DISCRIMINANT ANALYSIS (PROC DISCRIM) TO SEPARATE A STUDY POPULATION BY TREATMENT SUBGROUPS IN A CLINICAL TRIAL WITH A NEW ANTIDEPRESSANT

Lev Sverdlov, Ph.D., Innapharma, Inc., Park Ridge, NJ

ABSTRACT
Data were evaluated from a pilot phase 2 clinical trial (N=55) with a new antidepressant and multivariate discriminant analysis (PROC DISCRIM) was used to separate the drug-treated from placebo populations by treatment subgroups. The list of variables for discriminant analysis included the percent of change from baseline for the primary efficacy variable (21-item Hamilton Depression Rating Scale – HAMD-21) for 11 major time points from Day 3 (the first day of evaluation after treatment) to Day 40 (the last day of evaluation at the end of the trial). Separation with discriminant analysis was very effective for any combination of two of three treatment subgroups (from 82.4% to 86.7% of subjects with the correct classification by treatment subgroups) and for the three treatment subgroups together (80% of subjects with correct classification).

1. INTRODUCTION

We have previously reported [1] the use of four different approaches to separate a drug-treated from a placebo population after treatment with a new antidepressant [2]. All these approaches were developed to provide additional methodology for dealing with common statistical analysis of clinical data [3,4] and were very effective in separating the drug-treated population from placebo in two pilot clinical trials. We now report the use of an additional approach to separate drug-treated from placebo populations:

multivariate discriminant analysis, which uses data from all study assessment points rather than a single endpoint. The effect of separation using discriminant analysis was very strong for any combination of two of three treatment subgroups combined and for all three treatment subgroups combined. In contrast, the traditional currently used single endpoint statistical analysis (ANOVA at the end of observation) did not show the effect of separation between treatment subgroups.

2. METHOD

2.1. Study Design

The study design has been previously described in detail [5]. Fifty-five male and female physically healthy subjects, 18 years or older, diagnosed with non-psychotic major depression were enrolled in this pilot phase 2 study (outpatient, double-blind, randomized, placebo-controlled, parallel-design, single-center). The study investigated the efficacy and safety of a new drug administered subcutaneously for 10 days (two 5-day treatment cycles separated by 2 non-treatment days). Subjects were screened for enrollment and then returned to the facility between 3 to 7 days before the initial treatment to be randomized. At the end of the second 5-day treatment cycle, the subjects returned to the facility once weekly for the next 4 weeks for follow-up evaluations. The placebo group included 22 subjects who received lactose injections and the drug group included 33 subjects. Twenty-two of 33 subjects

treated with new drug received drug for both cycles, and 11 subjects received drug only in the first cycle and placebo (lactose injection) in the second cycle. The main objective of the one-cycle group was to compare safety profiles with those subjects receiving two cycles. The primary efficacy variable was the HAMD-21 score (change and % change from baseline). The last-observation-carried-forward approach (LOCF) was used for missing data. A retrospective pharmacokinetic analysis permitted the definition of the Minimum Projected Therapeutic Concentration (MPTC) and the subsequent division of the study population into three treatment subgroups: placebo subgroup (all subjects from the placebo group), drug-treated subgroup 1 with plasma drug concentrations in the therapeutic range (above MPTC) and drug-treated subgroup 2 with plasma drug concentrations below the therapeutic range (below MPTC).

2.2. Statistical Evaluation (Discriminant Analysis)

Discriminant analysis [6,7] is a multivariate statistical procedure which mathematically defines a special discriminant function to separate a study population by one classification variable (treatment subgroups). The numeric value of the discriminant function is different for each subject, and the treatment subgroup determined from discriminant analysis may or may not be the same as the actual treatment subgroup. The more subjects whose classified and actual treatment subgroups match, the better the effect of separation. The discriminant function can use several quantitative variables, each of which makes an independent contribution to the overall discrimination. Taking into consideration the effect of all quantitative variables, this discriminant function

produces the statistical decision for guessing to which subgroup of classification variable each subject belongs. The performance of discriminant analysis can be evaluated by estimating the error rate (probability of misclassification).

