

10 Appendix 1: Additional IVUS data analyses

10.1 Scope

This document provides an updated and shortened version of the IVUS summary report submitted to the FDA in the March 2005 NDA Amendment. New analysis included in this version are as follows:

- Additional IVUS selection bias assessments on baseline variables and few post-baseline variables (see section 2.1)
- The estimated odds ratio of exposure to the risk factor variable for IVUS patients relative to non-IVUS patients as the measurement of association of the risk factor to IVUS status.

These additional analysis further confirm the conclusions drawn in the March 2005 update.

10.2 Background information

12-month IVUS population

Study CRAD001 B253 demonstrated superiority of everolimus (RAD) over AZA for the primary efficacy endpoint. Moreover, everolimus patients studied by IVUS met predefined endpoints of having lower graft vasculopathy than AZA patients. The magnitude of the differences between everolimus and AZA were larger than previously demonstrated with other agents in randomized and blinded multicenter clinical trials. These data were based on N=211 patient who had evaluable IVUS data matched at baseline and at 12 months. However, the data collection was potentially affected by methodological issues including study center compliance, catheter recall, non-evaluable tapes, prospective design as an on-therapy evaluation and the selective exclusion of patients (by investigators) due to the fact that the intravenous contrast dye used in the IVUS procedure would be poorly tolerated by some patients. These and other reasons led to a reduction in baseline and 12 months IVUS data to 1/3 of the ITT population. Counterbalancing these issues is the fact the IVUS benefits were demonstrated in an otherwise adequate well-controlled blinded phase 3 heart study B253.

Feedback from Novartis heart Advisory Board meetings

Feedback from two Novartis heart transplantation advisory board meetings (May 2004) also recognized these methodological limitations. After assessing the following important characteristics and findings of the IVUS data (in study B253), the board concluded that these overall findings outweigh the weakness of IVUS data collection for the cardiac transplant community:

- Similar compliance with IVUS protocol in study B253 as with Roche MMF heart trial
- No apparent treatment-related patterns to the exclusion of patients.
- Unable to identify clinical factors used by investigators to include or exclude patients from IVUS evaluations that would reliably predict or prevent allograft vasculopathy in any population.

- The absence of a demonstrable effect of renal function affecting IVUS outcomes in the literature or in this study.
- Lack of influence of known potential confounders on conclusions about IVUS results.
- The superior methodology and quality of the IVUS data relative to other transplant trials
- The magnitude and clinical relevance of the treatment effect
- Predictive value of the chosen endpoint

Consultation with external statistical experts

Novartis also consulted with external statistical experts (LJ Wei and John Lewis) to review the issues of IVUS data collection and to identify approaches to address potential biases in IVUS treatment comparisons. The following additional aspects of the IVUS data were investigated:

- Distributions of patient disposition and patterns of the reasons for IVUS data loss among the treatment groups
- Comparability of the 12-month IVUS population to the original study population as well as to other IVUS populations
- The balance of the three treatment groups in the 12-month IVUS population with respect to baseline characteristics and post-baseline measurements (i.e. adverse events/infections, etc.) and with respect to anything that might lead to differential loss of IVUS patients
- Impact of treatment-related outcomes (i.e. efficacy failure, renal function, etc.) on selecting the 12-month IVUS population to check for potential patient selection bias
- Impact of renal function on IVUS outcome
- Robustness of the IVUS treatment difference to alternative assumptions and imputations for missing data

This report presents a detailed analyses plan for the above investigations (Section 2), summary of the analyses results (Section 3) and conclusions (Section 4).

10.3 Main conclusion

The 12-month IVUS population was a reasonable subset population for making inference about treatment effect on IVUS endpoints. Furthermore, it can be stated with a reasonable degree of conservatism that the benefit of everolimus treatment demonstrated in the IVUS study were representative for the subgroup of patients on study medication for one year. Importantly, there was no evidence of substantial differences between the treatment arms (in terms of variables known to be related to the IVUS outcome) that might explain the positive findings associated with everolimus. All sensitivity analyses supported the primary analysis.

