Circulating Cells: the natural sensors of plaque and patient destabilization
Circulating blood cells are essential mediators in various types of disease. Particularly in cardiovascular disease, different blood cell subtypes are mechanistically involved in each disease stage. For most leukocyte subtypes a causal role in atherosclerosis has been established, either as activator or inhibitor of the inflammatory reaction that drives atherosclerotic disease progression. Besides their direct local contribution, circulating cells are activated by local foci and therefore can serve as messengers of the disease state. Nevertheless, the circulating blood cell is a relatively unexplored biomarker source in cardiovascular medicine. Instead, the majority of biomarker research focuses on plasma or serum markers, despite being mostly surrogates of the disease rather than effectors. The rapid development in biomedical technology allows exploring new sources like the circulating cell as a source for strong predictive biomarkers in atherosclerotic disease progression.

The CTMM CIRCULATING CELLS program brought together private and public partners with complementary expertise in assay development, biomarker identification, bioinformatics and clinicians to develop new biomarker detection methods that are based on the altered function and expression patterns within the most active elements of the circulation: the cells.

For this purpose, an elaborate cell isolation and analysis protocol was established and >700 cardiovascular disease patients were included in 4 Dutch academic hospitals. The circulating blood cells were used for biomarker identification and validation. Simultaneously, the technical challenges in cell derived markers were addressed and validated.

All together, the CIRCULATING CELLS consortium provided new, cell based, biomarkers for atherosclerotic disease progression and novel analytical platforms allowing cellular biomarker measurements for diagnosis, prevention and treatment of cardiovascular disease.
Aim
This project aimed to investigate ‘circulating cells’ (e.g. white blood cells and platelets) to identify biomarkers suitable for discriminating patients with an increased risk of developing unstable plaques. Novel technologies were developed and validated to allow screening cell-based biomarkers for the assessment of atherosclerotic risk and disease progression. Candidate biomarkers were selected from each data type (e.g. gene expression proteomics) and correlated with clinical covariates. Both “omics” and non-“omics” results were used with different methods for candidate biomarker selection based on predefined clinical selection criteria (e.g. MACE = major cardiovascular events).

Atherosclerosis
Atherosclerosis is a chronic inflammatory disease with symptoms that may appear in very late stages of disease development. People with risk factors, like diabetes, high blood pressure, hypercholesterolemia, smoking and obesity, have an increased risk of suffering from severe complaints due to disease progression at a younger age. Clinical manifestations associated with atherosclerotic disease progression are myocardial infarction, stroke and peripheral artery disease. Atherosclerosis can progress very slowly over decades without giving rise to any symptoms. However, with an increasing number of atherosclerotic plaques, the risk increases that some of these plaques are ‘unstable’. These unstable plaques can rupture and give rise to local thrombus formation with an acute luminal occlusion as the ultimate consequence. Discrimination of patients that hide multiple of these so-called ‘unstable vulnerable plaques’ is still difficult. To overcome these hurdles, the CIRCULATING CELLS consortium was established.

Clinical Need
Biomarkers to identify patients with an increased risk for the development of multiple unstable plaques and plaque rupture.

Tools
Tools to measure cell-based biomarkers to allow screening in a general practitioner’s office to assess atherosclerotic risk and disease progression.
New devices for risk assessment and monitoring of Acute Coronary Syndrome (ACS) Patients
Organisation and Partners

Advisory board
ISAC CTMM

SteeringCie
Partner Representatives
CTMM

Project Team
PI: Prof. G. Pasterkamp, PI (UMCU)
PM: Dr. I. Hoefer (UMCU)
Prof. M. Prins (Philips)
Dr. K. Redekop (EUR)
Dr. E. Erdtsieck-Erns (CTMM)

Workpackage leaders
WP0: Dr. K. Redekop (EUR)
WP1: Prof. Jukema (LUMC)
WP2A: Dr. A Stubbs (EMC)
WP2B: Prof. A. Heck (UU)
WP3: Prof. Prins (Philips – TU/e)
WP4: Prof. Verrips (UU)
WP5: Dr. Lijtenberg (Orbus Neich)
WP6: Dr. Breek (CAVADIS)
WP7: Prof. Kuiper (LU)
WP8: Prof. Biessen (TU/e)
WP9: Dr. Scholten (UU)
WP10: Prof Biessen (MUMC)
WP11: Prof. Van Zonneveld (LUMC)

Imperial College (UK)
LUMC
Leiden University
Galapagos
FlexGen

UMCU
TU/e
Philips
FEI

Orbus Neich
Future Diagnostics
Beckman Coulter
ThermoFisher
EUMC
Dutch Heart Foundation

# including Catharina Hospital Eindhoven
Budget: CTMM manages the flow of funds

Funding:
- 25% Academia
- 25% Industrial
- 50% Government Subsidy

Project costs:
- Personnel
- Materials
- Use of existing equipment
- Investments
- Third parties
- Management (5%)
Facts & Figures

Distribution of the CIRCULATING CELLS budgets to the partners in order to perform the R&D activities.

