Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study

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Summary

Background TargomiRs are minicells (EnGeneIC Dream Vectors) loaded with miR-16-based mimic microRNA (miRNA) and targeted to EGFR that are designed to counteract the loss of the miR-15 and miR-16 family miRNAs, which is associated with unsuppressed tumour growth in preclinical models of malignant pleural mesothelioma. We aimed to assess the safety, optimal dosing, and activity of TargomiRs in patients with malignant pleural mesothelioma.

Methods In this first-in-man, open-label, dose-escalation phase 1 trial at three major cancer centres in Sydney (NSW, Australia), we recruited adults (aged ≥18 years) with a confirmed diagnosis of malignant pleural mesothelioma, measurable disease, radiological signs of progression after previous chemotherapy, Eastern Cooperative Oncology Group performance status of 0 or 1, life expectancy of 3 months or more, immunohistochemical evidence of tumour EGFR expression, and adequate bone marrow, liver, and renal function. Patients were given TargomiRs via 20 min intravenous infusion either once or twice a week (3 days apart) in a traditional 3 + 3 dose-escalation design in five dose cohorts. The dose-escalation steps planned were 5×10^9, 7×10^9, and 9×10^9 TargomiRs either once or twice weekly, but after analysis of data from the first eight patients, all subsequent patients started protocol treatment at 1×10^9 TargomiRs. The primary endpoints were to establish the maximum tolerated dose of TargomiRs as measured by dose-limiting toxicity, define the optimal frequency of administration, and objective response (defined as the percentage of assessable patients with a complete or partial response), duration of response (defined as time from the first evidence of response to disease progression in patients who achieved a response), time to response (ie, time from start of treatment to the first evidence of response) and overall survival (defined as time from treatment allocation to death from any cause). Analyses were based on the full analysis set principle, including every patient who received at least one dose of TargomiRs. The study was closed for patient entry on Jan 3, 2017, and registered with ClinicalTrials.gov, number NCT02369198, and the Australian Registry of Clinical Trials, number ACTRN12614001248651.

Findings Between Sept 29, 2014, and Nov 24, 2016, we enrolled 27 patients, 26 of whom received at least one TargomiR dose (one patient died before beginning treatment). Overall, five dose-limiting toxicities were noted: infusion-related inflammatory symptoms and coronary ischaemia, respectively, in two patients given 5×10^9 TargomiRs twice weekly; anaphylaxis and cardiomyopathy, respectively, in two patients given 5×10^9 TargomiRs once weekly but who received reduced dexamethasone prophylaxis; and non-cardiac pain in one patient who received 5×10^9 TargomiRs once weekly. We established that 5×10^9 TargomiRs once weekly was the maximum tolerated dose. TargomiR infusions were accompanied by transient lymphopenia (25 [96%] of 26 patients), temporal hypophosphataemia (17 [65%] of 26 patients), increased aspartate aminotransferase or alanine aminotranferase (six [23%] of 26 patients), and increased alkaline phosphatase blood concentrations (two [8%]). Cardiac events occurred in five patients: three patients had electrocardiographic changes, one patient had ischaemia, and one patient had Takotsubo cardiomyopathy. Of the 22 patients who were assessed for response by CT, one (5%) had a partial response, 15 (68%) had stable disease, and six (27%) had progressive disease. The proportion of patients who achieved an objective response was therefore one (5%) of 22, and the duration of the objective response in that patient was 32 weeks. Median overall survival was 200 days (95% CI 94–358). During the trial, 21 deaths occurred, of which 20 were related to tumour progression and one was due to bowel perforation.

Interpretation The acceptable safety profile and early signs of activity of TargomiRs in patients with malignant pleural mesothelioma support additional studies of TargomiRs in combination with chemotherapy or immune checkpoint inhibitors.

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Research in context

Evidence before this study

We searched PubMed with the terms "malignant pleural mesothelioma", "malignant mesothelioma", "mesothelioma", "first line", "second line", "third line", "phase 1", "phase 2", "phase 3", "relapsed", "refractory", "recurrent", "minicell", "microRNA" (miRNA), "miR-16", and "TargomiR" for articles published in English between Sept 28, 1999, and Sept 28, 2014. Malignant pleural mesothelioma, which is linked to asbestos exposure and deficiency of tumour suppressor microRNAs (miRNAs), is almost invariably fatal. Palliative chemotherapy has been the standard of care for more than 10 years, but usually less than 40% of patients obtain a partial response to the standard regimen of pemetrexed and cisplatin, and all patients eventually relapse. The addition of bevacizumab to standard chemotherapy increased overall survival by 2·7 months on average compared with chemotherapy alone in a 2016 study, which emphasised the need for better treatments for malignant pleural mesothelioma. The efficacy of the miR-16 mimic in cell culture and xenograft models provided evidence for translation of this novel treatment approach into the clinic.

Added value of this study

The results of this first-in-man study of TargomiRs showed that a dose of 5×10^9 TargomiRs per week was well tolerated and was accompanied by early signs of antitumour activity. This study provides important safety data and shows encouraging response and survival in patients with malignant pleural mesothelioma.

Implications of all the available evidence

Our results need to be confirmed in larger studies. Preclinical data have emerged that provide a rationale for combining TargomiRs with chemotherapy and immune checkpoint inhibitors. A randomised phase 2 study with TargomiRs added to standard chemotherapy is one potential way forward. Feasibility studies preceding a comparative trial could provide the opportunity to verify the suppression of proliferative signalling by TargomiRs in tumour tissue.

Introduction

Only 40% of patients with malignant pleural mesothelioma respond to standard platinum–pemetrexed combinations, and this response is usually short-lived.[1] Although addition of bevacizumab[2] to these regimens offers a potential survival gain of 2–3 months, malignant pleural mesothelioma remains profoundly resistant to therapy, and novel treatment approaches are urgently needed.[1] Non-coding RNAs have gained considerable attention for their crucial role in cell biology and their prevalent deregulation in human cancer. MicroRNAs (miRNAs), which belong to a highly conserved subclass of non-coding RNAs, are short sequences (18–24 nucleotides) that target messenger RNAs (mRNAs) through partial base pairing to sites found in their 3′ untranslated region.[3] More than 1000 human miRNAs have been identified,[4] each of which is capable of targeting multiple mRNAs,[5] and thus they have a major role in the control of gene and protein expression.

