Mechanisms of Plasma Therapy

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University of California, Berkeley

2017 NCCAVS Joint Users Group Meeting and Symposium
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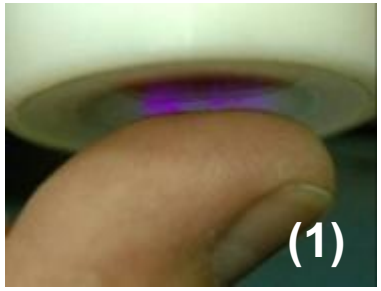
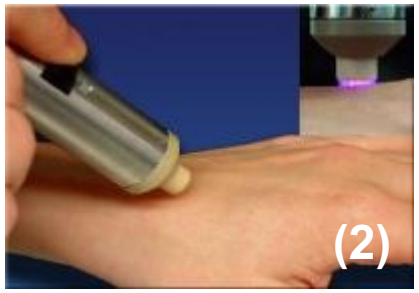
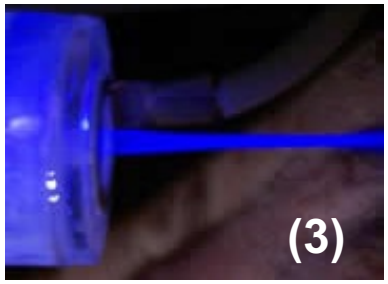
Dogan Guidon
I-Wei Chang

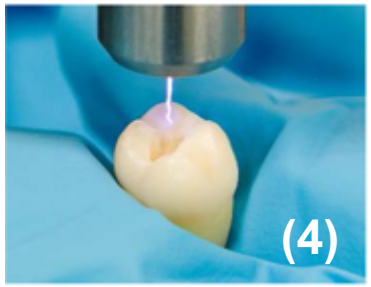
Plasma biomedicine/sources/chemistry

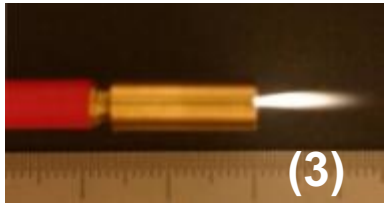
‘Cold’ Atmospheric Pressure Plasma Sources for Biomedical Applications

