Document Type:	Statistical Analysis Plan
Official Title:	A study to learn how safe the study drug intravitreal (given by an injection into the eye) aflibercept is in participants in India with diabetic macular edema
NCT Number:	NCT05511038
Document Date:	09-Apr-2025

Title Page

Protocol Title: A Phase IV interventional post approval trial to assess the safety of intravitreal aflibercept for the treatment of diabetic macular edema (DME) in patients in India.

Protocol Number: 21974

Compound Number: BAY 86-5321

Short Title: India IPAT (PASS) IVT aflibercept in DME

Sponsor Name: Bayer Zydus Pharma Private Limited

Legal Registered Address: Central Avenue, Hiranandani Estate, Thane – 400607, Maharashtra, India

Regulatory Agency Identifier Number(s): Not applicable

Date: 03 JUN 2024

Version: 2.0

Statistical Analysis Plan template version: 4.0

This Statistical Analysis Plan is produced on a word-processing system and bears no signatures. The approval of the Statistical Analysis Plan is documented in a separate signature document.

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Version History

This Statistical Analysis Plan (SAP) for Study BAY 86-5321 is based on the protocol Version 3.0 dated 08 Jun 2023.

SAP Version	Date	Change	Rationale
1.0	29SEP2023	Not applicable	Original version
2.0	03JUN2024	1. Added baseline definition to section 4.1.1 2. Added 2 step improvement information to section 4.3.4.2 3. Added treatment duration categories to section 4.5.1	Clarification of some details contained in corresponding outputs.

List of Abbreviations

AE	Adverse event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AR	Adverse Reaction
AST	Aspartate aminotransferase
ATE	Arterial thrombotic event
BCVA	Best Corrected Visual Acuity
BP	Blood Pressure
BUN	Blood urea nitrogen
CI	Confidence interval
CRF	Case Report Form (eCRF = electronic CRF)
CRT	Central Retinal Thickness
CTCAE	Common Terminology Criteria for Adverse Event
DME	Diabetic Macular Edema
DR	Diabetic Retinopathy
DRSS	Diabetic Retinopathy Severity Scale
ECG	Electrocardiogram
eCRF	electronic case report form
EDC	Electronic Data Capture
EOS	End of study
ETDRS	Early Treatment Diabetic Retinopathy Study
FAS	Full analysis set
FDA	Food and Drug Administration
FU	Follow-up
HbA1c	Haemoglobin A1c
hCG	Human chorionic gonadotropin
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonization
MCH	Mean corpuscular haemoglobin
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
TEAE	Treatment Emergent Adverse Events
TLF	Tables, Listings and Figures
WHO DD	World Health Organization Drug Dictionary

1. Introduction

The SAP describes the final analysis of the study. No statistical interim analysis will be performed. Table, figure and listing specifications are contained in a separate document.

This is a Phase IV interventional post approval trial to assess the safety of intravitreal aflibercept for the treatment of diabetic macular edema (DME) in patients in India.

The purpose of this Statistical Analysis Plan (SAP) is to provide details of the statistical analyses that have been outlined within the Protocol Version 3.0 dated 08-Jun-2023.

1.1. Study Objectives & Endpoints

Objectives	Endpoints
Primary	
• To investigate the safety of IVT aflibercept for the treatment of DME in a phase IV setting	• Frequency (number) of ocular and nonocular TEAEs
Secondary	
• To investigate the efficacy of use of IVT aflibercept for the treatment of DME in a phase IV setting	• The change in BCVA from baseline to week 52, as assessed using the ETDRS chart or equivalent. • Change in CRT from baseline to week 52 as measured by OCT, FA • Proportion of eyes that gain ≥ 5, 10 and 15 ETDRS letters from baseline to Week 52. • Proportion of eyes with a ≥2 step improvement in the ETDRS DRSS score

1.2. Study Design

This study is a phase IV, interventional, open label, single-arm, prospective, multi-center study, to investigate the safety of IVT aflibercept for the treatment of DME in Indian participants in routine clinical practice in India at a dosage of 2 mg according to label with the primary endpoint assessed at Week 52. The trial will be conducted at approximately 10-15 study sites across India. Approximately 100 participants will be recruited in the study.

The study duration will be up to 13 months covering 11 visits:

- Screening period (Visit 1 [Day-21 to Day-1]): The start of the study period is defined by signing of the informed consent form (ICF)
- Baseline visit (Visit 2 [Day 1]): HbA1c and ocular assessments check have to be confirmed at the baseline visit before the intravitreal administration of aflibercept.
- Visits 1 and 2 can be combined (as Visit 1+2) except in women of childbearing potential (see Section 1.3). Participants who met all of the inclusion and none of the exclusion criteria, will be assigned to aflibercept treatment on Day 1, Week 0 (at Visit 2 or Visit 1+2).

