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Efficacy and safety of avatrombopag in combination with immunosuppressive therapy in treatment-naïve and relapsed/refractory severe aplastic anaemia: protocol for the DIAAMOND-Ava-FIRST and DIAAMOND-Ava-NEXT Bayesian Optimal Phase II trials

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ABSTRACT

Introduction Immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and ciclosporin is standard of care for patients with severe aplastic anaemia (sAA) not eligible or suitable for allogeneic stem cell transplant. While patients respond to IST, few achieve complete responses and a significant proportion are refractory or relapse. The addition of eltrombopag, a thrombopoietin-receptor agonist (TPO-A), to IST has been shown to improve haematological responses in sAA. Avatrombopag is a second-generation TPO-A with potential advantages over eltrombopag. However, to date avatrombopag has not been studied in sAA.

Methods and analysis Investigator-initiated, single-arm registry-based Bayesian Optimal Phase II trial of avatrombopag conducted in two cohorts, patients with untreated sAA (FIRST cohort) and in patients with sAA that has relapsed or is refractory to IST (NEXT cohort). In the FIRST cohort, participants receive IST (equine ATG and ciclosporin) plus avatrombopag from day 1 until day 180 at 60 mg oral daily, with dose adjusted according to platelet count. Participants in the NEXT cohort receive avatrombopag at 60 mg oral daily from day 1 until day 180, with or without additional IST at the discretion of the treating clinician. For each cohort, two primary endpoints (haematological response and acquired clonal evolution) are jointly monitored and the trial reviewed at each interim analysis where a 'go/no-go' decision is made by evaluating the posterior probability of the events of interests.

Ethics and dissemination The trial has received ethics approval (Monash Health RES-18-0000707A). The trial conduct will comply with ICH-GCP and all applicable regulatory requirements. The results of the trial will be submitted to a peer-review journal for publication.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ First clinical trial evaluating efficacy and safety of avatrombopag for severe aplastic anaemia.
⇒ Bayesian Optimal Phase II trial design.
⇒ Protocol aligned with phase III trial of eltrombopag.
⇒ Coprimary outcomes prespecified and objectively assessed.
⇒ Single-arm, open-label study.

Trial registration number ACTRN12619001042134, ACTRN12619001043123.

INTRODUCTION

Aplastic anaemia (AA) is a rare bone marrow failure syndrome resulting from immune-mediated damage to haematopoietic stem cells, which results in bone marrow aplasia and pancytopaenia.[1 2] The standard upfront treatment of AA, depending on severity, age, comorbidities and matched sibling stem cell donor availability, is immunosuppressive therapy (IST) or haematopoietic stem cell transplant (HSCT).[3 4] IST is standard first-line therapy for patients older than 40 years with very severe/severe AA (sAA), younger patients without a suitable donor and patients with non-severe AA who are bleeding, transfusion-dependent or require treatment for other reasons.[3 4] Reported response rates with standard IST, composed of antithymocyte

globulin (ATG) and ciclosporin, are 50%–70%, with good long-term survival in many responders.[5] However, haematological recovery after IST is frequently suboptimal, and is poor in approximately 20% of patients, and one-third of responders relapse by 2 years.[5–7]

The thrombopoietin (TPO) receptor agonist eltrombopag has recently been shown to improve haematological responses in patients with sAA, including patients who are treatment naïve and who are refractory to IST.[8 9] This was first suggested by a phase II trial of 25 adults with sAA with thrombocytopaenia that persisted after one or more courses of treatment with equine or rabbit ATG and ciclosporin, who received eltrombopag at an initial dose of 50 mg daily and increased by 25 mg every 2 weeks to a maximum dose of 150 mg, depending on response to treatment.[8] Of the 25 patients, 11 (44%) had a haematological response in at least one lineage at 12 weeks and serial bone marrow biopsies showed normalisation of trilineage haematopoiesis in patients who had a response. A subsequent phase II trial in refractory sAA administered eltrombopag at fixed daily dose of 150 mg for 6 months in 39 patients.[10] In this trial, 19 (49%) patients met criteria for haematological response at 6 months, of whom 26% would have been deemed non-responders at 3 months of treatment. At median follow-up of 6 months, 15% developed bone marrow cytogenetic abnormalities, a rate comparable to previous cohorts not treated with a TPO receptor agonist. In treatment-naïve sAA, a phase I/II trial of eltrombopag in addition to standard IST conducted in 92 consecutive patients,[9] which included three consecutive cohorts who differed in the timing of initiation and duration of eltrombopag regimen, showed complete response (CR) rates of 26%–58% and overall response (OR) rates of 80%–94% across the three cohorts. Clonal cytogenetic evolution occurred in seven patients at 2 years (an incidence of 8%) and in five patients at 3–6 months, similar to the authors' historic experience with standard IST.[9]

The highest level of evidence for the use of eltrombopag in patients with sAA is from a recently published phase III randomised controlled trial, which enrolled 200 patients aged 15 years or older. In this trial, patients were allocated to receive upfront IST (equine ATG and ciclosporin) with or without the addition of eltrombopag 150 mg daily from day 14 for at least 3 months and a maximum of 6 months.[11] The authors reported a higher proportion of participants who had a CR at 3 months in the eltrombopag group compared with standard of care (22% vs 10%, OR: 3.2, 95% CI: 1.3 to 7.8, p=0.01), with no difference in severe adverse events, evolution to myelodysplastic syndrome (MDS) or clonal evolution as measured by somatic mutations.