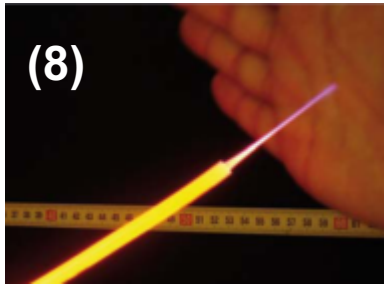


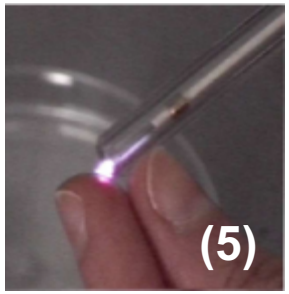


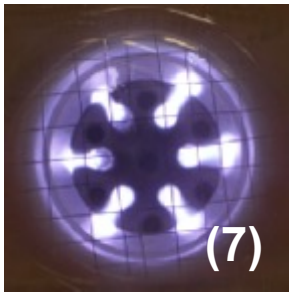


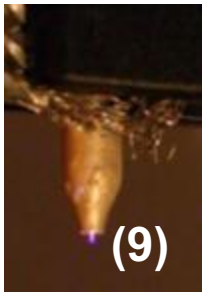


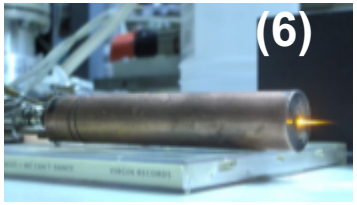


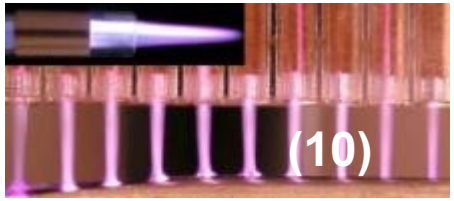


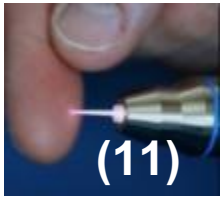










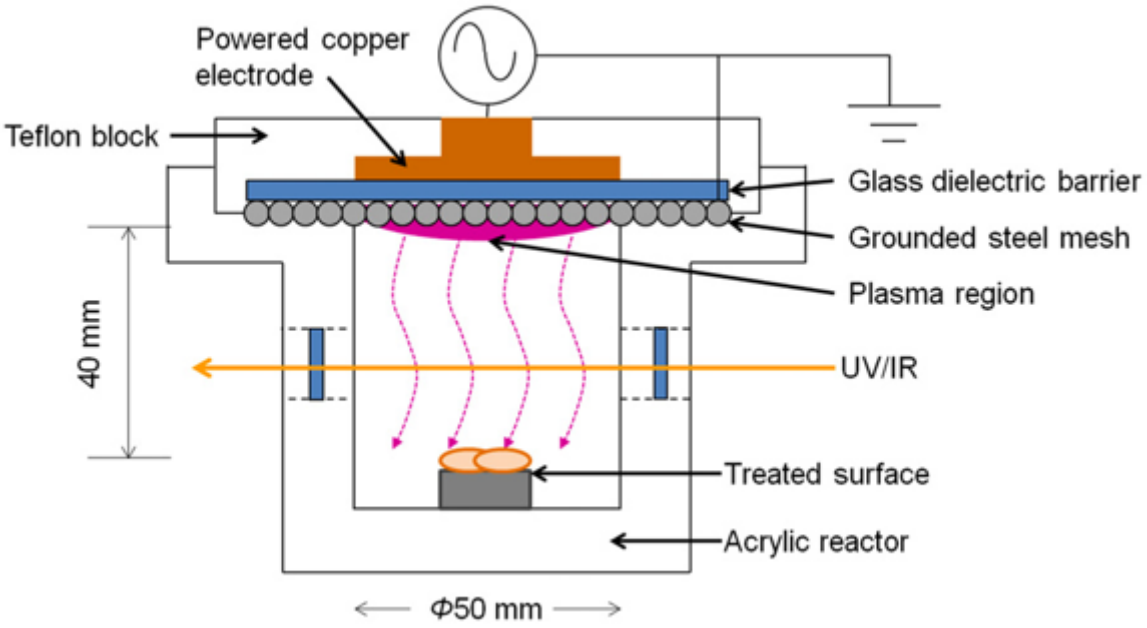


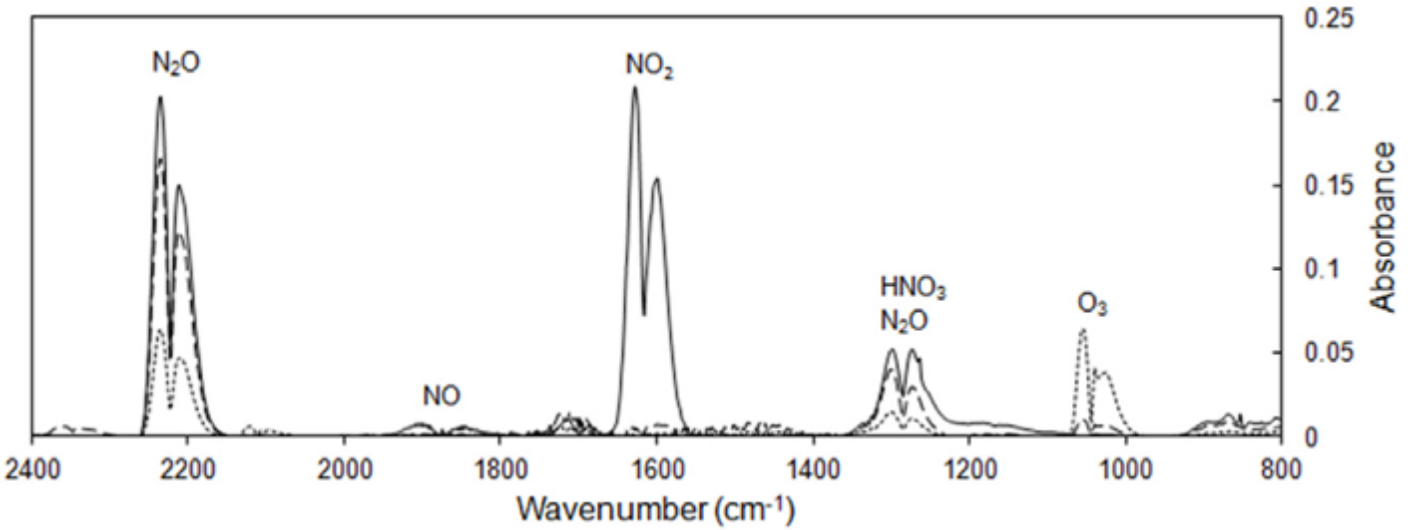
(1) Drexel University (USA)
(2) Cinogy GmbH (GER)
(3) Old Dominion University (USA)
(4) IOM Leipzig (GER)
(5) Eindhoven Univ. of Techn. (NED)
(6) New York University (USA)
(7) MPE Garching (GER)
(8) University of Orléans (FRA)
(9) McGill University, Montreal (CAN)
(10)Loughborough University (UK)
(11)INP Greifswald (GER)