Many studies have provided evidence that miRNA dysregulation is causal in the development of cancer and that miRNAs can act as tumour suppressors. The first link between loss of miR-15 and miR-16 and malignancy was made in leukaemia.[6] These miRNAs are also dysregulated in solid tumours, including lung cancer and prostate cancer.[7,8] The prime targets of miR-15 and miR-16 include the mRNAs of *BCL2, CDK1, ETS1,* and *JUN,* which are all involved in cancer progression. We showed that several members of the miR-16 family were downregulated in malignant pleural mesothelioma.[9] Restoration of expression of miR-16 with a miR-16 miRNA mimic led to a pronounced inhibition of growth of malignant pleural mesothelioma cells in vitro. In nude mice with human mesothelioma xenografts, miR-16

mimic was administered intravenously by loading the mimic into minicells known as EnGeneIC Dream Vectors (EDVs).[9] EDVs are a unique delivery system with the ability to transport cargoes of different types.[10] These minicells were targeted to EGFR, which is expressed in 44–97% of malignant pleural mesothelioma samples.[11,12] This formulation led to a substantial dose-dependent inhibition of xenograft tumour growth.

In this first-in-human study, we aimed to test a miR-16-based miRNA mimic packaged in TargomiRs—EDVs targeted to EGFR on the surface of mesothelioma cells by bispecific panitumumab-based antibodies[13]—in patients with malignant pleural mesothelioma in whom previous chemotherapy was unsuccessful.

Methods

Study design and participants

We did a first-in-man, open label, phase 1, dose-escalation study at three major cancer centres in Sydney (NSW, Australia; appendix p 3). Eligible patients were aged 18 years or older and had pathologically confirmed malignant pleural mesothelioma, radiological progression after one or more lines of therapy, a life expectancy of 3 months or more, immunohistochemical evidence of tumour EGFR expression, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, measurable disease per the modified Response Evaluation Criteria in Solid Tumors guidelines (modified RECIST),[14] and adequate bone marrow (ie, platelet count 100×10^9–800×10^9 per L, haemoglobin concentration ≥90 g/L, and absolute neutrophil count ≥$1·5 \times 10^9$ per L), liver (ie, total bilirubin concentrations ≤1·5 times the upper limit of normal [ULN], and alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase concentrations ≤2·5 times the

ULN), and renal function (glomerular filtration rate ≥60 mL/min per 1·73 m²). Exclusion criteria were previous or concurrent cancer (except cervical cancer in situ, non-melanoma skin cancer, and superficial bladder cancer [Ta, Tis, and T1]), known hypersensitivity to bacterial proteins, active infection, presence of major cardiac risk factors, and major surgical procedures in the preceding 4 weeks. A full list of inclusion and exclusion criteria is in the appendix.

The study was approved by institutional human research ethics committees at each centre, and all participants provided written informed consent. The protocol is provided in the appendix.

Procedures

TargomiRs were suspended in 20 mL of injectable saline and were administered via a peripheral vein cannula at a constant infusion rate (1 mL/min) by a Nikki T34 ambulatory syringe pump (Caesarea Medical Electronics, Caesarea, Israel) in outpatient chemotherapy wards. Patients were given 25 mg oral promethazine or 10 mg loratidine 60 min before each infusion and 1000 mg oral paracetamol and 8 mg intravenous dexamethasone 30 min before each infusion. We tested a starting miR-16-based miRNA mimic dose of 1·5 µg RNA in 5×10^9 EDVs. Because twice weekly doses were more effective than once weekly doses in xenografted mice,[9] we studied once and twice weekly dosing of TargomiRs (3 days apart) with a conventional 3 + 3 dose-escalation design. The initial planned dose-escalation cohorts were 5×10^9 TargomiRs once or twice weekly, followed by 7×10^9 once or twice weekly, followed by 9×10^9 once or twice weekly. On the basis of observations from Solomon and colleagues,[15] patients with increased interleukin-6 concentrations at baseline (>5 pg/mL) started treatment at 1×10^9 TargomiRs and escalated to the full dose for the dose level of their cohort after 2 weeks (known as the adapted scheme), whereas patients with serum interleukin-6 concentrations of less than 5 pg/mL commenced treatment directly at the dose level of their cohort (panel). However, on the basis of toxicity and cytokine data from the first eight patients, the protocol was amended on April 10, 2015, and the adapted scheme was used in all subsequent cohorts and patients received intensified ECG monitoring (echocardiograms and technetium-99m [^{99m}TC] sestamibi myocardial perfusion scans at baseline and if electro-cardiographic changes were noted; panel). On the basis of the lack of robust data on the effectiveness of dexamethasone in the management of anaphylaxis and the potential undesired immunosuppressive effect of dexamethasone, a protocol amendment was effectuated on the Sept 25, 2015, to explore the reduction of doses of dexamethasone prophylaxis. Cohort 5 was added to reduce the dose of dexamethasone prophylaxis (panel).

If treatment was well tolerated (assessed weekly), patients continued treatment (at the planned level or frequency) until reassessment by CT and PET–CT at 8 weeks (or earlier if patients were suspected to be unable

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Panel: Study dosing cohorts

Cohort 1

Patients with low interleukin-6 concentrations (<5 pg/mL): 5×10^9 TargomiRs weekly for 8 weeks

Patients with high interleukin-6 concentrations (≥5 pg/mL), adapted regimen: 1×10^9 TargomiRs weekly (week 1); 2×10^9 TargomiRs weekly (week 2); 5×10^9 TargomiRs weekly (weeks 3–8)

Cohort 2

Patients with low interleukin-6 concentrations (<5 pg/mL): 5×10^9 TargomiRs twice weekly

Patients with high interleukin-6 concentrations (≥5 pg/mL), adapted regimen: 1×10^9 TargomiRs twice weekly (week 1); 2×10^9 TargomiRs twice weekly (week 2); 5×10^9 TargomiRs twice weekly (weeks 3–8)