Avatrombopag is an orally administered small molecule TPO receptor agonist that has shown efficacy in the treatment of thrombocytopaenia in phase II and phase III clinical trials in chronic immune thrombocytopaenia (ITP)[12] and in thrombocytopaenic patients with chronic liver disease undergoing elective procedures.[13 14] Avatrombopag binding to the TPO receptor c-Mpl on haematopoietic stem cells activates intracellular signalling for megakaryocyte, platelet, haemoglobin and neutrophil production. Similar to eltrombopag, avatrombopag does not compete with endogenous TPO for receptor binding, as avatrombopag binds to the transmembrane domain of the TPO receptor,[15] suggesting avatrombopag may also be effective in AA.[15] Potential advantages of avatrombopag over eltrombopag relate to dosing, toxicities, pharmacokinetics and efficacy. Specifically, avatrombopag does not require dose reduction in patients of East Asian ancestry, may have less liver function toxicity and drug interactions[16] and may be efficacious in high endogenous TPO level contexts, such as chemotherapy-induced thrombocytopaenia and AA.[17] Furthermore, avatrombopag has been shown to have greater in vitro and in vivo pharmacological potency than eltrombopag.[15]

The aim of the diagnosis of aplastic anaemia, management and outcomes utilising a national dataset (DIAAMOND) trial is to evaluate the safety and efficacy of avatrombopag for patients with treatment naïve and relapsed or refractory sAA.

METHODS

Trial governance

The DIAAMOND trial is overseen by a Trial Steering Committee. A smaller Trial Management Committee oversees the day-to-day management of the trial. Monash University is the trial sponsor.

Study design

This trial is an investigator-initiated, non-randomised, single-arm registry-based Bayesian Optimal Phase II (BOP2) trial of avatrombopag which is being conducted in two cohorts, patients with untreated sAA (FIRST cohort) and in patients with sAA that has relapsed or is refractory to IST (NEXT cohort). The trial schema is shown in figure 1.

For each cohort, two primary endpoints (haematological response and acquired clonal evolution (ACE)) are jointly monitored and the trial reviewed at each interim analysis where a 'go/no-go' decision is made by evaluating the posterior probability of the events of interests.

Participants

In order to achieve adequate enrolment in this rare disease, the trial is open and recruiting participants from 11 hospitals across Australia (including most states and territories), which also allows cross-referral between hospitals in the same jurisdiction.

Both cohorts enrol patients aged 18 years or older with severe or very sAA, defined as bone marrow cellularity <30% (excluding lymphocytes) with at least two of the following: absolute neutrophil count $<0.5\times10^9$/L; a platelet count $<20\times10^9$/L; or an absolute reticulocyte count $<60\times10^9$/L. Participants in the FIRST cohort must not have had prior ATG-based IST and not be planned