Courtesy: K.D. Weltmann, Greifswald

Surface Microdischarge (SMD) in Air

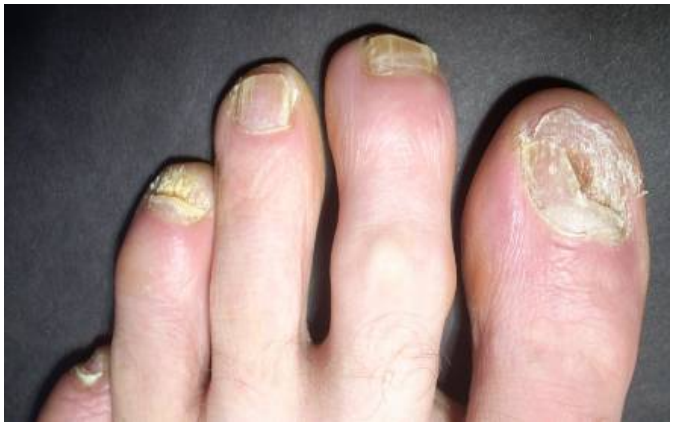
Pavlovich et al., Plasma Sources Sci. Technol. 23 (2014) 065036

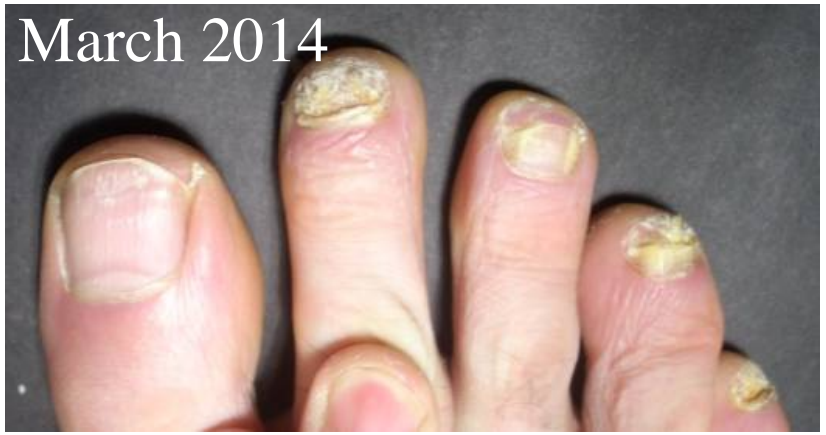


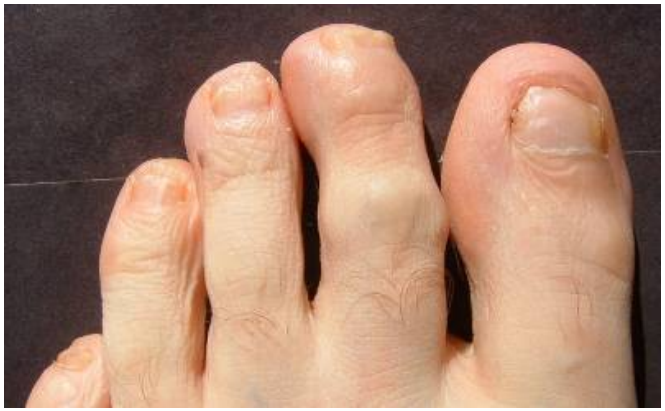


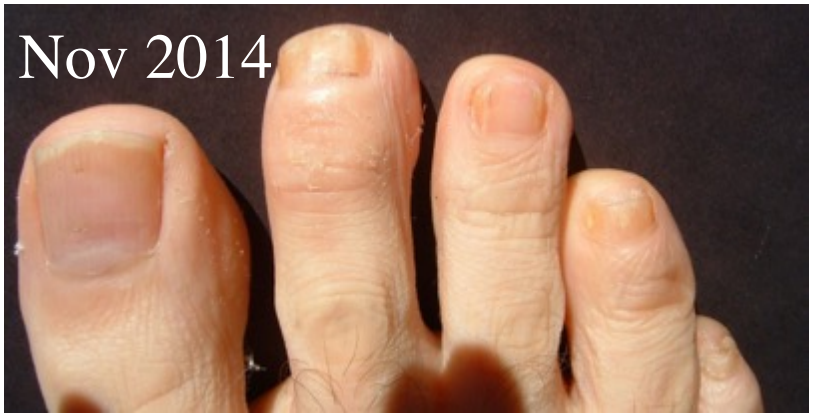
SMD Treatment of Onychomycosis

3 treatments of 45 min SMD exposure: 1 week









DeviceFarm
nail RENEWAL therapy

Toes of Jeff Roe, CEO of DeviceFarm

http://www.devicefarmtech.com/

Head and neck cancer treatment and physical plasma

Hans-Robert Metelmann [a,*], David S. Nedrelow [b], Christian Seebauer [a], Matthias Schuster [a], Thomas von Woedtke [c], Klaus-Dieter Weltmann [c], Stefan Kindler [a], Philine Henriette Metelmann [d], Steven E. Finkelstein [e], Daniel D. Von Hoff [f], Fred Podmelle [a,1]

