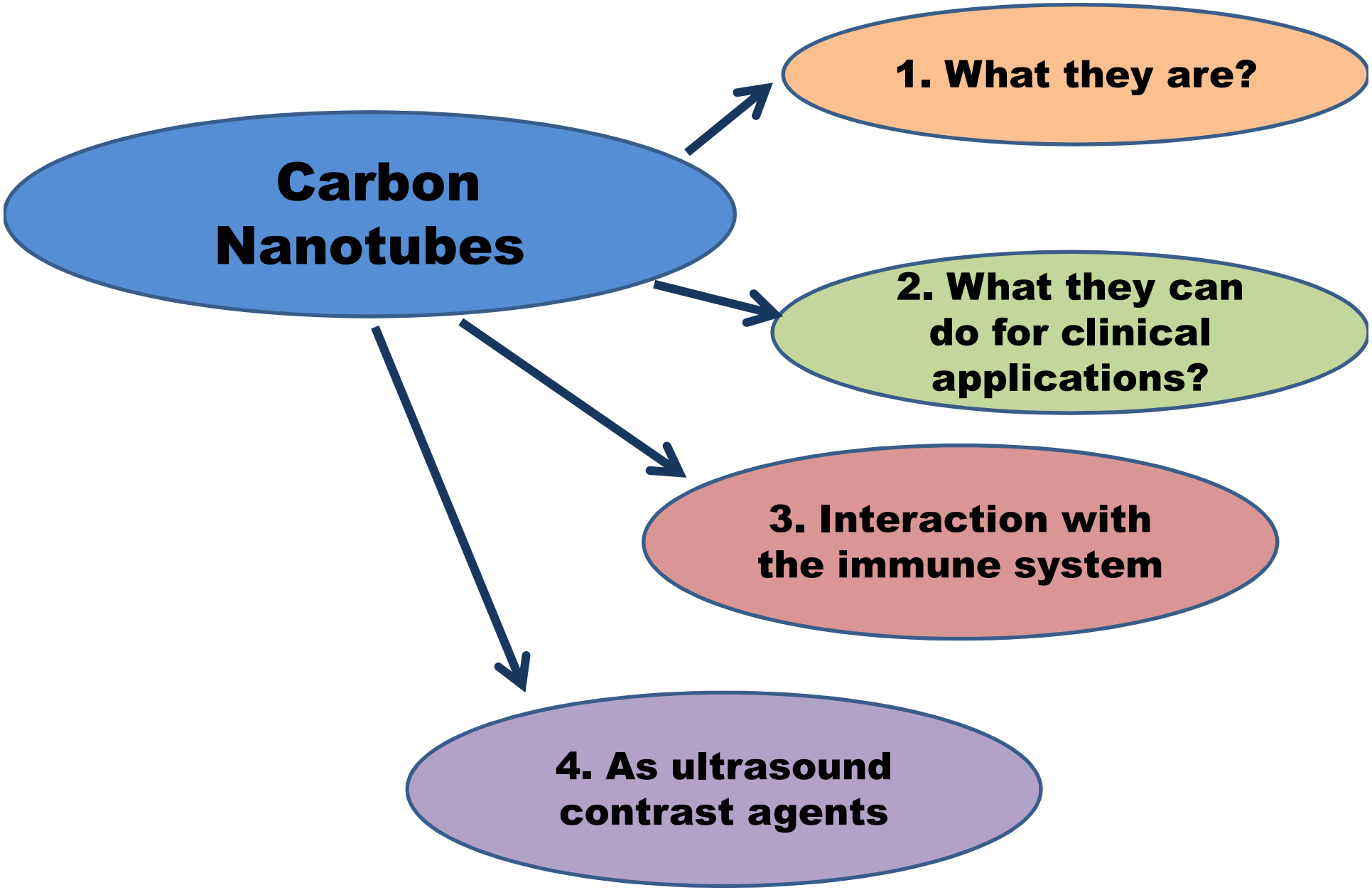
Functionalized carbon nanotubes as immunomodulator systems and ultrasound contrast agents

3rd International Conference and Exhibition on Clinical & Cellular Immunology
September 29 - October 01, 2014
Baltimore, USA

Lucia Gemma Delogu Ph.D.

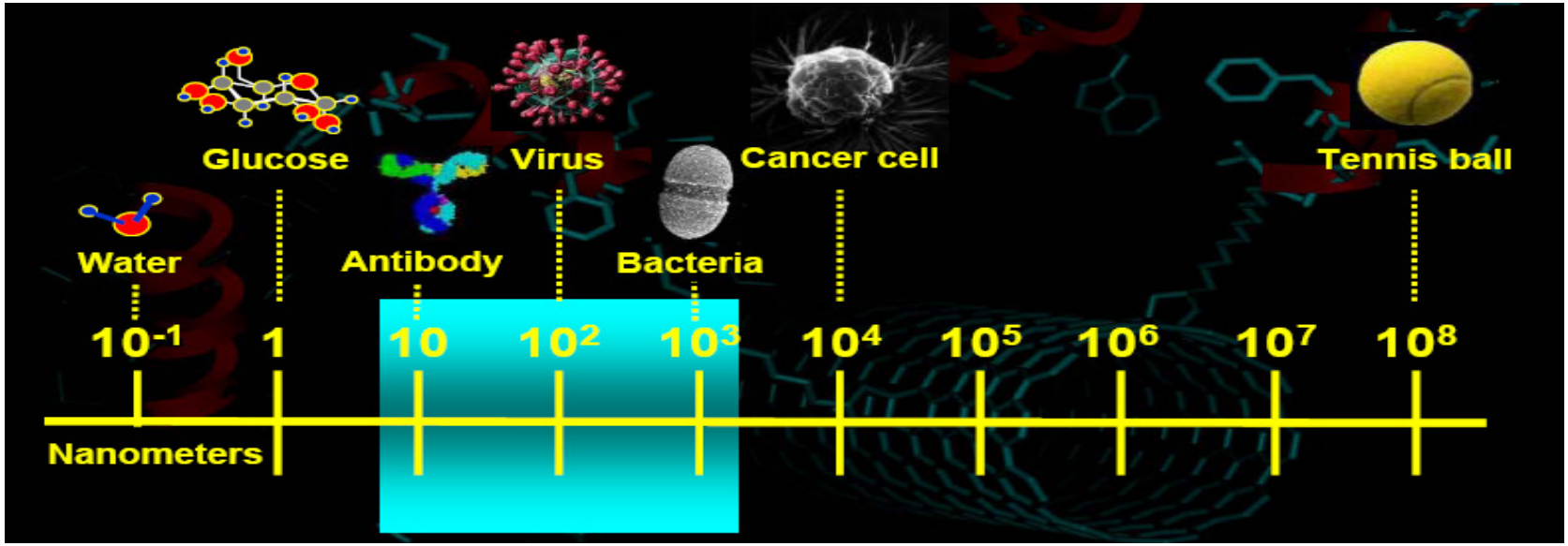
University of Sassari, Italy




Carbon Nanotubes
1. What they are?
2. What they can do for clinical applications?
3. Interaction with the immune system
4. As ultrasound contrast agents

Carbon Nanotubes

1. What they are?



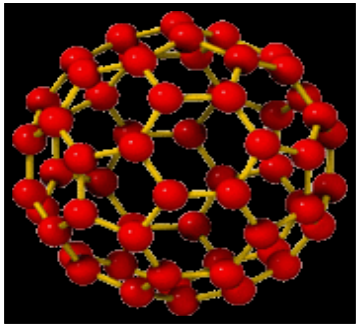
Nanomaterials dimensions

- Carbon Nanotubes

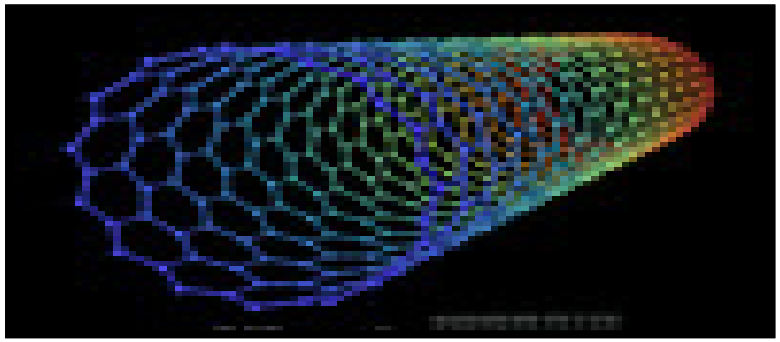
1. What they are?

Carbon Atoms





1991
Sumio Iijima





SWCNTs
Single-Walled Carbon Nanotubes



MWCNTs
Multi-Walled Carbon Nanotubes

- Carbon Nanotubes

1. What they are?

Biocompatible

- **Kam NWS** et al. *J Am Chem Soc* 2004
- **Cherukuri** [illegible] *J Am Chem* [illegible]004
- **Chen X** et al. *Ang*[illegible]
- **Kam N**[illegible]
- **Wu W** et al. *A*[illegible]
- **Dumortier H** et al. *N*[illegible]
- **Singh R** et al *PNAS* 2006
- **Cherukuri P** et al *PNAS* 2006
- **Chen X** et al. *J Am Chem Soc 2006*
- **Liu Z** et al. *ACS NANO* 2007
- **Liu Z** et al. *Angew Chem Int Ed* 2007
- **Chin SF** et al *Exper Biol Med* 2007
- **Yehia HN** et al *J Nanobiotech* 2007
- **Schipper ML** et al. *Nature Nanotech* 2008
- **Wu P** et al. *Angew Chem Int Ed* 2008
- **Ali-Boucetta H** et al. *Angew Chem Int Ed Engl.* 2013

…only when WELL FUNCTIONALIZED...