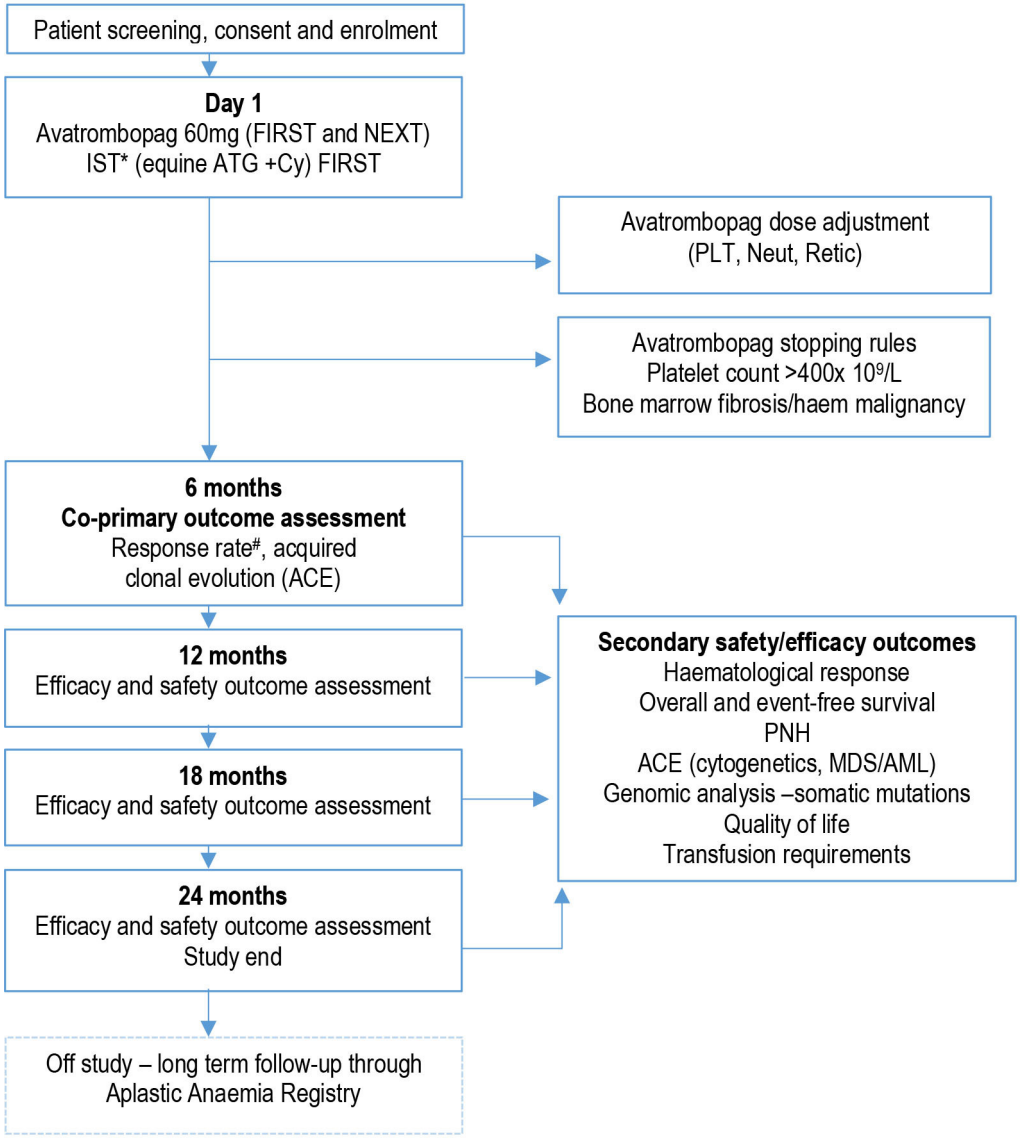

Figure 1 Trial schedule. *IST given for FIRST trial; IST given at the discretion of treating clinician in the NEXT trial cohort. #Efficacy endpoint for FIRST trial is complete response rate and for NEXT trial is overall response rate. ACE, acquired clonal evolution; AML, acute myeloid leukaemia; ATG, antithymocyte globulin; Cy, cylosporin; IST, immunosuppressive therapy; MDS, myelodysplastic syndrome; Neut, neutrophil; PLT, platelets; PNH, paroxysmal nocturnal haemoglobinuria; Retic, reticulocyte.

for a sibling allogeneic HSCT. Participants in the NEXT cohort must have refractory disease, defined as an incomplete response following at least one course of equine or rabbit ATG given ≥6 months ago or relapsed severe AA, defined as occurrence of one of the following after a haematological response to a prior course of equine or rabbit ATG given ≥6 months ago: meeting again criteria for sAA, requirement for transfusion support or neutrophil count of $<0.5\times10^9$/L or platelet count of $<20\times10^9$/L. Patients are excluded from both cohorts if they have evidence of MDS, known or suspected inherited bone marrow failure syndrome, diagnosis of a cancer within the last 5 years, are pregnant or breast feeding or have a known hypersensitivity to avatrombopag. All participants undergo germline testing for inherited bone marrow failure syndromes using a 37-gene next generation sequencing panel at study entry. The full inclusion and exclusion criteria are presented in table 1.

Intervention

Untreated (FIRST) cohort

Participants are treated with avatrombopag in addition to standard care (IST with equine ATG and ciclosporin). Avatrombopag is administered at 60 mg orally daily from day 1 to day 180. Avatrombopag dose may be adjusted according to platelet count, haemoglobin and neutrophil count (see online supplemental table 1). Avatrombopag is temporarily discontinued at any time during the treatment in the case platelet count $>400\times10^9$/L. Avatrombopag will be discontinued if new or worsening morphological abnormalities (eg, dysplasia, leucoerythroblastic film, circulating blast cells) or cytopaenia(s)

Table 1 Eligibility criteria for untreated (FIRST) study and relapsed or refractory (NEXT) cohort