[a] *Greifswald University Medicine, Department of Oral and Maxillofacial Surgery/Plastic Surgery, Ferdinand-Sauerbruch-Str. DZ 7, 17475 Greifswald, Germany*
[b] *University of Minnesota, Minnesota Dental Research Center for Biomaterials and Biomechanics, 16-212 Moos Tower, 515 Delaware St. SE, Minneapolis, MN 55455, USA*
[c] *Leibniz Institute for Plasma Science and Technology (INP), Felix-Hausdorff-Str. 2, 17489 Greifswald, Germany*
[d] *Greifswald University Dental School, Department of Orthodontics, Walter-Rathenau-Str. 42, 17475 Greifswald, Germany*
[e] *21st Century Oncology Translational Research Consortium (TRC), 7340E Thomas Road, Scottsdale, AZ 85251 USA*
[f] *Translational Genomics Research Institute (TGen), Cancer Drug Development Laboratory, 13208E Shea Blvd., Suite 106, Scottsdale, AZ 85259, USA*

Clinical Plasma Medicine 3 (2015) 17–23

kINPen med®
developed by the
Leibniz Institute for
Plasma Science and
Technology (INP),
Greifswald, Germany

Head and neck cancer treatment and physical plasma

Hans-Robert Metelmann [a,*], David S. Nedrelow [b], Christian Seebauer [a], Matthias Schuster [a], Thomas von Woedtke [c], Klaus-Dieter Weltmann [c], Stefan Kindler [a], Philine Henriette Metelmann [d], Steven E. Finkelstein [e], Daniel D. Von Hoff [f], Fred Podmelle [a,1]