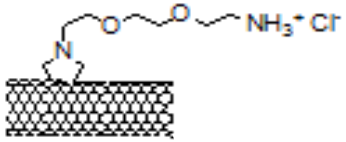
Biodegradable

- **K**[illegible] **VE** et al. *Nature Nanotech* 2010
- [illegible]*s Chem Soc* 2011
- [illegible]2011
- [illegible]*CC chem Res* 2012
- [illegible]*nond* 2012

i.e. Phospholipids absorption



i.e. 1,3 dipolar cycloaddition



Carbon Nanotubes

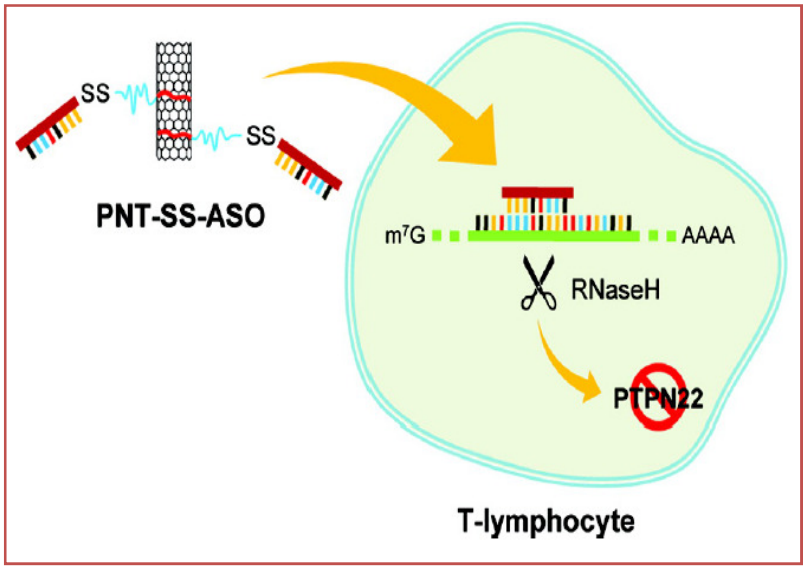
2. What they can do for clinical application?

In therapy

- **Drug Delivery**
- **Gene delivery**

Carbon Nanotubes (CNTs)

i.e. Delogu LG et al Bioconjugate chemistry 2009
Delogu LG et al JNN 2010



Carbon Nanotubes

2. What they can do for clinical application?

Carbon Nanotubes (CNTs)

In therapy

- **Drug Delivery**
- **Gene delivery**
- **Hyperthermia**
- **Tissue regeneration**
- **To increase the neuronal perfomance**

In diagnosis

- **Biosensors**
- **In imaging**
- **For enantioseparation of chiral drugs**

- Carbon Nanotubes

3. Interaction with the immune system

...on human immune cells

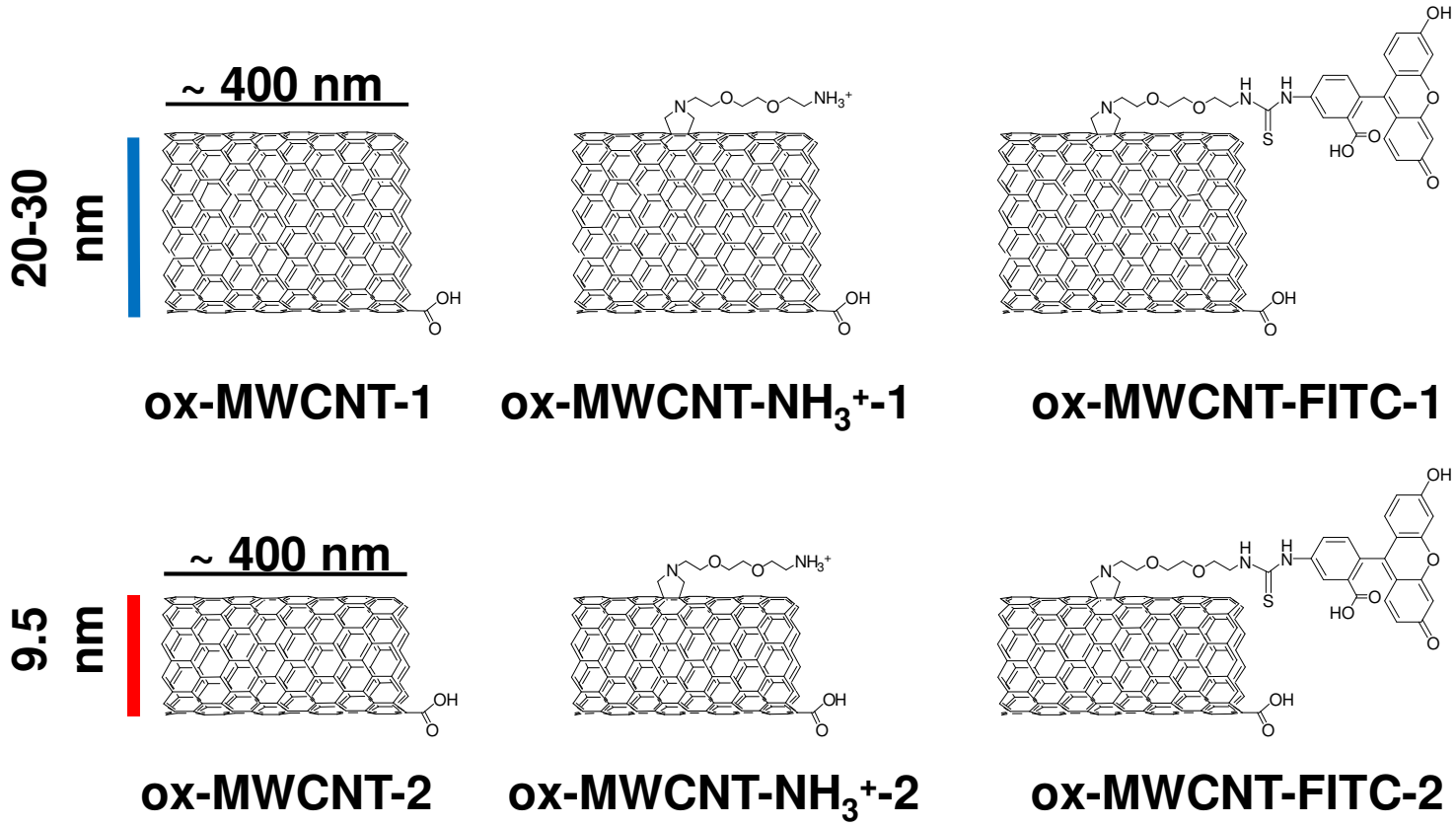
- Do differently functionalized carbon nanotubes with different diameters act differently with the immune cells?
- Do they interact with the immune system?.....
- .....if yes, how?
- Is the possible impact dependent on the cell type?

Delogu LG et al. Nanomedicine (London) 2012

- AAAAAA

- Carbon Nanotubes

3. Interaction with the immune system



ox-MWCNT-1 ox-MWCNT-NH_3^+-1 ox-MWCNT-FITC-1

ox-MWCNT-2 ox-MWCNT-NH_3^+-2 ox-MWCNT-FITC-2

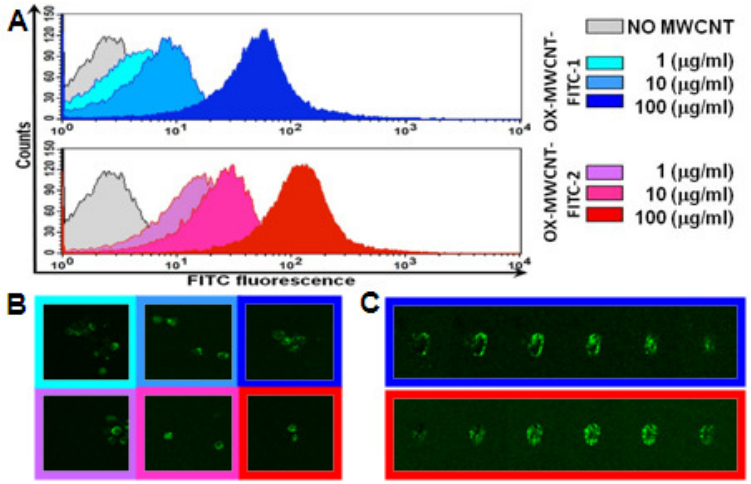
Delogu LG et al. Nanomedicine (London) 2012