SAS/STAT® [7] is a powerful tool for discriminant analysis with some options allowing selection of: parametric or non-parametric methods, linear or quadratic classified functions, equal or unequal prior probability for each level of classification variable, with or without calculation of new variables with canonical scores, et al.

The primary efficacy variable (percent change from baseline HAMD-21 score) was used to create a discriminant function. Because Day 1 cannot produce any percent change from baseline, only 11 out of 12 time points were used: pd3 (percent change from baseline for Day 3 - the first day of evaluation after two injections), pd5 (Day 5), pd6 (Day 6), pd8 (Day 8), pd10 (Day 10), pd12 (Day 12), pd13 (Day 13), pd19 (Day 19), pd26 (Day 26), pd33 (Day 33) and pd40 (Day 40 - the last day of evaluation after 4 weeks of follow-up). As mentioned above, three treatment subgroups (placebo subgroup, drug-treated subgroup 1 with plasma drug concentrations in the therapeutic range and drug-treated subgroup 2 with plasma drug concentrations below the therapeutic range) were included in the levels of the classification variable. The DISCRIM procedure in SAS/STAT® calculates the posterior probability of each individual subject belonging to each of three subgroups and assigns the subject to a corresponding subgroup according to the higher probability. In addition, the DISCRIM procedure summarizes the squared distance between subgroups, univariate and multivariate statistics,

canonical coefficients to derive canonical variables (a dimension-reduction technique*), list of misclassified observations, classification error-rate, the result of classification for each subject and total frequency of separation. Some additional procedures can be used to plot the results of classification in two dimensions. The classified function from the training (calibration) group of subjects can be saved and used for another (replication) group of subjects to estimate the replication effect of separation. Because of the relatively small sample size in this study, there was no replication group, and all of the subjects were included only in the training group

2.3. SAS Code

MACRO 1: Discriminant Analysis for Training Data Set

```
%macro da_train (datset_1) ;                          /* datset_1  - training data set */

proc discrim data=&datset_1 anova distance listerr method=normal
          ncan=2 out=testout outstat=teststat outd=outd ;
class plevel ;                                        /* classification variable */
priors proportional ;                       /* prior probabilities to the sample size */
var pd3  pd5  pd6  pd8  pd10  pd12  pd13
    pd19 pd26 pd33 pd40 ;                             /* 11 clinical variables */
title 'Discriminant analysis for 11 clinical variables for Training data set' ;
run ;

proc print data=testout ;
var subjid subjinit treat plevel pd3 pd5 pd6 pd8 pd10
    pd12 pd13 pd19 pd26 pd33 pd40 P H L _into_ ;
run ;

proc print data=outd ;
var subjid subjinit treat plevel pd3 pd5 pd6 pd8 pd10
    pd12 pd13 pd19 pd26 pd33 pd40 P H L  ;
run ;

proc print data=teststat ;
run ;
```

```
proc means data=testout n nmiss mean std stderr min max range ;
var can1 can2 ;
class plevel ;
title 'Canonical variables CAN1 and CAN2 for Training data set' ;
run ;
```

*The number of canonical variables equals the number of subgroups minus one. For example, for three subgroups there are two canonical variables.

```
proc plot data=testout ;
plot can2 * can1 = plevel / vaxis=-4.0 to 3.0 by 0.5
                            haxis=-3.0 to 5.0 by 0.5 ;
run ;

%mend da_train ;
```

MACRO 2: Discriminant Analysis for Replication Data Set

```
%macro da_replc (datset_1, datset_2) ;              /* datset_1  - training data set,
                                                        datset_2  - replication data set */
proc discrim data=&datset_1 testdata=&datset_2 anova distance
          listerr ncan=2 method=normal out=testout
          outstat=teststat outd=outd ;
class plevel ;                                       /* classification variable */
priors proportional ;                       /* prior probabilities to the sample size */
var pd3 pd5 pd6 pd8 pd10 pd12 pd13
    pd19 pd26 pd33 pd40 ;                            /* 11 clinical variables */
title 'Discriminant analysis for 11 clinical variables for Replication data set' ;
run ;

%mend da_replc ;
```

