging throughout the analysis of all the data. In very unstable patients, or in patients who must be treated for long periods of time, this can lead to quite unacceptable fits. Data must usually be broken up.

Because of this, our laboratory developed a sequential Bayesian interacting MM (IMM) method which now permits a patient's posterior support points to change from one population support point to another with each new dose or each new serum concentration, if doing so is calculated to be more likely [40]. This approach is well known in the aerospace community to track and hit most precisely targets taking evasive action.

We have implemented this approach in our clinical MM software [40]. In a simulated patient with a change in parameter values, it tracked drug behavior with less than half the error of either the MAP or the MM Bayesian methods. Further, in a study of over 130 patients on gentamicin and over 130 on vancomycin in a post-cardiac surgery unit, it tracked drug behavior significantly better than either the MAP or MM Bayesian methods [41]. This approach should also be useful in tracking drug behavior in patients over long periods of time, with few data points during that time, either during changes in gene expression over time, or as children grow up and their drug behavior changes.

Outcomes achieved with individualized drug regimens.

Several of the above tools have been employed in various MAP Bayesian software packages such as MW-Pharm [42], TDMS [43], the Abbottbase software [44], and the older USC*PACK software [45]. Using such approaches in the past, therapeutic efficacy of digitalis glycosides was preserved while toxicity was significantly reduced [46]. For lidocaine, much better arrhythmia control was achieved, with no increase in toxicity [47]. For aminoglycoside antibiotics, survival improved, toxicity was less, hospital stay was reduced by 6 days, and hospital costs were also significantly reduced [48]. For busulfan in children receiving bone marrow transplants, survival improved and veno-occlusive disease was reduced [49]. For cyclosporine in similar children, severe graft versus host disease (GVHD) was greatly reduced, and an average net cost savings of approximately 70,000 Euros was achieved for each episode of severe GVHD avoided [50]. The new MM software now provides more precise dosage regimens than the older MAP Bayesian approaches [22,32,37-39]. In addition, it has been useful in optimizing dosage individualization in children with HIV [51].

The FDA might be more useful in recognizing the value of these various approaches to TDM, and in encouraging pharmaceutical companies, and clinical and TDM laboratories, to adopt the approaches to errors in laboratory assay errors described in this report. The resulting models will be less biased and more precise. NP models are uniquely well suited to the development of the maximally precise MM dosage regimens described here. More precise dosage means reduced complications, and these translate into reduced hospital stays and reduced costs.

CONCLUSIONS

Genetic test results and measures of genetic expression are most useful approaches to obtain new covariate information about patients and the manner in which they handle drugs. They can provide further information to put into the overall structure of maximally precise MM Bayesian adaptive control of pharmacokinetic systems for optimal patent care with potentially toxic drug regimens.

Therapy with dangerous drugs cannot be individualized for each patient simply by looking up literature, by getting a general impression from the raw serum level data, or by using traditional clinical judgment. It requires a formal structure and specific quantitative tools. These tools are not only for modeling, but also specifically to best control those models to optimize the care of each individual patient. These tools have improved outcome in many instances (see above).

These tools need to be used and also need badly to be taught to medical and pharmacy students. The inertia of the medical and clinical pharmacology community to quantitative approaches may well be the reason that clinical pharmacology has so greatly declined as a specialty outside of the pharmaceutical industry. These methods, many of which have been around for decades, have still not been incorporated into medical school curricula and practice in any meaningful way. We continue to produce physicians who have no knowledge of or training in the techniques of optimal drug therapy with potentially dangerous drugs. The medical and the clinical pharmacology communities have failed markedly to adapt to the changes in drug therapy that have taken place in the last thirty years or so. A change badly needs to be made.

Modeling is not just for research, drug development, and general dosage guidelines. For potentially toxic drugs, it can and should also lead to precise clinical action. Good PK/PD software is the key tool for precise individualized therapy for each patient when it is needed. Most hospitals have perceived only the added cost of monitoring and analysis. This is usually reflected in the increased pharmacy budget. Hospital administrators have not yet perceived the reduction of therapeutic complications, the shortening of hospital stay and the significant reduction of overall hospital costs achieved by precise individualized

drug therapy. Hospital administrators, the FDA, and governmental health care administrators all should consider these important aspects of health care.

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DUALITY/CONFLICT OF INTERESTS
The authors have no financial interest in any matter discussed here. The MM-USCPACK software is made widely available by a license from the University of Southern California for a suggested nominal donation (often waived) which supports the work of the laboratory, not any salaries of the authors.

LIST OF ABBREVIATIONS
IMM – Interacting Multiple Model Sequential Bayesian Analysis.
MAP – Maximum Apposteriori Probability Bayesian Analysis.
MM – Multiple Model dosage design
NP – Nonparametric
PD – Pharmacodynamic
PK – Pharmacokinetic

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