

## Generate and Customize eDISH Plot to Identify Hy's Law Cases in Simple Steps

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### ABSTRACT

In clinical trial studies and research, the analysis and visualization of the data is crucial for assessing the efficacy and safety of the drug. It is always easier to extract valid information through a glance at the chart or the graph than going through the entire table and data. In this paper we are providing details for Hy's law related eDISH (Evaluation of Drug-Induced Serious Hepatotoxicity) plot which is not so common across clinical trial data analyses. It is mostly used for examining serious hepatotoxicity. Hy's Law states that hepatocellular drug induced liver injury (DILI) with jaundice indicates a serious reaction and is used to determine the risk of acute liver failure (ALF). Lab measurements for Bilirubin (BILI), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP) are divided by the upper limit of normal (ULN) and displayed in a scatterplot matrix annotated with Hy's Law reference lines for detecting potential hepatotoxicity for all subject across treatment arms. This paper will provide details from the SAS macro that is developed to simplify and automate the challenging and tedious process of creating an eDISH plot. This could immensely help programmers streamline the generation of eDISH plot and enhance reproducibility.

This paper is aimed at beginner to intermediate SAS programmers who already have familiarity of SAS Macro and wants to use it for creation of eDISH Plot.

### INTRODUCTION

Clinical trials go through the process of robust statistical analysis to measure the treatment effect on the patient's health. Safety is one of the major factors to be considered for any study. DILI is a major concern in clinical studies. DILI has been the most frequent single cause of safety-related drug marketing withdrawals for the past 50 years (e.g., iproniazid, ticrynafen, benoxaprofen, bromfenac, troglitazone, nefazodone)(FDA 2009). Another most cited reason for withdrawal of drug from the market are cases of acute liver injury, which accounts for about 15 % of the cases,(Aithal et al., 2011). Not only there has been instances of market withdrawals for these reasons, but there have been instances that even after the approval of marketing drug has been limited due to hepatotoxicity.

### DILI AND HY'S LAW

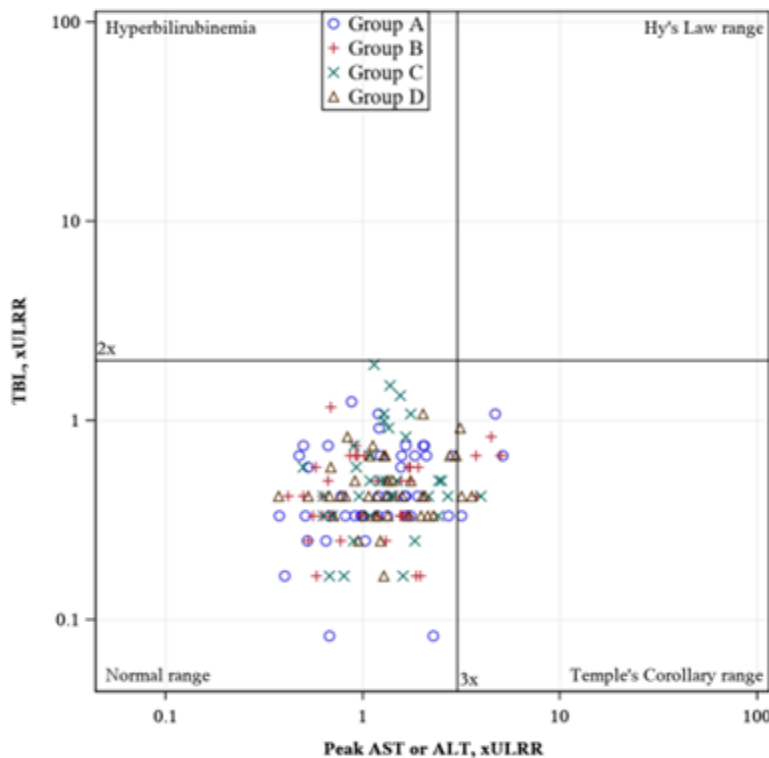
The type of liver injury that leads to severe DILI is hepatocellular injury, which is indicated by elevated ALT or AST levels in serum. However, the ability to cause hepatocellular injury does not necessarily predict a drug's potential for severe DILI, and many drugs that cause transient rises in serum aminotransferase(AT) activity do not lead to progressive or severe DILI. Only drugs that can significantly impair liver function by reducing bilirubin clearance or synthesizing coagulation factors cause severe DILI, and therefore it becomes important to identify these drugs as early as possible (FDA 2009).