Trial cohort	Participant inclusion	Participant exclusion
Untreated sAA (FIRST)	1. Severe or very severe AA characterised by bone marrow cellularity <30% (excluding lymphocytes) and at least two of the following: a. Absolute neutrophil count<0.5×10^9/L b. Platelet count<20×10^9/L c. Absolute reticulocyte count<60×10^9/L 2. No prior ATG-based IST 3. Age >18 years 4. Negative pregnancy test for women of childbearing potential	1. Planned for a sibling allogeneic stem cell transplant 2. Evidence of a MDS, defined by the presence of MDS features, excess of blasts or karyotypic abnormalities typical of MDS according to WHO 2017 criteria at the time of enrolment. Patients with AA with cytogenetic abnormalities that are recurrent in MDS, who do not meet the WHO diagnostic criteria for MDS, are also excluded. *Patients with del(20q), +8 and –Y are not included in this category and are therefore eligible for this trial* 3. Known diagnosis or clinical suspicion of IBMFS, including but not limited to Fanconi Anaemia, Dyskeratosis Congenita, Shwachman-Diamond syndrome and Diamond-Blackfan anaemia 4. Previous history of stem cell transplantation 5. Cancer diagnosis within the last 5 years (except for patients with resected basal cell carcinoma or squamous cell carcinoma of the skin) 6. Previous history of melanoma 7. Pregnant or breastfeeding patients 8. Active CMV disease 9. Participants with known hypersensitivity to any of the component medications (avatrombopag, ciclosporin, horse or rabbit ATG) 10. Concurrent hepatic, renal or cardiac disease of such severity that it would in the investigator's opinion, preclude the patient's ability to tolerate protocol therapy 11. Death anticipated within 14 days
Relapsed or refractory (NEXT) cohort	1. Refractory severe AA with an incomplete response following at least one course of equine or rabbit ATG given ≥6 months ago. Incomplete response defined as any one of the following: a. Absolute neutrophil count <0.5×10^9/L b. Platelet count <20×10^9/L c. Absolute reticulocyte count <60×10^9/L or ongoing requirement for red cell transfusion support (if not due to independent medical condition) OR 2. Relapsed severe AA, defined as the occurrence of any of the following, after a haematological response to a prior course of horse or rabbit ATG given ≥6 months ago: a. meeting again the criteria for sAA b. requirement for transfusion support (if not due to independent medical conditions) c. decrease in any of the peripheral blood counts as follows: i. absolute neutrophils<0.5×10^9/L ii. platelets<20×10^9/L 3. Age >18 years 4. Negative pregnancy test for women of childbearing potential	1. Evidence of a MDS, defined by the presence of MDS features, excess of blasts or karyotypic abnormalities typical of MDS according to the WHO 2017 criteria at the time of enrolment. Patients with AA with cytogenetic abnormalities, which are recurrent in MDS, who do not meet the WHO diagnostic criteria for MDS, are also excluded. *Patients with del (20q), +8 and –Y are not included in this category and are therefore eligible for this trial* 2. Known diagnosis or clinical suspicion of IBMFS, including but not limited to Fanconi Anaemia, Dyskeratosis Congenita, Shwachman-Diamond syndrome and Diamond-Blackfan anaemia 3. Cancer diagnosis within the last 5 years (except for patients with resected basal cell carcinoma or squamous cell carcinoma of the skin) 4. Previous history of melanoma 5. Pregnant or breastfeeding patients 6. Participants with known hypersensitivity to avatrombopag 7. Severe renal impairment (defined as creatine clearance ≤30 m/min) 8. Treatment with horse or rabbit ATG within 6 months of trial entry. Concurrent treatment with ciclosporin is permitted 9. Death anticipated within 14 days

AA, aplastic anaemia; ATG, antithymocyte globulin; CMV, cytomegalovirus; IBMFS, inherited bone marrow failure syndrome; IST, immunosuppressive therapy; MDS, myelodysplastic syndrome.

develop, until further investigation as clinically indicated to exclude development or progression of bone marrow reticulin fibrosis or haematological malignancy. This will be made based on clinical assessment, taking into account other secondary causes which may be contributing to cytopaenias (eg, concurrent infection or medication use).

Equine ATG is administered at a dose of 40 mg/kg/day on days 1, 2, 3 and 4. As prevention of ATG-related side-effects, corticosteroids are administered for at least 7 days and premedication with paracetamol and/or anti-histamine are allowed. Ciclosporin is administered at a dose of 5 mg/kg/day from day 1 to 365, with dose adjusted according to ciclosporin blood levels.

Participants who achieve a partial response at 6 months may receive an extended duration of avatrombopag for up to a total of 12 months. Participants who achieve a CR at 6 months and subsequently relapse within 6 months of discontinuation are able to restart avatrombopag for an additional 6 months of treatment.

Relapsed and refractory (NEXT) cohort

Avatrombopag is administered at 60 mg orally daily from day 1 to day 180, with dose adjusted as shown in online supplemental table 1, in addition to their standard of care. Participants may be on chronic ciclosporin therapy. For participants with relapsed sAA, co-administration of a course of equine or rabbit ATG is permitted if assessed as clinically indicated and appropriate by the treating clinician.

Justification for selection of avatrombopag dose

In the first version of our protocol, the initial dose for starting avatrombopag was 20 mg daily with uptitration to 60 mg daily over 12 weeks depending on the response. However, we amended the protocol to a starting dose of 60 mg after the initial 11 participants commenced treatment at the 20 mg dose. The initial dosing chosen in earlier versions of the protocol was based on the avatrombopag dose used for chronic administration in phase II and III studies in ITP, in which dosing was commenced at 20 mg daily and was uptitrated to a maximum dose of 40 mg daily.[12] In phase III studies in chronic liver disease, the avatrombopag dose was either 40 mg or 60 mg daily for 5 days, depending on baseline platelet count, with no signal for a higher incidence of serious adverse events (SAEs) in the 60 mg versus 40 mg daily group.[13] In the first phase II trial of eltrombopag, the starting dose was 50 mg, similar to that used in ITP, and titrated up to a maximum of 150 mg.[8] In that trial of 25 participants, all but one received the maximum eltrombopag dose. Subsequent studies of eltrombopag in AA, including the phase III trial, have commenced with the maximum dose of 150 mg daily, and then reduced the dose as needed based on platelet count to avoid thrombocytosis. Eltrombopag demonstrates accumulation after dosing at effective levels,[18] which may be necessary for the compound to exert its in vivo biological activity. This accumulation of dose may explain the slow platelet increment with eltrombopag.[15] The efficacy of avatrombopag in AA has not yet been tested, but similar issues may apply. As all 11 of our initial participants in the trial were up-titrated to the maximum dose of 60 mg without thrombocytosis at any lower interval doses, similar to what occurred with earlier studies of eltrombopag in AA, we amended the protocol to allow subsequent participants to commence on 60 mg and reduce the avatrombopag dose if required according to the platelet count response.

Monitoring of adherence to intervention protocol

Participants are reviewed by site investigators at regular study visits, where adherence to intervention is assessed and encouraged. Adherence is monitored via counting unused doses and review of the patient diary.

