MENARINI
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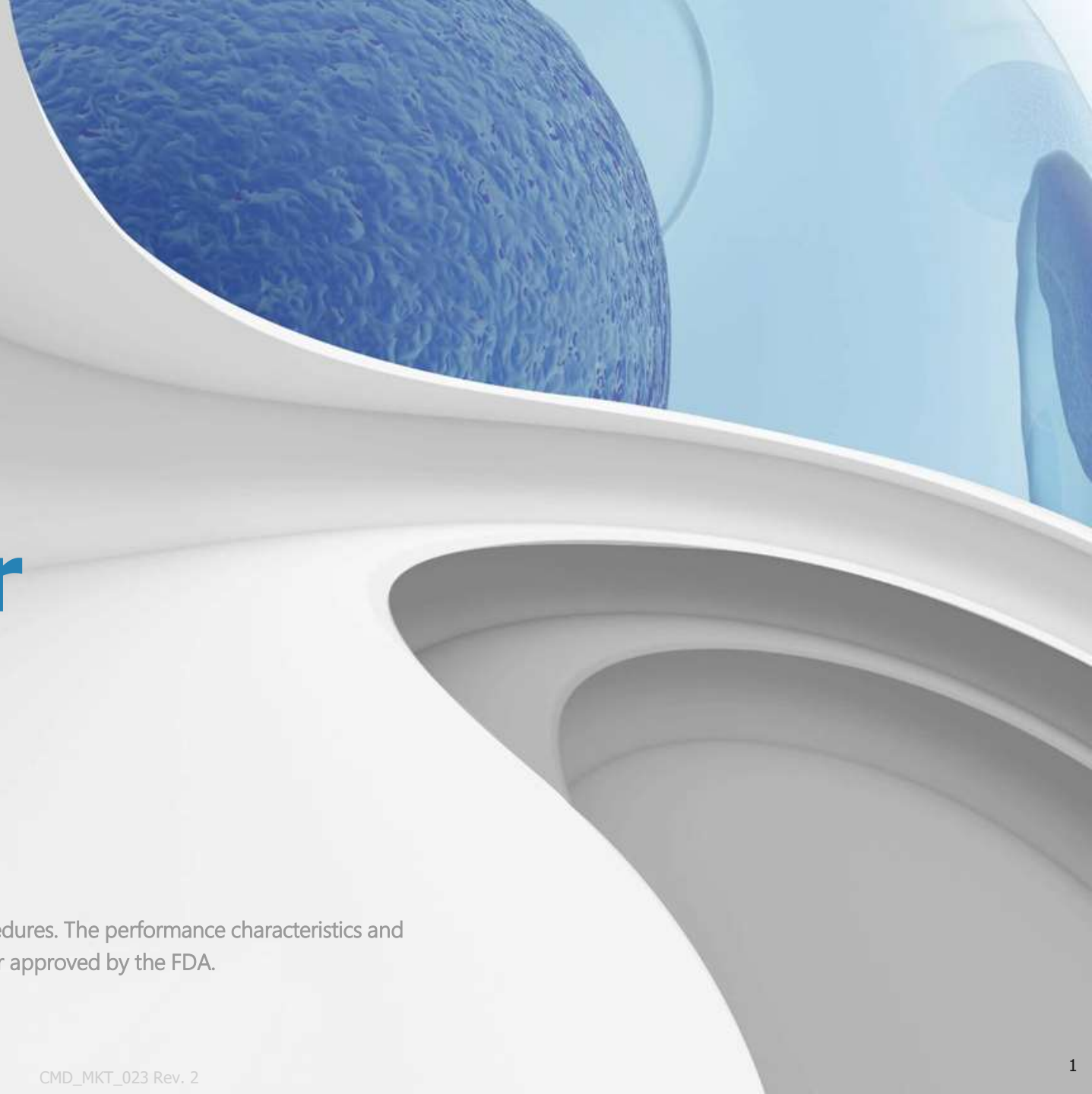


# Cellsearch technology for COVID-19

CELLSEARCH TECHNOLOGY for COVID-19

CEC Application is for Research Use Only. Not for use in diagnostic procedures. The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.

MENARINI silicon biosystems

# Rare Cells analysis with single cell precision

We offer unique **rare cell** technologies and solutions that provide clinical researchers with access to unparalleled **resolution** in the study of cells and their molecular characterization.