<table>
<thead>
<tr>
<th>Budget</th>
<th>20 M€</th>
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<tr>
<td>Start</td>
<td>2008</td>
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<tr>
<td>End</td>
<td>2014</td>
</tr>
<tr>
<td>Partners</td>
<td>21</td>
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</table>

Academic cash costs:
- PhD: 3,000,000
- PostDoc: 1,000,000
- Sen. Staff: 1,500,000
- Supp. Staff: 2,000,000
- IT-Staff: 500,000
- M&S: 1,000,000
- Investments: 1,500,000

Industrial cash costs:
- PhD: 500,000
- PostDoc: 1,000,000
- Sen. Staff: 1,500,000
- Supp. Staff: 2,000,000
- IT-Staff: 500,000
- M&S: 1,000,000
- Investments: 1,500,000

Academic in kind costs:
- PhD: 0
- PostDoc: 0
- Sen. Staff: 0
- Supp. Staff: 0
- IT-Staff: 0
- M&S: 0
- Investments: 0

Industrial in kind costs:
- PhD: 0
- PostDoc: 0
- Sen. Staff: 0
- Supp. Staff: 0
- IT-Staff: 0
- M&S: 0
- Investments: 0

CTMM investments:
- PhD: 0
- PostDoc: 0
- Sen. Staff: 0
- Supp. Staff: 0
- IT-Staff: 0
- M&S: 0
- Investments: 0
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<tr>
<td>Budget</td>
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<td>Output</td>
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<tr>
<td>Papers</td>
<td>21</td>
<td>21 papers in submission - mean impact factor all published  CIRCULATING CELLS papers:  4.3</td>
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<tr>
<td>Theses</td>
<td>8</td>
<td>7 planned for 2014-2016</td>
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<tr>
<td>Personal Grants</td>
<td>2</td>
<td>Prof. B. Prakken (UMCU) International UCAN Grant and Dutch Arthritis Foundation,  FP7 Marie Curie integrated Training network EUTRAIN grant (2010)</td>
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<tr>
<td>Patent (filings)</td>
<td>1</td>
<td>UU: tissue preparation method for combined light and electron microscopy</td>
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<td>Licenses</td>
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<td>FEI, license agreement on patent UU</td>
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<tr>
<td>Spin-off Companies</td>
<td>1</td>
<td>QVQ BV (Prof. T. Verrips)</td>
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<td>Raising Capital (&gt; 1 M€)</td>
<td>3</td>
<td>CVON grant GENIUS – Prof. J. Kuiper (LU)  NGI grant CardioLaborate - Prof. G. Pasterkamp (UMCU)  NHS grant Queen of Hearts – Prof G. Pasterkamp (UMCU)  large scale research facilities  NWO programme Proteins@Work – Prof. A. Heck (UU).</td>
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<tr>
<td>Public Media</td>
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Scientific Value Creation - Breakthroughs

- 2011: CTMM researchers developed a Oracle database for all clinical and experimental data of the Circulating Cells consortium (EMC, UMCU)
- 2012: Clinicians at the UMCU, LUMC, MUMC and Catharina Hospital establish a Dutch unique cohort of 500 ACS patients to find and test new circulating cell-based biomarkers.
- 2012: A new method was developed to quantify platelet-surface interactions by measuring a time-dependent calcium signaling in platelets (TU/e)
- 2012: Shear and platelet activation with collagen related peptide (CRP) triggered membrane blebbing, and extremely long Platelet Flow-Induced Protrusions (UMCU)
- 2012: In contrast to plaque macrophages, circulating monocytes display no clustering of genes expression (mRNA) and therefore do not yield any specific diagnostic information (MUMC)
- 2013: MicroRNA profiles of circulating CD14+ monocytes associate with MACE and reflect pro-inflammatory phenotypes in patients at risk for CAD (InteRNA/LUMC)
- 2013: The micro-vesicles proteome differs from patients with and without inducible ischemia (UMCU, TU/e)
- 2013: The incidence of cardiovascular events is reduced in patients with a high platelet density per monocyte (UMCU)

Highest Impact Papers – mean 7,2
5. Frese C.K. et al., Journal of Proteome Research 2011; 10, 2377–2388