Concomitant care

All participants receive antimicrobial prophylaxis according to local protocol, including antifungal agents active on mould infection. In the absence of local guidelines, antifungal prophylaxis with an agent active against mould infection until neutrophil recovery over 0.5×10^9/L is recommended, prophylaxis against *Pneumocystis jirovecii* is recommended for at least 2 months post-ATG and when CD4 cells are over 200 cells/µL, and antiviral prophylaxis with valaciclovir 500 mg once or two times per day daily is recommended until 2 weeks after ciclosporin is ceased.

All other aspects of care, including transfusion support, are according to local standard of care.

Trial outcomes

The primary efficacy outcome for the FIRST cohort is rate of CR at 6 months and for the NEXT cohort is the rate of OR (partial response (PR) and CR) at 6 months. For both cohorts, the primary safety outcome is ACE at 6 months. Clonal evolution is defined as a new clonal cytogenetic abnormality or bone marrow characteristics consistent with MDS or acute myeloid leukaemia (AML), as defined by the WHO classification of haematological malignancies 2016.[19]

Secondary trial outcomes are presented in table 2.

Participants will be considered as having a CR if achieving all the following peripheral blood counts:

1. Haemoglobin >100 g/L.
2. Absolute neutrophils $>1.0\times10^9$/L.
3. Platelets $>100\times10^9$/L.

Participants will be considered as having a PR if achieving all the following:

1. No longer meet criteria for diagnosis of sAA.
2. Do not meet criteria for CR above.
3. Transfusion independence (defined as no need for any RBC or platelet transfusion for at least 4 weeks).
4. Absolute neutrophils $>0.5\times10^9$/L.
5. Platelets (unsupported with transfusion) $>20\times10^9$/L.

Any participant not meeting the response criteria defined above for CR or PR will be classified as a non-responder.

Table 2 Trial outcomes

Trial cohort	Coprimary outcomes	Secondary outcomes
Untreated sAA (FIRST)	► Primary efficacy endpoint is rate of CR at 6 months ► Primary safety endpoint is ACE at 6 months	1. Time to first haematological response (CR or PR), described by cumulative incidence curve 2. Time to best haematological response, described by a cumulative incidence curve 3. Time to CR, described by cumulative incidence curve 4. Rates of haematological response (OR, CR and PR) at 6, 12, 18 and 24 months 5. OS probability; OS is defined as time from day 1 of trial treatment to death, or last follow-up for patients alive 6. EFS probability; EFS is defined as time from day 1 of trial treatment to either relapse, death, treatment failure or ACE (whichever occurs first), or last follow-up for patients alive in response 7. QOL as measured by the EORTC QLQ-C30 questionaries at 6, 12, 18 and 24 months 8. Cumulative incidence of PNH population occurrence and clinical haemolytic PNH occurrence 9. Need for and number of transfusions (RBC and platelet units) 10. Need for supportive care, including number and length of hospitalisations and ICU admissions 11. Rate of ACE at 12, 18 and 24 months 12. Rate of acquired somatic mutations detected on genomic testing at 6, 12, 18 and 24 months 13. Safety and tolerability of the avatrombopag, including serious adverse events
Relapsed or refractory (NEXT)	► Primary efficacy endpoint is rate of OR at 6 months. ► Primary safety endpoint is ACE at 6 months	1. Time to first haematological response (CR or PR), described by cumulative incidence curve 2. Time to best haematological response, described by cumulative incidence curve 3. Time to CR, described by cumulative incidence curve 4. Rates of haematological response (OR, PR and CR) at 6, 12, 18 and 24 months 5. OS probability; OS is defined as time from day 1 of trial treatment to death, or last follow-up for patients alive 6. EFS probability; EFS is defined as time from day 1 of trial treatment to either relapse, death, treatment failure or ACE (whichever occurs first), or last follow-up for patients alive in response 7. QOL as measured by the EORTC QLQ-C30 questionaries at 6, 12, 18 and 24 months 8. Rate of occurrence of PNH clones and clinical PNH haemolysis 9. Need for and number of transfusions (RBC and platelet units) 10. Need for supportive care, including hospitalisation and ICU admission 11. Safety and tolerability of the avatrombopag, including serious adverse events 12. Rate of ACE (defined in section 24.4) at 6, 12, 18 and 24 months 13. Rate of acquired somatic mutations detected on genomic testing at 6, 12, 18 and 24 months

ACE, acquired clonal evolution; CR, complete response; EFS, event free survival; ICU, intensive care unit; OR, overall response; OS, overall survival; PNH, paroxysmal nocturnal haemoglobinuria; PR, partial response; QOL, quality of life; RBC, red blood cell; sAA, severe aplastic anaemia.

The OR rate corresponds to the proportion of participants who have a CR or PR. These definitions are in accordance with the studies evaluating eltrombopag in AA,[11] which will ensure that our findings are directly comparable to previous trials.

Sample size and power calculations

Two binary endpoints, the CR or OR (depending on the cohort) rate at 6 months (ie, efficacy) and the incidence of ACE during the 6 month follow-up period (ie, safety) will be monitored jointly. θ_1 and θ_2 are denoted the true (unknown) parameter values for CR/OR and ACE, respectively. The thresholds for efficacy and safety were set prior to trial commencement, and were based on the phase II trials of eltrombopag and other literature published at the time.

