## 2. CLINICAL STUDY SYNOPSIS

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| **Name of Company:** Sanquin Plasma Products B.V. | **Volume:** (For national authority use only) |
| **Name of Finished Product:** Nanogam 50 mg/mL | **Page:** |
| **Name of Active Ingredient(s):** Human normal immunoglobulin | |
| **Title of Study:** Immunoglobulin therapy for patients with idiopathic cardiomyopathy and endomyocardial parvovirus B19 persistence - a prospective, double-blind, randomized, placebo-controlled clinical trial. | |
| **Protocol Number:** MD2009.01 | |
| **Study Period:** | **Phase of Development:** III |
| **Date of first enrolment:** 02 Nov 2009 | |
| **Date of last completed:** 12 Jan 2018 (Last Patient Last Visit)<br>06 Jun 2018 (Last Test Last Patient) | |
| **Reporting Period**: 6-months observational study period for each patient | |
| **Investigator(s):**<br>Coordinating investigator: S. Heymans, MD, PhD<br>Principal investigator: R. Dennert, MD, PhD (till 01 Oct 2010); C. Eurlings, MD (01 Oct 2010 - 01 Sep 2011); M. Hazebroek, MD, PhD (01 Sep 2011 - 01 Sep 2016, 01 Oct 2018 - 01 Oct 2019); and J. Merken, MD (01 Sep 2016 - 17 Jul 2018)<br>Department of Cardiology, Maastricht University Medical Centre+ (The Netherlands) | |
| **Study Centre:** Maastricht University Medical Centre+ (The Netherlands) | |
| **Publication(s):** Not applicable. | |
| **Background and Rationale for the Study:**<br>The link between myocarditis and idiopathic cardiomyopathy (ICM) is increasingly recognised. Myocarditis is an inflammatory cardiomyopathy with a variable natural history, ranging from spontaneous recovery, subclinical course with evolution to ICM or early death from multiple system failure. Viral persistence has been associated with diastolic and endothelial dysfunction in heart failure patients and to progressive cardiac dysfunction in ICM patients. Studies using viral polymerase chain reaction (PCR) in endomyocardial biopsies pointed towards parvovirus B19 (PVB19) as the most frequent virus found in patients with acute myocarditis or ICM.<br>Intravenous immune globulin (IVIg) is increasingly recognised as a safe and well tolerated standard therapy for the treatment of a number of autoimmune and systemic inflammatory diseases. Its anti-infectious properties are based on its ability to neutralize viruses and microbial toxins. Moreover, a single-cycle high dose of IVIg has been suggested to have a therapeutic effect in PVB19-induced diseases such as fetal hydrops, pure red-cell aplasia, polyarteritis nodosa and PVB19 infection during pregnancy.<br>This study is a prospective, randomised, double-blind placebo-controlled trial to evaluate the effect of IVIg (Nanogam) on virus presence and cardiac functional capacity before and 6 months after treatment with Nanogam for 4 consecutive days. The target population is patients with chronic ICM despite optimal conventional heart failure treatment and a significant PVB19 viral load in their hearts.<br>Current supportive heart failure treatment such as β-blockade or angiotensin-inhibition does not target the virus or related immune alterations. Therefore, a specific treatment that eliminates the cardiotrophic virus and modulates the immune response is mandatory in this select patient population. | |

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**Objectives:**

Primary Objective

The primary objective was to aim to investigate whether IVIg (Nanogam) therapy on top of conventional heart failure may improve cardiac systolic function as assessed by quantitative echocardiography (LVEF [Left Ventricular Ejection Fraction], LVEDD [Left Ventricular End-Diastolic Diameter], LVESD [Left Ventricular End-Systolic Diameter]).

Secondary Objectives

Secondary objectives included evaluation of the change in presence of cardiotrophic viruses (per µg DNA) of parvovirus B19 (PVB19), Human Herpes Virus-6 (HHV-6), Enterovirus (EV), ADV, Epstein-Barr Virus (EBV), inflammation (CD45-staining lymphocytes/$mm^2$), fibrosis (collagen volume fraction/$mm^2$), cardiac functional capacity (New York Heart Association [NYHA] functional class), 6-minute walk test, patient quality of life (Minnesota Living with Heart Failure Questionnaire).

Tertiary Objective

To analyse the used batches of Nanogam for neutralizing titres of B19-antibodies and to compare them to the presence and concentration of specific antibodies in the blood of the patients before and after infusion. Moreover, it was to be assessed whether there is a correlation between the change in specific PVB19 subtypes present in endomyocardial biopsies and the presence and titre of specific antibodies against PVB19.