3.   RESULTS AND DISCUSSION

Of the 55 enrolled and randomly assigned subjects (i.e., intent-to-treat data set) 51 subjects (92.7%) completed the initial treatment (i.e., evaluable data set). The MPTC for pharmacokinetic evaluation was defined as 45.7 ng/mL [8], and for subjects treated with drug this value was used to select subgroup 1 (13 subjects) and subgroup 2 (17 subjects) with plasma drug concentrations above or below the therapeutic range (9 subjects and 10 subjects correspondingly for the 10-day treatment group only). A summary of discriminant analysis and discriminant classification accuracy for all separations is presented in sections 3.1 – 3.4.

3.1. Separation of Subgroup 1 from Placebo

Ten of 13 subjects (76.9%) from subgroup 1 and 18 of 21 subjects (85.7%) from the placebo group were classified correctly and confirmed the prior known actual subgroups. The error rate of classification was 17.6%, which means 82.4% of correctly classified subjects were from both subgroups combined.

3.2. Separation of Subgroup 1 from Subgroup 2

Ten of 13 subjects (76.9%) from subgroup 1 and 16 of 17 subjects (94.1%) from subgroup 2 were classified correctly and confirmed the prior known actual subgroups. The error rate of classification was 13.3%, which means 86.7% of correctly classified subjects were from both subgroups combined.

3.3. Separation of Subgroup 2 from Placebo

Fourteen of 17 subjects (82.4%) from subgroup 2 and 18 of 21 subjects (85.7%) from the placebo group were classified correctly and confirmed the prior known actual subgroups. The error rate of classification was 15.8%, which means 85.2% of correctly classified subjects were from both subgroups combined

3.4. Separation of Subgroup 1 from Subgroup 2 (only 10-day treatment group) and from Placebo

Seven of 9 subjects (77.8%) from subgroup 1, 8 of 10 subjects (80.0%) from subgroup 2 and 17 of 21 subjects (80.9%) from the placebo group were classified correctly and confirmed the prior known actual subgroups. The error rate of classification was 20.0%, which means 80.0% of correctly classified subjects were from three subgroups combined. Clear separation was obtained using discriminant analysis. There was a significant difference for two canonical variables by treatment subgroup (ANOVA, two-tailed, 2 degrees of freedom, $P<0.01$). In contrast, the traditional currently used analysis of longitudinal data did not confirm the effect of separation between treatment subgroups on the basis of the single endpoint at the end of observation [5]. It is important to mention that after discriminant analysis the majority of responders were classified to subgroup 1 (above MPTC) versus the majority of nonresponders to subgroup 2 (below MPTC).

While our findings summarize the results from a pilot study with a limited sample size, discriminant analysis taking into account concentration of drug in plasma from pharmacokinetic evaluation successfully created a very comprehensive picture of the separation of the drug-treated population from the placebo population. We are now planning to use discriminant analysis to evaluate the results of future pivotal clinical studies.

4. CONCLUSIONS

Discriminant analysis (PROC DISCRIM) was very effective in separating a heterogeneous study population of subjects diagnosed with major depression into three treatment subgroups using HAMD-21 scores for all the available time points. Discriminant analysis demonstrated that from 82.4% to 86.7% of subjects had the correct classification for evaluation of any two of the three subgroups and 80.0% correct classification for evaluation of all three treatment subgroups combined. The results indicate that multivariate discriminant analysis is more reflective of the dynamics of drug effect than assessment at a single endpoint (particularly for atypical dose regimens) and provides a valid additional statistical approach to support conclusions of efficacy. All of the methodological aspects and SAS codes presented in this paper can be used during drug development in some pivotal studies in the CNS therapeutic area. These techniques allow for a greater sample size, while separating the study population into training (calibration) and replication groups.

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CONTACT INFORMATION

Lev Sverdlov, Ph.D.
Innapharma, Inc.
1 Maynard Drive, Suite 205
Park Ridge, NJ 07656
Phone: 201-505-1549 ext. 645
E-mail: LSVERDLOV@AOL.COM