- Treatment period (Visit 2 [Day 1, Week 0] to Visit 10 [Day 337, Week 48]) that include study intervention per month for five consecutive doses (Visit 2-6), followed by one injection every alternate month for rest of the duration (Visit 7-10).
- FU period: from month 11 to month 12 (visit 11)

Once patients complete Visit 11/Week 52, can be switched over to commercially available supply or other suitable treatment options at the discretion of the Investigator.

Only 1 eye per patient will be enrolled in the study. For patients who meet eligibility criteria in both eyes, the eye with the worst VA will be selected as the study eye. If a patient has DME with similar BCVA in both eyes, the eye with the clearest media will be selected as the study eye. If the ocular media of both eyes are similar in clarity, the patient's non-dominant eye (if identifiable) will be selected as the study eye. If neither eye is dominant, the right eye will be designated as the study eye.

2. Statistical Hypothesis

There is no hypothesis to confirm or reject in this study.

2.1. Multiplicity Adjustment

Not Applicable

3. Analysis Sets

For the purpose of analysis, the following analysis sets are defined:

Participant Analysis Set	**Description**
Full analysis set (FAS)	• All participants assigned to study intervention (aflibercept injection)
Per Protocol Set (PPS)	• All participants from FAS analysis and fulfill below criteria i) no violation of relevant inclusion / exclusion criteria, ii) non-missing baseline BCVA observation and at least one post baseline BCVA observation iii) completed all scheduled injections within ±7 days.
Safety analysis set (SAF)	• All participants who received at least one dose of study intervention (aflibercept injection)

The full analysis set and the per protocol set is used to analyze endpoints related to the efficacy objectives and the safety analysis set is used to analyze the endpoints and assessments related to safety.

Final decisions regarding the assignment of participants to analysis sets will be made during the review of study data and documented in the final list of important deviations, validity findings and assignment to analysis set(s).

4. Statistical Analyses

4.1. General Considerations

All outcomes will be analyzed descriptively by either frequency tables or summary statistics for the total study population. Whenever meaningful, continuous variables will also be categorized. If applicable, the analysis will be stratified by baseline factors. All analyses will be performed for each of the following cohorts:

- Naïve
- Pre-treated

and for the total study population.

The statistical evaluation will be performed by using the software SAS (release 9.4 or higher; SAS Institute Inc., Cary, NC, USA). All variables will be analyzed by descriptive statistical methods. The number of data available and missing data, mean, standard deviation, minimum, quartiles, median, and maximum will be calculated for metric data. Frequency tables will be generated for categorical data. For frequency tables, percentage calculation should be based on patients which do not have missing values.

All data will be presented in the participant data listing as they are recorded on the Case Report Form (CRF), i.e., partially missing data will appear as such.

4.1.1 Baseline definition and unscheduled visits

Baseline is defined as the last non-missing observation made prior to the first treatment administration. Unscheduled visits are not considered in baseline definition.

The assigned nominal visit will be used for by-visit summaries. Unscheduled measurements will not be included in by-visit summaries. Unscheduled visits are presented in listings only.

4.2. Primary Endpoint Analysis

Following is the primary endpoint of the study:

- Frequency (number) of ocular and non-ocular TEAEs

4.2.1. Definition of Endpoint(s)

- A TEAE is defined as any event arising or worsening after the start of study treatment administration until 30 days after the last administration of study treatment.
- In Adverse events CRF page, for TEAEs, if Adverse Event Category = “Ocular Event” then it will be considered as Ocular TEAEs. If Adverse Event Category = “Other” then it will be considered as non-ocular TEAEs.

4.2.2. Main Analytical Approach

AEs will be summarized for the SAF using the MedDRA coding system.

Occurrence rates for single AEs will be calculated based on the total number of patients valid for safety population and 95% confidence intervals (CIs) will be provided using Clopper Pearson method. AEs will be categorized according to ocular or non- ocular nature, relatedness, seriousness, discontinuation of therapy, action taken and outcome.

