Near-infrared image guidance in cancer surgery

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Chapter 4

Clinical trial of combined radio- and fluorescence-guided sentinel lymph node biopsy in breast cancer

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British Journal of Surgery 2013;100:1037-1044

ABSTRACT

Background

Combining radioactive colloids and a near-infrared (NIR) fluorophore permits preoperative planning and intraoperative localization of deeply located sentinel lymph nodes (SLNs) with direct optical guidance by a single lymphatic tracer. The aim of this clinical trial was to evaluate and optimize a hybrid NIR fluorescence and radioactive tracer for SLN detection in patients with breast cancer.

Methods

Patients with breast cancer undergoing SLN biopsy were enrolled. The day before surgery, a periareolar injection of indocyanine green (ICG)–^{99m}Tc-radiolabelled nanocolloid was administered and a lymphoscintigram acquired. Blue dye was injected immediately before surgery. Intraoperative SLN localization was performed using a gamma probe and the Mini-FLARETM NIR fluorescence imaging system. Patients were divided into two dose groups, with one group receiving twice the particle density of ICG and nanocolloid, but the same dose of radioactive ^{99m}Tc.

Results

Thirty-two patients were enrolled in the trial. At least one SLN was identified before and during operation. All 48 axillary SLNs could be detected by gamma tracing and NIR fluorescence imaging, but only 42 of them stained blue. NIR fluorescence imaging permitted detection of lymphatic vessels draining to the SLN up to 29 h after injection. Doubling the particle density did not yield a difference in fluorescence intensity (median 255 (range 98–542) versus 284 (90–921) arbitrary units; P = 0.590) or signal-to-background ratio (median 5.4 (range 3.0–15.4) versus 4.9 (3.5–16.3); P = 1.000) of the SLN.

Conclusion

The hybrid NIR fluorescence and radioactive tracer permitted accurate preoperative and intraoperative detection of the SLNs in patients with breast cancer. Registration number: NTR3685 (Netherlands Trial Register; http://www.trialregister.nl).

INTRODUCTION

Sentinel lymph node (SLN) biopsy is the standard of care for nodal staging in patients with breast cancer and clinically negative axillary lymph nodes.[1] To locate the SLNs, a combination of radioactive lymphatic tracers and blue dye staining is often preferred.[2–5] Radioactive tracers permit preoperative planning and retention in the first-echelon nodes, whereas blue dye allows direct intraoperative visualization of the lymphatics and SLNs.

Optical imaging using near-infrared (NIR; 700–900 nm) fluorescence has been tested extensively for SLN detection in breast cancer and in other cancers, such as melanoma.[6–15] The fluorescent tracer indocyanine green (ICG) is used for detection of SLNs up to several millimetres deep in tissue.[16–18] This tracer has outperformed blue dye staining for SLN identification in multiple clinical trials.[7,8,18,19] Nevertheless, radioactive colloids are still considered the standard of care for preoperative planning. Radioactive colloids are essential for localization of more deeply located SLNs, for example in patients with a higher body mass index (BMI).[8,20]

To combine radioactive and NIR fluorescence guidance in one 'package', the hybrid tracer ICG–^{99m}Tc-labelled nanocolloid (ICG-^{99m}Tc-nanocolloid) has been developed.[21] The fluorescent label ICG and the radioactive label ^{99m}Tc are integrated into the same colloidal particle. Unlike 'free' ICG, this nanoparticle is retained in the SLN for an extended period.[22] The feasibility and validity of use of this tracer has been shown in various tumour types.[23–25] However, it has not been studied in SLN biopsy procedures in patients with breast cancer.

The aim of this study was to evaluate the hybrid tracer for SLN detection in patients with breast cancer, and to assess the effect of increasing the particle density of the tracer on its nodal accumulation and the fluorescent signal intensity in the SLN.

METHODS

This clinical trial was approved by the medical ethics committee of Leiden University Medical Centre and performed in accordance with the ethical standards of the Helsinki Declaration of 1975. All patients with breast cancer scheduled for SLN biopsy between August 2011 and July 2012 were eligible to participate in the study. Included patients had clinically negative axillary nodes as assessed by palpation and ultrasonography. Exclusion criteria were pregnancy, lactation, or an allergy to iodine or ICG. Informed consent was given by all included patients and the acquired data were anonymized.