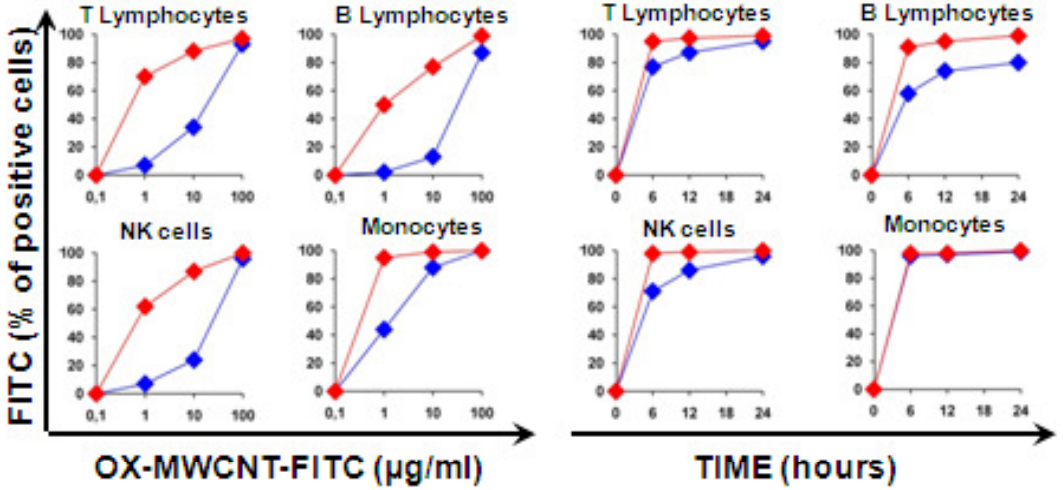
- Carbon Nanotubes

3. Interaction with the immune system

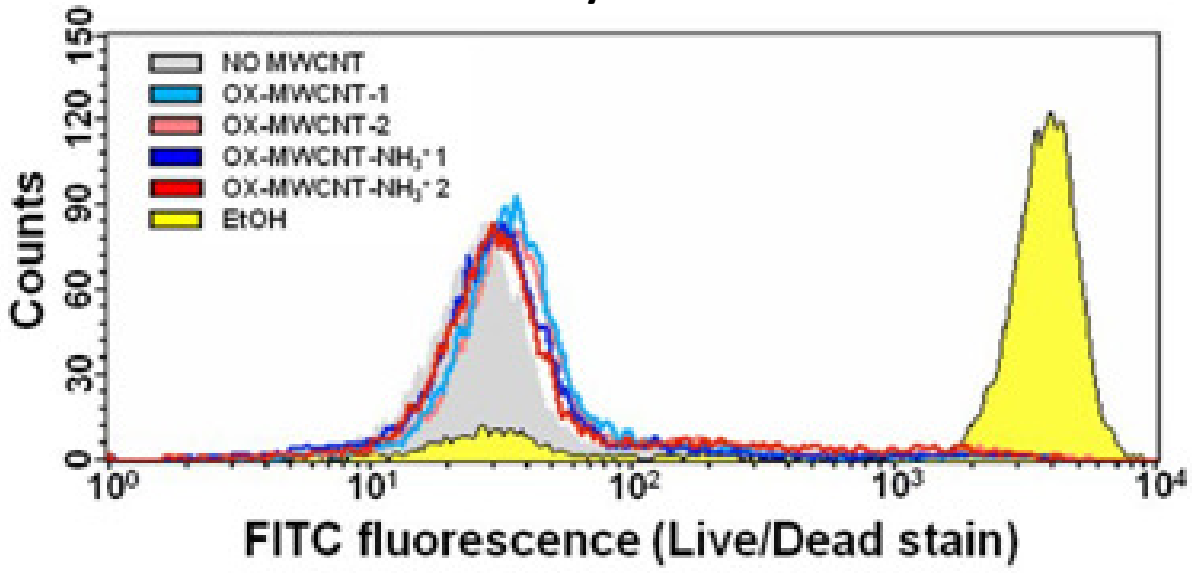
	Diameter
ox-MWCNT-FITC-1	20-30 nm
ox-MWCNT-FITC-2	9.5 nm