Cohort 3

All patients, adapted regimen: 1×10^9 TargomiRs weekly (week 1); 2×10^9 TargomiRs weekly (week 2); 5×10^9 TargomiRs weekly (weeks 3–8), plus electrocardiogram (ECG) monitoring (echocardiograms and technetium-99m [^{99m}TC] sestamibi myocardial perfusion scans at baseline and if electrocardiographic changes were noted)

Cohort 4

All patients, adapted regimen: $2·5 \times 10^9$ TargomiRs twice weekly, plus ECG monitoring (echocardiograms and technetium-99m [^{99m}TC] sestamibi myocardial perfusion scans at baseline and if electrocardiographic changes were noted)

Cohort 5

All patients, adapted regimen: 1×10^9 TargomiRs weekly (week 1); 2×10^9 TargomiRs weekly (week 2); 5×10^9 TargomiRs weekly (weeks 3–8), plus electrocardiographic monitoring (echocardiograms and technetium-99m [^{99m}TC] sestamibi myocardial perfusion scans at baseline and if electrocardiographic changes were noted). Prophylactic dexamethasone tapering as follows: 4 mg dexamethasone (weeks 1–4); 2 mg dexamethasone (weeks 5–6); 1 mg dexamethasone (weeks 7–8).

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to complete the first 8 treatment weeks). Patients without disease progression after 8 weeks of treatment were permitted to continue TargomiR treatment for another 8 weeks, followed by another reassessment, ongoing. Patients were followed up weekly. All adverse events were monitored throughout the study period (until 30 days after participants' last TargomiR dose) and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE; version 4.03). Dose-limiting toxicity was defined as a grade 3 or 4 adverse event that was attributable to study treatment. Grade 3 or 4 haematological and biochemical abnormalities that were not associated with symptoms and reversed within 24 h were not considered dose limiting. Resumption of treatment for patients with a dose-limiting toxicity was permitted (when clinically appropriate) if the severity of the toxicity fell to grade 1 or lower and treatment was interrupted for no more than 3 weeks. All events occurring during the first 8 weeks of treatment were included in our toxicity analyses.[16]

Full clinical histories were taken at screening. Anatomical (CT) and functional (PET–CT) tumour imaging were done at baseline and after 8 weeks of TargomiR treatment (appendix p 1). CTs were independently reviewed by an

expert radiologist (LR) on the basis of modified RECIST criteria for malignant pleural mesothelioma. If treatment discontinuation was anticipated before 8 weeks, patients were encouraged to undergo CT and PET–CT earlier. Pulmonary function testing (forced expiratory volume in 1 s and forced vital capacity) was mandatory at baseline but optional at follow-up. Laboratory tests for haematology, chemistry, urinalysis, and cytokine measurement were done within the 14 days before the first dose of TargomiRs, and then repeated 1 h before every TargomiR infusion, 3 h and 24 h after infusion for the first 4 weeks, and 3 h after infusion for the remaining treatment weeks. Physical examinations, assessments of ECOG performance status, and administration of quality-of-life questionnaires were done weekly. The quality-of-life assessments were based on answers to question 30 of the European Organisation for Research and Treatment of Cancer (EORTC) QLQ-C30 questionnaire: How would you rate your overall quality of life during the past week? Scores were between 1 (very poor) and 7 (excellent).

After a protocol amendment on June 14, 2016, PD-L1 expression in diagnostic biopsies taken before study entry was assessed by immunohistochemistry (E1L3N clone, Cell Signaling Technology; Danvers, MA, USA) and the percentage of tumour cells with positive membranous staining was scored by an experienced pathologist (WAC). The rationale for assessment of PD-L1 expression was based on findings suggesting a potential predictive value of PD-L1 testing for immunotherapy-targeting checkpoint inhibitors in malignant pleural mesothelioma.[17] We considered the immune-activating capacity of EDVs a valid reason to explore the potential predictive role of PD-L1 expression. Anti-lipopolysaccharide IgG antibody titres were assessed weekly in the first five patients enrolled. They were established by reacting serial dilutions of serum with 96-well microtitre plates coated with *Salmonella typhimurium* lipopolysaccharide and detected with goat anti-human IgG-Fc horseradish peroxidase conjugate. The antibody titre was quantified by reading the absorbance at 450 nm and determining the serum dilution that gave the half maximal A_{450} nm reading. Sampling for anti-*Salmonella* antibodies was done at screening and weekly during TargomiR therapy. The antibody titre cutoff was established from patient samples at screening and calculated by adding 3 SDs to the mean of the titre. Antibody titres to the EGFR-targeting single-chain bispecific antibody were determined by reacting serial dilutions of serum with 96-well microtitre plates coated with the anti-EGFR single-chain bispecific antibody and detected with protein G–horseradish peroxidase conjugate. The antibody titre was quantitated by reading the absorbance at 450 nm and establishing the serum dilution that gave a half-maximal A450 nm reading.

Outcomes
The primary endpoints were to establish the maximum tolerated dose of TargomiRs as measured by dose-limiting toxicity, define the optimal frequency of administration, and objective response (defined as the percentage of assessable patients with a complete or partial response), duration of response (defined as time from the first evidence of response to disease progression in patients who achieved a response), time to response (ie, time from start of treatment to the first evidence of response) and overall survival (defined as time from treatment allocation to death from any cause). Secondary endpoints were quality of life, change in ECOG performance status scores from baseline to reassessment by CT and PET–CT, and changes in pulmonary function (from baseline to assessment at 8 weeks). Changes in immune and cytokine markers were exploratory endpoints.

Statistical analysis
Analysis of tolerability and toxicity was based on all patients in each dosing cohort who received at least one dose of the study drug. Response analysis was done in patients who underwent a baseline assessment and at least one scheduled post-baseline tumour assessment by CT and PET–CT. The maximum tolerated dose was based on the dose-limiting toxicities noted during the first 8 weeks of treatment. We used descriptive statistics to summarise data. Categorical data are expressed as counts and percentages, and continuous data as mean (SD) or median (IQR). We used the Kaplan-Meier method to analyse overall survival; patients still alive were censored at the date last contacted. Median overall survival was estimated with the Kaplan-Meier method. Potential prognostic factors for overall survival (whole blood cell counts, neutrophil-to-lymphocyte ratio, PD-L1 expression, and blood concentrations of C-reactive protein, interleukin 6 and TNFα) were explored in post-hoc univariate Cox regression analyses (both adjusted and unadjusted for cohort). The proportional hazard assumption was verified with the Schoenfeld residuals test. All statistical tests were two-sided and assumed a two-sided significance level of 0·05. We used (version 12.1) for all analyses. The trial was registered with ClinicalTrials.gov (NCT02369198) and the Australian Registry of Clinical Trials (ACTRN12614001248651).