Dr Hyman Zimmerman observed the change in liver functioning and liver damage caused by certain drugs resulted to higher levels of liver enzymes called ALT. However, the bilirubin increase is due to cholestasis rather than hepatocellular damage. His observation of the critical importance of altered liver function has been referred to as Hy's Law. Hy's Law is now the rule of thumb that a patient is at high risk of fatal DILI, if given a medication that causes hepatocellular injury with jaundice.

### EDISH PLOT

The FDA has set up several guidance for the clinical trial to identify the signals for liver safety. The graphical tool developed by FDA to easily identify the signals for liver safety in clinical trials is called eDISH, also known as Evaluation of Drug Induced Serious Hepatotoxicity. The graph displays the peak

level for each subject. The eDISH plot helps in better detection and reporting of the Hy's law cases in clinical trials. eDISH plot is a valuable tool to visually represent the laboratory data for the ALT/AST along with Total Bilirubin.



**Figure 1. Plot Mockup**

Hy's law is recommended by FDA, where it states that there is a potential of DILI, given ALT (alanine aminotransferase) is  $> 3x$  ULN (Upper limit of normal and total bilirubin) and total bilirubin  $> 2x$  ULN (FDA 2009). The plot in Figure 1 shows the highest recorded result for laboratory tests including ALT, AST, and BILI.

The plot is divided into four planes. The two-reference line drawn to divide the plane in four quadrants are  $2x$ ULN for the Total Bilirubin, and  $3x$  ULN for ALT or AST. Each point in the plot represents a unique subject in clinical study. The graph plots the peak serum total bilirubin (TBL) times the upper limit of the reference range against the peak of the upper limit of reference range of the ALT. The values of TBL and ALT are in the multiples of ULN. The scale used for the plot is log 10 scale (Guo et al., 2009).

The normal cases will lie in the lower left quadrant. Any point in the upper left quadrant will reflect the possible cases of hyperbilirubinemia. Here the total bilirubin is  $> 2x$  ratio but ALT/AST is  $< 3x$  ratio of the AVAL/UNL. The data points falling in the lower right quadrant, which is exceeding the threshold for ALT but below the threshold value of TBL, also suggests an increased risk for liver injury but not as high as the subject lying in the upper right quadrant. This quadrant is also known as the Temple's Corollary. The subject falling in this quadrant will be subjected to further investigation. The subject falling in the upper right-hand quadrant will be the ones potentially at risk for the Hy's Law threshold. It is important to note that the upper right quadrant is referred as Hy's Law quadrant, including potentially DILI cases. Any subject that falls in the Hy's Law quadrant is subject to further special investigation to adjudicate the case (Guo et al., 2009). Here the ALT and the total bilirubin both are above the threshold level of  $3x$  UNL and  $2x$  UNL respectively, not necessarily on the same test day.

## DEVELOPMENT OF THE MACRO

The SAS system provides a platform to do basic calculations and plot graphs. But when we are dealing with complex plots like eDISH, usually a considerable amount of time is required on doing a repetitive job. Here we have attempted to develop a macro, most of which can be easily used for any related study, to prepare an eDISH plot. Each point in this plot can be directly linked with each subject clinical and laboratory data, thus giving a full picture of the related hepatic abnormality (Regev & Björnsson, 2014). The SAS macro template introduced in this paper facilitates the generation of the eDISH plot by automating the necessary data manipulation and data visualization steps. The input SAS datasets which contain one observation per participant per laboratory test, must contain the following variables and values in lab datasets:

1. Paramcd: This variable is needed to plot value on x axis and y axis. Paramcd value PASLT(Peak AST or ALT) will plot on x axis and paramcd value BILPASLT(BILI at Peak AST or ALT) will plot on y-axis. Definition for deriving PASLT and BILPASLT paramcd may vary across different studies however standard definition in one of our studies is as follows:

For PARAMCD="PASLT", take the post treatment peak ratio (AVAL/ANRHI) from PARAMCD in ("AST", "ALT") for each subject. If the peak ratio values are same across different days, then take the day with maximum BILI ratio. If the "BILI" ratio values are same across different days, then take earliest day value.

For PARAMCD="BILPASLT", take the corresponding ratio (AVAL/ANRHI) from PARAMCD="BILI" record where the peak "AST" or "ALT" ratio is identified for PARAMCD="PASLT".