Uptake





Viability

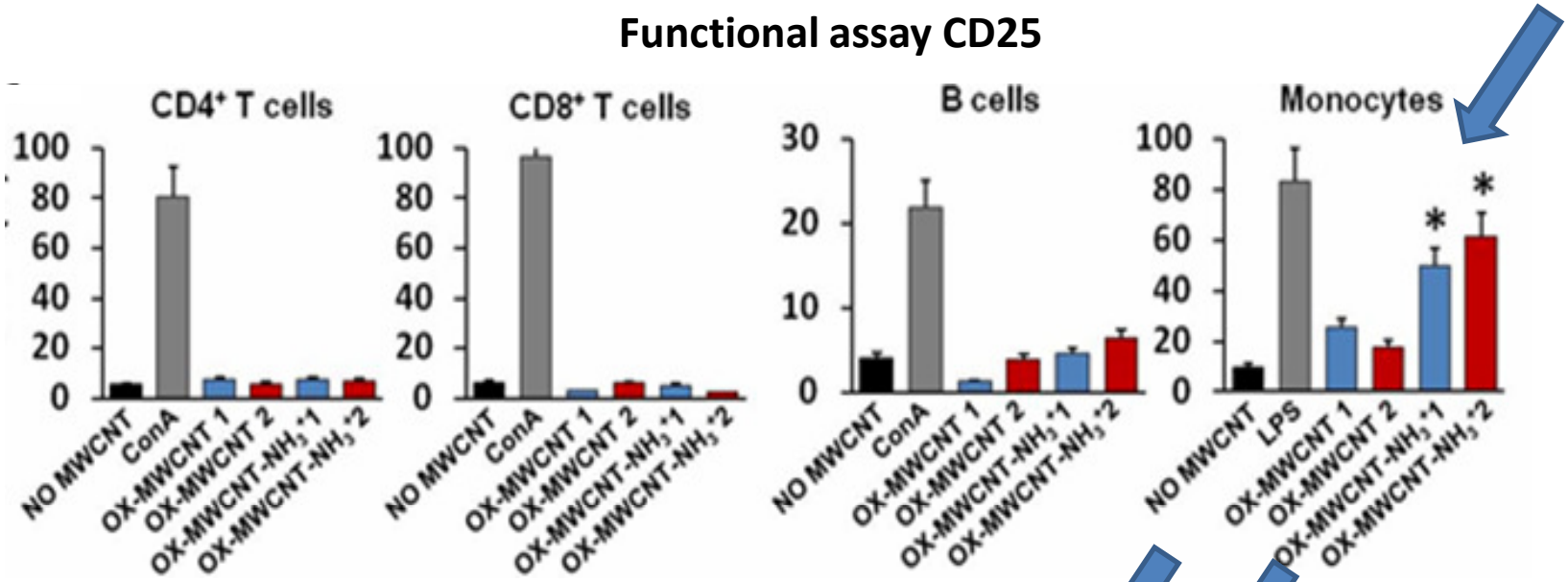


Delogu LG et al. Nanomedicine (London) 2012

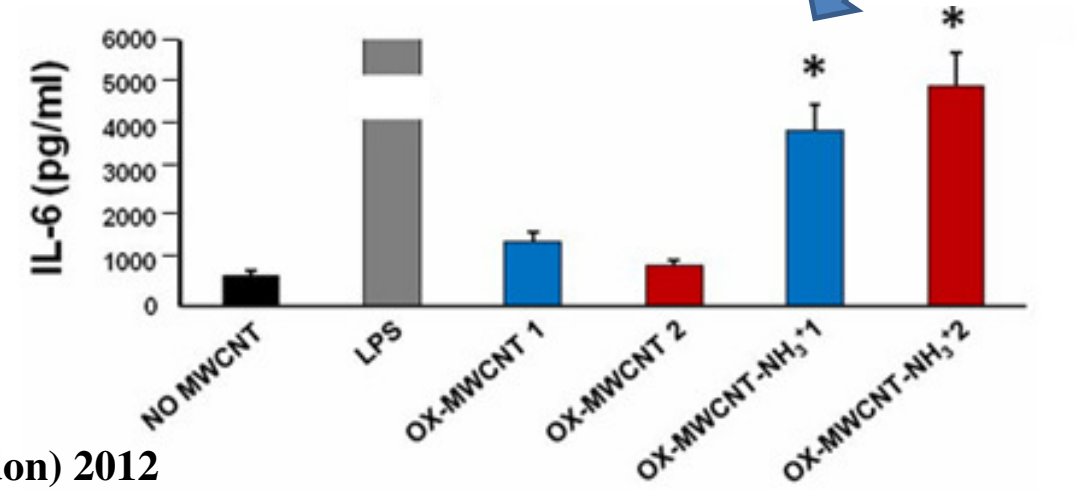
- Carbon Nanotubes

3. Interaction with the immune system

Functional assay CD25



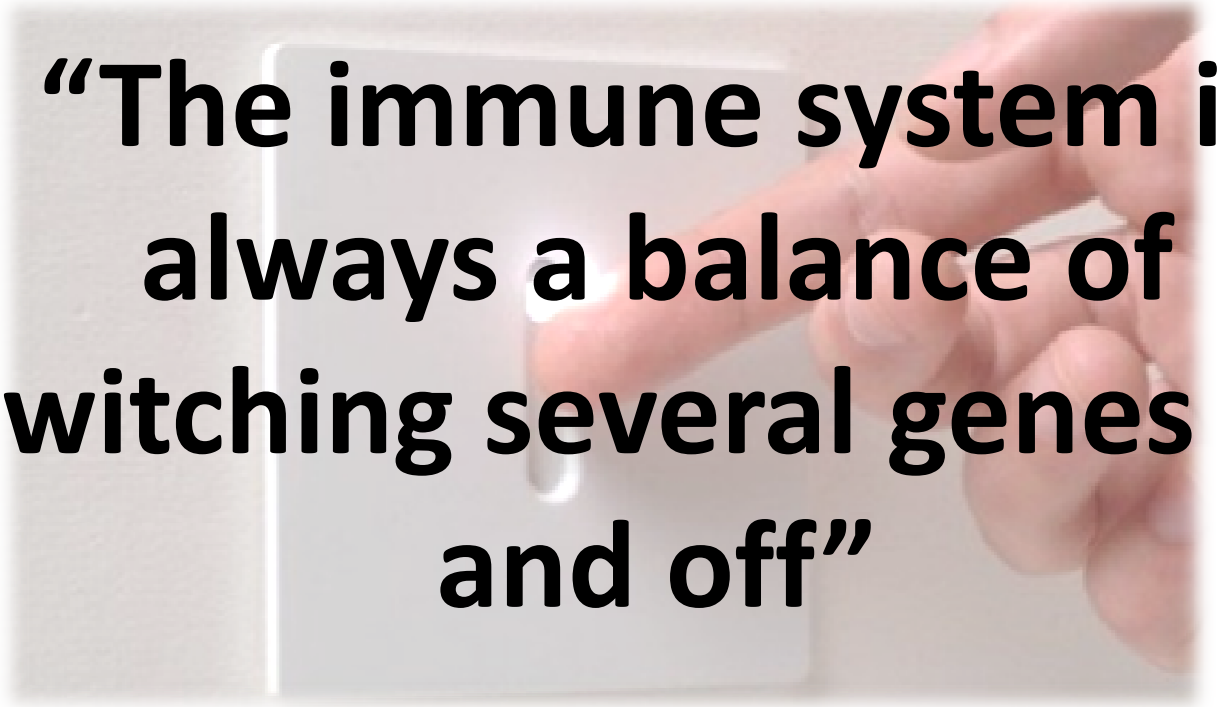
Monocytes



Delogu LG et al.
Nanomedicine (London) 2012

- Carbon Nanotubes

3. Interaction with the immune system



“The immune system is always a balance of switching several genes on and off”

- Carbon Nanotubes

3. Interaction with the immune system

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Leading opinion

Functionalized carbon nanotubes as immunomodulator systems

Mario Pescatori [a,b], Davide Bedognetti [c], Enrica Venturelli [d], Cécilia Ménard-Moyon [d], Camilla Bernardini [e], Elena Muresu [f], Andrea Piana [f], Giorgio Maida [f], Roberto Manetti [g], Francesco Sgarrella [h], Alberto Bianco [d,*], Lucia Gemma Delogu [h,*]

[a] *Department of Surgery, Section Surgical Oncology, Laboratory Experimental Surgical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands*
[b] *Crosslinks BV, Rotterdam, The Netherlands*
[c] *Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center and Trans-National Institutes of Health Center for Human Immunology, National Institutes of Health, Bethesda, MD 20892, USA*
[d] *Centre National de la Recherche Scientifique, Institut de Biologie Moléculaire et Cellulaire, Laboratoire d'Immunopathologie et Chimie Thérapeutique, 67000 Strasbourg, France*
[e] *Institute of Anatomy and Cell Biology, Università Cattolica S. Cuore, Rome, Italy*
[f] *Dipartimento di Scienze Biomediche, Università degli Studi di Sassari, 07100 Sassari, Italy*
[g] *Dipartimento di Medicina Clinica, Sperimentale e Oncologica, Università degli Studi di Sassari, 07100 Sassari, Italy*
[h] *Dipartimento di Chimica e Farmacia, Università degli Studi di Sassari, 07100 Sassari, Italy*

- Carbon Nanotubes

3. Interaction with the immune system

Pescatori M et al. Biomaterials 2013

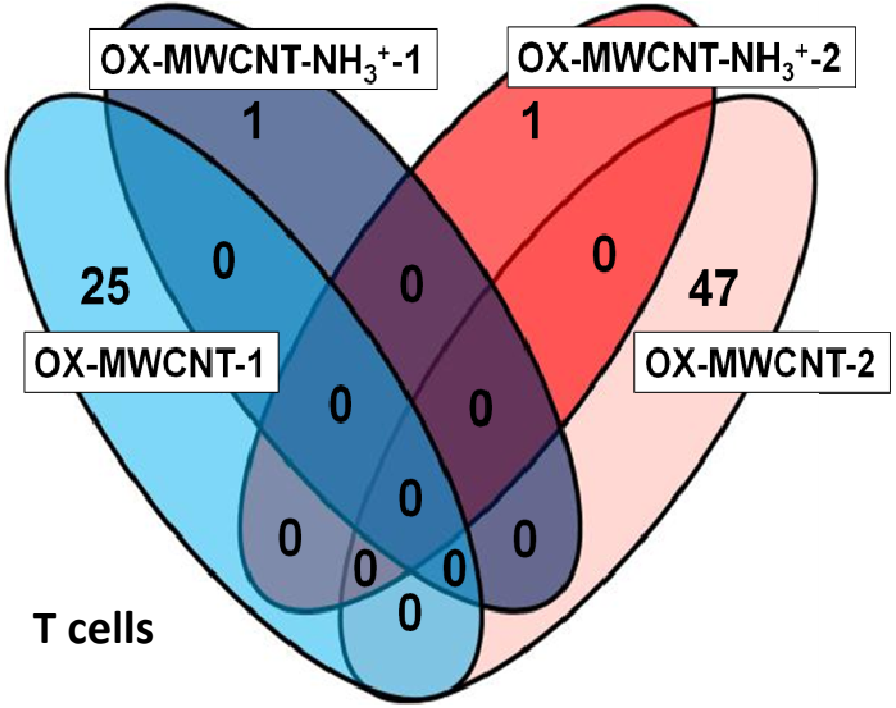
Affymetrix technology to analyze the expression of 32 020 transcripts

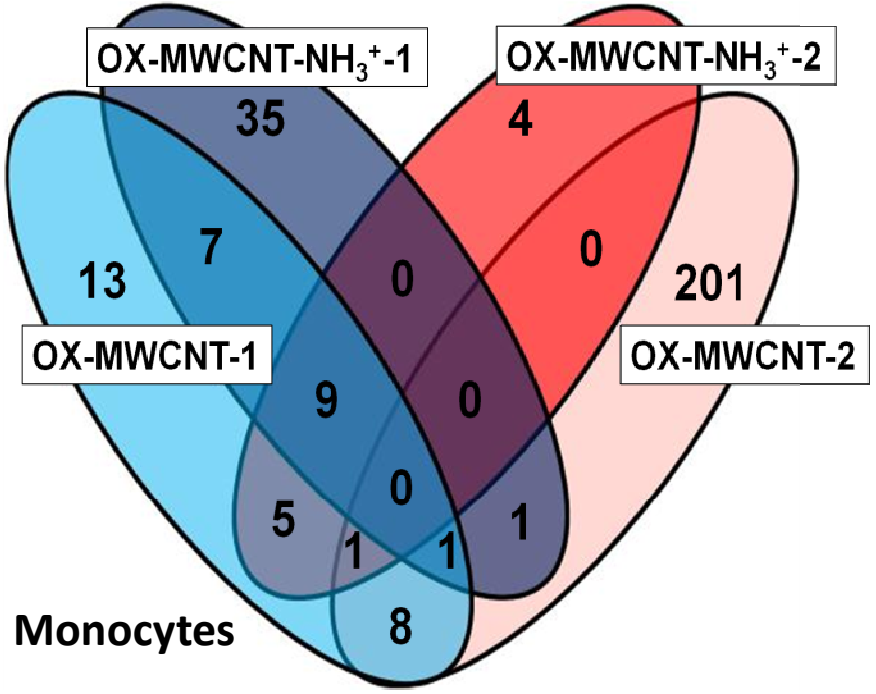
Diameter 20-30 nm

9.5 nm

P< 0.001

	T cells	Monocytes
OX-MWCNTs-1	25	44
OX-MWCNTs-2	47	212
OX-MWCNTNH$_3^+$-1	1	53
OX-MWCNTNH$_3^+$-2	1	19