The definition of ACE is the same definition as previous eltrombopag studies. The rate of clonal evolution in the upfront phase II eltrombopag trial was 8% at 2 years, detected at 3–6 months after commencing treatment in five (5%) participants and seen at 30 months in seven (8%) participants.[9] In the randomised controlled trial, which was not yet published at the time of our protocol development, clonal evolution occurred at 6 months in 2 (2%)/84 participants on the eltrombopag arm and 1 (1%)/86 in the standard of care arm.[11] In participants refractory to IST, in a phase II trial of eltrombopag, ACE

was reported in 2 (8%)/25 participants. In a combined analysis of two phase II studies of eltrombopag in refractory sAA, ACE was reported in 13 (16%)/83 participants at 6 months.[20]

In studies of IST without TPO receptor agonists in sAA with normal karyotype at diagnosis, ACE has been reported in 14 (11%)/122[21] and 19 (11%)/170 participants.[22] In long-term follow-up of a trial of 84 AA (severe and non-severe) participants randomised to ATG with or without ciclosporin, at a median of 11 years of follow-up, ACE was reported in 8%.[5] In a retrospective analysis of 127 AA (severe and non-severe) patients, at a median follow-up of 46.8 months, acquired cytogenetic abnormalities were reported in 3.1%.[23] In a retrospective analysis of 802 AA (severe and non-severe) patients, 28/788 (3.5%) developed MDS/AML or new cytogenetic abnormalities at 5 years.[24] These ACE rates vary due to differences in trial design, inclusion criteria (eg, severe vs non-severe), treatment and length of follow-up. However, they are comparable to the 8% rate reported in the eltrombopag phase II trial, on which we based our safety monitoring.

For the FIRST cohort, the null hypothesis for efficacy is H_0: $\theta_1 \leq 0.15$ as a CR below 15% is considered futile. The null hypothesis for safety is H'_0: $\theta_2 > 0.16$ as an ACE rate of 8% was observed for the first generation agent, eltrombopag, in untreated sAA and doubling the rate was deemed unacceptable. The trial will be terminated at each interim analysis if either (1) *the posterior probability of* $\theta_1 \leq 0.15$ *given the interim data are greater than a cut-off* C_1*—lack of efficacy*; or (2) *the posterior probability of* $\theta_2 > 0.16$ *given the interim data are greater than another cut-off* C_2*—safety concern*. C_1 and C_2 are cutoffs close to 1 depending on the interim sample size n; more exactly they are set as a spending function of the information n/N where N=50 is the maximal sample size. The parameters of this function are optimised to maximise power under a prespecified alternative.

For the NEXT cohort, the null hypothesis for efficacy is H_0: $\theta_1 \leq 0.15$ as an OR rate (CR or PR) below 15% is considered futile. The null hypothesis for safety is H'_0: $\theta_2 > 0.22$ as ACE rates of approximately 11% overall were observed in the combined trials of eltrombopag including IST-refractory patients, and more than 22% was deemed unacceptable. The trial will be terminated at each interim analysis if either (1) *the posterior probability of* $\theta_1 \leq 0.15$ *given the interim data are greater than a cut-off* C_1 *– lack of efficacy*; or (2) *the posterior probability of* $\theta_2 > 0.22$ *given the interim data are greater than another cut-off* C_2 *– safety concern*. As for the FIRST cohort, C_1 and C_2 are cutoffs close to 1 depending on the interim sample size n.

Stopping boundaries at each look are given by the software in terms of number of participants experiencing each event. They have been *enumerated before the onset of the trial*, and shown in table 3, making the monitoring clear for the Data Safety and Monitoring Committee (DSMC), who are responsible for recommending a 'go/no-go' decision. Boundaries were calculated assuming a maximum of five looks carried out after collecting data on respectively 10, 20, 30, 40, 50 participants and for a global alternative (θ_1=40% and θ_2=8% for first cohort and θ_1=40% and θ_2=11% for the NEXT cohort). Parameter values have been observed for eltrombopag.[9] A similar activity and safety profile is expected for avatrombopag. Rates θ_1 and θ_2 can be estimated by maximising their posterior at the analysis at the time the trial was stopped. Ninety-five per cent credible intervals will be provided.

Table 3 Stopping boundaries for Data Safety and Monitoring Committee

FIRST study cohort		
No. of participants completed 6-month assessment	**Stop if N with CR at 6 months is equal to or less than:**	**Stop if N with ACE at 6 months is equal to or greater than:**
10	0	4
20	2	5
30	3	6
40	6	7
50	8	8
NEXT study cohort		
Number of participants treated and completed 6-month assessment	**Stop if number with ORR at 6 months is equal to or less than:**	**Stop if number with ACE at 6 months is equal to or greater than:**
10	0	4
20	2	6
30	4	8
40	6	10
50	8	11

ACE, acquired clonal evolution.

These stopping boundaries were calculated using the BOP2 application provided by the originators of the approach[25] on the MD Anderson website: http://www.trialdesign.org/. A target false-positive rate of 10% was chosen under the global null as recommended in phase II studies. The stopping boundaries are calculated under the assumption that exactly 10, 20, 30, 40, 50 participants with complete data will be available at looks 1, 2, 3, 4, 5, respectively. If for some reason, these numbers differ by more than ±1, the stopping boundaries will be adjusted according to the actual number of evaluable participants at the interim analyses.