10.4 Analysis plan for heart CRAD001 B253 IVUS data

This section describes in detail the additional analysis plan of the IVUS data to assess the potential for bias in the IVUS patient population. The 12-month database of study B253 was used to analyze IVUS, efficacy and renal function data. The cut-offs for 12-month data analyses are Day 450 for safety and Day 381 for efficacy.

10.4.1 Assessment of potential biases in selecting 12-month IVUS patient population

The IVUS patient selection process was done while patients and investigators were completely blinded to treatment assignments. However, the selection of patients to undergo an IVUS procedure may depend on baseline characteristics and on an individual patient's current medical status (such as efficacy and toxicity). These factors could potentially produce in the 12-month IVUS subpopulation an unintentional selection bias. To address this concern, the following variables were investigated:

1. To identify variables, among the baseline characteristics and post-baseline measurements, that might drive the IVUS patient selection in such a biased way that the 3 treatment groups became unbalanced in the 12-mo IVUS subpopulation.
2. To determine if the impact of selection process was in favor of everolimus arms.

The IVUS subpopulation was alive at 12 months and hence in some respects had better prognoses than non-IVUS population, so one might not be able to show comparability of the IVUS population to the original study population. Therefore, the goal of this investigation was to rule out systematic IVUS patient selection bias (in favor of everolimus arms) based on the potential risk factors available in the database, and to demonstrate that the three treatment groups that make up the total of 211 IVUS patients were selected in comparable fashion into the 12-month IVUS population.

The following baseline and post-baseline measurements were investigated to determine if the impact of selection process favored everolimus arms:

- Baseline variables: recipient age, recipient gender, recipient race, donor age and gender, GFR < 29 mL/min/1.73m², diabetes and hypertension status at baseline, BMI > 33, PRA and CMV
- Post-baseline variables (at 12 months): adverse/infections, premature discontinuation of study medication, efficacy (such as biopsy-proven acute rejection (BPAR), BPAR+ hemodynamic compromise (HDC), treated acute rejection, renal function (creatinine clearance; CrCl), total cholesterol, triglyceride, post-transplant diabetes mellitus (PTDM) and statin use

The impact of each of the above factors on IVUS status was assessed as follows:

- Summary statistics of baseline characteristics was provided by IVUS status and treatment group to identify those baseline factors that could potentially influence who would be in the 12-month IVUS population.
- Logistic regression model was used for binary response variable (such as BPAR) with covariates: patient IVUS status (yes/no) at 12 months, indicator variables for two treatment comparisons (everolimus vs. AZA) and interaction terms of IVUS status by

treatment. The odds ratio of exposure to the risk factor variable for IVUS patients relative to non-IVUS patients was estimated as the measurement of association of the risk factor to IVUS status. It was then compared among the three treatment groups to see if the everolimus arms are comparable to AZA in the way they were selected for the IVUS population. This comparison was done by checking if there was any treatment by selection process interaction in the logistic regression model. A statistically significant interaction (at 0.10 level) may suggest a selection process bias either in favor of or against everolimus arms depending on the direction of the interaction.

- General linear model procedure was used for continuous response variable (such as renal function and CsA trough level) with covariates: patient IVUS status (yes/No) at 12 months, indicator variables for two treatment comparisons (everolimus vs. AZA) and interaction terms of IVUS status by treatment

10.4.2 Sensitivity analyses to assess the robustness of the positive IVUS results

Among IVUS ITT population, IVUS data was imputed for those patients who did not have 12 month IVUS data and were still alive and in the study. Two different ways of imputing missing IVUS data were described below to assess the robustness of the positive 12-month IVUS results.

Assigning IVUS data from AZA arm to patients with missing IVUS data at 12 months

For a patient with missing 12-month IVUS evaluation (either everolimus or AZA patient), a demographically matched (by age only) AZA patient from the 12-month IVUS population was randomly selected. The 12-month IVUS outcome observed from this AZA patient was assigned to the patient with the missing 12-month IVUS data. For example, for an everolimus patient who was ≥ 50 years of age, then an IVUS patient would be randomly selected from the corresponding subset of the AZA patients of the same age group with 12-month IVUS data, and the IVUS outcome of the AZA patient would be assigned to that everolimus patient. The idea was to assign missing values to patients who were of similar baseline information. Note, missing values were also imputed for AZA who had no 12-month IVUS data. This imputation method was then applied first, to all patients of the IVUS ITT population with missing 12-month IVUS data. Secondly, it was applied to only patients with missing 12-month IVUS data due to a renal issue.