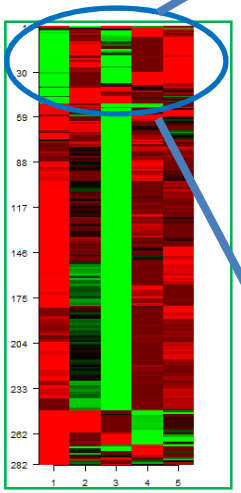


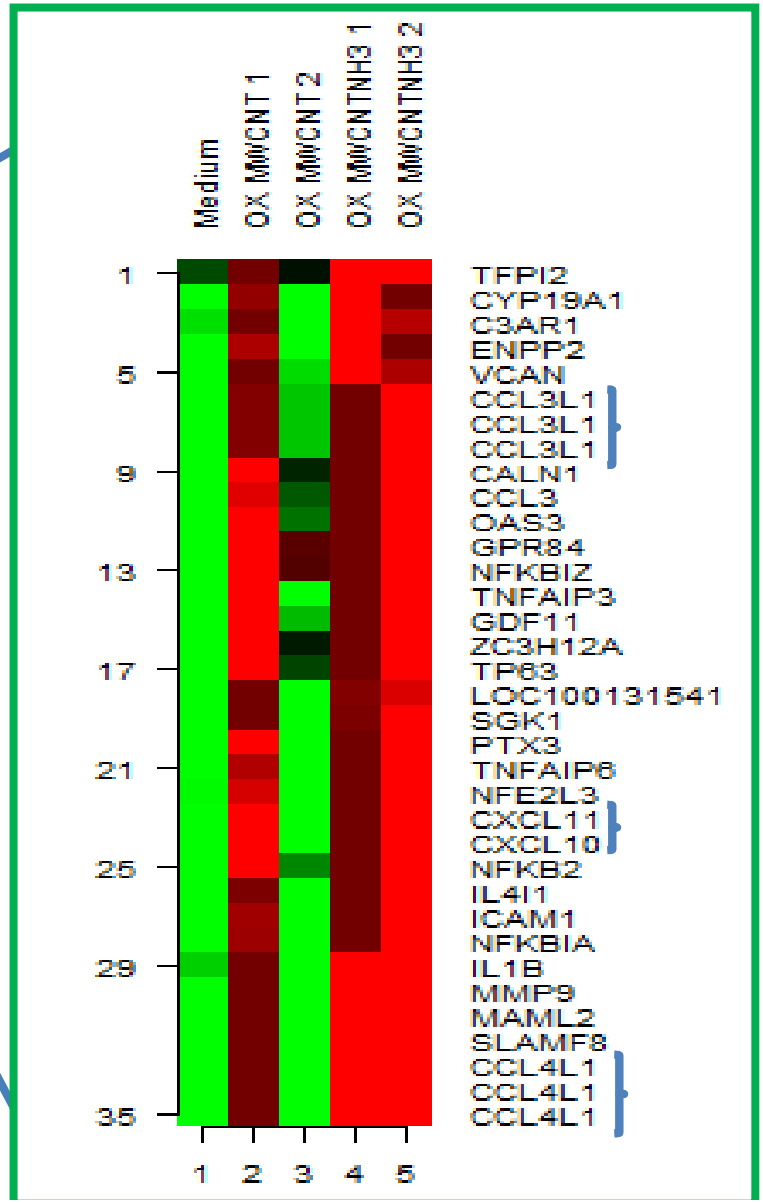


- Carbon Nanotubes

3. Interaction with the immune system

Monocytes activators MA-CNTs





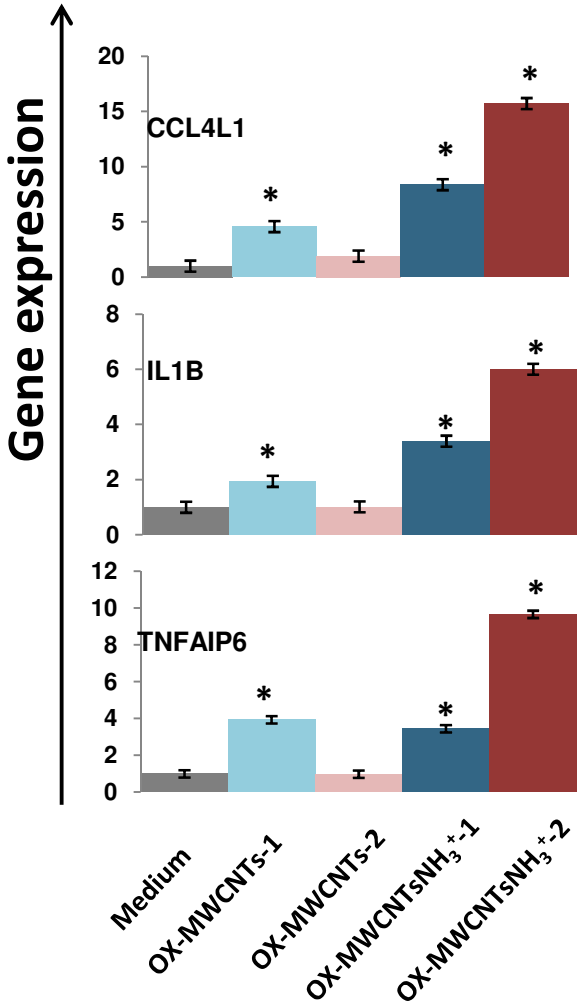
Pescatori M et al. Biomaterials 2013

- Carbon Nanotubes

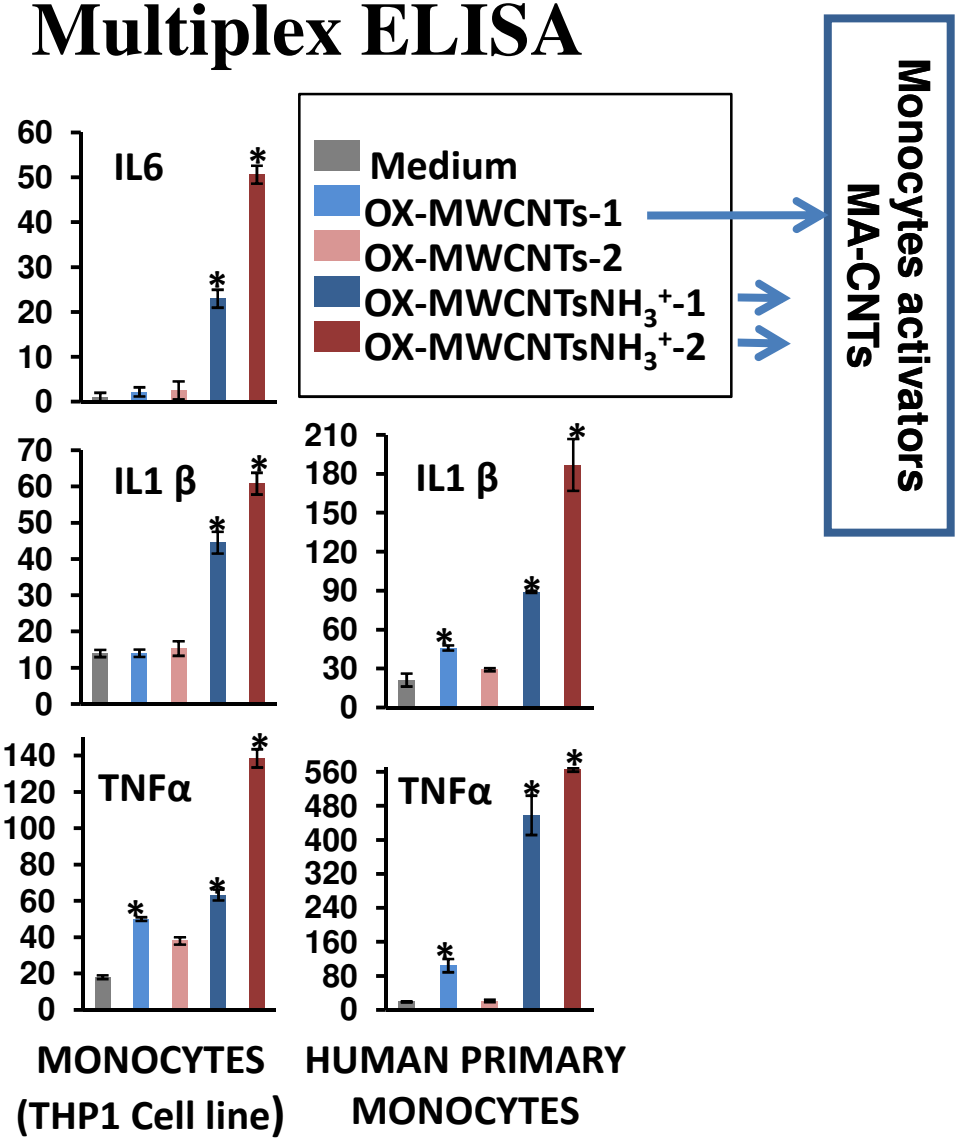
3. Interaction with the immune system

Pescatori M et al. Biomaterials 2013

Real Time PCR



Multiplex ELISA



- Carbon Nanotubes

3. Interaction with the immune system

- A cell specific action on monocytes from 3 types of nanotubes (MA-CNTs) with the selective induction of innate activation pathways.

We have the opportunity to choose the activators CNTs…

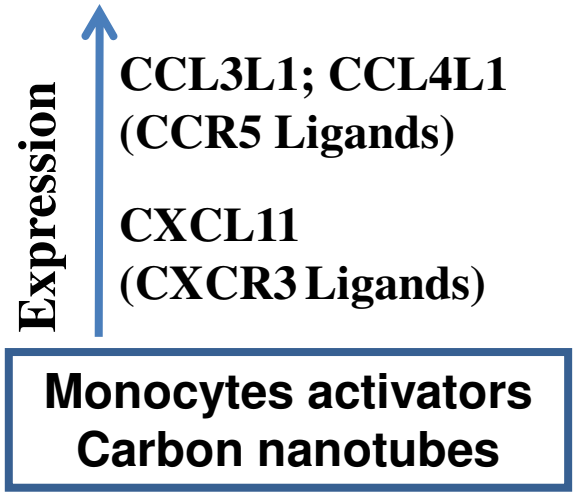
Future perspectives:

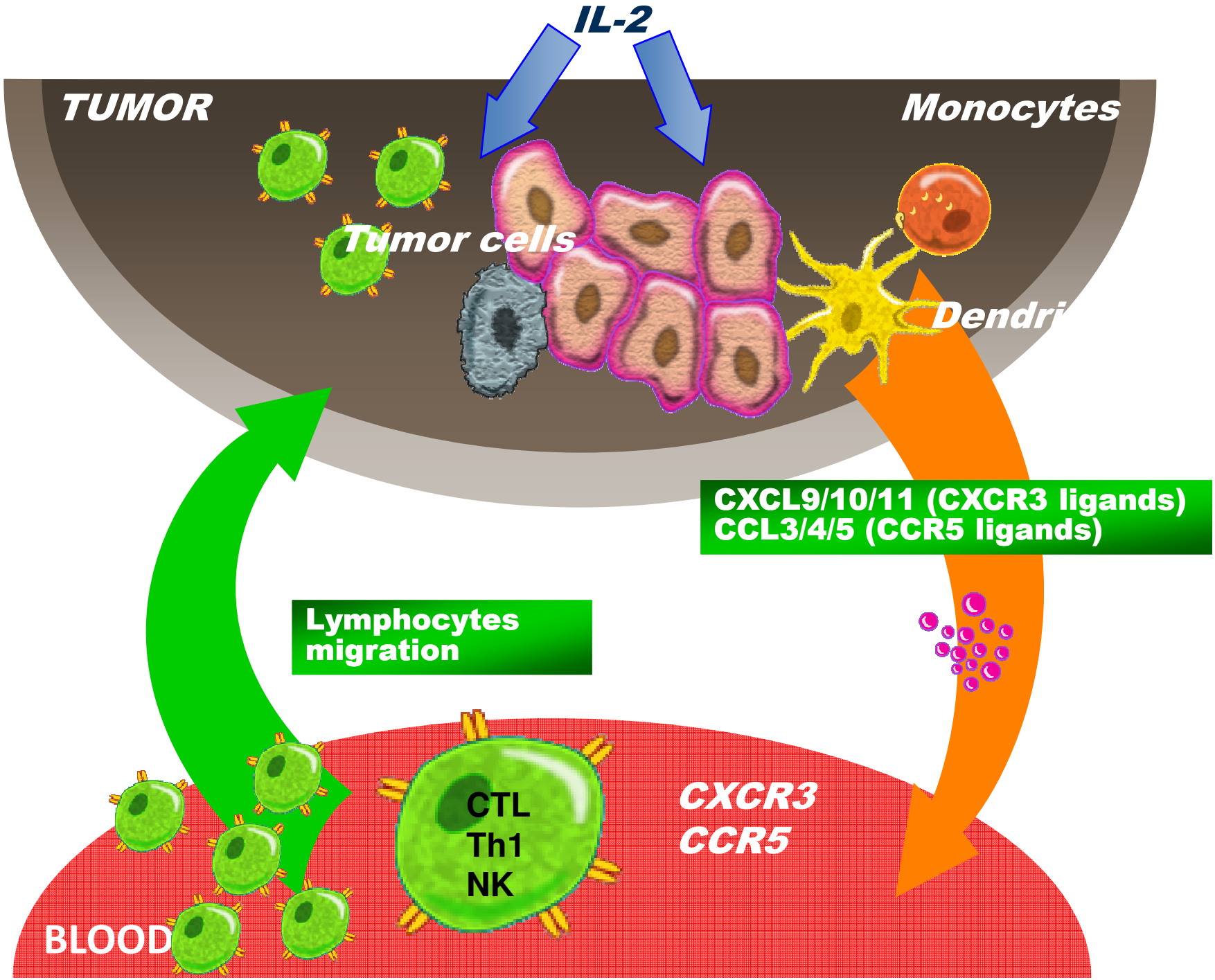
A carrier of a vaccine able also to act as a adjuvant

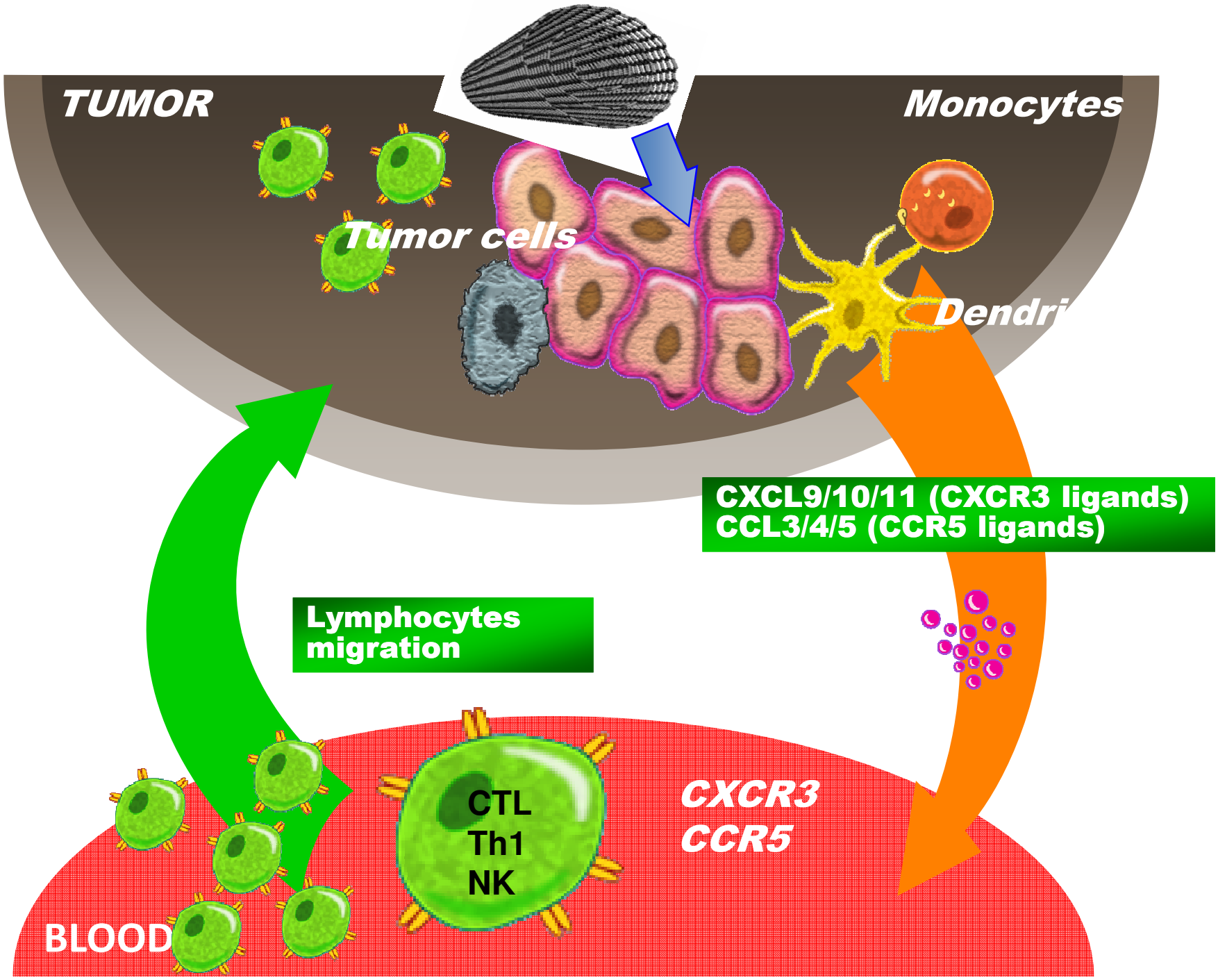
- Carbon Nanotubes

3. Interaction with the immune system

We have the opportunity to choose the activators CNTs…
Future perspectives:

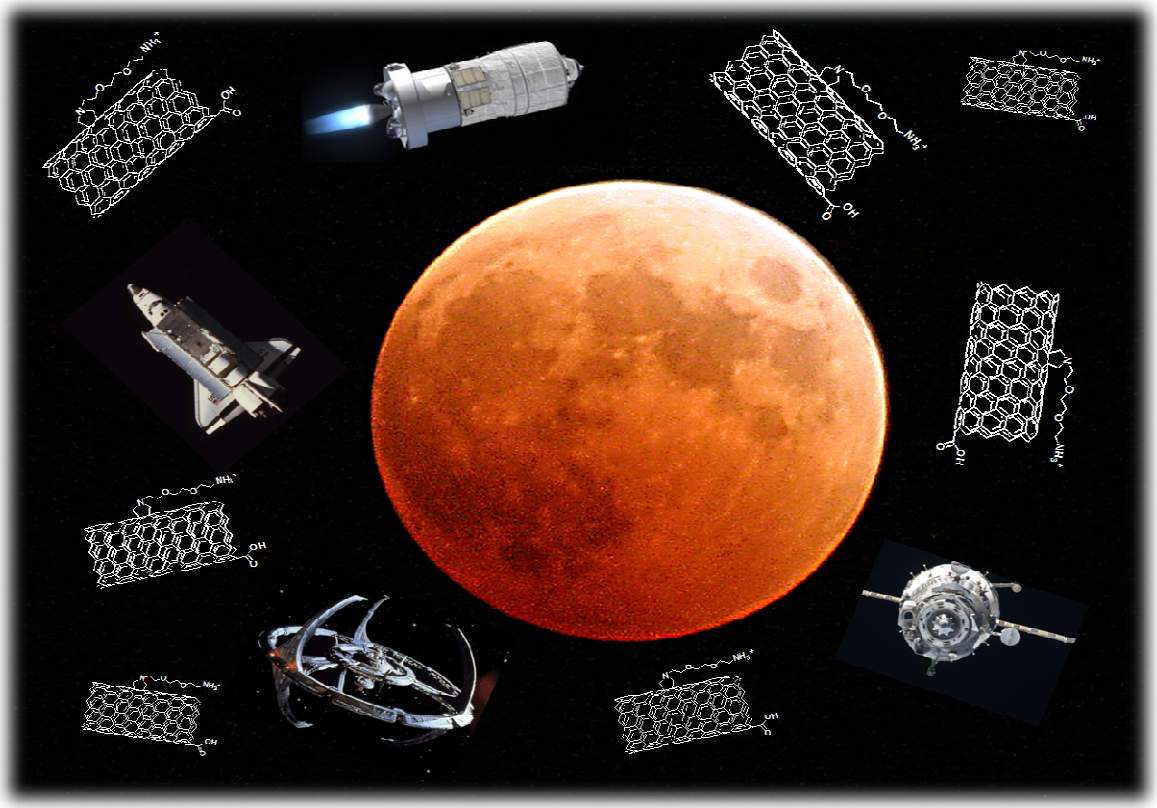



IL-2
TUMOR
Monocytes
Tumor cells
Dendri
CXCL9/10/11 (CXCR3 ligands)
CCL3/4/5 (CCR5 ligands)
Lymphocytes migration
CTL
Th1
NK
CXCR3
CCR5
BLOOD