Mean Impact Factor

<table>
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<th>Category</th>
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<td>CTMM - cardiovascular</td>
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2 - Mean impact factor based on 106 papers from the CTMM cardiovascular first call projects.
## Scientific Value Creation - Theses

<table>
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<tr>
<th>TRIUMPH Thesis</th>
<th>Partner</th>
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<tr>
<td>Coen van Solingen</td>
<td>LUMC</td>
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</tr>
<tr>
<td>Jan-Willem Sels</td>
<td>TU/e</td>
<td>2013</td>
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<tr>
<td>Loes van Zijp</td>
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<td>Thijs Holten</td>
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<td>Claudia Tersteeg</td>
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<td>Matthias Irmscher</td>
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<td>Ellen Elsenberg</td>
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<td>Jeroen Otten</td>
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<td>Thijs Zweers</td>
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<td>Agnese Ravetto</td>
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<td>Laura Burgers</td>
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<td>Patrick Wijten</td>
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<tr>
<td>Edwin Bredewold</td>
<td>LUMC</td>
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**Images:**
- [Platelets in Atherosclerosis](image1)
- [Circulating Monocytes in Atherosclerosis](image2)
- [Mechanics of the contact interface between cells and functionalized surfaces](image3)
- [The role of microRNA-126 in vascular homeostasis](image4)
- [Toll-like receptor response and circulating cells in cardiovascular disease](image5)
- [Platelet adherence during inflammatory diseases](image6)
- [Inducible myocardial ischemia in the systemic circulation](image7)
- [Local plaque characteristics and circulating cells in atherosclerosis](image8)
- [Study of methods for platelet function testing in the perspective of lab-on-chip applications](image9)
- [On platelet biology and cardiovascular disease](image10)
Scientific Value Creation - Infrastructure

Animal Models

• Col18 double knock outs: mouse model with increased angiogenic activity, due to lack of endostatin resulting in with massive plaque microvessels (MUMC+)
• Fibrillin-1/Apo E knock-out: mouse model with an increased risk of aneurysm formation, IPH and plaque rupture (MUMC+)
• Fibrillin-1/LDLR knock-out: under investigation (MUMC+)
• ApoE3 Leiden mouse model exposed to a western type of diet either or not in combination with statin treatment (LUMC)

Molecular Diagnostics-1

Cohorts
• Multicenter cohort of 700 PCI patients (CIRCULATING CELLS). Blood and blood cells isolated at hospitalization. Follow-up 9 months (UMCU, TU/e, LUMC, MUMC+)
• Cohort of >1000 PCI patients (UCORBIO). Blood samples collected at hospitalization. Follow-up 1 year (UMCU)
• Cohort of n=120 patients undergoing carotid endarterectomy (AtheroExpress). Both plaque material as well as blood samples have been isolated during operation (UMCU)
• Children's obesity cohort (AIMOB), n = 96 (UMCU)

Biobanks

Data-driven Methods
• Bioinformatics platform with clinical and experimental data in Oracle with Spotfire® portal for integration (EMC)
• HTA Methods: Mathematical Markov model (EUR)

Molecular Diagnostics-2

• Sample pipeline for routine proteomic analysis of both low abundant (monocytes, lymphocytes) and high abundant (platelets, granulocytes) blood cell fractions (UU)
• Nanobody libraries for VHH selection against antigens blood cells (monocytes/macrophages, platelets) (UU)
• Set of assays to measure platelet-leukocyte complexes (UMCU, TU/e, LUMC, MUMC)
• Platelet activation assay (UMCU)
• Leukocyte responsiveness assay (UMCU)
• Exosome proteomics (UMCU, TU/e, UU)
• A new method to study the dynamics of phagocytosis in real-time (TU/e)
• Microfluidics assay for mechanical property testing of activated leukocytes (TU/e)

Cohorts

• Sample pipeline for routine proteomic analysis of both low abundant (monocytes, lymphocytes) and high abundant (platelets, granulocytes) blood cell fractions (UU)

Data-driven methods
• A high content screening platform with 6 miniaturized assays to monitor the atherosclerosis signature of candidate genes: platelet activation, macrophage lipid accumulation, oxidative stress, apoptosis activation, phagocytosis assay, macrophage 3D migration, efferocytosis (MUMC+)
• Flow cytometry antibody panels for monocyte and lymphocyte marker measurements (LU, LUMC, UMCU, TU/e, MUMC)
• Gene expression profiling of peripheral blood monocytes (MUMC)
• Genotyping (UMCU)
• Advanced PCR miRNA screening for high throughput measurements (LUMC, InteRNA)
• Selection of microRNA subsets that reflect phenotypic alterations in circulating CD14, CD4 and CD8 cells (LUMC)
Clinical and Economic Value Creation of CIRCULATING CELLS