The performance of the trial was assessed via 10 000 simulations. Table 4 displays the operating characteristics of the trial under various scenarios for both cohorts. For example, scenario A for the FIRST cohort corresponds to the global null hypothesis and as expected, the trial is stopped early 83.7% of the time and the average sample size is 27. The false-positive rate is 9.8%, very close to the 10% nominal level.

Table 4 Operating characteristics of the study

Scenario	θ_1	θ_2	Early stopping (%)	Claim acceptable (%)	Average sample size
FIRST study cohort					
A*	0.15	0.16	83.7	9.8	27
B†	0.40	0.08	6.5	92.0	48.5
C	0.40	0.10	13.7	82.6	46.9
D	0.35	0.08	8.0	90.3	47.9
E	0.45	0.12	23.5	69.2	44.8
NEXT study cohort					
A*	0.15	0.22	84.0	9.2	24.7
B†	0.40	0.11	4.9	94.3	48.4
C	0.40	0.13	9.1	88.9	47.2
D	0.35	0.10	5.5	94.0	48.2
E	0.50	0.13	7.4	90.7	47.8
F	0.50	0.16	17.3	76.5	45.1

Scenario A corresponds to the global null hypothesis. For the FIRST cohort, as expected, the trial is stopped early 83.7% of the time and the average sample size is 27. The false-positive rate is 9.8% very close to the 10% nominal level. Scenario B corresponds to the alternative hypothesis under which the boundaries were generated (θ_1=40% and θ_2=8% for FIRST cohort and θ_1=40% and θ_2=11% for the NEXT cohort). The probability of claiming that treatment is effective and safe (ie, the Bayesian equivalent of power) is displayed in the fifth column and reaches 92.0% for an average sample size of 48.5 participants for the FIRST cohort and 94% for an average sample size of 48.4 for the NEXT cohort. Scenario C assumes a higher ACE rate generating a higher chance of earlier stopping but power is still high (>82% for FIRST cohort and >88% for NEXT cohort). Scenario D is a variant with a lower treatment activity (35%) and the original safety rate, which maintains good power (90.3% for FIRST and 94% for NEXT cohort). The last scenario assumes higher CR and ACE rates, where treatment is stopped more often due to safety concerns (23.5% for FIRST and 17% for NEXT cohort) but the design still has power of 69.2%–76.5% to detect efficacy. These results were obtained using the stopping rules of the original publication[24] implemented in the initial version of the application.
*Null hypothesis.
†Main alternate hypothesis.

Statistical analysis plan

Participant characteristics will be summarised using number (%), mean (SD) or median (IQR) as appropriate. The primary outcome, rate of CR or OR at 6 months, will be reported as number (%) with 95% CI. ACE during the first 6 months is also a critical endpoint monitored jointly with CR or OR. As the BOP2 methodology followed here is based on Bayesian statistics, a point estimate defined as the mean of the posterior distribution and a 95% credible interval will also be provided for the rates of CR or OR and ACE. The prior is a Dirichlet distribution with all parameters set at 1/2. This choice was made to generate a uniform prior for the efficacy and safety rates given the lack of evidence on their distribution in this population.

Secondary outcomes of rate of OR and PR at 6, 12, 18 and 24 months and CR at other timepoints than 6 months will be reported as number (%) with 95% CIs. Time to first haematological response (either complete or partial, whichever occurs first), time to best haematological response and time to CR will be described using cumulative incidence curve.

Overall survival (OS) will be defined as time from day 1 of trial treatment to death, or last follow-up for participants alive, and will be estimated by the Kaplan-Meier method. Event-free survival (EFS) will be defined as time from day 1 of trial treatment to either relapse, death, treatment failure or ACE (whichever occurs first), or last follow-up for participants alive in response. EFS will be estimated by the Kaplan-Meier method.

In addition to the monitoring of ACE, a cumulative incidence curve of ACE will be displayed with death treated as a competing risk. Cumulative incidence of paroxysmal nocturnal haemoglobinuria (PNH) occurrence (both PNH population occurrence and clinical haemolytic PNH occurrence) will also be calculated using the same methodology.

Global health status, physical functioning, cognitive functioning, social functioning, fatigue and dyspnoea scales at 6, 12, 18 and 24 months will be calculated and transformed from the EORTC QLQ-C30 Questionnaires. Scores will be summarised using appropriate statistics and changes over trial duration will be modelled using generalised linear models.

Proportion of participants requiring RBC and platelet transfusions at 6, 12, 18 and 24 months will be reported as number (%). Total number of RBC and platelet transfusions given will be calculated for each participant and reported as mean (SD) or median (IQR), as appropriate.

Number of hospitalisations and intensive care unit admissions will be reported calculated for each participant and reported as mean (SD) median (IQR) as appropriate.

Number of adverse events, adverse reactions and SAEs will be reported.

Data management and data monitoring

Data are entered directly into an electronic case report form (eCRF) with inbuilt range checks for data values, and stored in a REDCap Database within Monash University servers. Primary source documents are collected (results of pathology including cytogenetics, molecular and bone marrow reports) for the coprimary outcomes. Outcome data (efficacy and safety) are monitored by a medical monitor to confirm the correct classification for the primary endpoints based on the source documents. Monitoring by the trial sponsor is conducted following the first participant enrolled at each site, and at least annually thereafter. Additional visits (either on-site or remote) are scheduled as required. Participants who have discontinued the intervention continue to be followed up according to trial protocol. Participants who withdraw from the trial are continued to be followed up for survival and clonal evolution through the national registry (see below).