2. Therapy group variable: treatment planned variable for randomized population(e.g., TRT01P) or treatment actual variable (e.g., TRT01A) will be required to plot legend values for actual treated population.
3. Treatment group code: numeric treatment planned variable (e.g., TRT01PN) or numeric treatment actual variable (e.g., TRT01AN)
4. Aval: It is the analysis value, which is in numeric

USUBJID	SUBJID	RANDFL	TRTFL	TRT01P	TRT01PN	TRT01A	TRT01AN	PARAM	PARAMCD	AVAL	AVALC
1234-xx1_xxxx2xxxx1	2x31x4	Y	Y	ABC-1234 50 mg	1	ABC-1234 50 mg	1	BILI at Peak AST or ALT	BILPASLT	0.5833333333	0.5833333333
1234-xx1_xxxx2xxxx1	2x31x4	Y	Y	ABC-1234 50 mg	1	ABC-1234 50 mg	1	Peak AST or ALT	PASLT	0.675	0.675
1234-xx1_xx1xxxx4	2x1x27	Y	Y	ABC-1234 300 mg	3	ABC-1234 300 mg	3	BILI at Peak AST or ALT	BILPASLT	0.6666666667	0.6666666667
1234-xx1_xx1xxxx4	2x1x27	Y	Y	ABC-1234 300 mg	3	ABC-1234 300 mg	3	Peak AST or ALT	PASLT	1.2708333333	1.2708333333
1234-xx1_x4xxxxxx1	2x1934	Y	Y	ABC-1234 300 mg	3	ABC-1234 300 mg	3	BILI at Peak AST or ALT	BILPASLT	1.25	1.25
1234-xx1_x4xxxxxx1	2x1934	Y	Y	ABC-1234 300 mg	3	ABC-1234 300 mg	3	Peak AST or ALT	PASLT	0.875	0.875

**Figure 2. Partial Dataset**

Figure 2 shows example of partial datasets which was used to create macro for eDISH plot.

Dataset contains subject id, study id, randomization flag, treatment flag, visit number, visit names, age, gender, BMI, parameter name, parameter results, limits of the normal range, and other demographic related variables. Once we have all the required parameters and variables in ADLB(Analysis Dataset Laboratory) dataset, our next step is transposing the dataset by USUBJID. Once dataset is transposed by USUBJID, it needs to be taken care that our final dataset has only one record per usubjid per parameter.

After completing the final dataset with non-missing values, the next steps can be carried out to form a macro template for the plot.

During the creation of the macro template, we identified four parameters that are crucial for the plot. These parameters must contain a value and cannot be empty. The input\_dataset, x\_param, y\_param and source\_domain are the mandatory parameters for the macro. However, the remaining macro parameters are not mandatory and can be left without a value as they have default values already assigned in the program. Below is an example of a macro call containing a list of macro parameters developed during our study. A detailed explanation of each macro parameter can be found in the section titled "PARAMETERS" later in this document.

### Example of Macro Call (optional):

```
%macro hyslawplot(  
input_dataset      = /* required */  
, observation_where =  
, therapy_des_var  =  
, therapy_cd_var   =  
, x_param          = /* required*/  
, y_param          = /* required*/  
, graph_parameters =  
, style            =  
, title_out        =  
, footnote_out     =  
, x_label          =  
, y_label          =  
, page_orientation =  
, page_margins     =  
, plotheight       =  
, plotwidth        =  
, display_grid     =  
, legend_location  =  
, display_legend   =  
, halign           =  
, valign           =  
, output_hysplot_ds =  
, output_hysplot_fl =  
, source_domain    = /*required*/  
);
```

The paper focuses on how to create a macro that generates an eDISH plot. Since the main macro calls upon several interrelated macros, providing detailed information on each macro parameter is not feasible. Users can develop their own utility macros for each parameter to assign default values and can link them to the main eDISH macro function.

### PARAMETERS:

Below are the details of the macro parameters which were created to make this macro robust.