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[b] *University of Minnesota, Minnesota Dental Research Center for Biomaterials and Biomechanics, 16-212 Moos Tower, 515 Delaware St. SE, Minneapolis, MN 55455, USA*
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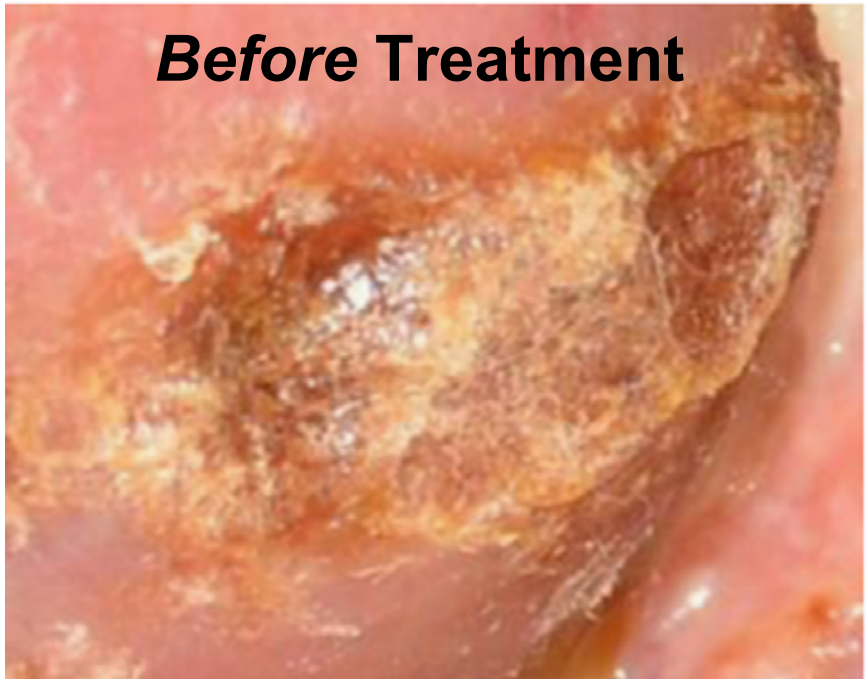
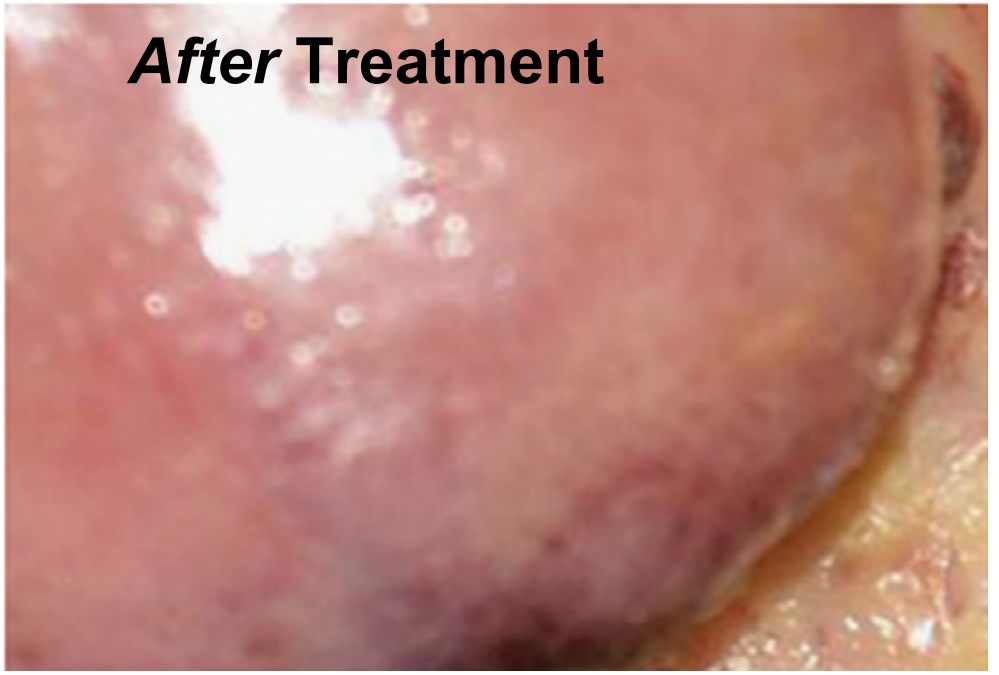
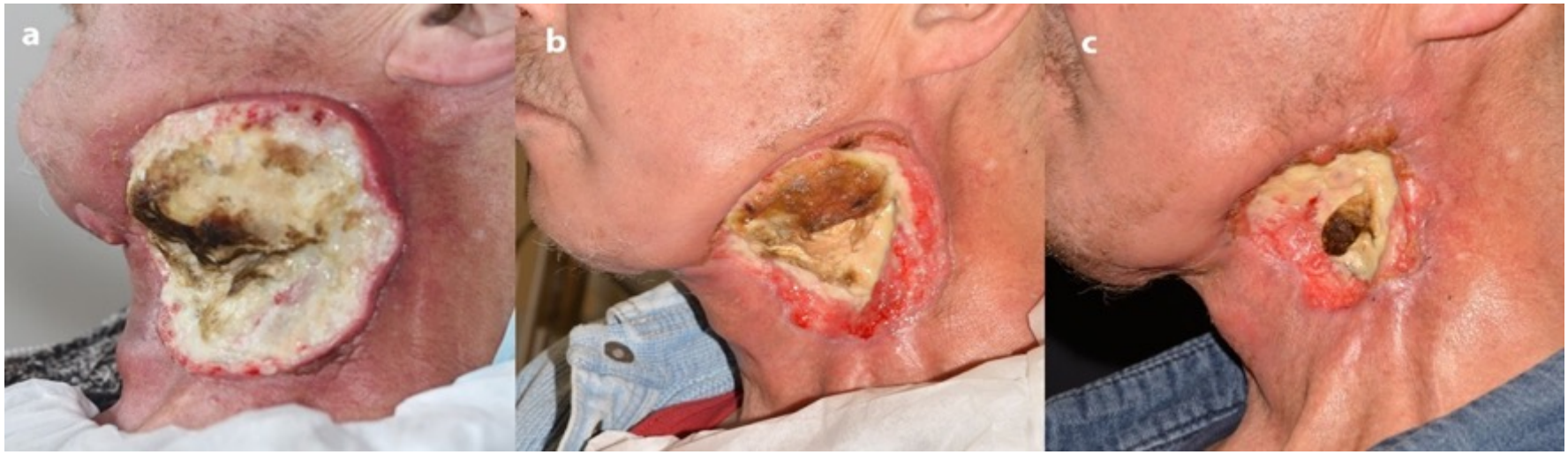
Clinical Plasma Medicine 3 (2015) 17–23