Including **circulating tumor cells** and **circulating endothelial cells.**

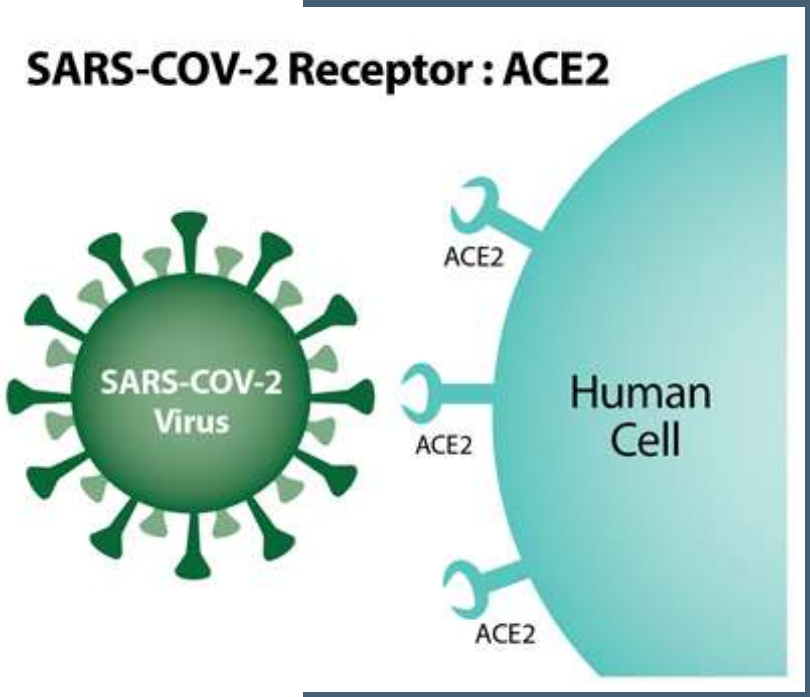
# COVID-19 and Endothelium

MENARINI silicon biosystems

CELLSEARCH TECHNOLOGY for COVID-19

- COVID-19 enters into cells through the **ACE2 receptor** (1)
- ACE2 = angiotensin-converting enzyme 2

- The surface expression of **ACE2 protein is NOT only limited to lung alveolar epithelial cells** and enterocytes of the small intestine.
- **ACE 2 is also present** in arterial and venous **endothelial cells** (2)




1. Zhang et al. Intensive Care Med, 2020
2. Hamming et al. J Pathol, 2004

# COVID-19 and Endothelium

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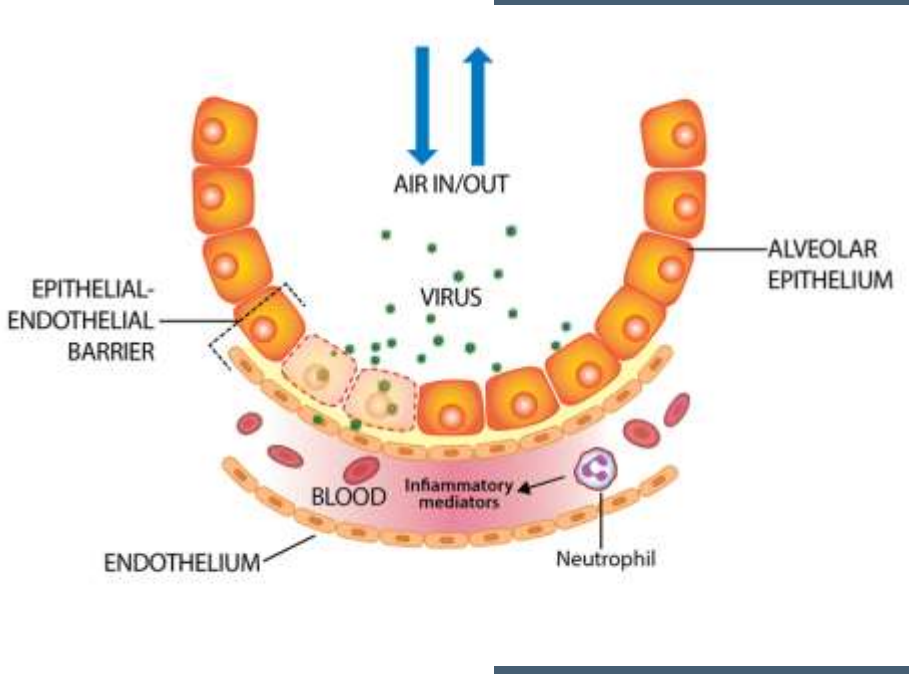
CELLSEARCH TECHNOLOGY for COVID-19

- Acute respiratory distress syndrome (ARDS), a severe complication of some viral infections such as influenza, is mainly caused by damage of the epithelial–endothelial barrier.
- **COVID-19 is directly targeting both components of the barrier** (3)
- **The damaged and/or activated endothelium could greatly contribute to the pro-inflammatory cascade** which perpetuates and amplifies lung damage and respiratory distress. (4, 5)