Except for the reason of “Not done due to renal issue”, the reasons for missing 12-month IVUS data were fairly comparable among the treatment arms., Since more patients on everolimus arms have this as a reason for not having the 12-month value the second imputed analysis population was derived. Thus, imputing method was applied to all patients with missing 12-month IVUS data and to just the subset of patients with missing 12-month IVUS data with the reason for not having the data given as “Not done due to renal issues”.

Assigning AZA patients’ IVUS outcome to everolimus patients was considered conservative. Based on the imputed values, the IVUS data were re-analyzed to see if positive findings or at least trends still hold.

Assigning worst IVUS outcome to all patients with missing IVUS data at 12 months

Even more conservative than the above imputing method was to assign all patients missing IVUS data with the worst IVUS outcome (vasculopathy=yes) as follows:

- For all patients of the IVUS ITT population with missing 12-month IVUS data

Among the IVUS ITT population, assign IVUS outcome vasculopathy=yes to all patients with missing IVUS data at 12 months. This was done for all treatment groups.

- For patients with missing 12-month IVUS data due to renal issue

Instead of imputing all missing values, this imputing method was performed only for patients without IVUS data due to reason of “Not done due to renal issues”.

10.4.3 Assessment of relationship of renal function to IVUS outcome

Renal dysfunction was one of the main reasons that prevented everolimus patients from having IVUS evaluations at 12-months. Thus, was imperative to check if renal function (calculated creatinine clearance) was related to IVUS outcomes, to see if the exclusion of more everolimus patients from the IVUS population due to renal issues would bias IVUS outcome in favor of everolimus patients. A logistic regression model was used to assess the relationship of renal function to IVUS outcome of vasculopathy=yes/no. A simple regression model was used to explore impact of renal function to IVUS outcome of the largest maximal intimal thickness (MIT) increase from baseline.

10.5 Summary results of IVUS data analyses

10.5.1 Assessment of impact of baseline variables on IVUS patient selection status

Table 10-1 below summarizes the impact of baseline variables on IVUS selection to see if any are associated with a selection bias in favor of everolimus as indicated by the p-values for testing main effect (association of the risk factor with the 12-month IVUS status) and treatment by IVUS status interactions.

The assessments indicate that the impact of the baseline variables on IVUS selection was either similar among the three treatment groups (indicated by the non-significant treatment by IVUS status interactions) or not in favor of everolimus arms.

Table 10-1 Summary of assessment of impact of baseline variables on IVUS selection status (whether or not 2 matched IVUS studies were performed)

| Baseline variables | P-value for testing | |
|---------------------------------------|------------------------------|-------------------------------------|
| | Association with IVUS status | Treatment x IVUS status interaction |
| Recipient age | 0.237 | 0.850 |
| Recipient gender | 0.784 | 0.542 |
| Recipient race | 0.999 | 0.372 |
| Donor age | 0.132 | 0.695 |
| Donor gender | 0.242 | 0.737 |
| GFR < 29 (mL/min/1.73m ²) | 0.647 | 0.549 |
| Coronary artery disease | 0.965 | 0.868 |
| Diabetic at baseline | 0.080 | 0.074 |
| BMI > 33 | 0.432 | 0.248 |
| LVAD | 0.709 | 0.830 |
| Hypertension | 0.410 | 0.751 |
| PRA > 20 | 0.956 | 1.000 |
| CMV | 0.467 | 0.494 |

With respect to baseline diabetic status, there was a significant treatment by IVUS status interaction (at 0.10 level). The everolimus 3mg group had a statistically significant higher rate of diabetic patients being selected into the IVUS population (35% for the everolimus 3mg arm versus 14% and 19% for everolimus 1.5mg and AZA groups, respectively). That is, patients with diabetes at baseline in everolimus 3mg group were more likely to be selected into the 12-month IVUS subpopulation than AZA and everolimus 1.5mg patients. As diabetes is a potential risk factor for vasculopathy, the data suggests that the selection bias is not in favor of everolimus 3mg arm.