TUMOR
Monocytes
Tumor cells
Dendri
CXCL9/10/11 (CXCR3 ligands)
CCL3/4/5 (CCR5 ligands)
Lymphocytes migration
CTL
Th1
NK
CXCR3
CCR5
BLOOD

- Carbon Nanotubes

3. Interaction with the immune system



- Carbon Nanotubes

3. Interaction with the immune system

Nanoscale

COMMUNICATION

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Immunomodulatory properties of carbon nanotubes are able to compensate immune function dysregulation caused by microgravity conditions†

Claudia Crescio,[a] Marco Orecchioni,[b] Cécilia Ménard-Moyon,[c] Francesco Sgarrella,[b] Proto Pippia,[a] Roberto Manetti,[d] Alberto Bianco*[c] and Lucia Gemma Delogu*[b]

Cite this: DOI: 10.1039/c4nr02711f

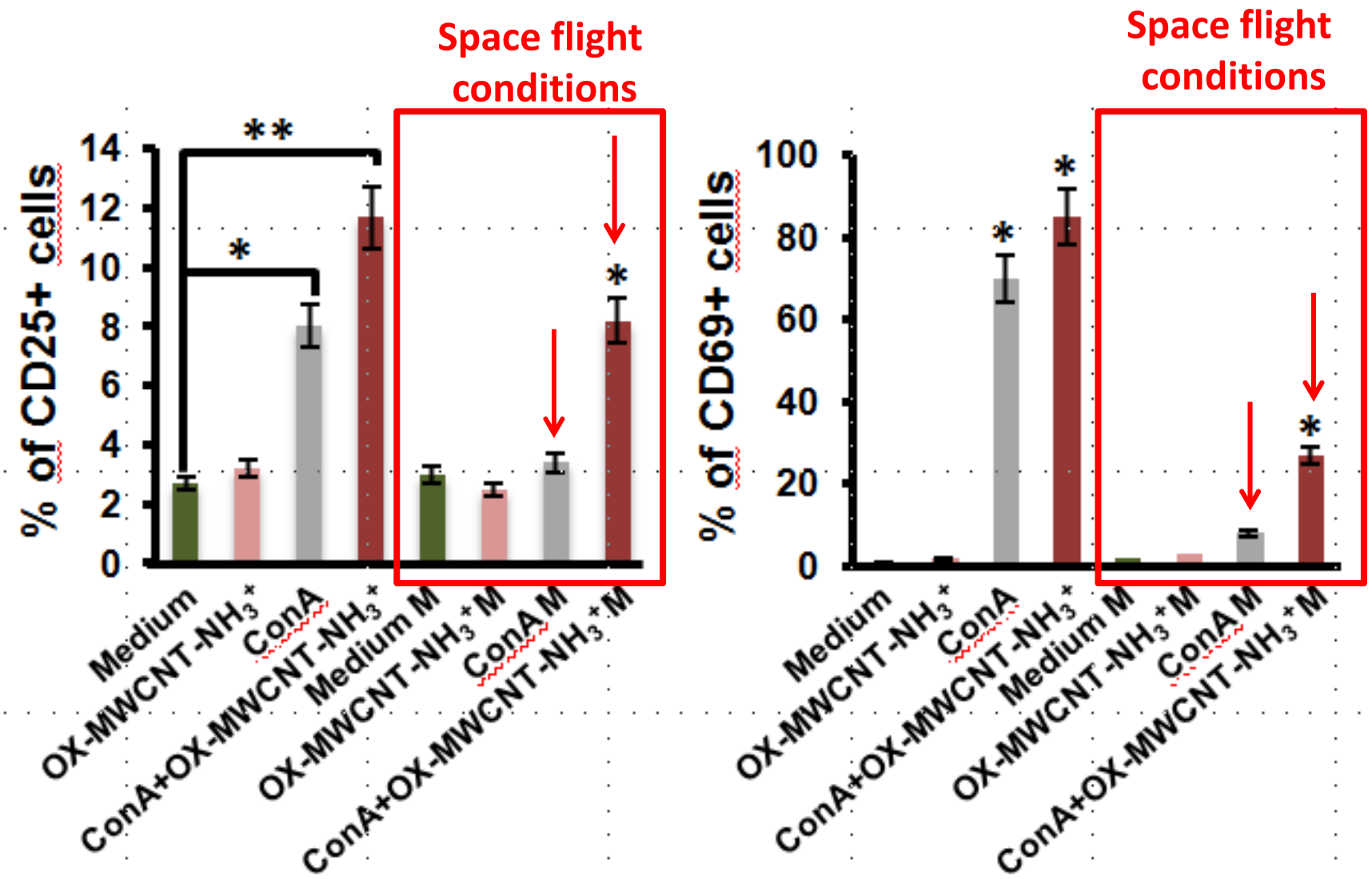
Received 17th May 2014
Accepted 14th June 2014

DOI: 10.1039/c4nr02711f

www.rsc.org/nanoscale

- Carbon Nanotubes

3. Interaction with the immune system



Possible future applications:

- in immunotherapy

- as vaccine adjuvants

- as possible fighters to contrast spaceflight immune cell dysregulation.

- Carbon Nanotubes

4. As ultrasound contrast agents

PNAS PNAS

Functionalized multiwalled carbon nanotubes as ultrasound contrast agents

Lucia Gemma Delogu[a,1], Gianpaolo Vidili[b], Enrica Venturelli[c], Cécilia Ménard-Moyon[c], Maria Antonietta Zoroddu[a], Giovannantonio Pilo[d], Paola Nicolussi[d], Ciriaco Ligios[d], Davide Bedognetti[e,f], Francesco Sgarrella[a], Roberto Manetti[b], and Alberto Bianco[c,1]

[a]Dipartimento di Chimica e Farmacia and [b]Dipartimento di Medicina Clinica, Sperimentale e Oncologica, Università degli Studi di Sassari, 07100 Sassari, Italy; [c]Centre National de la Recherche Scientifique, Institut de Biologie Moléculaire et Cellulaire, Laboratoire d'Immunologie et Chimie Thérapeutiques, Unité Propre de Recherche 9021, 67000 Strasbourg, France; [d]Istituto Zooprofilattico Sperimentale della Sardegna, 07100 Sassari, Italy; [e]Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center and Trans-National Institutes of Health Center for Human Immunology, National Institutes of Health, Bethesda, MD 20892; and [f]Dipartimento di Oncologia, Biologia, e Genetica e Dipartimento di Medicina Interna, Universita' degli Studi di Genova, 16132 Genoa, Italy

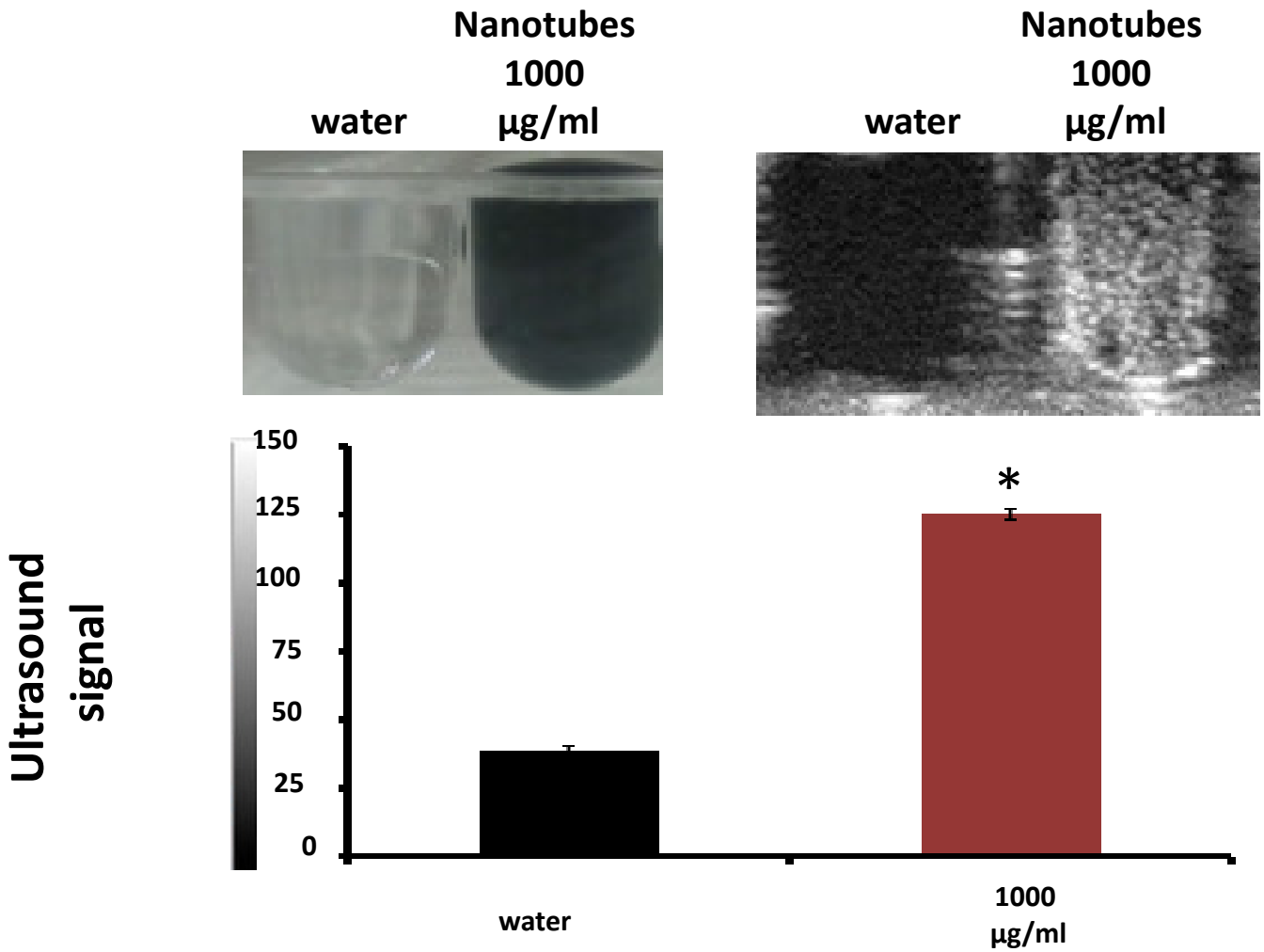
Edited[†] by Sumio Iijima, Meijo University, Nagoya, Japan, and approved August 31, 2012 (received for review May 16, 2012)