Role of the funding source
EnGeneIC, which provided the TargomiRs and did the cytokine and antibody assessments, had a role in study design. The funders did not have roles in data collection, analysis, or interpretation, or in writing of the final report. NvZ, YH, and AC had access to all study data, and NvZ had final responsibility for the decision to submit for publication.

Results
We screened 39 patients with malignant pleural mesothelioma between Sep 29, 2014, and Nov 24, 2016, 12 (31%) of whom were excluded (figure 1). The remaining 27 patients were eligible for inclusion in our study (table 1). One patient assigned to cohort 5 died before receiving

treatment and was therefore excluded from subsequent analyses. Six patients were enrolled in cohort 1, of whom three had increased interleukin-6 concentrations at baseline and received the adapted regimen. One patient in this cohort had dose-limiting severe non-cardiac chest pain after their first dose of 5×10^9 TargomiRs (table 2). After a temporary dose reduction, this patient continued with 5×10^9 TargomiRs weekly, which was well tolerated. Four patients enrolled in cohort 2, and dose-limiting toxicities were reported in two—a substantial inflammatory reaction and coronary ischaemia, respectively (table 2). We thus extended our study with the addition of six patients in cohort 3, in which all patients received the adapted regimen and had intensified cardiac monitoring. No dose-limiting toxicities were noted in this cohort.

Two patients enrolled in cohort 4 and no dose-limiting toxicities were noted (table 2). However, after reviewing both twice-weekly dosing cohorts together (cohorts 2 and 4), we decided that twice-weekly administration was too substantial a burden (in terms of both adverse events and the need for twice weekly clinic visits) to continue that dosing schedule. We then investigated the consequences of reducing dexamethasone prophylaxis in eight patients in cohort 5 (excluding the patient who died). A dose-limiting Takotsubo (stress) cardiomyopathy occurred after dose two in one patient (table 2). Enrolment was stopped after a patient in this cohort developed a dose-limiting anaphylactoid reaction (table 2). We established that 5×10^9 TargomiRs once weekly was the maximum tolerated dose.

A median of eight TargomiR doses (IQR 7–15) was received by 26 patients. TargomiR infusions were followed by rigors and fever within 60–90 min in all patients (table 2). Inflammatory symptoms were self-limiting and usually lasted 30–50 min (data not shown). Adverse event frequency and severity by dose is shown in the appendix (pp 2, 4). 14 (54%) of 26 patients reported transient non-cardiac chest pain after TargomiR infusion. The pain was localised at or near the known sites of pleural disease and best described as an exacerbation of tumour pain in all patients. A temporary 2-week dose reduction was judged necessary for one patient in cohort 1 who had severe chest pain after the first TargomiR dose. In one patient in cohort 2 a temporary 2-week dose reduction was advised after a pronounced inflammatory reaction after the second TargomiR dose during the first week. Inflammatory toxicity and non-cardiac chest pain in weeks 1–3 are shown in table 3 in the first eight patients in the study. With adapted doses, infusion-related reactions were less severe and less frequent (table 3); this trend was also noted upon continuing treatment. Chest pain intensity also seemed to decrease when therapy continued. TargomiR infusions were accompanied by transient lymphopenia (25 [96%] of 26 patients), temporal hypophosphataemia (17 [65%] of 26 patients), increased aspartate aminotransferase or alanine aminotranferase (six [23%] of 26 patients), and increased alkaline phosphatase blood concentrations (two [8%]; table 2).

Abnormal cell counts (25 [96%] of 26 patients) and low plasma phosphate (17 [65%] of 26 patients) resolved within 24 h and were not considered dose limiting.

Electrocardiographic changes, which were noted in three patients, were transient and did not recur upon continuation of treatment (table 4). Takotsubo
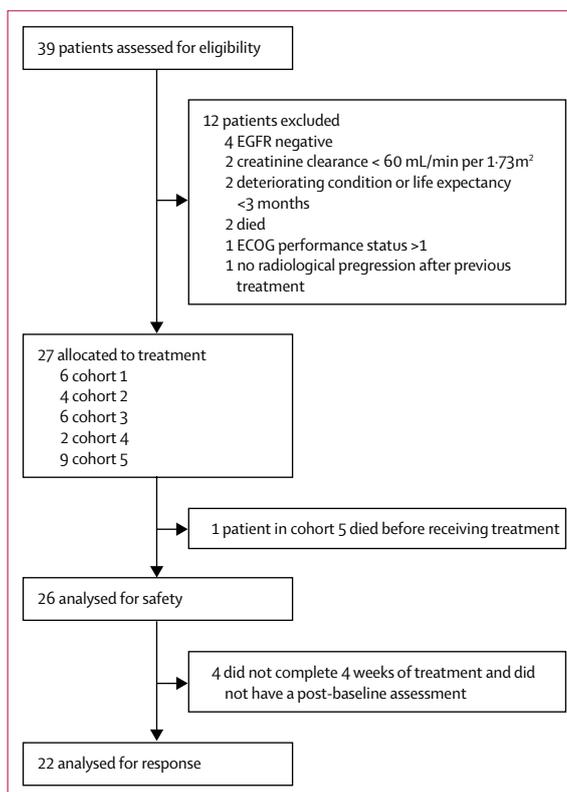
Figure 1: Trial flow diagram

	Patients (n=27)
Median age (IQR), years	66 (IQR 60–74)*
Sex	
Male	21 (78%)
Female	6 (22%)
Tumour histology	
Epithelioid	24 (89%)
Sarcomatoid	2 (7%)
Mixed	1 (4%)
Previous treatment	
Chemotherapy	27 (100%)†
Surgery	5 (19%)
Radiotherapy	7 (26%)
Eastern Cooperative Oncology Group performance status	
0	8 (30%)
1	19 (70%)

Data are n (%), unless otherwise specified. *Four patients were older than 75 years. †Participants had a median of two previous chemotherapy lines (IQR 1–2).