10.5.2 Assessment of impact of post-baseline outcomes on IVUS patient selection status

Table 10-2 shows the summary results for all post-baseline measurements, such as, BPAR \geq 3A, BPAR+HDC, treated acute rejection (AR), triglycerides, total cholesterol, CsA level and post transplant diabetes mellitus. For these post-baseline variables, there were no treatment by IVUS status interactions (except for renal function which will be explained below), indicating that everolimus patients who were selected into the 12-month IVUS population were comparable to AZA patients with respect to the influence of these variables.

Table 10-2 Assessment of impact of post-baseline 12-month outcomes on IVUS patient selection status

| Post-baseline outcomes | P-value for testing | |
|------------------------|------------------------------|-------------------------------------|
| | Association with IVUS status | Treatment x IVUS status interaction |
| BPAR >=3A | 0.026 | 0.741 |
| BPAR + HDC | 0.0004 | 0.685 |
| Treated AR | 0.001 | 0.122 |
| Renal function (CrCl) | 0.345 | <0.01 |
| Triglyceride | 0.807 | 0.969 |
| Cholesterol | 0.041 | 0.523 |
| CsA trough level | 0.496 | 0.610 |
| PTDM | 0.621 | 0.509 |
| Statin use | 0.001 | 0.647 |

n.s. - not statistically significant

10.5.2.1 Impact of renal function on 12-month IVUS status

With respect to renal function (see Table 10-3), there was a significant evidence of treatment by IVUS status interaction (P-value < 0.01), indicating renal function was different among the three treatment groups for IVUS patients compared with non-IVUS patients: the IVUS everolimus patients had similar mean CrCl to the non-IVUS everolimus patients (mean CrCl of 53.6 to 50.8 for everolimus 1.5mg and, mean CrCl of 48.6 to 55.8 for everolimus 3mg), while for AZA patients, renal function was clearly better among those who had IVUS values (mean CrCl of 70.3) than those who didn't (mean CrCl of 60.4). This finding suggests that the treatment-related selection bias exists but works against everolimus arms, since patients with relatively better renal function were selected in the AZA arm than in everolimus groups.

Table 10-3 Mean creatinine clearance (ml/min) at 12 months by patient 12-month IVUS status (Yes/No) vs. its complement relative to the original study population (N=211 vs. N=634-211)

| TRT | IVUS patients status at 12 months | | | | Difference (95% CI) | p-value for TRT by IVUS status interaction |
|------------------|-----------------------------------|--------|-----|--------|----------------------------|---|
| | No | | Yes | | | |
| | N | Mean | N | Mean | | |
| everolimus 1.5mg | 76 | 50.833 | 66 | 53.583 | -2.75 (-9.1, 3.599) | |
| everolimus 3mg | 69 | 55.826 | 66 | 48.65 | 7.176 (-2.583, 16.936) | 0.0095 |
| AZA | 81 | 60.381 | 72 | 70.301 | -9.921 (-17.11, -2.727) | |

Not all patients have 12-month creatinine clearance value

10.5.2.2 Impact of efficacy (BPAR \geq 3A) on 12-month IVUS status

Table 10-4 below shows that:

- 1) There was a significant association between IVUS status and patient’s BPAR status: IVUS patients had lower BPAR rate than non-IVUS patients, or equivalently speaking, patients with BPAR were less likely to be selected into the IVUS subpopulation.
- 2) However, this association (described by the odds ratio below) was very similar among the three treatment groups, indicating that everolimus patients who were selected into the 12-month IVUS population were comparable to AZA patients with respect to BPAR status.