Quaternary and Safety Objectives

To monitor the tolerability and safety profile of IVIg (Nanogam) in the population at large. The safety and clinical tolerance were to be monitored by adverse event (AE) surveillance and laboratory measurements. Also, to monitor viral safety of Nanogam, pre-treatment blood samples were to be taken and stored until 1 year after the last patient is out of the study.

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**Endpoints:**

*Efficacy:*

Primary Endpoint

- Absolute change in LVEF (%), using biplane Simpson methods at baseline versus 6 months follow-up.

Secondary Endpoints

- Absolute changes in:
  - LVEDD (mm) at baseline versus 6 months follow-up
  - LVESD (mm) at baseline versus 6 months follow-up
  - Left Ventricular End-Diastolic Volume (LVEDV) (mL) at baseline versus 6 months follow-up
  - LVESV (mL) at baseline versus 6 months follow-up
  - Left Ventricular Mass Index (LVMI) ($g/m^2$) at baseline versus 6 months follow up
- Absolute changes in endomyocardial biopsy (EMB)'s presence of:
  - PVB19 at baseline versus 6 months follow-up
  - HHV-6, EV, ADV, EBV at baseline versus 6 months follow-up
- Absolute changes in EMBs of:
  - CD3, CD8, CD20, CD45-staining lymphocytes (cells/$mm^2$) and CD68 staining macrophages (cells/$mm^2$) at baseline versus 6 months follow-up
  - Collagen volume fraction (%) at baseline versus 6 months follow-up
- Absolute change in clinical functional capacity (NYHA functional class) at baseline versus 6 months follow-up
- 6-minute walk test at baseline versus 6 months follow-up
- Changes patient quality of life (the Minnesota Living with Heart Failure Questionnaire) at baseline versus 6 months follow-up
- Changes in blood laboratory values at baseline versus 6 months follow-up
  - N-terminal prohormone of brain natriuretic peptide (NT-proBNP) (pmol/L)
  - Cardiac troponin T (µg/L)
  - Cystatin-C
  - High-sensitivity C-reactive protein (mg/L)
  - Soluble interleukine-2 (pg/mL)
  - Neopterin (ng/mL)
  - Antiheart autoantibodies

Tertiary Endpoints

- Presence and titre of neutralizing antibodies against PVB19 antigens (viral capsid proteins VP1/VP2) (U/mL) in the blood serum
- Presence of antibodies against non structural protein 1 (NS1) (ratio) to monitor PVB19 persistence
- Immunoglobulin G (IgG) trough levels (g/L)

*Safety:*

Safety Endpoints

- AEs and serious adverse events (SAEs)
- Clinical laboratory measurements: blood count, haematology, clinical chemistry
- Physical signs
- 12-lead electrocardiograms (ECG)

**Study Design:** Prospective, double-blind, randomised, placebo-controlled clinical trial

**Number of Patients (planned and analysed):**
Planned: A maximum of 60
Analysed: 26 for Nanogam group and 24 for placebo group.
Full analysis set (FAS), per protocol set (PPS), and safety set (SAF) included 50 patients who were treated with study medication; all 3 analysis sets included the same population.

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**Diagnosis and Main Criteria for Inclusion and Exclusion:**

Inclusion Criteria:

1. ICM (LVEF <45%) >6 months.
2. Optimal conventional heart failure medication >3 months.
3. PVB19 viral load >200 copies/µg DNA in endomyocardial biopsies (EMBs).
4. Signed informed consent.
5. Aged between 18 and 75 years.

Exclusion Criteria:

1. Other causes for heart failure:
   - Significant coronary artery disease (lesions >70% stenosis)
   - Significant valvular disease
   - Untreated hypertension (systolic blood pressure >140 mmHg)
   - Substance abuse
   - Chemotherapy induced
2. Significant titre of other cardiotrophic viruses (EV, ADV, HHV6, EBV).
3. Pregnancy or lactation.
4. Systemic diseases such as sarcoidosis, giant cell myocarditis, hemochromatosis, or systemic autoimmune diseases.
5. Treatment with any other investigational drug within 7 days before study entry or previous enrolment in this study.
6. Known with allergic reactions against human plasma or plasma products.
7. Having an ongoing progressive terminal disease, including human immunodeficiency virus (HIV) infection.
8. Having renal insufficiency (plasma creatinine >115 µmol/L or creatinine clearance <20 mL/min).
9. Having an ongoing active disease causing general symptoms e.g. chronic active hepatitis, persistent enterovirus infection with ongoing systemic complaints.
10. Having detectable anti-immunoglobulin A (IgA) antibodies.
11. Active systemic lupus erythematosus.