Tracer preparation

^{99m}Tc-labelled nanocolloid was prepared by adding sodium pertechnetate (approximately 1000 MBq) in 2 ml saline to a vial containing 0.5 mg human serum albumin nanocolloid (GE Healthcare, Eindhoven, The Netherlands). After 30 min of incubation at room temperature, 50 µl 6.4 mmol/l (0.25 mg) ICG (Pulsion Medical Systems, Munich, Germany) was dissolved in water for injection to obtain ICG-^{99m}Tc-nanocolloid at a final ICG concentration of 160 µmol/l and pH of 6.0–7.0.[24,25] To obtain a twofold higher concentration of ICG-nanocolloid, 99mTc-labelled nanocolloid was prepared by adding pertechnetate (approximately 500 MBq) in 1 ml saline to 0.5 mg nanocolloid, while the amount of ICG (0.25 mg) was kept the same, resulting in a final ICG concentration of 320 µmol/l. This preparation resulted in doubling of the ICG and nanocolloid concentration, without changing the dose of radioactivity (approximately 100 MBq). All procedures were performed under current good manufacturing practice and under supervision of the institution's pharmacist.

Study design

The day before surgery, a periareolar injection of ICG-^{99m}Tc-nanocolloid was given intracutaneously in the quadrant in which the tumour was located (at one site in a total volume 0.2 ml; 100 MBq). Patients were divided into groups with a low and high ICG-99mTc-nanocolloid particle density; the first consecutive half of the patients were assigned to the low dose, and the second half to receive a twofold higher dose. Anterior, lateral and anterior oblique planar images were obtained at 15 min and approximately 3 h after injection, by the detector of a single- or two-headed gamma camera (Symbia T6, Siemens, Erlangen, Germany; or Toshiba GCA-7200PI/7200DI/7100UI, Toshiba, Tokyo, Japan). The percentage of tracer in the SLN was calculated from the anterior oblique images by dividing the counts in the SLN(s) by the sum of counts at the injection site and all lymph nodes. Simultaneously with the gamma-camera images, percutaneous NIR fluorescence images were acquired using the Mini-FLARE™ NIR fluorescence imaging system, as described previously.[26] A SLN was defined as a lymph node on a direct lymphatic drainage pathway from the primary tumour as detected by lymphoscintigraphy.[27] During surgery lymph nodes with a gamma count of 10 per cent or more compared with the most radioactive SLN were also designated as SLNs.[28]

Directly before surgery, a total of 1 ml patent blue dye (Bleu Patenté V; Guerbet, Brussels, Belgium) was injected at multiple sites around the areola. Gentle pressure was applied to the injection site for 1 min. The surgical field was illuminated using the white light source of the Mini-FLARE™ imaging system. During surgical exploration, the combined radioactive and fluorescence signature of the SLNs defined

before surgery was visualized by means of a hand-held gamma probe (Europrobe, Euromedical Instruments, Le Chesnay, France) and the Mini-FLARE™.

The primary endpoint of the study was the SLN identification rate. Secondary endpoints were the number of SLNs identified per patient, the percentage of tracer accumulated in the SLN based on scintigraphy, the fluorescence intensity, and the fluorescence signal-to-background ratio (SBR) of the SLNs. The SBR of the SLN was calculated by dividing the fluorescence intensity of the SLN by the fluorescence intensity of the fatty tissue directly surrounding the SLN. Blue dye staining was used to provide additional optical guidance.

SLNs were fixed in formalin and embedded in paraffin for routine haematoxylin and eosin staining, and immunohistopathological staining for cytokeratin (AE1/AE3). This was done at three levels, with an interval of 150–250 μm, according to the Dutch guidelines for SLN analysis in breast cancer.

Statistical analysis

For statistical analysis, SPSS statistical software package (Version 20.0, Chicago, IL) was used. To compare categorical characteristics between the two groups of patients, the Fisher's Exact test was used for binary data and the chi-square test for non-binary data. Continuous data was tested for normal distribution using the Shapiro-Wilk test. Normal distributed continuous data (age and tumour size) was tested using the independent-sample t- test and not normally distributed data was tested using the Mann-Whitney test. Continuous data is presented as median and range. $P < 0.05$ was considered significant.