New ‘products’ for clinical care
Main Product Pipelines

**Technology/Assay Development**

- **Biosensor**
  - Philips

- **Platelet Function**
  - UMCU

- **Leucocyte-platelet complexes**
  - UMCU

- **Flow cytometry**
  - Beckman Coulter

- **miRNA megaplexing**
  - InteRNA

- **mRNA profiling**
  - Flexgen

- **Protein multiplex**
  - Cavadis, ACS-B

**Clinical & Experimental Verification**

- New & existing plasma markers
- Platelet reactivity
- Platelet density per monocyte
- FACS of 2 monocyte-selective, 3 lymphocyte selective panels
- 92 CD14+ selective miRNAs.

**Markers predictive for future events**

- troponin I
- Platelet reactivity
- Platelet density per monocyte
- FACS of 2 monocyte-selective, 3 lymphocyte selective panels
- 92 CD14+ selective miRNAs.

**Transfer to Technology**

- portfolio prognostic /diagnostic miRNA profiles
- UCORBIO cohort

**Clinical Validation**

- Not yet financed
- Not yet financed
- Not yet financed
- Not yet financed

---

**ILEM**

iLEM combines a fluorescence light microscope and an electron microscope into a single instrument for dedicated cell research

**Treatment targets plaque stabilization**

genes favor M2 or M1 polarisation of macrophages

---

**ACSM Risk calculator**

EMC, UMCU

---

**Gene expression**

Not yet financed

---

**Platelet reactivity**

Not yet financed

---

**Platelet density per monocyte**

Not yet financed

---

**FACS of 2 monocyte-selective, 3 lymphocyte selective panels**

Not yet financed

---

**92 CD14+ selective miRNAs.**

Not yet financed

---

**UCORBIO cohort**

Not yet financed
Biosensor, protein panel to stratify ACS patients

The CIRCULATING CELLS project aims to identify a new panel of protein biomarkers for measurement on a new point-of-care biosensor for the determination of the risk to suffer a second major cardiovascular event (hospitalization for heart attack, stroke) in Acute Coronary Syndrome patients.

Hand held device
A surface sensitive technique involving frustrated total internal reflection is used to detect the presence of magnetic particle labels attached to the sensor surface via a sandwich immunoassay. The optics and actuating electromagnets are integrated in a compact portable analyzer. Fully integrated, plastic disposable cartridges allow for easy use.

Leading Company
Royal Philips

Current Diagnostics
Risk factors, cTnI, NT-proBNP

Main results in CTMM
• **Biomarker selection**: A literature analysis was performed on biomarkers for primary and secondary cardiovascular events. Thereafter markers were selected and tested in the CTMM Circulating Cell clinical ACS cohort. It was shown that patients with elevated NT-proBNP levels have an elevated hazard ratio for MACE (major adverse coronary event) after correction for the Framingham risk score characteristics.
• **Demonstrator development**: In collaboration with the TRIUMPH consortium, B-type natriuretic peptide and troponin were applied on the biosensor as model markers. Sensitive detection of both markers in parallel on one cartridge demonstrated multiplexing feasibility of the platform.

Future Outlook
After protein marker selection and assay development on the biosensor, thorough technical testing and validation in patient groups will be required before marker and/or test release.

Expected impact of the product
**General**: Rapid diagnostic testing near the patient has the potential to enable on-the-spot decision making and thereby play a major part in improving patient outcomes and reducing healthcare costs.

**Quality of Life**: Accurate prognosis of ACS patients could stratify higher risk patients for delayed hospital release or more stringent follow-up after release, which may reduce the number of secondary events. Furthermore, more closely monitoring ACS treatment through measurements at home may improve compliance.

**Accessibility**: The handheld and easy-to-use system aims to be suitable for applications outside of the hospital, e.g. in the general practitioner’s office or at a patient’s home.
Platelet reactivity is increased in patients with coronary artery disease (CAD). We studied whether the platelet reactivity to specific triggers with the severity of CAD in patients who underwent percutaneous coronary intervention (PCI).

Assay
Reactivity of platelets was quantified by the median fluorescence intensity (MFI) of the platelet-specific activation marker CD62P on the surface of platelets, using flow cytometry.

Main results in CTMM
- Measurement were performed in leucocytes and platelets of 484 CAD patients admitted for PCI Netherlands. Patients with nSTEMI and unstable angina had a higher platelet reactivity for ADP than patients with unstable angina (figure).