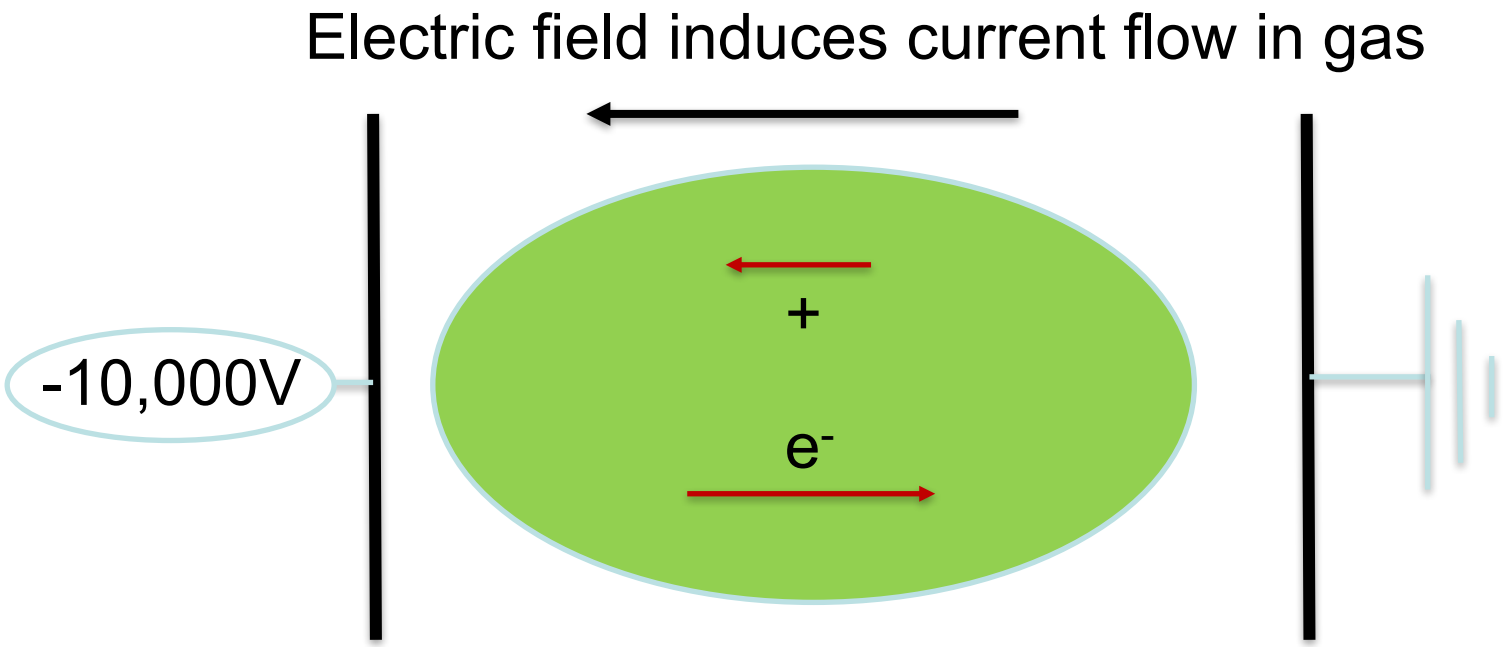
Another kINPen med® Application



Images from one patient with squamous cell carcinoma treated with the kINPen Med device three times per week, 1 minute/cm^2. (a) image from treatment commencement in April 2016; (b) image from June 2016; (c) image taken in August 2016. (Courtesy Dr. Hans Metelmann, Germany)

What mechanism(s) might explain the observed biomedical effects of cold atmospheric plasma?

Key Elements of Discharge Physics and Chemistry



Gas is *weakly* ionized (<10^{-6})

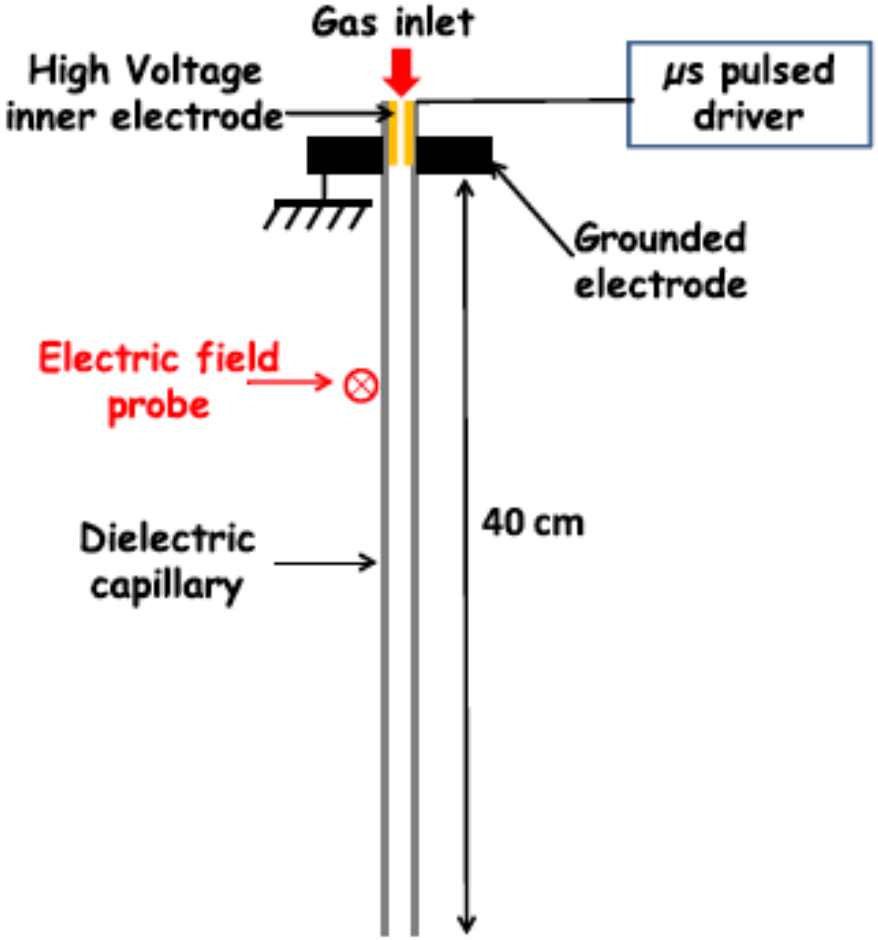
Electrons are HOT (~10^4 K); ions/neutrals ~ 300-600 K