RESULTS

Thirty-two consecutive patients with breast cancer undergoing SLN biopsy were included in the study (Fig. 1). The median age was 56 (34–82) years and median BMI was 24 (18–39) kg/m^2. The first 16 patients were assigned to the low dose of ICG-^{99m}Tc-nanocolloid and the following 16 received the twofold higher dose. There were no differences in patient, tumour and treatment characteristics between the two treatment groups (Table 1). The median time between injection of ICG-99mTc-nanocolloid and surgery was 24 (19–29) and 22 (20–25) h for the low- and high-dose treatment groups respectively. Similar to the use of 'free' ICG.[7,8,26] no adverse reactions were associated with the use of ICG-^{99m}Tc-nanocolloid.

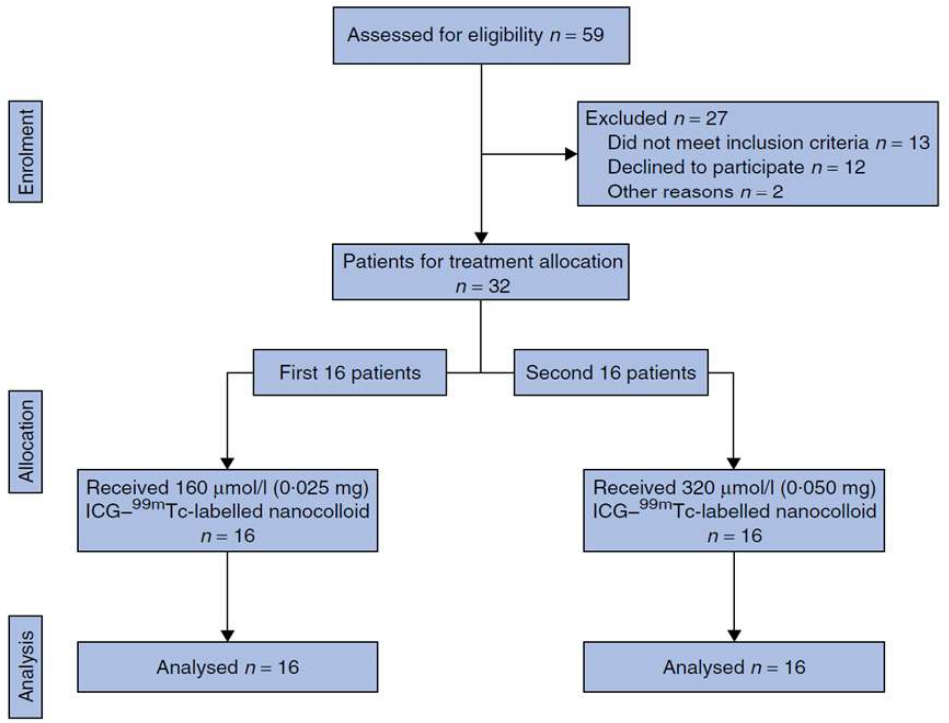

Fig. 1. Patient enrolment. ICG, indocyanine green

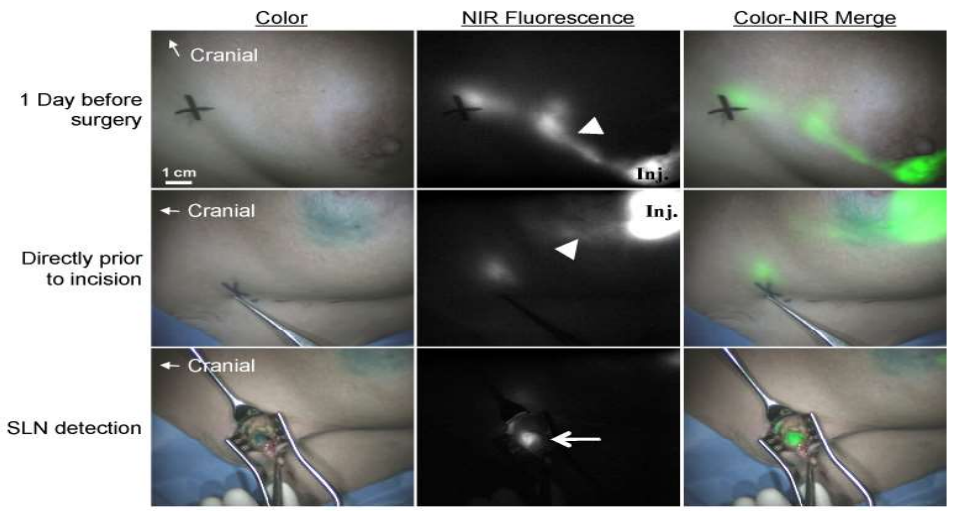

Fig. 2. Near-infrared (NIR) fluorescence imaging during sentinel lymph node (SLN) mapping in breast cancer. The periareolar injection site (inj.) and an afferent lymphatic channel (arrowhead) are clearly visualized the day before surgery (upper panel). Directly before surgery, patent blue is injected around the areola. The lymphatic channels (arrowhead) can still be visualized percutaneously using NIR fluorescence (middle panel). The SLN (arrow) is identified by NIR fluorescence and blue staining. The black cross indicates the presumed position of the SLN. Camera exposure times were 100 ms (upper and middle panels) and 40 ms (lower panel).

Table 1. Patient and tumour characteristics

Characteristic	Low-dose group (16 patients)	High-dose group (16 patients)	P
	N	N	
Age in years (median; range)	59 (44 – 82)	55 (34 – 77)	0.322
Body mass index, kg/m2 (median; range)	25 (21 – 39)	23 (18 – 37)	0.043
Previous breast procedure			0.635
Breast augmentation	1	1	
Lumpectomy	0	1	
Neoadjuvant hormonal / Chemotherapy	4	2	
Previous axillary surgery	0	0	
Multifocality	1	5	0.172
Type of Operation			0.458
Mastectomy	7	4	
Wide local excision	9	12	
Hours between injection and surgery (median; range)	24 (19 - 29)	22 (20 - 25)	0.196
Pathological tumour size in mm (median; range)	14 (3 – 34)	15 (4 – 30)	0.681
Histological type			0.717
Infiltrating ductal adenocarcinoma	13	13	
Infiltrating lobular adenocarcinoma	2	1	
Ductal Carcinoma In Situ (DCIS)	1	2	
Histological grade			0.058
I	2	3	
II	10	4	
III	1	7	
No grading possible (DCIS)	3	2	