#### ***Required parameters where values cannot be leave blank:***

- 1 Input\_dataset: Name of final input dataset after transposing record by usubjid. This input dataset should have only one record per usubjid. The dataset must contain variables paramcd , treatment planned/actual variable and numeric aval variable.
- 2 X\_param: Enter only 1 paramcd to be plotted on X axis. Preferably paramcd value which contains Peak AST or ALT value is plotted on X axis.
- 3 Y\_param: Enter only 1 paramcd to be plotted on X axis. Preferably paramcd value which contains BILI at Peak AST or ALT value is plotted on Y axis.
- 4 Source\_domain: Data source text written outside plot area to indicate the source of dataset used to create eDISH plot. Source dataset my contain the value as "ABC1234: ADAM-ADLB" where ABC1234 is the name of study with protocol ID and ADAM\_ADLB is the source dataset.

#### ***Permissible variables where user has the option to leave these parameter blank.***

If user do not want to follow the default value, then user can define their default values in these parameters. We will discuss about these variables in details as follows:

- 5 observation\_where: As ADLB dataset may contains other parameter other than BILIPASLT and PASLT , user can create this macro variable to subset final dataset for required treatment group and parameter values. As this is not a required variable, use can keep it blank.
- 6 therapy\_des\_var/ therapy\_cd\_var: The input dataset therapy/treatment group variable based on population subset criteria. If the plot is for actual treatment group population, then use can pass TRT01A /TRT01AN variable or if the plot is for randomized population, then user can pass TRT01P/TRT01PN variable. The value should be entered in the format of dataset variable (e.g., adlb.trt01a/ adlb.trt01an).
- 7 graph\_parameters :This parameter is created to standardize the style of template per submission requirement. User can control style and attributes of rtf output file along with graph style using Proc Template define style statement to standardize values such as page margin, plot height, plot width, style fonts for title fonts, style graph fonts for graph title font, graph data font. This parameter can be a default parameter if no value defined in call macro for this variable. The following code shows how to modify parent style and defined the color and line style for each element:

```

Proc Template;
  define style styles.wmastyle; parent = styles.rtf;
  ...
  ...
  ...
  class GraphData1 / contrastcolor=red color=red linestyle=1
  markersymbol=circle
  class GraphData2 / contrastcolor= black color=black linestyle=1
  markersymbol=plus;
  class GraphData3 / contrastcolor=green color=green linestyle=1
  markersymbol=x;
  class GraphData4 / contrastcolor=blue color=blue linestyle=1
  markersymbol=triangle;
  . . .
run;

```

For detail information on controlling style attributes , user can refer Graph Template Language: User's Guide(2016) for detail on how to customize a style template.

- 8 Style :As mentioned above, graph\_parameters will be the default style template for creating eDISH plot. By default, the above template will output element symbol in gray color, however if user wants to modify only symbol color and line style then they can create their own style and pass this style in style parameter.
- 9 title\_out : Plot titles written out of the plot area, separated by |.
- 10 x\_label : Default X axis label is the paramcd name of &x\_param parameter. User can change the default parameter value by x\_label parameter.
- 11 y\_label : Default Y axis label is the paramcd name of &y\_param parameter. User can change the default parameter value by y\_label parameter.

Following code explains how user can override the default x and y label value using proc template:

```

Proc Template;
  define statgraph hysplot;
    beginngraph;
      layout overlay /
xaxisopts=(type=log logopts=(base=10 viewmin=0.05 viewmax=100) %if
%sysevalf(%superq(x_label)^=,boolean) %then %do;
label="&x_label"
%end;
      display=all griddisplay=&display_grid.)
      yaxisopts=(type=log logopts=(base=10 viewmin=0.05 viewmax=100)
%if %sysevalf(%superq(y_label)^=,boolean) %then %do;
label="&y_label"

```

```
%end;
```

- 12 page\_orientation : page orientation can be landscape or portrait depending upon the requirement and plot appearance.
- 13 page\_margins : Customize page margins, four number in inch in the order of top, bottom, left, right page margins.

User can define page\_orientation and page\_margins parameter in proc template as follows:

```
options orientation=&page_orientation. topmargin=&page_margin1.in  
bottommargin=&page_margin2.in  
leftmargin=&page_margin3.in rightmargin=&page_margin4.in nodate  
nonumber;
```

Before calling values from any macro variables in parameters topmargin, bottommargin, leftmargin, rightmargin, users has to define macro variable for each margin.

- 14 plotheight : Adjust plot height for RTF output format . This parameter is useful to control the plot height to fix orphan page issue when there are multiple lines of footnote and title.
- 15 plotwidth :It will adjust the plot width in inches.