3. Varga Z. et al, Lancet, 2020
4. Short KR et al. Lancet infectious disease, 2014
5. Guo et al. J Am Heart Assoc 2020

# COVID-19 Clinical Manifestations

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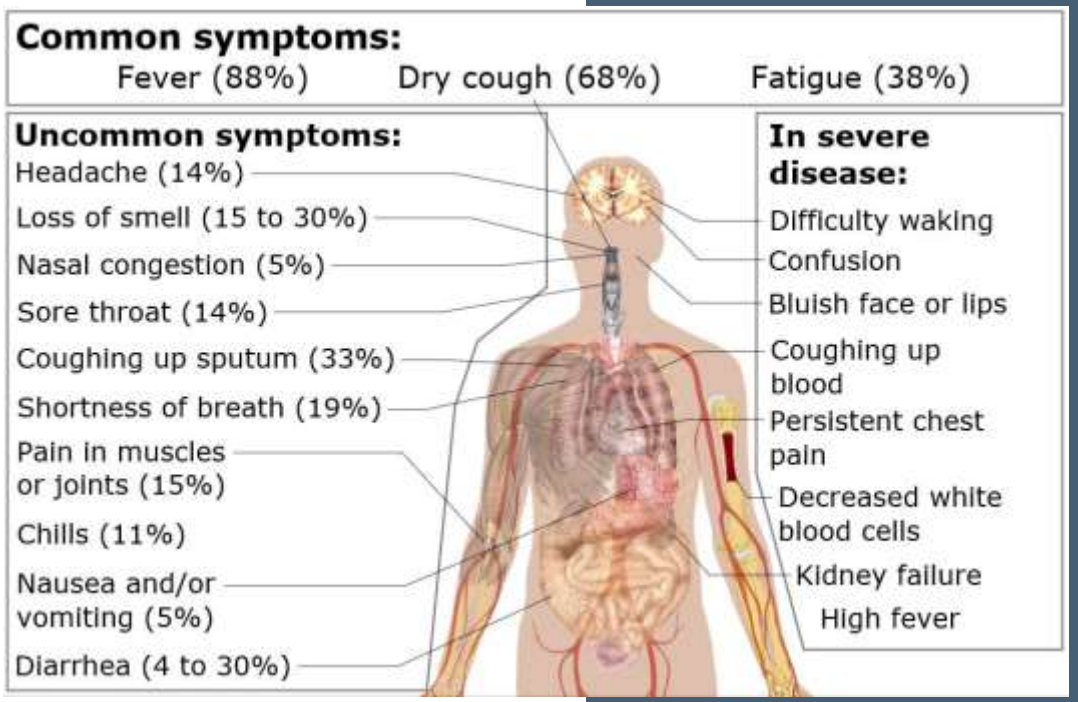
CELLSEARCH TECHNOLOGY for COVID-19

- The main severe clinical manifestation is **interstitial pneumonia** which can evolve in a **severe respiratory distress**
- It has been reported that **some patients first went to see a doctor because of cardiovascular symptoms rather than respiratory**. Among the people who died from COVID-19 reported by the NHC, 11.8% of patients without underlying CVD had substantial heart damage (6)




By Mikael Häggström, used with permission. CC0.

6. Zeng et al., Nature Reviews Cardiology, 2020

# COVID-19 and Circulating Endothelial Cells (CECs)

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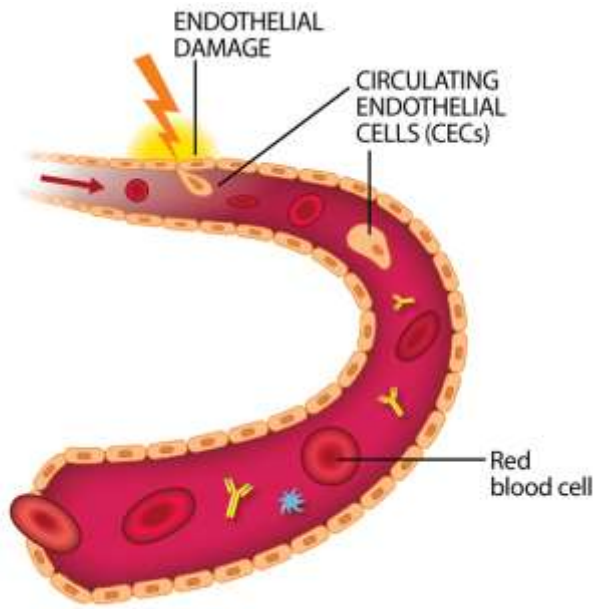
- CECs are considered a specific and sensitive marker of endothelial damage in a variety of pathological conditions (7-12)
- **CEC count increase is reported to be linked to cardio-vascular events** (13)
- CEC count may resemble also the pulmonary endothelial damage
- CEC count increase may predict the extent of pro-inflammatory cascade activation




7. Dignat-Gearge F. et al Eur J Hematology, 2000
8. Rowand et al Cytometry 2007
9. Erdbruegger et al Curr Stem cells Therapy and Research 2010
10. Almici et al. Transplantation 2014
11. Almici et al. Bone Marrow Transplantation 2017
12. Almici et al. Scientic Report 2019
13. De Simone C et al. J Eur Acad Dermatol Venerol 28 (5) 590-6;2014

# CELLSEARCH®
# for Circulating Endothelial Cell & COVID-19

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# The CELLSEARCH® System for enumeration of Circulating Endothelial Cells from whole blood

MENARINI silicon biosystems

Blood is collected into a **CellSave Preservative Tube.**

**CELLSEARCH System Test reagents**, and controls.

**The CELLTRACKS AUTOPREP System** automatically prepares the sample, ready for analysis.

The enriched sample is transferred and incubated in **the Magnest** Cartridge Holder.