Ultrasonography is a fundamental diagnostic imaging tool in everyday clinical practice. Here, we are unique in describing the use of functionalized multiwalled carbon nanotubes (MWCNTs) as hyperechogenic material, suggesting their potential application as ultrasound contrast agents. Initially, we carried out a thorough investigation to assess the echogenic property of the nanotubes in vitro. We demonstrated their long-lasting ultrasound contrast properties. We also showed that ultrasound signal of functional

source, but the conventional ultrasound introduces high-frequency acoustic waves and detects their echo; (*ii*) the photoacoustics is broadband, containing frequencies up to 50 MHz or more, but ultrasound usually uses a single frequency. In addition, despite the high potential of photoacoustic imaging, ultrasonography remains the most popular medical imaging modality because of to the low price per examination and safety (11). There are few

- Carbon Nanotubes

4. As ultrasound contrast agents

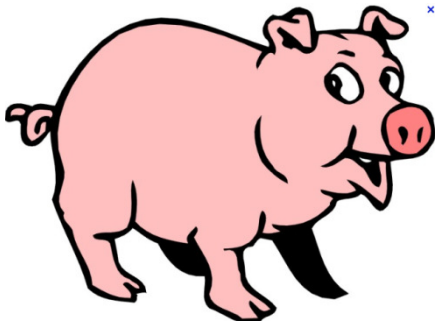


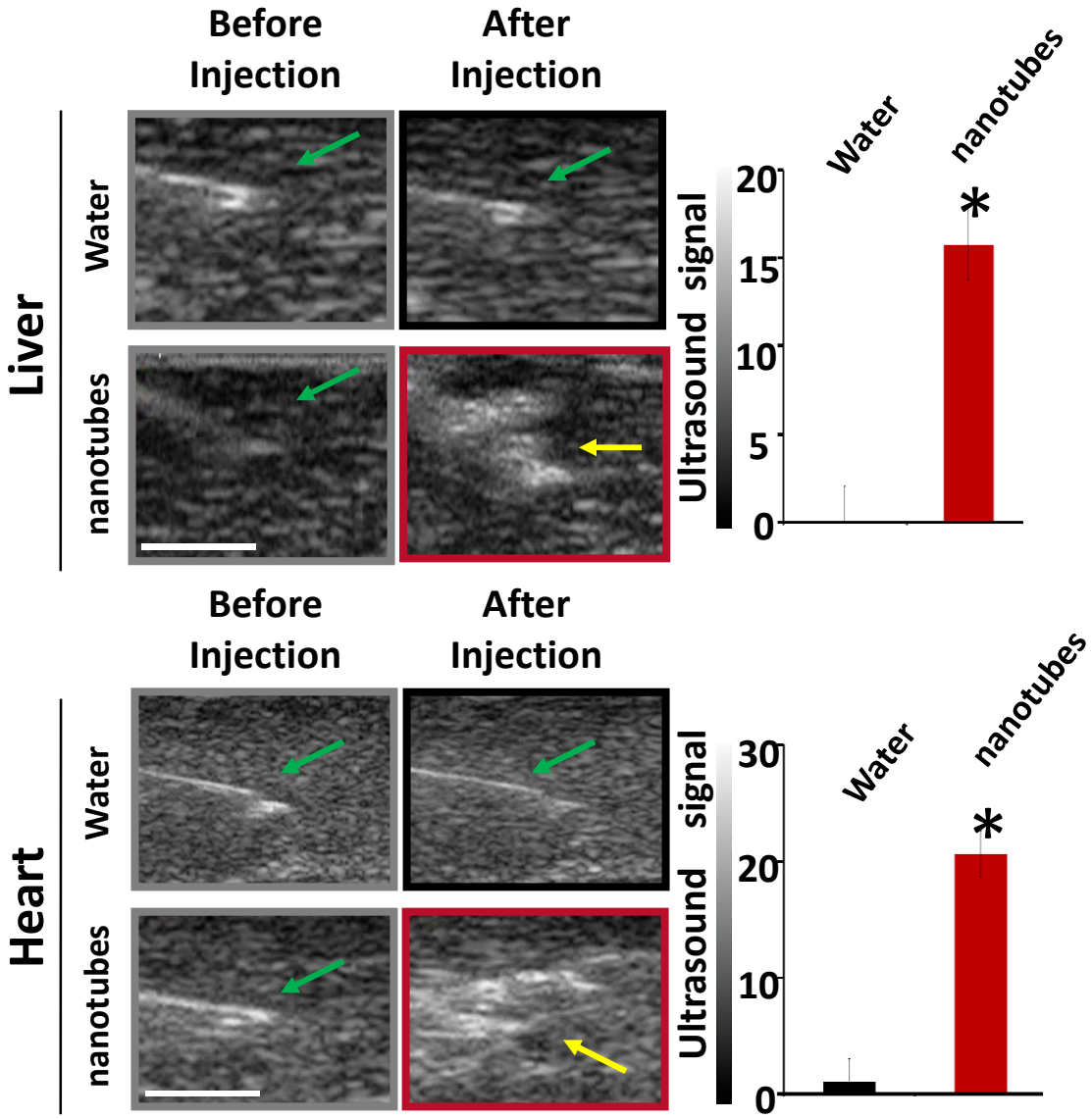
Delogu LG et al. PNAS 2012

* Pvalue < 0.05

- Carbon Nanotubes

4. As ultrasound contrast agents

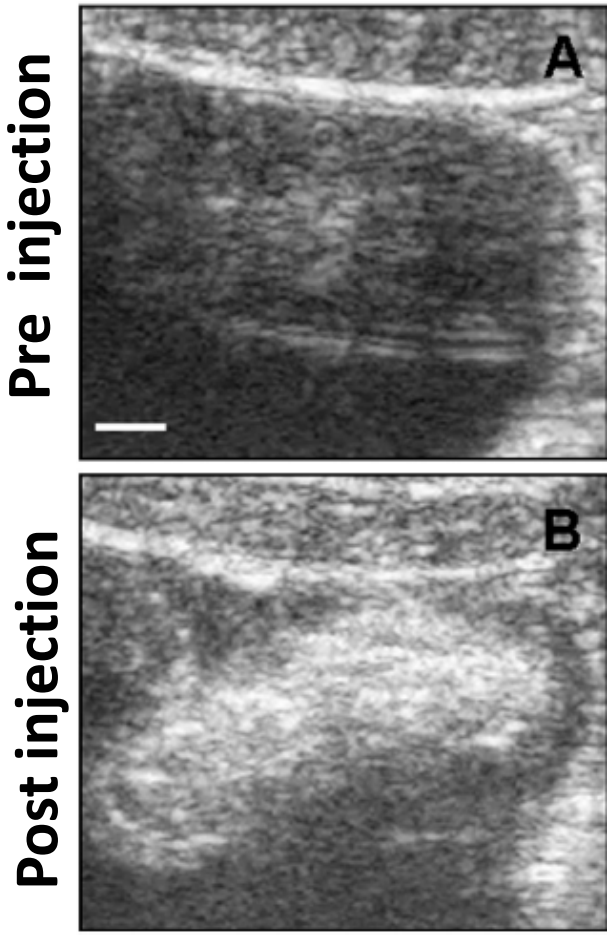




- Carbon Nanotubes

4. As ultrasound contrast agents

IN VIVO PIG BLADDER



- Carbon Nanotubes

4. As ultrasound contrast agents

MICROBUBBLES

- Big size 1-8 µm; purely intravascular flow tracers
- Are classified as long half-life agents when the signal persists for more than 5 min after injection
- Research as drug delivery system is still at an early stage

CARBON NANOTUBES

- Small enough to reach a tumor region (vascular pore up to 780 nm)
- Long lasting signal (are eliminated in 24-48 hours by renal clearance)
- Can be functionalized with therapeutic molecules

Working on carbon nanotubes



University of Sassari

Francesco Sgarrella
Roberto Manetti
Proto Pippia
Giampaolo Vidili
Claudia Crescio
Marco Orecchioni

CNRS, Strasbourg

Alberto Bianco

Rotterdam, Netherlands

Mario Pescatori

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Ciriaco Ligios
Giovannantonio Pilo

NIH Bethesda USA

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Thanks for the attention

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