Table 1: Patient demographics and clinical characteristics

	Grade 1–2					Grade 3					Grade 4				
	Cohort 1 (n=6)	Cohort 2 (n=4)	Cohort 3 (n=6)	Cohort 4 (n=2)	Cohort 5* (n=8)	Cohort 1 (n=6)	Cohort 2 (n=4)	Cohort 3 (n=6)	Cohort 4 (n=2)	Cohort 5* (n=8)	Cohort 1 (n=6)	Cohort 2 (n=4)	Cohort 3 (n=6)	Cohort 4 (n=2)	Cohort 5* (n=8)
Cardiac															
Cardiomyopathy (with hypoxia)	..	..	..	..	..	..	..	..	..	1 (13%)*	..	..	..	..	..
Electrocardiographic (T-wave) changes	..	2 (50%)	1 (17%)	..	..	..	..	..	..	..	..	..	..	..	..
Ischaemia‡	..	..	..	..	..	..	1 (25%)*	..	..	..	..	..	..	..	..
General															
Fatigue or malaise	1 (17%)	..	1 (17%)	..	4 (50%)	..	1 (25%)*	1 (17%)	..	..	..	..	..	..	..
Infusion-related (cytokine-release) reactions (including chills, shivering, rigor, fever, tachycardia, increased blood pressure, and night sweats)	6 (100%)	4 (100%)	5 (83%)	2 (100%)	8 (100%)	..	..	..	..	..	..	..	..	..	..
Dyspnoea	2 (33%)	2 (50%)	1 (17%)	1 (50%)	6 (75%)	..	..	1 (17%)	..	..	..	..	..	..	..
Headache	2 (33%)	1 (25%)	2 (33%)	..	3 (38%)	..	..	..	..	..	..	..	..	..	..
Pain (non-cardiac)	3 (50%)	2 (50%)	4 (67%)	1 (50%)	4 (50%)	1 (17%)*	..	..	..	..	..	..	..	..	..
Other															
Anaphylactoid reaction (with bronchospasm and hypoxia)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1 (13%)*
Anorexia	2 (33%)	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Lethargy	3 (50%)	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Insomnia	..	1 (25%)	1 (17%)	..	..	..	..	..	..	..	..	..	..	..	..
Cough	..	1 (25%)	1 (17%)	..	1 (13%)	..	..	..	..	..	..	..	..	..	..
Hoarseness	..	1 (25%)	1 (17%)	..	..	..	..	..	..	..	..	..	..	..	..
Laboratory abnormalities															
Increased alkaline phosphatase	..	..	1 (17%)	..	..	..	..	1 (17%)	..	..	..	..	..	..	..
Increased alanine aminotransferase	1 (17%)	..	1 (17%)	..	..	..	..	1 (17%)	..	..	..	..	..	..	..
Increased aspartate aminotransferase	1 (17%)	..	2 (33%)	..	..	..	..	..	..	..	..	..	..	..	..
Increased γ-glutamyl transferase	4 (67%)	3 (75%)	2 (33%)	1 (50%)	1 (13%)	..	..	3 (50%)	..	1 (13%)	..	..	..	..	..
Hypokalaemia	..	..	2 (33%)	..	..	..	..	..	..	..	..	..	..	..	..
Hyperkalaemia	3 (50%)	1 (25%)	..	..	2 (25%)	..	2 (50%)	..	..	..	..	..	..	..	..
Hypophosphataemia	2 (33%)	2 (50%)	4 (67%)	1 (50%)	3 (38%)	1 (17%)	..	2 (33%)	1 (50%)	1 (13%)	..	..	..	..	..
Lymphopenia	..	..	..	..	..	2 (33%)	1 (25%)	1 (17%)	2 (100%)	3 (38%)	4 (67%)	3 (75%)	5 (83%)	..	4 (50%)

Data are n (%). Grade 1–2 events that occurred in fewer than two patients across all cohorts are not included. *Toxicity was considered to be dose limiting. ‡Sestamibi perfusion study.

Table 2: **Summary of treatment-related adverse events in first 8 weeks of treatment**

cardiomyopathy with typical echocardiographic and electrocardiographic findings and pulmonary oedema were noted shortly after the second dose of TargomiRs in a 74-year-old woman (table 4). The patient improved after treatment with an angiotensin-converting enzyme inhibitor and diuretics but discontinued treatment. Asymptomatic coronary ischaemia, as evidenced by T-wave inversion and ST depression, was noted shortly after the seventh dose of TargomiRs in a 70-year-old man; an increased Troponin T was recorded at 48 h and anteroseptal ischaemia (sestamibi scan) was noted 5 days later (table 4). This patient (patient nine) discontinued treatment due to this serious adverse event. Data for all patients who discontinued treatment due to adverse events is shown in the appendix (p 2).

Tapering dexamethasone dose before treatment in cohort 5 was associated with anaphylactoid symptoms in one patient and potentially contributed to cardiomyopathy in a second patient. Anaphylactoid symptoms (stridor and swollen tongue) responded quickly to hydrocortisone, antihistamines, and salbutamol. We were unable to confirm increased cytokine responses after reduction of dexamethasone doses (appendix pp 5–7).

Anti-lipopolysaccharide antibody titres gradually fell after a modest rise during the first 3 weeks of treatment in the first five patients. Titres were low in the patient who developed anaphylaxis (patient 25; appendix p 9). Antibody titres to the EGFR-targeting single-chain bispecific antibody monitored in the first four patients did not show any appreciable change (data not shown).

Patients were followed up for a median of 184 days (IQR 76–358). At the data cutoff date (March 17, 2017), five (19%) patients were alive, with two (8%) surviving more than 2 years after their first TargomiR dose (figure 2A). Seven patients (27%) received treatment for longer than 8 weeks.