The sample is analyzed on the **CELLTRACKS ANALYZER II**, and images are presented to the operator for classification.

Watch the VIDEO for further information on CELLSEARCH technology

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# CELLSEARCH® Advantages for the Study of CECs in the COVID-19 Setting

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**The CELLSEARCH Circulating Endothelial Cell Test** is well established, reliable and robust:

- 30 papers have been published analyzing CECs with the CELLSEARCH System, using both CELLSEARCH CEC Kit and CELLTRACKS® CEC Profile Kit.
- CECs have been evaluated in multiple research applications, from different settings in **oncology** to **autoimmune disease**, from **acute myocardial infarction** to **Graft vs Host Disease** (GvHD).
- The **CELLSEARCH Circulating Endothelial Cell kit** is available for CEC research projects requiring the capture & enumeration of CECs and potentially downstream analysis.

**The acquired experience of CELLSEARCH technology in oncology demonstrates its applicability and feasibility in clinical research. This experience can be easily transferred to COVID-19 offering a feasible reproducible approach to study CECs as a predictive biomarker of cardiovascular event and ARDS risk.**

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# CELLSEARCH® CECs Kit

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## For the enumeration and characterization of circulating endothelial cells (CECs)

**Sample Collection:** CellSave® Preservative tube, stable for up to 72 hours at room temperature

**Ferrofluid Capture:** CD146-positive cells

**Secondary Staining reagents:**

- Anti-CD105-PE (identify endothelial cells)
- Anti-CD45 –APC (identify WBCs)
- DAPI (stains cell nucleus)

**Spare Channel:** FITC for user definable antibody

**Number of tests/Kit:** 16 Tests

**Results Reported:** Number of CECs per **4ML** of blood
**Quality Control:** The CELLSEARCH® CEC/CMC Control Kit should be run each day or when using a new lot of reagents. Control kit contains 24 tests with two levels of cells at 50 & 1000 cells.

**Instructions for use:**

- **CellSave Preservative Tube** (Cat No. 7900005)
- **CELLSEARCH® CEC Kit** (Cat No. 7900009)
- **CELLSEARCH® CEC/CMC Control Kit** (Cat No. 9572V):

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# CELLSEARCH® CECs Kit

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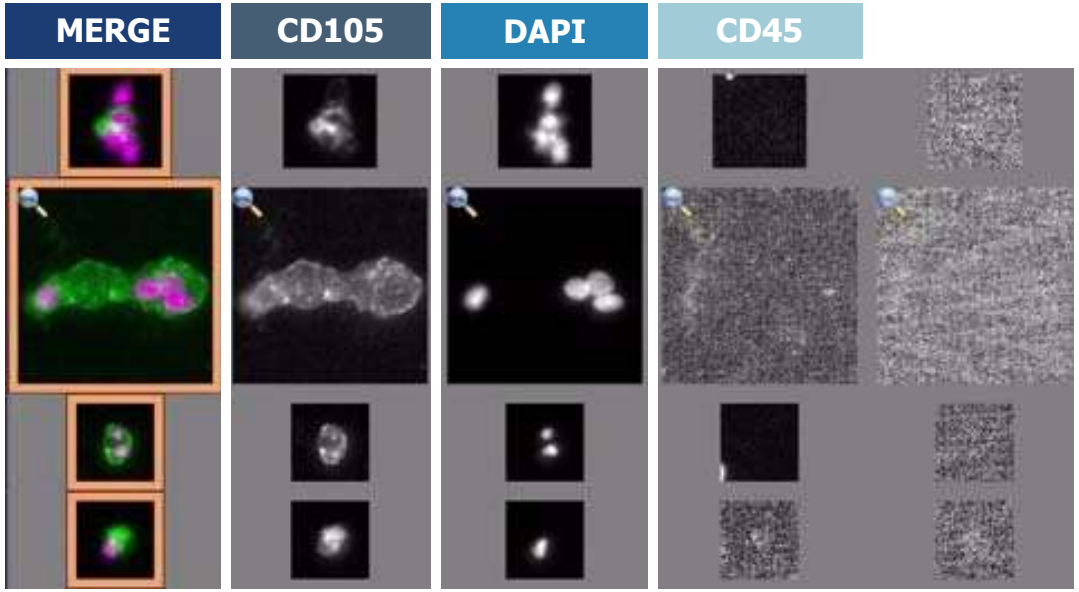
## For the enumeration and characterization of circulating endothelial cells (CECs)




CEC Application is for Research Use Only. Not for use in diagnostic procedures. The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.