**Test Product, Dose and Mode of Administration, and Lot Number(s):** Total dose of 2 g/kg bodyweight of intravenous immunoglobulin product Nanogam, over a period of 6 hours on each of 4 consecutive days.
Lot numbers: 09D22H464A, 09D25H464A, 10J26H464A, 11I01H464A, 11J19H464A, 13C01H464A, 13I24H464A, 15C12H464A, 15L12H464A, and 16D28H464A

**Control Product, Dose and Mode of Administration, and Lot Number(s):** Intravenous pasteurised plasma protein solution G.P.O./Albuman, over a period of 6 hours on each of 4 consecutive days.
Lot number for G.P.O: 08F26H100A
Lot numbers for Albuman: 10A13H150D, 12E30H150A, 13E29H150A, 16A07H150A

**Duration of Treatment:** 4 days

**Criteria for Evaluation:**

*Efficacy:* Cardiac systolic function (primary objective) as assessed by quantitative echocardiography (LVEF [%], LVEDD [mm], LVESD [mm]), change in presence of cardiotrophic viruses (per µg DNA) of PVB19, HHV-6, EV, ADV, EBV, inflammation (CD45-staining lymphocytes/$mm^2$), fibrosis (collagen volume fraction/$mm^2$), cardiac functional capacity (NYHA functional class), 6-minute walk test, patient quality of life (Minnesota Living with Heart Failure Questionnaire), and the titre of neutralizing antibodies against PVB19 antigens (viral capsid proteins VP1/VP2), the titre of antibodies against NS1, and the presence of the cardiotrophic virus PVB19 in the EMBs.

*Safety:* Adverse Event, Haematology, Clinical Chemistry, Vital Signs, 12-lead-ECG, Physical Signs

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| **Statistical Methods:**<br>*Efficacy:* Repeated measurements ANCOVA modelling including the treatment group, visit, and the interaction term as fixed effects and baseline as the covariate, Mann-Whitney-U-Test, and 95% confidence interval from the Hodges-Lehmann estimation<br>*Safety:* Descriptive Statistics | | |
| **Patient Disposition:** Of 53 patients who were randomised, 50 patients (94.3%) were treated with study medication and completed the treatment according to protocol and the 6 months observational study period after study treatment.<br>One (1) patient (3.7%) in the Nanogam group and 2 patients (7.7%) in the placebo group were withdrawn from the study without taking any study medication, all due to patients' preferences. No patient was withdrawn due to adverse drug reaction, clinical failure, noncompliance with therapy, complication, AE, death or other reason. | | |
| **Demography and Baseline Characteristics:** Overall, mean (SD) age was 53.9 (11.44) years, mean (SD) height was 177.5 (10.71) cm and mean (SD) body weight was 86.53 (16.135) kg; 78% of patients were male. Demographic characteristics were generally similar between Nanogam and placebo groups. | | |

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**Efficacy Results:**

**Primary Efficacy Endpoint: absolute change in LVEF (%), at baseline versus 6 months follow-up**

- The superiority of Nanogam over placebo for the primary efficacy endpoint was not established for the FAS set (p = 0.547 [Mann-Whitney-U-Test] and 95% Hodges-Lehmann CI: -7.00, 4.00).
- The Subgroup analyses (i.e. viral PBV19 load at entry level and regression analysis of LVEF [6 months follow-up] against CD45 count and treatment). There were no notable differences observed between treatments (after 6-months follow-up in the corresponding sub groups, p = 0.5950 [high PBV19 viral load] and p = 0.5634 [low PBV19 viral load] [T-test]).

**Secondary Efficacy Endpoints:**

- After 6-months follow-up, no notable difference was observed between Nanogam treatment and placebo for LVEDD (p = 0.630 and 95% CI: -2.00, 4.00), LVESD (p = 0.619 and 95% CI: -3.00, 2.00), LVEDV (p = 0.205 and 95% CI: -11.00, 42.00), LVESV (p = 0.267 and 95% CI: -8.00, 26.00) and LVMI (p = 0.186 and 95% CI: -4.00, 19.00) (Mann-Whitney-U-Test and 95% Hodges-Lehmann CI).
- The Subgroup analyses (by viral PBV19 load and timepoint) of LVEDD, LVESD, LVEDV, LVESV, and LVMI did not identify any significant treatment-subgroup interaction.
- No notable difference was observed between Nanogam treatment and placebo for absolute change in clinical functional capacity (assessed by NYHA functional class, p = 0.0961 [CMH test]), 6-minute walk test after 6-months follow-up (p = 0.5670 [T-test]), the Patient quality of life (as assessed by the Minnesota Living with Heart Failure Questionnaire) (p = 0.6836 [T-test]).
- No notable difference for blood laboratory parameters were observed between treatments (the 6-months follow-up for NT-proBNP (p = 0.1694), cardiac troponin (p = 0.4950), cystatin-C (p = 0.2975), high-sensitivity C-reactive protein (p = 0.2178), soluble interleukine-2 (p = 0.4979), neopterin (p = 0.5185) (T-test) and anti-heart antibodies (p = 0.7456) (CMH test).
- The treatment with Nanogam did not eliminate the PVB19 virus more effectively than placebo (after 6 months follow up p = 0.2582). The results were similar for the HHV-6 and ADV after 6-months follow-up (p = 0.3419 and p = 0.3028, respectively) (T-test). The other virus types EV and EBV were never present at the start.
- No notable treatment difference was observed in the absolute changes in the EMBs in presence of CD3 (p = 0.9290), CD8 (p = 0.0813), CD20 (p = 0.9389), CD45-staining lymphocytes (p = 0.1411) (cells/$mm^2$) at baseline versus 6 months follow up. But, following the Nanogam treatment, notable difference (p = 0.0060) was observed for the CD68 staining macrophages (cells/$mm^2$). No notable difference was observed for the collagen volume fraction (%) between the treatments (p = 0.1195) (T-test).