The analyses will be performed on incident TEAE. Events which are not treatment-emergent will be tabulated without further stratification. An overview table will provide overall incidences for patients with any TEAE, any study drug related TEAE, any serious TEAE, any serious study drug related TEAE, any TEAE leading to change of treatment regimen with aflibercept (treatment interrupted, dose reduced, dose increased), any TEAE leading to discontinuation of aflibercept, any TEAE with outcome death. Similar overview tables will be produced for ocular and non-ocular events, with ocular events also reported separately for the study eye, treated fellow eye, untreated fellow eye, and all fellow eyes. Incidence proportion tables by MedDRA SOC and PT will be presented for all categories of TEAEs from the overview tables. Reasons for serious AEs, actions taken with IVT aflibercept due to AE and outcomes of AEs will be reported event based for all TEAEs and for ocular and non-ocular events separately.

All non-TEAEs and all serious non-TEAEs will be presented in incidence tables by MedDRA system organ class (SOC) and preferred term (PT).

Same analysis will be performed for the serious adverse events.

4.2.3. Sensitivity Analysis

Sensitivity analysis will be performed considering all the lost to follow up cases. TEAE incidence rates per person per year and per injection will be calculated as follows:

(i)TEAE Incidence rates per person per year= Sum of TEAE's / Total patient time in study
Here, Incidence rates will be shown based on 100 patient years.

The 95% is based on Poisson distribution and calculated via the normal approximation using below formula:

$$\lambda \pm Z_{97.5} * \mathrm{sqrt}(\lambda/n)$$

Where λ= Incidence rates per person per year for TEAEs

$Z_{97.5}$ = The 97.5% quartile of the normal distribution (i.e. $Z_{97.5} = 1.96$)

(ii) TEAE Incidence rates per injection= Sum of TEAE's/ Number of all injections

The 95% is based on Poisson distribution and calculated via the normal approximation using below formula:

$$\lambda \pm Z_{97.5} * \mathrm{sqrt}(\lambda/n)$$

Where $\lambda =$ Incidence rates per injection for TEAEs

4.2.4. Supplementary Analysis

Not Applicable

4.3. Secondary Endpoint Analysis

Following is the secondary endpoint of the study:

4.3.1. First Endpoint

Following is the secondary endpoint of the study:

- The change in best corrected visual acuity (BCVA) from baseline to week 52, as assessed using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart or equivalent.

4.3.1.1. Definition of Endpoint(s)

- Total Visual Acuity Score (ETDRS letters for study eye)
- Approximate SNELLEN acuity equivalent (smallest line with 1 or fewer errors) for study eye

Approximate SNELLEN acuity equivalent scores will be converted into ETDRS score [1].

Snellen Fraction	ETDRS Equivalent Snellen Fraction	logMAR*	ETDRS Letter Score	Approximate ETDRS Letter Score†
1/200 (CF)		2.30		0
2/200		2.00		2
	20/800	1.60	5	5
6/200		1.52		9
	20/640	1.51	10	10
	20/500	1.40	15	15
20/400	20/400	1.30	20	20
	20/320	1.20	25	25
20/300		1.18		26
	20/252	1.10	30	30
20/250		1.10		30
20/200	20/200	1.00	35	35
	20/160	0.90	40	40
	20/125	0.80	45	45
20/100	20/100	0.70	50	50
20/80	20/80	0.60	55	55
20/70		0.54		58
	20/63	0.50	60	60
20/60		0.48		61
20/50	20/50	0.40	65	65
20/40	20/40	0.30	70	70
	20/32	0.20	75	75
20/30		0.18		76
	20/25	0.10	80	80
20/20	20/20	0.00	85	85
	20/16	−0.10	90	90
20/15		−0.12		91
	20/13	−0.19	95	95
	20/10	−0.30	100	100

*Snellen converted to logMAR = −1 × log(Snellen fraction).
†Snellen converted to approximate ETDRS letters = 85 + 50 × log(Snellen fraction), which may be rounded to the nearest letter.
CF, counting fingers.

Round(85 + 50 * (log10 (Snellen fraction))) if Snellen scale was used. If the collected or derived score is below 0 then it will be set equal to 0, if it's above 100 then it will be set equal to 100.

4.3.1.2. Main Analytical Approach

Total visual acuity (BCVA) score and change from baseline for study eye will be analyzed descriptively by cohorts and total. Summary statistics mean, standard deviation, minimum, percent quartiles, median, maximum and 95% confidence intervals for absolute BCVA values and respective changes from baseline will be analyzed. Analysis will be based on the full analysis set.