# CECs in Myocardial Infarction (MI)

**For the enumeration and characterization of circulating endothelial cells (CECs)**

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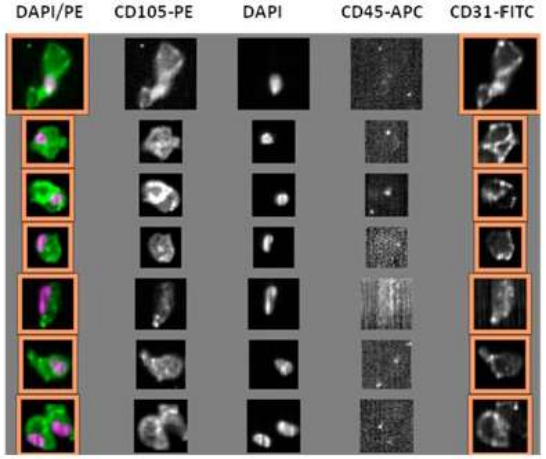
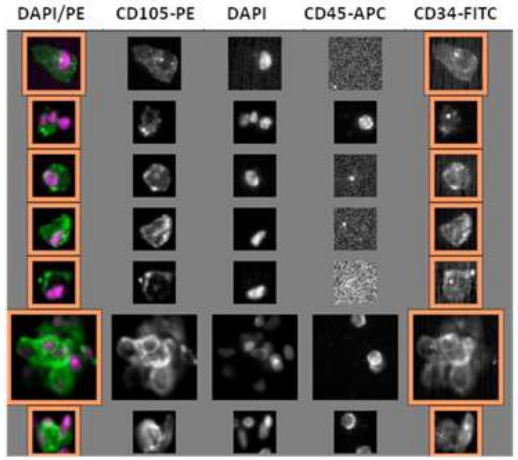
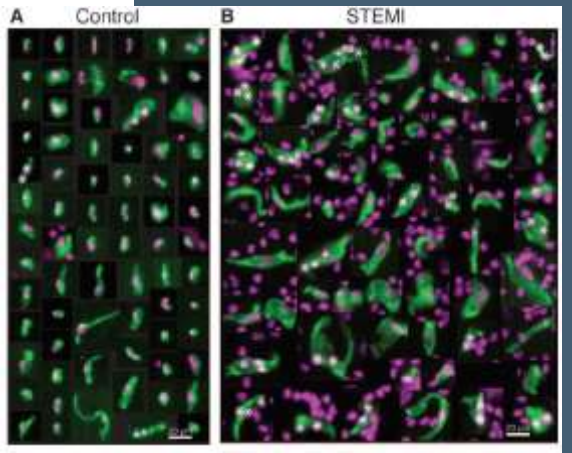
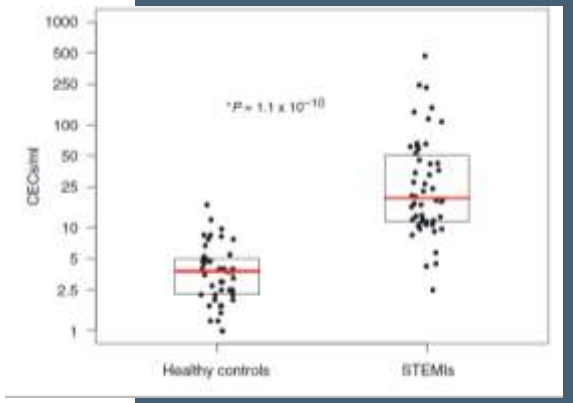
- CEC in STEMI (ST-elevation myocardial infarction) patients vs control: «pathological CECs» are abnormally large, misshapened, and may have multiple nuclei
- The median number of CECs in healthy controls was 4 CECs/ml, whilst the median number of CECs in STEMI patients was 19 CECs/ml
- Additional phenotype marker CD31-FITC (left) or CD34-FITC (right) was added to the samples during staining step taking advantage of the fourth CELLSEARCH channel




Damani S et al.,

Sci Transl Med. 2012

**CEC Application is for Research Use Only. Not for use in diagnostic procedures. The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.**

# CELLTRACKS® CEC Profile Kit

## For immunomagnetically capture CD146+ cells from whole blood

**Sample Collection:** CellSave® Preservative tube, stable for up to 72 hours at room temperature

**Ferrofluid Capture:** CD146-positive cells

**Secondary Staining reagents:** N/A

**Number of tests/Kit:** 16 Tests

**Results Reported:** N/A

**Quality Control:** The CELLSEARCH® CEC/CMC Control Kit should be run each day or when using a new lot of reagents. Control kit contains 24 tests with two levels of cells at 50 & 1000 cells.

**Molecular Analysis:**

Use EDTA tubes if samples will be processed for isolation of RNA or DNA. Follow the EDTA tubes manufacturer's instructions. Process samples as soon as possible to maximize nucleic acid integrity and recovery. A 2-3 fold loss of mRNA from endothelial cells may result if processing is delayed up to 36 hours when compared to immediate processing as measured by real-time quantitative RT-PCR. Blood samples may be stored or transported in EDTA tubes for up to 36 hours at room temperature (15–30 °C or 59–86 °F) prior to processing. Do not refrigerate or freeze.