Progress obtained in translational pipeline
- Discovery: Pathways biomarkers
- Selection: Pathways biomarkers
- Demonstrator: Development device
- Clinical Evaluation: Cohorts
- Market access

Future Outlook
- A large-scale study is needed to investigate whether monitoring of on treatment platelet reactivity reduces MACE incidence after PCI. If platelet reactivity improves treatment accuracy then it might represent a novel therapeutic tool to prevent recurrence of MACE after PCI.

Expected impact of the product
We have introduced a novel high throughput platelet activation assay in cardiovascular research. The test monitors the major agonist dependent activation pathways and it can handle high numbers of patient samples. The clinical data show that platelet activation is not a major biomarker of future MACE.

Future research should focus on the measurement of on platelet inhibitor treatment platelet function as a biomarker of therapeutic efficiency.
Platelet-Leucocyte Complex Assay in ACS

**PRODUCT**

Binding of circulating platelets to leukocytes is increased in patients with coronary artery disease (CAD). We studied whether the platelet density per leukocyte and the percentage of platelet-leucocyte complexes are associated with the incidence of major adverse cardiovascular events (MACE) in patients who underwent percutaneous coronary intervention (PCI).

In red monocyte staining, in green staining of the platelets and in blue nucleus staining. Examples of complex formation in patients with from the high MACE group (left) and low MACE group (right)

**Assay**

Binding of multiple platelets per leukocyte, also referred to as the platelet density per leukocyte, was quantified by the median fluorescence intensity (MFI) of the platelet-specific marker CD42b on the surface of leukocytes using flow cytometry.

**Leading Partner**

UMCU

**Current Diagnostics**

Risk factors, cTnl, NT-proBNP

**PARTNERSHIP**

**Progress obtained in translational pipeline**

- Discovery Pathways biomarkers
- Selection Pathways biomarkers
- Demonstrator Development device
- Clinical Evaluation cohorts
- Market access

**Main results in CTMM**

- We introduce a measurement of platelet density per monocyte as biomarker of vaso-active monocytes.
- We have shown that higher density of platelets on monocytes is associated with more endothelial cell interaction.
- Measurement were performed in leucocytes and platelets of 263 CAD patients admitted for PCI in 4 medical centers in the Netherlands.
- Patients with a high platelet density per monocyte (MFI>6.16) exhibited a 5-fold reduced incidence of MACE after PCI ... independent of established cardiovascular disease risk factors, antiplatelet therapy and disease severity at inclusion.

**Future Outlook**

A large-scale study is needed to investigate whether the platelet density per monocyte reduces MACE incidence after PCI. If a high platelet density per monocyte plays a role in vascular repair, it might represent a novel therapeutic target to prevent recurrence of MACE after PCI.

**PATIENT**

**Number CHD patients**

648,400

**Yearly Costs**

1397 M€

Possible impact of the product

Platelet density per monocyte gives an indication of increased numbers of vaso-active monocytes. These specific complexes may be important for the regulated communication between monocytes and endothelial cells. Our first data suggest that this may be beneficial for patients with a history of Myocardial infarction.
Flow Cytometry to assess risk in ACS

Flow cytometry enables fast, simultaneous analysis of multiple cellular markers using multi-color panels. Modern CE-IVDD flow cytometers allow quantitative detection of up to 10 markers simultaneously, which can be used for subclassification of blood cells and identification of rare populations carrying important biological information and potential prognostic value in cardiovascular disease.

Device/Assay
Flow cytometry protocol with various monocyte and lymphocyte panels with 7 parameters each that were amended with up to 3 additional equivalent markers and marker combinations.

Leading Partner
Beckman Coulter

Current Diagnostics
Risk factors, cTnl, NT-proBNP

Main results in CTMM
- In CTMM “Circulating Cells” we extensively tested multi-marker flow cytometry panels to identify optimal cell based marker combinations predictive for future cardiovascular events. For this purpose, 4 flow cytometers were installed at the participating clinical centers that contributed to the clinical study. We acquired flow cytometry data from more than 500 patients, focusing on monocyte and lymphocyte subpopulations and analyzed their correlation with clinical characteristics and their predictive value for secondary events.
- From the initial 16 different panels for classification of monocyte and lymphocyte populations, 4 monocyte markers have been selected with the highest predictive value and those that highly correlate with relevant clinical characteristics.