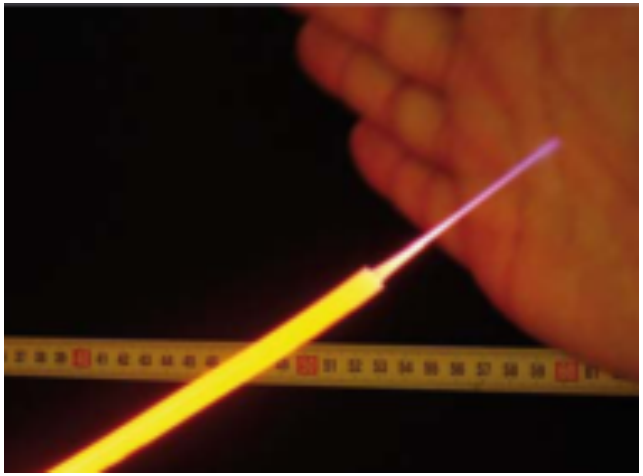
Electron-impact ionization creates new charge (electrons/ions)
Electron-impact molecular dissociation creates radicals (e.g. O atoms)
Electron-impact excitation (and relaxation) creates UV/VUV photons

New insights on the propagation of pulsed atmospheric plasma streams: From single jet to multi jet arrays

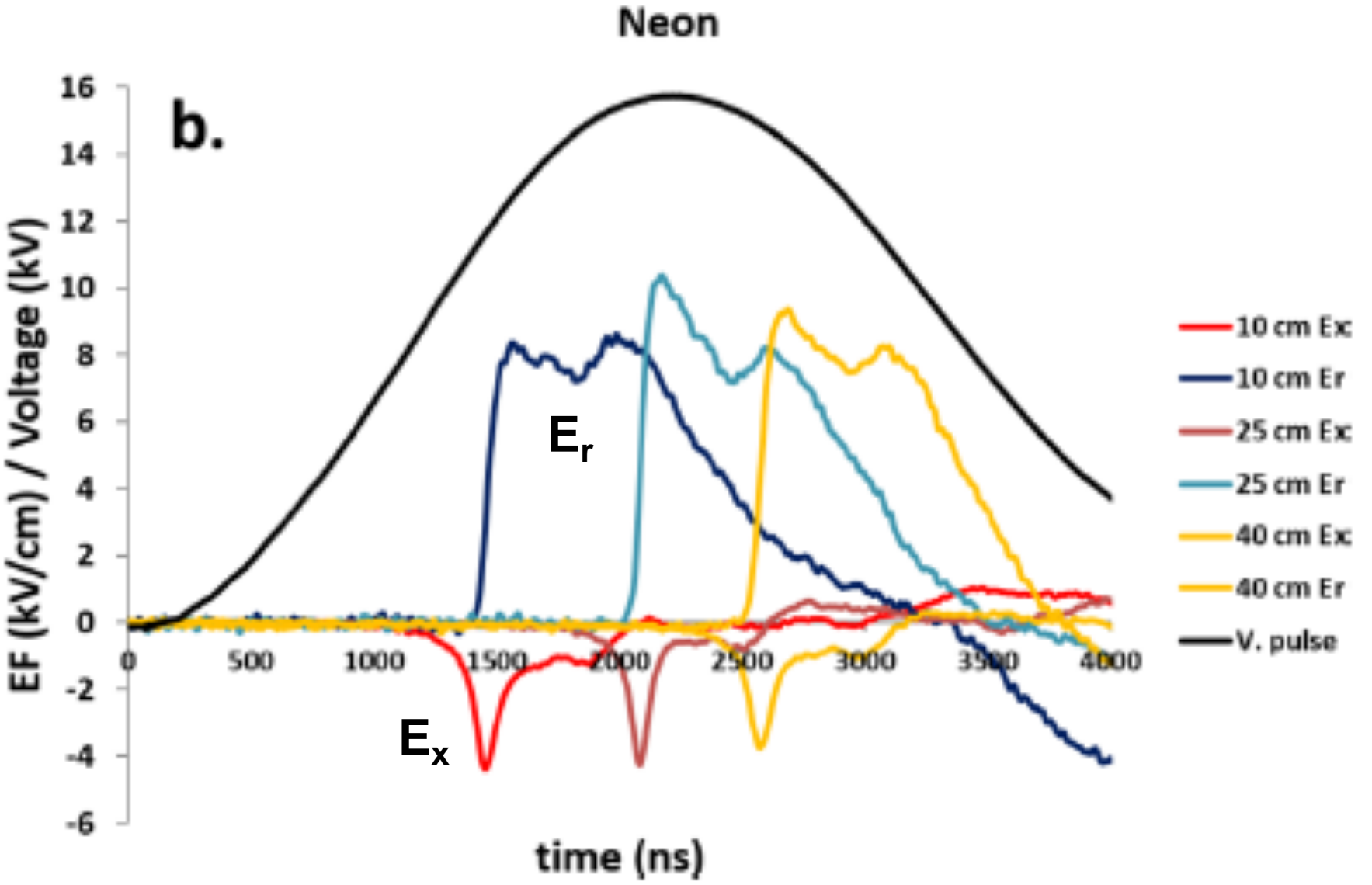
E. Robert, T. Darny, S. Dozias, S. Iseni, and J. M. Pouvesle
GREMI, UMR 7344, CNRS/Université d'Orléans, BP 6744, 45067 Orléans Cedex 2, France