**Instructions for use:**

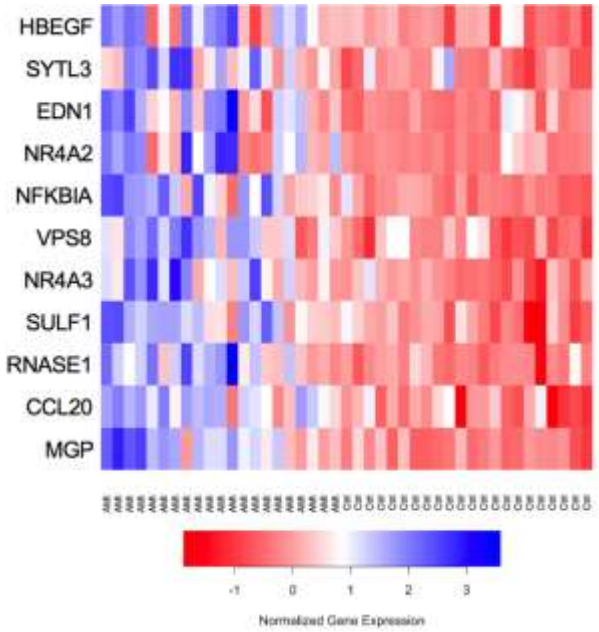
- **CellSave Preservative Tube** (Cat No. 7900005)
- **CELLSEARCH® CEC Profile Kit** (Cat No. 9564V)
- **CELLSEARCH® CEC/CMC Control Kit** (Cat No. 9572V)

**CEC Application is for Research Use Only. Not for use in diagnostic procedures. The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.**

# Gene Expression

## Microarray Analysis of Enriched CECs

An 11-gene signature for acute myocardial infarction (AMI) was determined from **microarray gene expression analysis of CECs** enriched with CELLSEARCH CEC profile kit from healthy control and AMI patients.




Muse ED et al., Sci Rep. 2017

CEC Application is for Research Use Only. Not for use in diagnostic procedures. The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.

# CECs Kits Overview

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| | CELLSEARCH® Circulating Endothelial Cell Kit | CELLTRACKS® Circulating Endothelial Cell Profile Kit |
|---|---|---|
| **Cell Type** | Endothelial | Endothelial |
| **Ferrofluid Capture Reagent** | Anti-CD146 | Anti-CD146 |
| **Staining Reagent** | anti-CD105-PE<br>anti-CD45-APC | NA |
| **Marker Reagent** | FITC | NA |
| **# tests** | 16 | 16 |
| **Control Kit** | yes | yes |

**CEC Application is for Research Use Only. Not for use in diagnostic procedures. The performance characteristics and safety and effectiveness have not been established and are not cleared or approved by the FDA.**

# Most relevant CECs Paper - Oncology




- Vasseur A et al., *Prognostic value of CEC count in HER2-negative metastatic breast cancer patients treated with bevacizumab and chemotherapy: a prospective validation study (UCBG COMET)*. Angiogenesis. 2020 May;23(2):193-202 PMID: 31773439
- Ueno T et al., *A multicenter phase II trial of neoadjuvant letrozole plus low-dose cyclophosphamide in postmenopausal patients with estrogen receptor-positive breast cancer (JBCRG-07): therapeutic efficacy and clinical implications of CECs*. Cancer Med. 2018 Jun;7(6):2442-2451. PMID: 29733541
- Rahbari NN et al, *Prognostic value of CEC in metastatic colorectal cancer*. Oncotarget. 2017 Jun 6;8(23):37491-37501. PMID: 28415583
- Sánchez Hernández A et al., *Quantification of CECs as predictor of response to chemotherapy with platinum and pemetrexed in patients with advanced non-squamous NSCLC*. Clin Transl Oncol. 2015 Apr;17(4):281-8. PMID: 25236392
- Ilie M et al., *Clinical value of circulating endothelial cells and of soluble CD146 levels in patients undergoing surgery for non-small cell lung cancer*. Br J Cancer. 2014 Mar 4;110(5):1236-43. PMID: 24473396
- Ali AM et al., *Determining CECs using CellSearch system during preoperative systemic chemotherapy in breast cancer patients. Eur J Cancer*. 2011 Oct;47(15):2265-72. PMID: 21737256
- Matsusaka S et al., *Circulating endothelial cells predict for response to bevacizumab-based chemotherapy in metastatic colorectal cancer. Cancer*. Chemother Pharmacol. 2011 Sep;68(3):763-8. PMID: 21170650
- Mostert B et al., Detection of circulating tumor cells in breast cancer may improve through enrichment with anti-CD146. Breast Cancer Res Treat. 2011 May;127(1):33-41. PMID: 20379845
- Bidard FC et al., *Clinical value of circulating endothelial cells and circulating tumor cells in metastatic breast cancer patients treated first line with bevacizumab and chemotherapy*. Ann Oncol. 2010 Sep;21(9):1765-71. PMID: 20233745
- Rowand JL et al, *Endothelial cells in peripheral blood of healthy subjects and patients with metastatic carcinomas*. Cytometry A. 2007 Feb;71(2):105-13. PMID: 17226859
- Smirnov DA et al., *Global gene expression profiling of circulating endothelial cells in patients with metastatic carcinomas*. Cancer Res. 2006 Mar 15;66(6):2918-22. PMID: 1654063