Expected Impact of the product
The product is expected to be an affordable panel of up to 5 fluorescently labeled antibodies that will allow the identification of both numbers and subsets of white blood cells. The use of this panel will contribute to the stratification of the patient in terms of severity of ACS and contribute to the prognosis of the patient and this risk assessment will contribute to the quality of life. We expect that this product will be easily accessible and can be applied in a large number of clinical settings.
miRNA chip/PCR assays to stratify ACS patients

Circulating CD14+ monocyctic cells can differentiate into macrophages and dendritic cells to control infections and repair tissue damage and contribute to formation and remodeling of new blood vessels. In patients at risk for cardiovascular disease, circulating CD14+ cells are skewed to a more macrophage-like, CD16+ pro-inflammatory phenotype. Phenotype-selective miRNA profiles of these cells may predict risk and may affect the intention to treat subjects at risk.

**Main results in CTMM**

- We have identified miRNA profiles in circulating CD14+ cells or monocytes characterized as intermediate or non-classical monocytes and that are predictive of inflammation.

- Selective miRNAs that are modulated or differentially expressed have been identified using deep sequencing and microarray analyses and have been validated on CD14+ cell samples from a unique cohort consisting of 441 patients with coronary artery disease that were extensively characterized for their cardiovascular phenotype.

**Future Outlook**

Efforts are underway to find a diagnostic development partner to further validate the miRNA profiles on a second patient cohort and development of the miRNA profiles into (a) screening/diagnostic product(s).

**Expected impact of the product**

The differential miRNAs expression profiles acquired are surprisingly indicative of monocyte phenotype. Since miRNAs can be detected by highly sensitive e.g. PCR-based assays from very low volumes of blood, our technology can be employed to develop assay to select patients that are predisposed to develop cardiovascular disease. Such assays may help to select which patients should (or should not) receive preventive medication at an early age. For instance the use of statin therapy can be extremely beneficial but is also expensive and not without adverse side effects.
FEI’s Tecnai with iCorr is the first integrated light and electron microscope that combines a fluorescence light microscope and electron microscope into a single, harmonized instrument, thereby enabling a faster more accurate approach to correlative microscopy.

The Tecnai with iCorr is designed to automate and accelerate CLEM experiments, resulting in large overview light microscopy images pin pointing to the cells of interest based on fluorescent markers which can then be analyzed by electron microscopy to determine ultrastructural changes. Moreover, the software automatically places the ultra-structural detail in the light microscopy context.

Sample preparation protocols were developed and optimized in order to retain fluorescence and still have enough contrast for ultra-structural investigation by EM.

**Expected impact of the product**
The Tecnai with iCorr will be an important product for the characterization and validation of new bio markers in cardiovascular diseases and other diseases.

These new biomarkers can then be used for early diagnosis and have the potential to save lives and optimize treatment.
Macrophages with a profound, alternatively-activated phenotype known as M2, contribute to fibrosis by secreting profibrotic substances and activating other cells which contribute to fibrosis, such as fibroblasts. While pro-inflammatory M1 macrophages predominate in rupture prone plaques, their re-polarisation into M2 macrophages could well lead to plaque stabilisation.

**Device**
A high-throughput assay will be developed suitable to identify novel target genes involved in the polarization of macrophages towards the profibrotic M2 phenotype using the SilenceSelect® adenoviral shRNA library. Identified M2 markers can be potential new treatment targets or diagnostic - prognostic markers for atherosclerosis.

**Main results in CTMM**
- A robust assay for M2 polarization which can be transduced efficiently with adenoviral shRNA constructs without observing any adverse effect of the virus on the read-out and viability.
- Furthermore, we identified shRNA viruses that target known signaling pathways as positive controls. This M2 polarizing assay is therefore a disease-relevant assay that... genome-wide screening using a SilenceSelect® shRNA library led to the identification of a large number of candidate genes.
- Over ten candidate genes (not yet to be disclosed) have been selected and are currently further characterized.

**Expected impact of the product**
Plaque stabilization in patients at immediate risk of or that had just suffered an AMI is expected • to improve the clinical prognosis • to reduce recurrent cardiovascular events

**Future Outlook**
- Targets will be further validated
- Expression profile of target genes in atherosclerosis and fibrosis patients will be determined
- Targets will be selected for drug discovery

**Figure**
CCL-18 production is one of the Hallmarks to identify M2 phenotypic cells

**Leading Company**
Galapagos

**Current Diagnostics**
Risk factors, cTnI, NT-proBNP

**Number CHD patients**
648,400

**Yearly Costs**
1397 M€
The CIRCULATING CELLS project aims to identify a new panel of blood biomarkers for which a new risk calculator will be developed that can be used to determine the risk of a second major cardiovascular event (hospitalization for heart attack, stroke, etc.) in Acute coronary Syndrome patients.