Sentinel lymph node detection

Preoperative scintigraphy identified at least one SLN in all patients, with a median of 1 (1–2) per patient. All SLNs were located in the axilla. In 22 patients superficial lymph drainage could be at least partially visualized percutaneously by NIR fluorescence at the time of preoperative scintigraphy or before surgery (Fig. 2).

Surgical excision of the SLNs was guided by a combination of radioactivity and fluorescence. After initial guidance from the preoperative scintigram and the gamma probe, the axilla was explored using NIR fluorescence imaging. The gamma probe was used for additional intraoperative guidance only when NIR fluorescence did not directly point to the SLN. The results of the SLN biopsy procedures are summarized in Table 2. The NIR fluorescence-based detection of lymphatic vessels that drained to the SLN contributed to their identification (Fig. 3). At least one SLN was identified and resected in all patients. In addition to the SLNs detected by lymphoscintigraphy,

ten lymph nodes were considered as SLN during surgery, with a median of 1 (1–3) SLNs harvested from each patient. All 48 radioactive SLNs were detected by NIR fluorescence and the gamma probe, but only 42 of 48 SLNs stained blue. In all patients the NIR fluorescence signal in the SLN was detected before patent blue was visualized. Histological analysis of the SLNs showed lymph node metastases in 13 of 32 patients. Macrometastases (larger than 2 mm) were found in eight patients and isolated tumour cells or micrometastases (2 mm or smaller) in five.

Table 2. Results of sentinel lymph node identification

Characteristic	Total (32 patients)	Low-dose (16 patients)	High-dose (16 patients)	P
	N	N	N	
Preoperative SLN identification				
Identification rate	32 / 32	16 / 16	16 / 16	
Number of SLNs detected per patient (median; range)	1 (1 – 2)	1 (1 – 2)	1 (1 – 2)	0.564
Percutaneous fluorescence lymph drainage visualization	22 / 32	10 / 16	12 / 16	0.704
Intraoperative SLN identification				
Identification rate	32 / 32	16 / 16	16 / 16	
Total number of SLNs removed	48	21	27	
Method of intraoperative detection				
Radioactive	48 / 48	21 / 21	27 / 27	
Blue	42 / 48	17 / 21	25 / 27	0.383
Fluorescent	48 / 48	21 / 21	27 / 27	
Histology sentinel lymph node				0.686
Negative	19 / 32	10 / 16	9 / 16	
Micrometestases / ITC	5 / 32	3 / 16	2 / 16	
Macrometastases	8 / 32	3 / 16	5 / 16	