22 patients had a baseline CT and at least one post-baseline CT: 16 (73%) had repeat scans after 8 weeks, and six (27%) who discontinued treatment early were scanned between weeks 4 and 7 (figure 2B). One (5%) patient had a partial response, 15 (68%) had stable disease, and six (27%) had progressive disease. The proportion of patients who achieved an objective response was therefore one (5%) of 22. The duration of the objective response in that patient was 32 weeks. The patient with a partial response showed substantial improvement in pulmonary function: forced vital capacity increased by 28% and forced expiratory volume in 1 s increased by 20%. Repeat pulmonary function data were not available for other patients. PET–CT was done in 15 patients (58%) a mean of 65 days (SD 7) after the baseline PET–CT scan (figure 2B). At the time of the post-treatment scan, after the first cycle of treatment, reduced maximum pleural

|	Full dose (n=4)		Adapted dose (n=4)	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Infusion-related reaction*				
Week 1	3 (75%)	1 (25%)	4 (100%)	0 (0%)
Week 2	3 (75%)	0 (0%)	3 (75%)	0 (0%)
Week 3	2 (50%)	0 (0%)	2 (50%)	0 (0%)
Non-cardiac chest pain				
Week 1	0 (0%)	1 (25%)	0 (0%)	0 (0%)
Week 2	1 (25%)	0 (0%)	1 (25%)	0 (0%)
Week 3	1 (25%)	0 (0%)	0 (0%)	0 (0%)

Data are n (%) and are for patients in cohorts 1 and 2. Only eight patients are included because dose adaptation was introduced for every patient from cohort 3. *Chills, shivering, rigor, fever, tachycardia, increased blood pressure, hot flush, or night sweats.

Table 3: Treatment-related inflammatory symptoms and pain in full dose and adapted regimen groups

	Cohort	Sex	Age (years)	Cardiac history	Event (timepoint)	ECG	Troponin T	Echocardiogram	Sestamibi scan
7	2	Female	60	None	ECG change (week 1, dose 2)	Intraventricular conduction delay at 0 h, T-wave flattening* at 4 h	0·003 µg/L at 8 h, 0·006 µg/L at 20 h	Not done	Not done
9	2	Male	70	Hypertension (2005), surgery for abdominal aortic aneurysm (2010)	Ischaemia (week 4, dose 1)	T-wave inversion and ST depression at 24 h*	0·057 µg/L at 48 h	Not done	Positive stress sestamibi scan for ischaemia (no chest pain) and normal LVEF at 7 days
13	3	Male	53	High cholesterol (2011)	ECG change (week 2)	Intraventricular conduction delay at 0 h, T-wave flattening* at 3 h	0·015 µg/L at 0 h, 0·012 µg/L at 24 h	LVEF >50% at baseline and 48 h	No ischaemia and normal LVEF at baseline
14	3	Male	65	None	ECG change (week 3)	Non-specific T wave abnormality* at 4 h	0·004 µg/L at 0 h, 0·003 µg/L at 3 h, 0·006 µg/L at 24 h	LVEF >50% at baseline and 48 h	No ischaemia and normal LVEF at baseline and 24 h
26	5	Female	74	Atrial fibrillation (2010)	Takotsubo cardiomyopathy (week 2)	T-wave inversion and sinus tachycardia at 4 h, biphasic T-wave at 24 h, atrial fibrillation at 6 days	0·017 µg/L at 0 h, 0·039 µg/L at 3 h, 0·080 µg/L at 24 h, 0·028 µg/L at 7 days	LVEF >50% at baseline, 30% with apical akinesis at 22 h, and >50% at 7 days	No ischaemia and normal LVEF at baseline

ECG=electrocardiogram. LVEF=left ventricular ejection fraction. *ECG normalised within 24 h.

Table 4: Description of cardiac events, by patient number
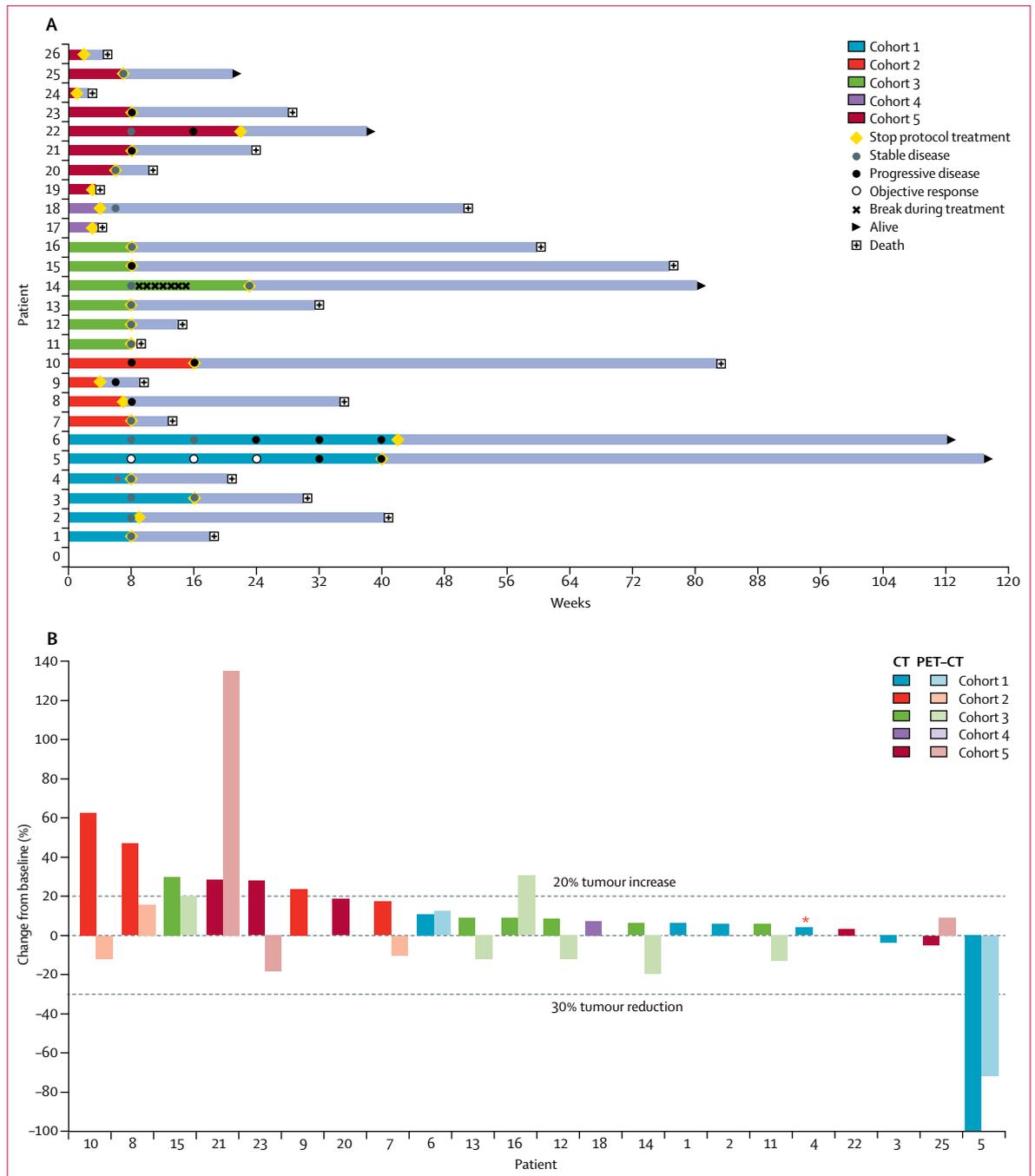
***Figure 2*: Swimmer plot of time on study treatment, response status, and survival (A); and waterfall plot of pleural CT and PET–CT findings (B)**
In (B), CT findings are presented for 22 patients according to modified Response Evaluation Criteria in Solid Tumors. PET–CT findings show the mean percentage change in maximum standardised uptake value from five pleural regions of interest in the affected hemithorax after one cycle of treatment (appendix). *Patient four got a second primary cancer diagnosis at 7 weeks.