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# Myocardial Infarction


- Muse ED et al., *A Whole Blood Molecular Signature for Acute Myocardial Infarction*. Sci Rep. 2017 Sep 25;7(1):12268. PMID: 2894774
- Damani S et al., *Characterization of circulating endothelial cells in acute myocardial infarction*. Sci Transl Med. 2012 Mar 21;4(126):126ra33. PMID: 22440735


# Endocrinology


- Tanaka S et al., *Alterations of circulating endothelial cell and endothelial progenitor cell counts around the ovulation*. J Clin Endocrinol Metab. 2012 Nov;97(11):4182-92. PMID: 22948762
- Milardi D et al., *CECs as marker of endothelial damage in male hypogonadism*. J Androl. 2012 Nov-Dec;33(6):1291-7. PMID: 22700760


# Autoimmune disease


- De Simone C et al., *CEC levels in psoriatic patients and their modification after an anti-TNF-alpha (Etanercept) treatment*. J Eur Acad Dermatol Venereol. 2014 May;28(5):590-6. PMID: 23506557


# Graft vs Host Disease


- Almici C et al., *Counting CECs in allo-HSCT: an ad hoc designed polychromatic flowcytometry-based panel versus the CellSearch System*. Sci Rep. 2019 Jan 14;9(1):87. PMID: 30643152
- Almici C et al., *CEC count: a reliable marker of endothelial damage in patients undergoing hematopoietic stem cell transplantation*. Bone Marrow Transplant. 2017 Dec;52(12):1637-1642. PMID: 28892085
- Almici C et al., *Changes in circulating endothelial cells count could become a valuable tool in the diagnostic definition of acute graft-versus-host disease*. Transplantation. 2014 Oct 15;98(7):706-12. PMID: 25119132

# CELLSEARCH®
# for Circulating Tumor Cells (CTCs)

# CELLSEARCH® At the Forefront of Circulating Tumor Cells (CTC) Technology

MENARINI silicon biosystems

The only FDA cleared system for **the capture and detection of CTCs in metastatic breast, prostate or colorectal cancer**

The only NMPA cleared system for **the capture and detection of CTCs in metastatic breast cancer**

## Leading through industry firsts

Regulatory approvals: **US FDA** (first and only FDA-cleared product), **EU CE** marked according to IVDD and China **NMPA**

## Pioneering the automated detection, capture, and enumeration of CTCs

75 Issued patents and 10 current applications

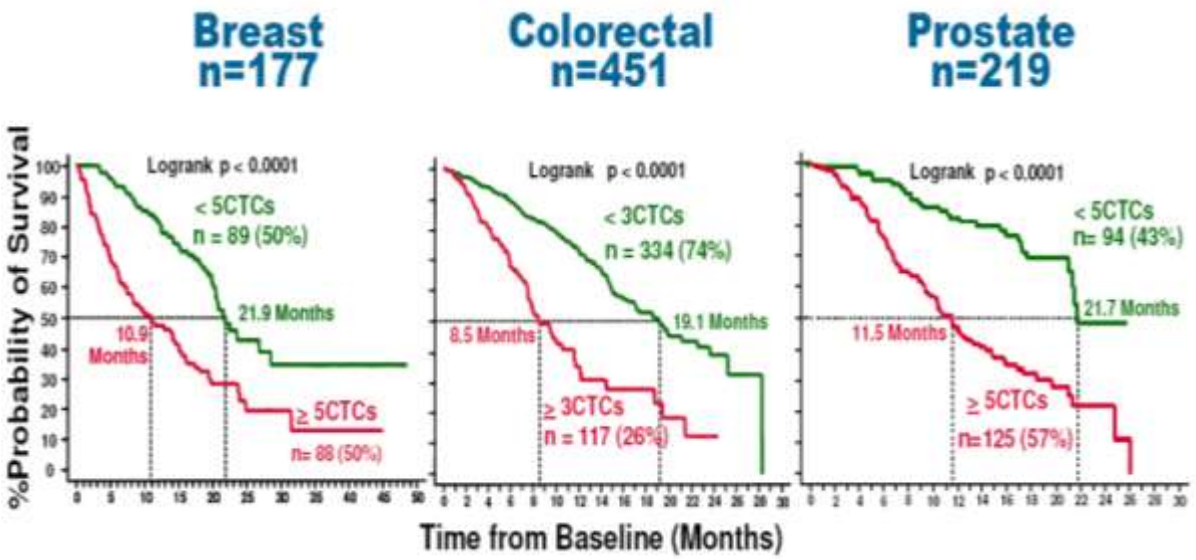
## Setting the standard

- 650 peer-reviewed publications
- Over 35 prospective clinical trials in metastatic breast, prostate and colorectal cancer

# CELLSEARCH in the clinical setting

MENARINI silicon biosystems

**The first and only** actionable test for detecting CTCs of epithelial origin in cancer patients with metastatic breast, prostate or colorectal cancer*