An analysis of covariance (ANCOVA) model with baseline BCVA as covariate will be performed to estimate the baseline adjusted BCVA change. Change from baseline values of BCVA will be considered as a response variable and baseline BCVA values and visit will be considered as an independent variable. p-value and 95% CI will be presented for the analysis. Missing data will be imputed using LOCF method. Only post baseline values will be carried forward if missing.

4.3.1.3. Sensitivity Analysis

Further sensitivity analyses like subgroup analysis by baseline BCVA will be performed. BCVA values cut-off for groups division for subgroup analysis: VA letter score ranging from78 to 69, or 20/32 to 20/40 Snellen; and those with fewer than 69 letters, equivalent to about 20/50 or worse.

4.3.1.4. Supplementary Analysis

Not Applicable

4.3.2. Second Endpoint

Following is the secondary endpoint of the study:

- Change in CRT from baseline to week 52 as measured by OCT, FA

4.3.2.1. Definition of Endpoint(s)

- Central retinal thickness of study eye

4.3.2.2. Main Analytical Approach

Summary statistics for absolute CRT values and respective changes from baseline will be displayed by cohort. Change from baseline in CRT on baseline and week 52 will be analyzed by Mean, standard deviation, minimum, percent quartiles, median, maximum and 95% confidence intervals for the full population. Missing CRT values will be imputed using LOCF method. Only post baseline values will be carry forward if missing.

4.3.3. Third Endpoint

Following is the secondary endpoint of the study:

- Proportion of eyes that gain ≥ 5, 10 and 15 ETDRS letters from baseline to Week 52.

4.3.3.1. Definition of Endpoint(s)

Total Visual Acuity Score (ETDRS letters) of study eye

4.3.3.2. Main Analytical Approach

Proportion of eyes that gain ≥ 5, 10 and 15 ETDRS letters from baseline to Week 52 will be analyzed as frequency and percentage by cohort and total. 95% CI will be provided by Clopper Pearson method. Missing values will be imputed using LOCF method. Only post baseline values will be carry forward if missing.

4.3.4. Fourth Endpoint

Following is the secondary endpoint of the study:

- Proportion of eyes with a ≥2 step improvement in the ETDRS DRSS score

4.3.4.1. Definition of Endpoint(s)

Diabetic Retinopathy Severity Score of Study Eye

4.3.4.2. Main Analytical Approach

Proportion of eyes with a ≥2 step improvement in the ETDRS DRSS score will be analyzed as frequency and percentage by cohort and 95% CI will be provided by Clopper Pearson method.

The DRSS scores have numbers between 10 to 90, every level is 1 step besides levels 14, 15 and 20 which are considered to be the same level.

Combined DR severity levels (used to determine step change in DRSS)
10
14, 15, 20
35
43
47
53
61
65
71
75
81
85
90

For Example:

1) if a patient goes from level 35 to 14 this would be 1 step improvement.
2) If a patient goes from level 71 to 61 this would be 2 steps.
3) If a patient goes from level 20 to 14 this would be 0 steps.

For each visit the step improvement is calculated relative to Baseline.

4.4. Other Endpoint Analysis

Frequency (number and percentage) of IVT aflibercept injections in the study eye over 12 months of the study period will be provided with 95% CI. The summary statistics for the number of injections will be provided.

4.5. Other Safety Analyses

Following is the safety analysis for the study:

4.5.1. Extent of Exposure

Extent of exposure can be calculated with reference to start and end date of study intervention. Extent of the exposure can be calculated as follows:

Extent of Exposure = End date of study intervention- Start date of study intervention+1

Extent of exposure will be summarized as Mean, standard deviation, minimum, percent quartiles, median, maximum by cohort and total. Dose modification and dose modification results will be summarized as frequency and percentages by cohort. Listing will be provided for the exposure.

Treatment duration in weeks will also be presented. Treatment duration category is 0 to 4 weeks, > 4 to 8 weeks, > 8 to 12 weeks, > 12 to 16 weeks, > 16 to 20 weeks, > 20 to 24 weeks, > 24 to 32 weeks, > 32 to 36 weeks, > 36 to 40 weeks, > 40 to 44 weeks, > 44 to 48 weeks, > 48 to 52 weeks, > 52 weeks.

4.5.2. Adverse Events

All adverse events (AEs) will be summarized using the Medical Dictionary for Regulatory Activities (MedDRA, the current version at the time of analysis) preferred terms (PTs) grouped by system organ class (SOC) for cohorts.

An AE is any untoward medical occurrence in a clinical study participant, associated with the use of study intervention, whether or not considered related to the study intervention.