Table 10-4 BPAR \geq 3A rate at 12 months by patient 12-month IVUS status (Yes/No) vs. its complement relative to the original study population (N=211 vs. N=643-211)

| TRT | IVUS patients status at 12 months | | | | Odds ratio (95% CI) | p-value for TRT by IVUS status interaction |
|---------------------|-----------------------------------|-----|-----|-----|------------------------|--|
| | No | | Yes | | | |
| | N | % | N | % | | |
| everolimus 1.5mg | 139 | 35% | 70 | 23% | 0.56 (0.29, 1.08) | 0.7608 |
| everolimus 3mg | 142 | 24% | 69 | 16% | 0.60 (0.28, 1.28) | |
| AZA | 142 | 48% | 72 | 42% | 0.78 (0.44, 1.38) | |

10.5.3 Sensitivity analyses to assess the robustness of the positive IVUS results

Results of sensitivity analyses of IVUS outcomes are summarized below using two different imputing methods:

- assigning AZA patients’ outcome to patients with missing values
- assign vasculopathy=“yes” to patients with missing values

Results are presented for two different analysis populations: IVUS ITT population and 12-month IVUS population plus those who had missing IVUS at 12 months due to renal issue.

Vasculopathy: defined as the largest MIT increase ≥ 0.5 mm from baseline (yes/no)

Using both imputing methods for both analysis populations, the vasculopathy rates were obtained and compared between everolimus and AZA groups (see Table 10-5 below):

- Low dose of everolimus 1.5mg showed numerically lower vasculopathy rates than AZA group but the difference was not statistically superior.
- High dose of everolimus 3mg was either borderline significantly or statistically significantly (at 0.05 level) superior to AZA.

Table 10-5 Sensitivity analyses of IVUS outcome vasculopathy: the largest MIT ≥ 0.5 mm from baseline using different imputation methods for two different analysis populations

| Analysis population | Imputation method | Treatment group | #event/N | % vasculopathy | P-value (Fisher's exact) |
|---|---------------------------|------------------|-----------|----------------|--------------------------|
| 12-mo IVUS | None | everolimus 1.5mg | 25 / 70 | 35.7% | 0.045 |
| | | everolimus 3mg | 21 / 69 | 30.4% | 0.010 |
| | | AZA | 38 / 72 | 52.8% | |
| IVUS ITT | Assign AZA outcome | everolimus 1.5mg | 60 / 173 | 43.8% | 0.150 |
| | | everolimus 3mg | 59 / 142 | 41.5% | 0.073 |
| | | AZA | 74 / 140 | 52.9% | |
| | Assign vasculopathy="yes" | everolimus 1.5mg | 92 / 137 | 67.2% | 0.143 |
| | | everolimus 3mg | 94 / 142 | 66.2% | 0.089 |
| | | AZA | 106 / 140 | 75.7% | |
| *12-mo IVUS + patients with missing values due to renal issue | Assign AZA outcome | everolimus 1.5mg | 35 / 85 | 41.2% | 0.116 |
| | | everolimus 3mg | 28 / 81 | 34.6% | 0.016 |
| | | AZA | 41 / 76 | 53.9% | |
| | Assign vasculopathy="yes" | everolimus 1.5mg | 40 / 85 | 47.1% | 0.345 |
| | | everolimus 3mg | 33 / 81 | 40.7% | 0.080 |
| | | AZA | 42 / 76 | 55.3% | |

*The analysis population included 12-month IVUS patients plus those with missing value due to renal issues

The change in average MIT from baseline

Similarly by assigning for IVUS outcome average MIT of AZA patients for patients who had missing IVUS data for both analysis populations, the mean change in average MIT from baseline were obtained and compared between everolimus and AZA groups (see Table 10-6 below):

- Both low and high everolimus dose groups were still superior to AZA with respect to this IVUS endpoint (p-values <0.05)
- There was still a positive dose-response relationship: the treatment effect on the change in average MIT was greater in the higher everolimus dose group than the low dose group.