**Tertiary Efficacy Endpoints:**

- Titre of neutralizing antibodies against PVB19 antigens (anti viral capsid proteins VP1/VP2) at Day 4 after last infusion increased from baseline in the Nanogam group. A notable difference between treatment groups was observed at Day 4 after last infusion (p = 0.0000), after 2 weeks (p = 0.0405) and 6-months follow-up (p = 0.0236). Note that a difference was already present at baseline (p = 0.0393) (T-test). The antibodies against NS1 at Day 4 after last infusion, increased from a non-statistical difference at baseline (p = 0.3830) to statistically significant higher levels in the Nanogam group (p = 0.000). This difference was not significant at other timepoints: after 2 weeks (p = 0.5024) and at 6-months follow-up (p = 0.5571) (T-test).
- Following administration of Nanogam, the IgG trough levels peaked on the Day 4 after infusion and returned to baseline levels by 6-months follow-up visit. Following administration of placebo, no notable changes were observed in IgG trough levels.

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**Safety Results:**

- Eighteen (18) patients (69.2%) out of 26 experienced 34 AEs (all were TEAEs) in the Nanogam group, and 13 patients (54.2%) out of 24 experienced 21 AEs (all were TEAEs) in the placebo group. Note that the 2 TEAEs of viral myocarditis reported in the Nanogam group (viral heart disease with or without inflammation/myocarditis) should not have been reported as AEs as the conditions were specified in the inclusion criteria.
- No TEAE led to interruption or discontinuation of the study treatment.
- Two (2) patients (7.7%) experienced 2 SAEs in the Nanogam group, and 1 patient (4.2%) experienced 1 SAE in the placebo group. None of them were considered related to the study medication.
- The most common TEAEs included headache (11/26 patients [42.3%] in the Nanogam group and 4/24 [16.7%] in the placebo group), nausea (2 patients [7.7%] in the Nanogam group and 4 patients [16.7%] in the placebo group), and influenza like illness (1 patient [3.8%] in the Nanogam group and 2 patients [8.3%] in the placebo group).
- Of the most common TEAEs listed above, only headache and nausea were considered related to the study medication. Headache was reported more frequently in the Nanogam group (42.3%) than in the placebo group (16.7%); the difference between the groups for headache was statistically significant ($p = 0.0481$). Nausea was reported more frequently in the placebo group than in the Nanogam group; the difference between the groups was not statistically significant ($p = 0.3293$) (Chi-square test). Both headache and nausea are recognised as most common AEs for Nanogam.
- In the Nanogam group, headache was considered probably/definitely related to the study medication for 6 patients (23.1%), possibly related for 3 patients (11.5%), and not related for 2 patients (7.7%). In the placebo group, headache was considered probably/definitely related for 1 patient (4.2%), possibly related for 1 patient (4.2%), and not related for 2 patients (8.3%).
- Nausea was considered probably/definitely related to the study medication for 2 patients (7.7%) in the Nanogam group and for 2 patients (8.3%) in the placebo group; 2 other cases in the placebo group were considered as not related to the study medication.
- Three (3) SAEs were reported; salivary gland cancer and deep vein thrombosis in the Nanogam group and vessel puncture site haematoma in the placebo group. None of them were considered related to the study medication. The worst intensity of each SAE was severe for salivary gland cancer, moderate for deep vein thrombosis, and mild for vessel puncture site haematoma.
- None of the clinically relevant laboratory measurements were reported as AEs, and they all were related to concomitant disease.
- No clinically significant changes in vital signs were observed.
- No clinically significant changes from baseline in 12-lead ECG results and rhythm assessments/abnormalities were observed in 6-months follow-up.

**Conclusions:**
The superiority of Nanogam treatment over placebo for the efficacy endpoints was not established. Safety results showed no new findings which will affect the current safety profile of Nanogam.

**Date of this Report**: 01 Nov 2019