Treatment-Emergent Adverse Event (TEAE): A TEAE is defined as any event arising or worsening after the start of study treatment administration until 30 days after the last administration of study treatment.

If in adverse event form, Serious =" Yes" then it will be considered as a serious adverse event. An overview summary will be presented for adverse events (n=number of subjects, m=number of events and %=percentage of subjects) by cohort and total. AEs, TEAEs and SAEs will be summarized by SOC and PT. Summary of TEAEs will be provided by Intensity, Action taken with Study Drug, Outcome. All adverse events will be listed for SAF population.

Note: If there are uncoded adverse events, then the uncoded category will be added in the AEs by SOC/PT summary tables.

4.5.3. Additional Safety Assessments

4.5.3.1 Laboratory Assessments

Summaries of all the laboratory tests will include descriptive statistics of the following:

- Actual and change from baseline (number of patients, Mean, standard deviation, minimum, percent quartiles, median, maximum) (for quantitative measurements)
- Frequencies and percentages (n and %) (for qualitative measurements)

Summary will be provided by cohort.

All the laboratory tests will be listed separately and listing of abnormal laboratory test will be presented. Listing will be provided for routine analysis parameters and pregnancy testing.

4.5.3.2 Vital Signs

Summaries of all the vital sign parameters will include descriptive statistics of the following:

- Actual and change from baseline (number of patients, Mean, standard deviation, minimum, percent quartiles, median, maximum)

Summary will be provided by cohort.

Listing will be provided by subject and by cohort for safety population.

4.5.3.3 12-Lead ECG

Summaries of all the 12-lead ECG parameters will include descriptive statistics of the following:

- Actual and change from baseline (number of patients, Mean, standard deviation, minimum, percent quartiles, median, maximum) (for quantitative measurements)
- Frequencies and percentages (n and %) (for qualitative measurements)

Summary will be provided by cohort.

Listing will be provided by subject and by cohort for safety population.

4.6. Other Analyses

4.6.3.1 Other Variables and/or Parameters

Not Applicable

4.6.3.1 Subgroup Analysis

Baseline demographics and whenever applicable, related efficacy analysis will be performed on below subgroups:

- Overall: Consider all reported patients
- BL BCVA
    - BL BCVA 0-69

  - BL BCVA 70-100
- BL CRT
  - BL CRT <400
  - BL CRT >=400
- BL BCVA and CRT
  - BL BCVA 0-69 AND BL CRT <400
  - BL BCVA 0-69 AND BL CRT >=400
  - BL BCVA 70-100 AND BL CRT <400
  - BL BCVA 70-100 AND BL CRT >=400
- BL Hba1c:
  - <8%
  - >=8%

4.7. Interim Analyses

Not Applicable

4.8. Changes to Protocol-planned Analyses

Not Applicable

5. Sample Size Determination

The study aims to enroll 100 patients from 10-15 sites.

The sample size of 100 patients is the agreed sample size with the Indian health authority to describe the safety profile of the study intervention. In an analysis of the data from the clinical trials VIVID and VISTA, the reported proportion for any ocular SAE was 1.7% over the course of 52 weeks.

6. Supporting Documentation

6.1. Population Characteristic

6.1.1. Subject Disposition

Subject disposition will be summarized for all screened patients by premature discontinuation reasons. Listing will be provided for full population by subject.

6.1.2. Protocol Deviation

Protocol deviation categories will be summarized by frequency and percentage. Listing will be provided for the full population by subject and by cohort.

6.1.3. Demographics and Baseline Characteristics

Demographic parameters will be summarized descriptively. Age, height, and weight will be analyzed as mean, standard deviation, minimum, percent quartiles, median, maximum and sex and race will be summarized as frequency and percentage by cohort. Listings will be provided for full population by subject and by cohort.

6.1.4. Medical History

Medical history will be summarized with their frequency and percentage by system organ class (SOC), preferred term (PT) and cohort. Subject data listing also provided for full population by subject and by cohort.

6.1.5. Prior and Concomitant Therapy

All medications taken before study start (initiated and stopped before first intravitreal aflibercept injection) are termed prior medications.

All medications taken in addition to aflibercept for any indication (either initiated at the time of enrollment or during the study) are termed concomitant medications.

Prior and concomitant medication will be summarized with their frequency and percentage by ATC class. Listings will be provided for the full population.

7. References

1. NOVEL METHOD FOR ANALYZING SNELLEN VISUAL ACUITY MEASUREMENTS Gregori, Ninel Z MD*†; Feuer, William MS*; Rosenfeld, Philip J MD, PhD*