ITC; Isolated tumour cells, SLN; Sentinel lymph node

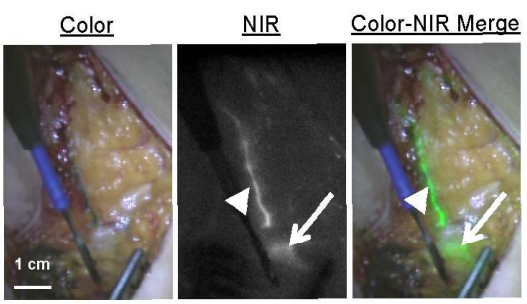

Fig. 3. Intraoperative detection of lymphatic vessels using near-infrared (NIR) fluorescence imaging. The lymphatic vessel (arrowhead) draining to the sentinel node (arrow) located more deeply in the tissue is clearly identified by NIR fluorescence

Comparison between treatment groups

The number of preoperative identified SLNs by radioscintigraphy was not different between the low dose group, median one (1 – 2), and high dose group, median one (1 – 2), P = 0.564. Despite doubling the particle density, the percentage of the amount of injected tracer accumulated in the SLN per patient based on the scintigraphy did not improve, median 1.7 (0.2 – 7.3) per cent vs. median 2.6 (0.4 – 30.4) per cent, P = 0.402. Nevertheless, the higher particle density group had SLNs that showed a higher accumulation of the tracer (Fig. 4). Between the groups no difference was observed in the amount of higher echelon nodes during lymphoscintigraphy.

Intraoperatively, the number of identified SLNs did not differ between the low dose group, median one (1 – 2), and high dose group, median one (1 - 3), P = 0.323. No significant difference was found for the fluorescence intensity, median 255 (98 – 542) vs. median 284 (90 – 921), P = 0.590 or signal-to-background ratio of the SLN, median 5.4 (3.0 – 15.4) vs. median 4.9 (3.5 – 16.3), P = 1.000, between the low dose group and higher dose group, respectively (Fig. 4).

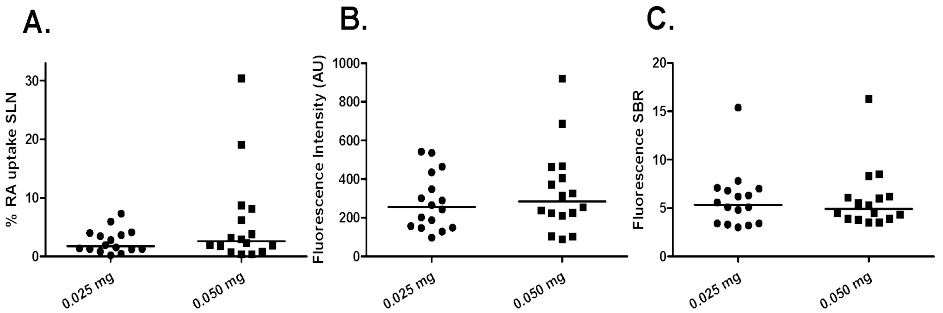

Fig. 4. Influence of indocyanine green-labelled nanocolloid dose on a percentage of radioactive injected dose of hybrid tracer in the sentinel lymph node (SLN) approximately 3 h after injection, b fluorescence intensity of the SLN measured during surgery and c fluorescence signal-to-background ratio (SBR) of the SLN during surgery. Circles represent individual patient values and the line indicates the median. AU, arbitrary units

DISCUSSION

The present study has shown that the use of ICG-^{99m}Tc-nanocolloid for SLN biopsy is feasible in patients with breast cancer. This tracer permits preoperative imaging and intraoperative guidance. By combining NIR fluorescence and radioactivity in a single tracer, discrepancies between the two imaging modalities used for SLN localization are less likely to occur. In the present study all SLNs could be detected surgically by both gamma radiation and NIR fluorescence imaging. Lymphatic vessels draining to the SLN were fluorescent the day after tracer administration and contributed to detection of the SLN.