Table 10-6 Sensitivity analyses of IVUS outcome: change in average MIT from baseline using two imputation methods for two different analysis populations

| Analysis population | Imputation method | Treatment group | N | Mean change in average MIT | p-value (Wilcoxon rank-sum) |
|---|-----------------------|------------------|-----|----------------------------|-----------------------------|
| 12-month IVUS | None | everolimus 1.5mg | 70 | 0.04 | 0.014 |
| | | everolimus 3mg | 69 | 0.03 | 0.003 |
| | | AZA | 72 | 0.10 | |
| IVUS ITT | Assigning AZA outcome | everolimus 1.5mg | 137 | 0.07 | 0.010 |
| | | everolimus 3mg | 142 | 0.07 | 0.011 |
| | | AZA | 140 | 0.10 | |
| 12-month IVUS + patients with missing values due to renal issue | Assigning AZA outcome | everolimus 1.5mg | 85 | 0.05 | 0.027 |
| | | everolimus 3mg | 81 | 0.04 | 0.007 |
| | | AZA | 76 | 0.10 | |

10.5.4 Assessment of relationship of renal function to IVUS outcome at 12 months

Renal function data (CrCl) at 6 and 12 months were evaluated to assess the relationship with 12-month IVUS outcomes. For the yes/no variable, vasculopathy, the p-value was obtained from the logistic regression model (in which vasculopathy was the response variable, creatinine clearance and treatment group indicator variables were covariates). For the continuous variable defined by the largest MIT increase from baseline, the p-value was obtained from the linear regression model (in which largest MIT was the response variable, creatinine clearance and treatment group indicator variables were covariates).

Table 10-7 provides the summary results, indicating there was no evidence suggesting renal function data using creatinine clearance at 6 and 12-months was a risk factor for IVUS outcome at 12 months. Everolimus treatment effects remained positive after adjustment for CrCl.

Table 10-7 Assessment of impact of 6 and 12 months creatinine clearance on IVUS outcome at 12 months

| Creatinine clearance | IVUS outcome at 12 months (response variable) | p-value for testing association with renal function(covariate) | p-value for testing treatment effect: everolimus 1.5mg vs. AZA everolimus 3mg vs. AZA |
|----------------------|---|---|--|
| At 6 months | Vasculopathy | 0.1996 | 0.0364 |
| | Largest MIT increase from baseline | | 0.0045 |
| At 12 months | Vasculopathy | 0.8848 | 0.0252 |
| | Largest MIT increase from baseline | | 0.0030 |
| | | | 0.0898 |
| | | | 0.0083 |
| | | | 0.0538 |
| | | | 0.0076 |

10.6 Conclusions

It should be noted that all the analyses performed were necessary to further identify evidence of selection bias and discuss its effect on everolimus treatment effects on IVUS outcomes. The data appears to be robust as regards any differences observed in the 211 patients who had matched IVUS evaluations:

- There was a potential selection bias that affected entry into the 12-month IVUS patient population and that was driven primarily by the treatment related outcomes of the patients (efficacy and renal function, etc.). However, the bias was either consistent across the three treatment groups or it did not favor everolimus patients:
 - There was a significant association between IVUS status and patients’s BPAR status (patients with BPAR are less likely selected into the IVUS population), indicating a selection bias in the IVUS population, however, the bias was similar across the three treatment groups

- The CrCl of everolimus patients in the 12-month IVUS population was no better than the CrCl of everolimus patients without IVUS, and was much lower than AZA patients either with or without 12-month IVUS.
- There was no statistical evidence that renal function was associated with 12 months IVUS outcome
- Sensitivity analyses using conservative imputing methods for missing values, confirmed the positive trend of IVUS outcome for everolimus patients.

Despite the limitations of IVUS data collection in heart study B253, the 12-month IVUS population appeared to be a reasonable subset population for making inference about IVUS endpoints. Furthermore, it can be stated with a reasonable degree of conservatism that the benefit of everolimus treatment demonstrated in the IVUS study was representative for the subgroup of patients who able to remain on study medication for one year. Importantly, there was no evidence of substantial differences between the treatment arms (in terms of variables known to be related to the IVUS outcome) that might explain the positive findings associated with everolimus.

11 Appendix 2: Position Paper: Everolimus in Heart Transplantation

12 Appendix 3: Selected References