Adverse event reporting

All adverse events that occur between the first trial-related procedure (ie, screening) and 30 days following last avatrombopag dose and all SAEs relating to clonal evolution until the last follow-up visit, or after this date if the investigator feels the event is related to the trial treatment, are recorded and reported. Those meeting the definition of a SAE are reported using a separate SAE Report form.

Investigators record in the eCRF and the participant clinical notes their opinion concerning details of nature, onset, duration, severity of the adverse and assess any relationship to the investigational medicinal product.

Any SAEs, serious adverse reactions or unexpected (but not serious) adverse reactions are reported to the sponsor (Monash University) within 24 hours of the site becoming aware of the event.

Data and Safety Monitoring Committee

An independent DSMC, comprising experts in haematology and statistics, was established before participant enrolment. The DSMC is responsible for safeguarding the interests of trial participants and assessing their safety during the trial. In addition to reviewing the interim analyses (as described in the sample size section), the DSMC monitors evidence for treatment harm (including adverse events as above) and compliance with the protocol and with previous DSMC recommendations.

Post-trial care, AA registry and long-term follow-up

The DIAAMOND trial is a registry-based trial utilising the infrastructure of the Aplastic Anaemia and Other Bone Marrow Failure Syndromes Registry (AAR). The AAR was established in 2012, through a collaboration between Monash University's Department of Public Health and Preventive Medicine and partner hospitals, clinicians and patients. The Registry employs an opt-out approach for participant recruitment and the regulatory operations are overseen by a multidisciplinary steering committee.

Registry data collection is performed at baseline, 6 months and ongoing annually following diagnosis. This includes:

1. Participant demographics, comorbidities, performance status at diagnosis.
2. Disease characteristics: disease type, stage, specific risk scores, laboratory diagnostic data.
3. Treatment: drug/dose of treatment, number of lines of therapy, reasons for alteration or discontinuation.
4. Outcomes: progression-free survival, OS.

Additional data specific to the DIAAMOND trial are collected separately, with storage of identifiers maintained in the database for the AAR and the DIAAMOND trial to allow linkage.

The registry will facilitate long-term follow-up of DIAAMOND trial participants after completion of the trial schedule of assessments.

Following completion of the trial schedule of assessments, or early trial withdrawal, post-trial care will be returned to the local treating clinician according to routine standard of care.

Biological specimens

Peripheral blood samples are collected prior to commencing therapy, and at 1, 2, 3, 6, 12, 18 and 24 months and are processed and stored for later correlative substudies. Bone marrow samples are also collected prior to commencing therapy and at 6, 12, 18 and 24 months and are processed and stored centrally for later correlative substudies.

Patient and public involvement

A consumer representative has been involved in all stages of the study design, including study design (including research question and outcome measures), preparation of patient information and trial governance. The DIAAMOND trial is a registry-based trial using the AAR, which is part-funded by Maddie Riewoldt's Vision, a charitable foundation supporting patients and families with AA and other bone marrow failure syndromes. Trial results will be made available to participants on the AAR and DIAAMOND trial website and will be disseminated more broadly to patients and families with AA through Maddie Riewoldt's Vision.

ETHICS AND DISSEMINATION

The trial has received ethics approval (Monash Health RES-18–0000707A) and registered on ANZCTR (ACTRN12619001042134, ACTRN12619001043123). Any amendments to the protocol are reviewed and approved by the TSC, Human Research Ethics Committee and DSMC. Informed consent, including for the collection and storage of biological samples, is sought from participants by the Principal Investigator or appropriately trained delegates. The method of obtaining and documenting the informed consent and the contents of the consent complies with ICH-GCP and all applicable

regulatory requirements. The trial has an independent DSMC.

The results of the trial will be submitted to a peer-review journal for publication.

Access to data and confidentiality

Trial data are securely stored on Monash University servers, with access limited to named investigators and Monash staff as listed on the ethics application. The final trial dataset will be made available to the trial investigators. Following trial conclusion and publication of primary results, deidentified participant data and study protocol may be made available on request to the DIAAMOND principal investigators by email to the corresponding author. Investigators whose proposal has been reviewed and approved by the DIAAMOND investigators and relevant ethical review committees will be able to undertake analyses to achieve the aims specified in the approved proposal by accessing data through a web-based data portal safe-haven based at Monash University, Australia.

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Contributors ZM, ST, EW and SH conceived the study and initiated the trial design and implementation. ZM wrote the first draft of the protocol. LF and PB designed the genomic testing correlative studies. ZM and SH designed the statistical analysis. EW is the grant holder. KW provided a consumer perspective on study design and participant information. ZM, SH, LF, VF, LY, PB, IC, JC, AH, DKH, RF, FF, PL, KM, AM, DP, SP, WS, JS, NW, KW, ST and EW contributed to refinement of the trial protocol and approved the final version.

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Competing interests Sobi Pharmaceuticals have supplied avatrombopag for this trial. JS has been a consultant and member of Speakers Bureau for Sobi Pharmaceuticals. AM has served on an Advisory Board for Swedish Orphan Biovitrum and served on an Advisory Board and received speaker fees from Novartis. The other authors have no other competing interests to declare.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

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