User can define plot height and width in ods statement as follows:

```
ods graphics on / noborder imagefmt=png height=&plot_height.in  
width=&plot_width.in. ;
```

- 16 display\_grid : User can choose if they want to display grid in graph using display\_grid parameter. The value for display\_grid parameter can be used in axisopts and yaxisopts as follows:

```
axisopts=(type=log logopts=(base=10 viewmin=0.05 viewmax=100) %if  
%sysevalf(%superq(x_label)^=,boolean) %then  
%do;  
label="&x_label"  
%end;  
display=all griddisplay=&display_grid.)axisopts=(type=log  
logopts=(base=10 viewmin=0.05 viewmax=100) %if  
%sysevalf(%superq(y_label)^=,boolean) %then  
%do;  
label="&y_label"  
%end;  
display=all griddisplay=&display_grid.);
```

- 17 legend\_location/ display\_legend/halign/valign : User can control the position of treatment legend with option halign and valign parameter. For this plot we have fixed the position of legend inside the graph on top quadrant. However, user can give more flexibility to display legend values outside the plot using halign and valign statement. For more detail on legend location user can refer SAS(R) 9.3 Graph Template Language: User's Guide.
- 18 output\_hysplot\_ds/ output\_hysplot\_fl : Name of the output sas dataset used to plot if user want to save it permanently whereas output\_hysplot\_fl will save final plot in rtf file.
- 19 footnote\_out : When the plot saved to an RTF file, the footnotes in this parameter would written outside of graph body.

### SAS CODE SNIPPETS:

The following template is defined using parameters explained above to create the eDISH plot for our program:

```
ods escapechar='^';
```

```

Proc Template;
    define statgraph hysplot;
        begingraph;
            layout overlay /
                xaxisopts=(type=log logopts=(base=10
viewmin=0.05 viewmax=100) %if %sysevalf(%superq(x_label)^=,boolean) 10
%then %do;

                label="&x_label" 10
                %end;

                display=all griddisplay=&display_grid.) 16
                yaxisopts=(type=log logopts=(base=10
viewmin=0.05 viewmax=100) %if %sysevalf(%superq(y_label)^=,boolean) 11
%then

                    %do;
                        label="&y_label" 11
                    %end;

                display=all griddisplay=&display_grid.); 16
group=&therapy_des_var;
                scatterplot x=&x_param y=&y_param. / 3 name='r' 2
                entry "Hy's Law range" / autoalign=(topright);
                entry 'Hyperbilirubinemia' /
autoalign=(topleft);
                entry "Temple's Corollary range" /
autoalign=(bottomright);
                entry 'Normal range' / autoalign=(bottomleft);
                referenceline x=3 / xaxis=x curvelabel='3x'
curvelabellocation=inside curvelabelposition=min lineattrs=(pattern=1
color=black);
                referenceline y=2 / yaxis=y curvelabel='2x'
curvelabellocation=inside curvelabelposition=min lineattrs=(pattern=1
color=black);

                    17
                %if &legend_location=INSIDE %then
                    %do;
                        discretelegend "r"/ border=true
title='' location=inside across=1 halign=0.3 valign=1; 17
                    %end;

                %if &legend_location=OUTSIDE %then 17
                    %do;
                        discretelegend 'r'/title=''
location=OUTSIDE halign=&halign. valign=&valign. order=rowmajor 17
across=&display_legend. border=false; 17
                    %end;

                endlayout;
            endgraph;
        end;
run;

quit;

**** page margins;

```

```

12
options orientation=&page_orientation. topmargin=&page_margin1.in
bottommargin=&page_margin2.in
    leftmargin=&page_margin3.in rightmargin=&page_margin4.in nodate
nonumber;

%if %sysevalf(%superq(_ttlout)=1,boolean) %then
    %do;
        %let ftitle=%qsysfunc(tranwrd(&title_out, %str(,),
9
%str(^{newline})));
        title "&ftitle";
    %end;

/*title &title_in.*/
ods _all_ close; /* closes all open ODS output destinations before ods
rtf to make sure only the rtf output destination is open */
ods rtf file="%sysfunc(pathname(fptog,f))/&output_hysplot_fl..rtf" 18
bodytitle nogtitle nogfootnote
8 style=&style. dpi=&rtf_dpi. &wma_rtf_hf.;
ods graphics on / noborder imagefmt=png height=&plot_height.in 14
width=&plot_width.in; 15

18
Proc Sgrender data=&output_hysplot_ds. template=hysplot;
    %if %sysevalf(%superq(footnote_out)^=,boolean) %then
        %do;
            %do i = 1 %to &_fntout0;
                footnote&i. j=left
19
"%nrbrquote(&&footnote_out_&i.)";
            %end;
        %end;
        %let p=%eval(&_fntout0+1);
        footnote&p. j=center "&source_domain";
run;

ods rtf close
file="%sysfunc(pathname(fptog,f))/&output_hysplot_fl..rtf";
ods graphics off;
ods graphics /reset=all;
ods listing;
title;
footnote;