glucose metabolism compared with baseline was noted in nine (60%) of 15 patients (mean 19·3; range 0·3–71·8).

Median overall survival was 200 days (95% CI 94–358; figure 3). During the trial, 21 deaths occurred (not including the patient who died before treatment). 20 deaths were related to tumour progression and one

patient died as a result of a bowel perforation (due to a second primary tumour).

Post-hoc univariate analysis of the association of C-reactive protein concentration, the neutrophil-to-lymphocyte ratio, and PD-L1 expression with overall survival are shown in table 5. Additional post-hoc

univariate analyses of overall survival are shown in the appendix (p 2). Quality-of-life and ECOG performance status scores are shown in the appendix (pp 8–9).

Discussion

In this first-in-man phase 1 study of TargomiRs, a novel minicell-based formulation for a miR-16-based miRNA mimic, 5×10⁹ TargomiRs per week after dose escalation was well tolerated and was accompanied by early signs of antitumour activity in patients with malignant pleural mesothelioma. Dose-limiting toxicities were noted in five patients and were associated with non-adapted or twice weekly TargomiR dosing and reduced dexamethasone prophylaxis. Stepwise increases in dose and continued treatment seemed to confer a degree of tolerance to the effects of TargomiR infusions.

The transient (non-cardiac) chest pain reported by more than half the patients after TargomiR infusion was a notable occurrence. In experimental animal models, EDVs accumulate rapidly in xenograft tumours, which is probably a passive process occurring as a result of leaky tumour vasculature.[18] Thus, the chest pain might have resulted from TargomiRs accumulating at the tumour site. Evidence from animal studies suggests that small interfering RNAs lead to target knockdown.[19] We could not, however, confirm that the TargomiRs delivered the miRNA mimic to tumour cells because additional biopsies were not done in our study. We also could not do pharmacological studies because EDVs disappear rapidly from the circulation (within minutes after injection) and cannot be reliably used for pharmacological analyses.[13,20] Additionally, concentrations of cell-free miRNAs—and particularly miR-16—are abundant in the blood and vary little between individuals, or between patients and controls.[21] Even a theoretical loss of the entire mimic cargo of 5×10⁹ EDVs into the circulation would not lead to the detectable presence of the miRNA mimic in plasma. Detection of our miR-16-based mimic in tumour biopsies will be needed to verify delivery in future studies, and will be aided by its unique sequence.

The haematological and biochemical abnormalities noted after TargomiR infusion resemble those reported in earlier studies of EDVs.[15,22] Rapid-onset lymphopenia and hypophosphataemia suggest a self-limiting immune reaction. Rapidly reversible hypophosphataemia, probably caused by depletion of ATP stores of immune cells, has been noted in the early stages of sepsis.[23] Because of the association between hypophosphataemia and arrhythmia,[24] we recommend prophylactic intravenous phosphorus in future studies. The transient increases in aminotransferases and alkaline phosphatase that we noted were probably also part of the TargomiR-induced immune reaction. Reassuringly, concentrations of these enzymes decreased upon continuation of therapy.

We paid special attention to cardiovascular toxicity because evidence suggests that inhibition of miR-15 can protect against cardiac ischaemic injury and assist in
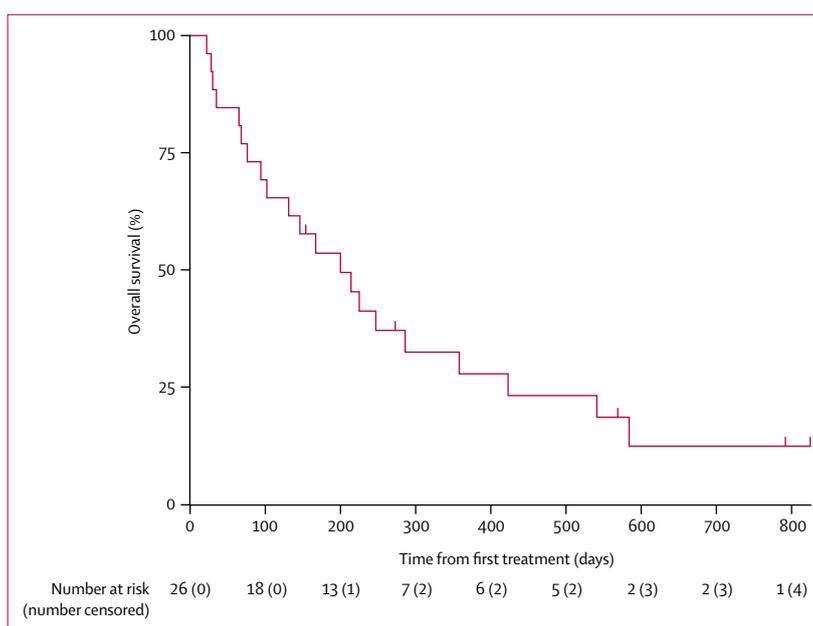
Figure 3: Kaplan-Meier graph of overall survival (all-cause mortality)
n=26. 21 deaths were recorded.