Modern statistical analysis techniques are utilized for accurate predictions and classifications. In addition to traditional clinical measurements, several types of biological data have been analyzed simultaneously to obtain a global picture of patients’ health. This integrated analysis has provided a panel of biomarkers that can be used to assess the risk of a second cardiac event.

Leading Partners
UMCU, EMC

Current Diagnostics
TIMI risk score
GRACE risk score

Main results in CTMM

- Data for this project are derived from clinical metadata associated with each patient, routine biochemical assays (e.g., CRP); “omics” platforms for mRNA, miRNA, and protein; and non-high throughput assays (FACS, ELISA). The data are stored in the Translational data warehouse (Circucel DB) for use in assay specific analysis which includes approx. 700 patients
- Candidate biomarkers are selected from each data type and correlated with clinical covariates.
- Analysis was based on linear models and pathway testing.
- Integrative analysis has revealed a basic prognostic value based on the currently clinically used diagnostics as well as new multivariate marker panel with added predictive value (not yet to be disclosed)

Future Outlook
Further analysis and new studies with independent cohort are necessary to develop and validate this risk prediction model.

Expected impact of the product
Patients diagnosed with stable or unstable coronary syndromes or ST-elevation MI are usually treated with medication with or without revascularization (PCI or CABG). More accurate risk calculations would assist in reclassifying patients into low-risk or high-risk groups and thereby help clinicians to decide whether to intensify medication therapy. This approach would improve both health and cost-effectiveness by ensuring that intensified treatment is only used on patients who will benefit from it.

In addition, by receiving a better prediction about their risk of another cardiovascular event, patients might improve their adherence to treatment.
**Current care setting**
The European Society of Cardiology guideline recommends that individuals with an intermediate 10-year risk (3-15%) of developing cardiovascular disease (CVD) receive statin treatment if their total cholesterol level ≥5 mmol/L and/or low-density lipoprotein cholesterol level ≥3 mmol/L.

**Model development**
A Markov model was developed to simulate the future costs and quality-adjusted life-years of different age- and gender-specific cohorts of individuals with an intermediate risk. Events in the model include: myocardial infarction, stroke, revascularization, myopathy, rhabdomyolysis and death over a 10-year time horizon. The model was then used to compare four strategies: treat all with statins, treat none with statins, treat according to the European guidelines, and test first to select individuals for statin treatment. Furthermore, the influence of uncertainty is incorporated in the model.

**Patient stratification**
Methods to stratify patients into risk categories already exist and are used in practice. They can separate patients into categories such as low, intermediate and high risk very accurately. However, only a fraction of intermediate-risk patients will actually have a cardiovascular event in the next 10 years. A new test should be able to reclassify intermediate-risk patients into low-risk or high-risk patients.

**New care setting**
A risk prediction test could be used to reclassify some intermediate-risk individuals into a low-risk or high-risk category and therefore help to improve decision-making about whether or not to use statins.

**Performance evaluation**
A risk prediction test was found to be more effective and less costly than the other strategies if it was 100% accurate and cost no more than €237. The test and the treat-all strategy were equally effective but the test led to lower costs by avoiding unnecessary statin use and reducing side-effects. A test with an accuracy lower than 100% could still be cost-effective as long as its performance was not too poor and its cost not too high.

**Treatment allocation**
Statins are often used to reduce the risk of a cardiovascular event amongst individuals who do not have cardiovascular disease. A new risk prediction test should help to determine which intermediate-risk patients are actually low-risk patients and therefore do not necessarily need statins.

**Potential of personalized treatment**
Use of a highly accurate prediction test could reduce overall CVD risk, frequency of drug side-effects and lifetime costs. However, given the effectiveness and relatively low costs of currently available statins, the price of this test cannot be excessive. Besides cost-effectiveness considerations, patients who receive a better estimate of their 10-year CVD risk may be more motivated to make lifestyle modifications to reduce their risk.

**Implications**
There is an opportunity to improve risk stratification in patients with an intermediate 10-year risk of a cardiovascular event since only 3-15% of the patients with an intermediate risk actually have a cardiovascular event in the next 10 years.

**Commercial headroom available**
The test strategy could reduce unnecessary statin use and thereby reduce its complications (e.g. myopathy) and costs. Furthermore, with this test patients who actually are at risk will be treated and thus cardiovascular events can be prevented. However, the cost of the test cannot be excessive. The degree of headroom depends on the performance of the test; a poorer performing test must have a lower price to compensate for prediction errors.
Early HTA: Potential Impact of New Technologies

Model development
A Markov model was developed to simulate the future costs and quality-adjusted life-years of patients with cardiovascular disease. Events in the model include: myocardial infarction, cardiac arrest, stroke, revascularization, and death. The model can be used to compare current practice (based on the Circulating Cells cohort) with risk-stratified treatment in which treatment intensity is varied depending on the results of a test that estimates the risk of a future cardiovascular event.