In an attempt to optimize nodal accumulation of the tracer and the NIR fluorescence signal, a twofold higher particle density of ICG and nanocolloid was administered. In the higher-dose group, multiple outliers with greater accumulation of tracer in the SLN were observed, although there was no statistically significant increase in tracer accumulation. The results were similar for the fluorescence-based identification. In contrast to intratumoural tracer deposition[29], an increase in particle density does not appear to have a significant influence on SLN identification when periareolar administration is used. In the present study the dose of ^{99m}Tc was kept the same for both groups (100 MBq). Globally, different doses of ^{99m}Tc, varying from 10 to 370 MBq, are used for SLN detection.[30] As the lymphatic distribution of ICG-^{99m}Tc-nanocolloid is mainly determined by the large nanocolloid particles, no significant differences in sensitivity of the fluorescence signal are to be expected when the amount of 99mTc is varied while maintaining the nanocolloid dose.

In previous studies using ICG alone, 25 times more ICG was injected during surgery.[7,8] In addition, the volume of these injections was eight times higher, thereby increasing the interstitial fluid pressure and lymphatic drainage. These two factors contributed to a SBR of approximately nine.[7,8] Here, a lower SBR (approximately six) was observed when ICG-^{99m}Tc-nanocolloid was used. This decrease is relatively small because the lower dose and smaller injection volume of ICG was probably offset by the longer time between injection and imaging, which would have aided in the concentration of ICG in the SLN. Importantly, this decrease did not influence surgical guidance, as ICG-^{99m}Tc-nanocolloid permitted accurate SLN identification during surgery in all these patients and lymphatic vessels were still clearly visible.

In the present study 42 of 48 SLNs stained blue, which is comparable to the identification rate of blue dye in large multicentre studies.3 NIR fluorescence identified more SLNs than blue dye staining and detected SLNs that were more deeply located in the tissue. Similar results were obtained in previous studies[7,26], and blue dye showed no benefit when NIR fluorescence imaging was used.8 When ICG-^{99m}Tc-nanocolloid is used and blue dye omitted, no lymphatic tracer has to be injected directly before surgery; this, combined with the enhanced detection by NIR fluorescence compared with blue dyes, can improve logistics and reduce the duration of surgery.

Various techniques are being evaluated to improve the depth penetration of NIR fluorescence contrast agents[31,32] and to study when radioactivity can be omitted.[8] If in the future depth penetration of NIR fluorescence contrast agents can be increased, radioactive colloids may possibly be omitted, thereby improving logistics and costs. At present, NIR fluorescence is mainly used in addition to radioactive colloids. Widespread clinical dissemination of this technique requires a cost-effective approach. ICG-^{99m}Tc-nanocolloid is based on a lymphatic tracer that is in regular use in Europe and only needs addition of a small amount (0.025 mg) of ICG, at a cost of

approximately €50–80 for 25 mg. A variety of relatively low-cost commercial camera systems are already available for clinical use.[33]

The hybrid optical–nuclear agent ICG-^{99m}Tc-nanocolloid has been used successfully as a tracer for image-guided SLN biopsy in patients with breast cancer. Use of ICG-^{99m}Tc-nanocolloid provides fully integrated preoperative and intraoperative radioactive and NIR fluorescence guidance, with no need for an injection immediately before surgery, producing intraoperative findings comparable to those provided by use of ICG alone. As no difference was observed between the two ICG-^{99m}Tc-nanocolloid doses, a particle density of 160 μmol/l ICG-^{99m}Tc-nanocolloid injected in a volume of 200 μl the day before surgery could be recommended.

ACKNOWLEDGMENTS

The authors thank G. Ranke, E. Krol-Warmerdam (Breast Cancer Unit), K. Schimmel (Central Pharmacy) and H. Putter (Medical Statistics) for their contribution to this study, and D. Burrington Jr for editing.

This work was supported in part by National Institutes of Health (grants R01-CA-115296 and R21-CA-130297), the Dutch Cancer Society (grants UL2010-4732 and PGF 2009-4344), and a VIDI grant from the Dutch national research council (NWO-STW-11272). This research was performed within the framework of the Centre for Translational Molecular Medicine, project MUSIS (grant 03O-202). J.R.v.d.V. is an MD-medical research trainee funded by the Netherlands Organization for Health Research and Development (grant 92003593).

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