```

### SAMPLE CALL PROGRAM:

```

%hyslawplot(
    input_dataset           =adlb
    ,observation_where      =paramcd in ('PASLT' 'BILPASLT')
    ,x_param                =PASLT
    ,y_param                =bilpaslt
    ,style                  =mystyle
    ,output_hysplot_ds      =example030hypldata
    ,output_hysplot_fl      =example030hysplot
    ,source_domain          =%str([ABC-1234: adam-adlb])
);

```

The sample call program provided above, including the four required parameters(input\_dataset, x\_param, y\_param and source\_domain), whereas all other parameters not displayed will take default values. The



resulting output from executing above sample call program is displayed in the Figure 3. Output for the Sample Call Program below.

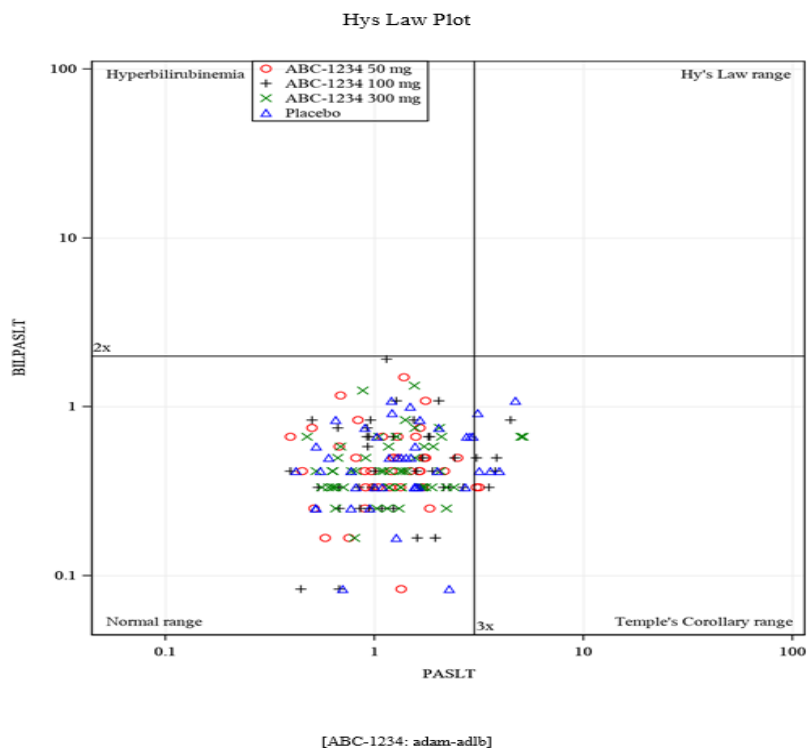


Figure 3. Output for the Sample Call Program

## CONCLUSION

We continue to see that DILI and HY's Law related eDISH plot are becoming popular in the study analysis reporting and the content we have shared in this paper will immensely help users to efficiently generate the plot and automate it. Prior to creating and utilizing the eDISH macro template provided in the paper, it is recommended that users perform bulletproofing, validation and assign default values to non-required parameters ensuring regulatory compliance and high-quality deliverables are generated. While the proposed workflow covers the primary steps of creating an eDISH plot, we acknowledge that the implementation of the workflow may differ across studies. We trust that this procedure can offer valuable guidance for utilizing a SAS macro in clinical data analysis and generating meaningful reports and figures.

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## ACKNOWLEDGMENTS

The authors would like to thank subject matter experts from the BARDS TA standard management teams from Merck & Co., Inc., Rahway, NJ, USA, for their advice and guidance on this paper/presentation. The authors are also grateful for reviewing the paper and providing feedback by Erica Davis, Shi Changhong, Abhilash Vasu Chimbirithy, Su Chao and our department head Amy Gillespie.

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