Performance evaluation
Since the final results of the Circulating Cells study are not yet known, only preliminary what-if analyses have been performed. If the overall risk of an event is low, this may reduce the added value of a new test. Specifically, the benefit of identifying the few patients who will have an event may be limited if the effectiveness of intensified treatment is limited and if the cost of the test is not low (since all patients undergo the test). This could be resolved by using the test on a preselected patient subgroup.

Patient stratification
Patients diagnosed with coronary artery disease can be stratified according to the TIMI score or the GRACE risk model. Both models predict short term and longer term cardiovascular events but not adequately stratify patients. A new test would help to improve the discriminative power and therefore optimize treatment.

Treatment allocation
Patients with a recent cardiac event often receive medication to prevent another event. A new risk prediction test would help to determine which of these patients are at increased risk of another event and would therefore benefit from a more intensive medication regime. It is also possible that other patients could be classified as low risk and undergo a relatively less intensive regimen as a result.

Commercial headroom available
A test that predicts the risk of a cardiovascular event could help to identify which patients will benefit from intensification of treatment. The headroom available depends on several factors including current treatment patterns. In addition, a less accurate test will have to have a lower price to compensate for prediction error.

Implications
Decision analytic modeling can be useful in assessing the potential clinical utility and budget impact of risk-stratified treatment in patients with cardiovascular disease. Preliminary analyses can help to determine if it is worthwhile to continue developing risk stratification tests or helping to consider how a new test could be best be implemented in clinical practice. For example, a low overall risk of an event may limit the cost-effective of a new test. Patient pre-selection would then be worth considering.

Potential of personalized treatment
Use of a highly accurate prediction test could reduce cardiovascular events and lifetime costs of patients with coronary artery disease. Besides cost-effectiveness considerations, patients who receive a better estimate of their risk may be more motivated to make lifestyle modifications to reduce their risk.

Current care setting
Patients diagnosed with stable or unstable coronary syndromes or ST-elevation MI are usually treated with medication with or without revascularization (PCI or CABG).

New care setting
A risk prediction test could be used to reclassify some patients into a low-risk or high-risk category and therefore help to improve decisions about whether or not to intensify medication therapy. Furthermore, adherence to treatment could be improved if patients are more aware of their risk.
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List of Publications

1. Imo E. Hoefer• Jan-Willem Sels• J. Wouter Jukema • Sander Berghena• Erik Blessen• Elizabeth McClellan• Mat Daemen• Pieter Doevendans• Philip de Groot• Marieke Hillaar• Sebastiaan Horsman• Mustafa Ilhan• Johan Kuper• Nico Pijs• Ken Redekop• Peter van der Speel• Andrew Studbs• Eric van de Veen• Johannes Wattenberger• Anton-Jan van Zonneveld• Gerard Pasterkamp (2013) Circulating Cells as Predictors of Secondary Manifestations of Cardiovascular Disease – Design of the CIRCULATING CELLS Study, Clin Res Cardiol; DOI 10.1007/s00392-013-0607-9


Others sources


<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>ACS</td>
<td>Acute Coronary Syndrome</td>
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<tr>
<td>CABG</td>
<td>Coronary artery bypass grafting</td>
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<td>CAD</td>
<td>Coronary Artery Disease</td>
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<td>CHD</td>
<td>Coronary Heart Disease</td>
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<td>CRP</td>
<td>Collagen Related Peptide</td>
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<td>CTnI</td>
<td>Cardiac-Troponine</td>
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<td>CTMM</td>
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<td>CVD</td>
<td>Cardiovascular Disease</td>
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<td>FACS</td>
<td>Fluorescence-Activated Cell Sorting</td>
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<td>GRACE</td>
<td>Global Registry of Acute Coronary Events</td>
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<tr>
<td>LDLR</td>
<td>Low Density Lipoprotein Receptor</td>
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<td>LEM</td>
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<tr>
<td>MACE</td>
<td>Major Adverse Cardiovascular Events</td>
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<tr>
<td>MFI</td>
<td>Median Fluorescence Intensity</td>
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<tr>
<td>nSTEMI</td>
<td>none- ST elevated myocardial infarct</td>
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<td>NT-proBNP</td>
<td>N-terminal pro Brain Natriuretic Peptide</td>
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<td>PCI</td>
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<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>STW</td>
<td>Stichting voor de Technische Wetenschappen</td>
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<tr>
<td>VHH</td>
<td>Variable domain of heavy chain of camelid antibodies</td>
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