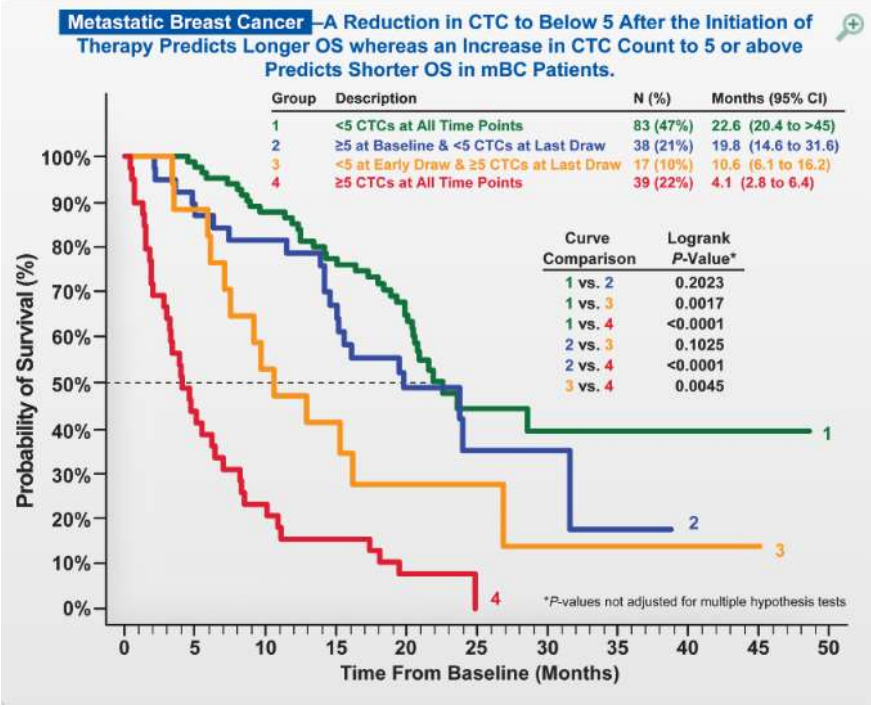

Cristofanilli M, Hayes DF, Budd GT, et al. J Clin Oncol. 2005;23(7):1420-1430.
de Bono JS, Scher HI, Montgomery RB, et al. Clin Cancer Res. 2008;14:6302-6309.
Cohen SJ, Punt CJA, Iannotti N, et al. J Clin Oncol. 2008;26(19):3213-3221.

**Circulating tumor cells (CTCs), as measured by the CELLSEARCH® CTC Test, are a strong, independent predictor of overall and progression-free survival in metastatic breast, prostate, and colorectal cancer**

*For more information on the full intended use and limitations please see the Instructions for Use

# Dynamic information on CTC count

## Longitudinal patient monitoring




**Throughout therapy, CELLSEARCH CTC testing can be used to monitor a patient's status by showing you whether their prognosis on any given therapy is favorable or unfavorable at any given time.**

* For more information on the full intended use and limitations please see the **Instructions for Use**

# CELLSEARCH for Research Use

MENARINI silicon biosystems

## Enrichment of rare cell types from blood

| | CELLSEARCH® Circulating Epithelial Cell Kit | CELLSEARCH® Circulating Endothelial Cell Kit | CELLTRACKS® Circulating Melanoma Cell Kit |
|---|---|---|---|
| **Cell Type** | Epithelial | Endothelial | Melanoma |
| **Ferrofluid Capture Reagent** | Anti-EpCAM | Anti-CD146 | Anti-CD146 |
| **Staining Reagent** | Anti-CK-PE/<br>Anti-CD45-APC | Anti-CD105-PE<br>Anti-CD45-APC | Anti-Mel-PE<br>Anti-CD45-APC<br>Anti-CD34-APC |

**Further information:**
www.cellsearchruo.com/ruo-products/kits

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CELLSEARCH TECHNOLOGY for COVID-19

# Menarini Silicon Biosystems
# Company Overview

# Rare Cells analysis with single cell precision

We offer unique **rare cell technologies** and solutions that provide clinical researchers with access to unparalleled **resolution** in the study of cells and their molecular characterization.

# Milestones

COMPANY OVERVIEW

**1999** Silicon Biosystems was founded

**2004** CELLSEARCH FDA cleared for IVD use

**2010** DEPArray V1 launched & opening of USA branch

**2011** CELLSEARCH NMPA (China) approval

**2013** Menarini acquires Silicon Biosystems

**2014** DEPArray V2 launched

**2016** DEPArray NxT launched

**2017** CELLSEARCH acquisition

**2020** DEPArray PLUS

# Menarini Silicon Biosystems in the World

**Menarini Silicon Biosystems Inc.**
Huntington Valley – North America Operations

**Menarini Silicon Biosystems SpA**
Bologna – Corporate HQ

**Menarini APAC**
Singapore - Commercial Office

www.**siliconbiosystems**.com