		Unadjusted		Adjusted for cohort	
	n	HR (95% CI)	p value	HR (95% CI)	p value
Neutrophil-to-lymphocyte ratio*					
≤4·3†	13	··	0·035	··	<0·0001
>4·3†	13	2·61 (1·07–6·36)	··	11·38 (3·05–42·39)	··
C-reactive protein*					
≤3429 nmol/L†	12	··	0·049	··	0·067
>3429 nmol/L†	12	2·6 (1·00–6·72)	··	3·37 (0·92–12·37)	··
PD-L1 expression					
<5%	16	··	0·15	··	0·033
≥5%	9	1·93 (0·79–4·72)	··	3·75 (1·12–12·58)	··

The proportional hazard assumption of Cox regression was satisfied for all factors. HR=hazard ratio. *Baseline values for neutrophil-to-lymphocyte ratio and C-reactive protein were assessed in a blood sample taken <1 h before the start of treatment. †Median (ie, median cutoff point).

Table 5: Univariate analyses of potential prognostic or predictive factors for overall survival

mammalian heart regeneration.[25] Although the transient increase in Troponin T concentrations and subtle electrocardiographic changes might have been caused by miRNA-mimic-related myocardial injury, the cardiac events could plausibly also have resulted from the inflammatory reaction elicited by EDVs. In experimental preclinical models of sepsis, structural myocardial changes have been noted.[26] Although cardiac toxicities were not recorded in two earlier phase 1 trials[15,22] of EDVs carrying docetaxel or paclitaxel, subtle electrocardiographic changes might have escaped detection because ECG follow-up was less stringent in those studies than in ours. The coronary ischaemia that occurred 5 days after protocol treatment was unlikely to have been directly related to TargomiR infusion. Pre-existing coronary artery disease is a more

plausible explanation. Tapering of dexamethasone might have contributed to the occurrence of stress-induced cardiomyopathy. Furthermore, the anaphylactoid symptoms noted in patient 25 underline the importance of full dexamethasone prophylaxis. ST elevations, myocardial infarction, or stroke were not recorded in patients receiving prolonged TargomiR treatment. Nonetheless, in view of the high prevalence of cardiotoxicity associated with oncological therapies and the advanced age of patients with malignant pleural mesothelioma, strict selection criteria and cardiac-monitoring protocols should be implemented in future studies of TargomiRs.

The efficient and safe delivery of nucleic acids remains the Achilles heel of gene therapy.[27] To our knowledge, our trial is the second phase 1 study of miRNA mimic-based therapy. MRX34, a miR-34a mimic carried by lipid nanoparticles, was studied in the first,[28] but major immune-related organ toxicities led to closure of this trial in September, 2016 (NCT01829971). Phase 1 studies of carrier-mediated miRNA mimic delivery do not allow definite conclusions to be drawn about the cause of adverse events (ie, they could be caused by the carrier or the mimic). However, the amount of mimic per dose reported in the miR-34a study was at least 1000 times greater than that used in our study. Double-stranded RNAs elicit a strong innate immune response,[29] and thus the mimic component could have contributed to the serious adverse events. Because the overall range of serious adverse events in our study was similar to those reported in earlier phase 1 studies with cytostatic-loaded EDVs, the carrier seems likely to have been primarily responsible for the inflammatory toxicities noted. In fact, the inflammatory response meant that the maximum amount of miRNA mimic that could be administered per week was 1·5 µg, which is only five times the dose capable of inducing tumour suppression in mice. Arguably, higher doses might be needed in human beings and other routes of TargomiR administration should be considered. The intrapleural route is feasible in malignant pleural mesothelioma, and might help to improve the therapeutic window of TargomiRs.[30,31]

In our previous work, we showed that miR-16 could sensitise malignant pleural mesothelioma tumour cells to pemetrexed and gemcitabine.[9] Therefore, investigation of the feasibility of the combination of TargomiRs with the standard cisplatin–pemetrexed combination in the clinic would also be appropriate. Additionally, translational studies done by our group have provided evidence for an inverse relation between miR-16 and PD-L1 expression in malignant pleural mesothelioma.[32] This interaction, which has also been reported for miR-34a,[33] provides a rationale for combination of immune checkpoint inhibitors with TargomiRs and investigation of whether the somewhat poor efficacy of checkpoint inhibitors in malignant pleural mesothelioma[34] can be boosted by combination with miRNA mimic. The exploratory analyses of biomarkers, which had limited power because

of our small dataset, suggested a potential prognostic value of the neutrophil-to-lymphocyte ratio, C-reactive protein, and PD-L1 expression in pretreated patients with malignant pleural mesothelioma, and underline the importance of including carefully designed biomarker studies when further investigating TargomiRs.

The results of second-line therapy in recurrent malignant pleural mesothelioma are moderate,[35] and only restricted clinical benefit can be expected from phase 1 exploration of new drugs.[36] The response and disease stabilisation data from our phase 1 study are therefore encouraging. The partial response in one patient, which has been previously reported in detail,[37] continued for 32 weeks. Long-term survival after a short treatment period was also noted. However, our study has several limitations. The sample size is too small to draw firm conclusions about time-to-event variables (eg, overall survival). We could not confirm the knockdown of miR-16 targets in tumour cells after TargomiR treatment because post-treatment biopsies were not taken. A tissue biopsy after treatment could also have addressed the possibility that our promising outcomes resulted from patient selection rather than treatment. The introduction of comparative trials early in the development of TargomiRs is expected to assist in solving this issue, as was the case in a study[38] of pegylated arginine deiminase in patients with malignant pleural mesothelioma. Comparative studies in which additional tissue biopsies are done would seem the ideal way forward with TargomiRs.

In conclusion, our findings show that TargomiRs, at a dose of 5×10^9 per week with full dexamethasone prophylaxis, were well tolerated by patients with refractory malignant pleural mesothelioma. We suggest that the safety and early signs of activity of TargomiRs warrant further clinical investigation.

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