PHYSICS OF PLASMAS **22**, 122007 (2015)

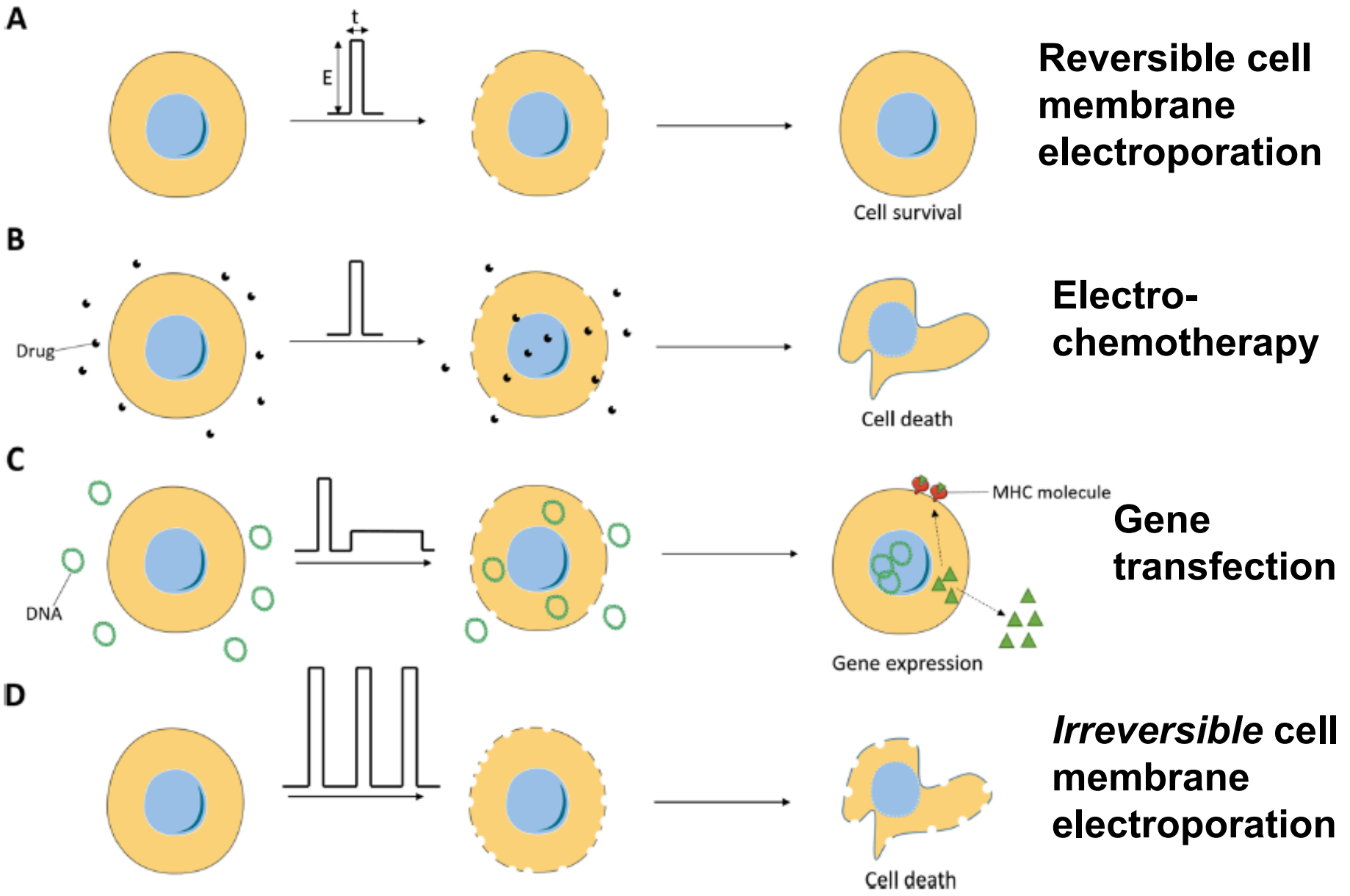




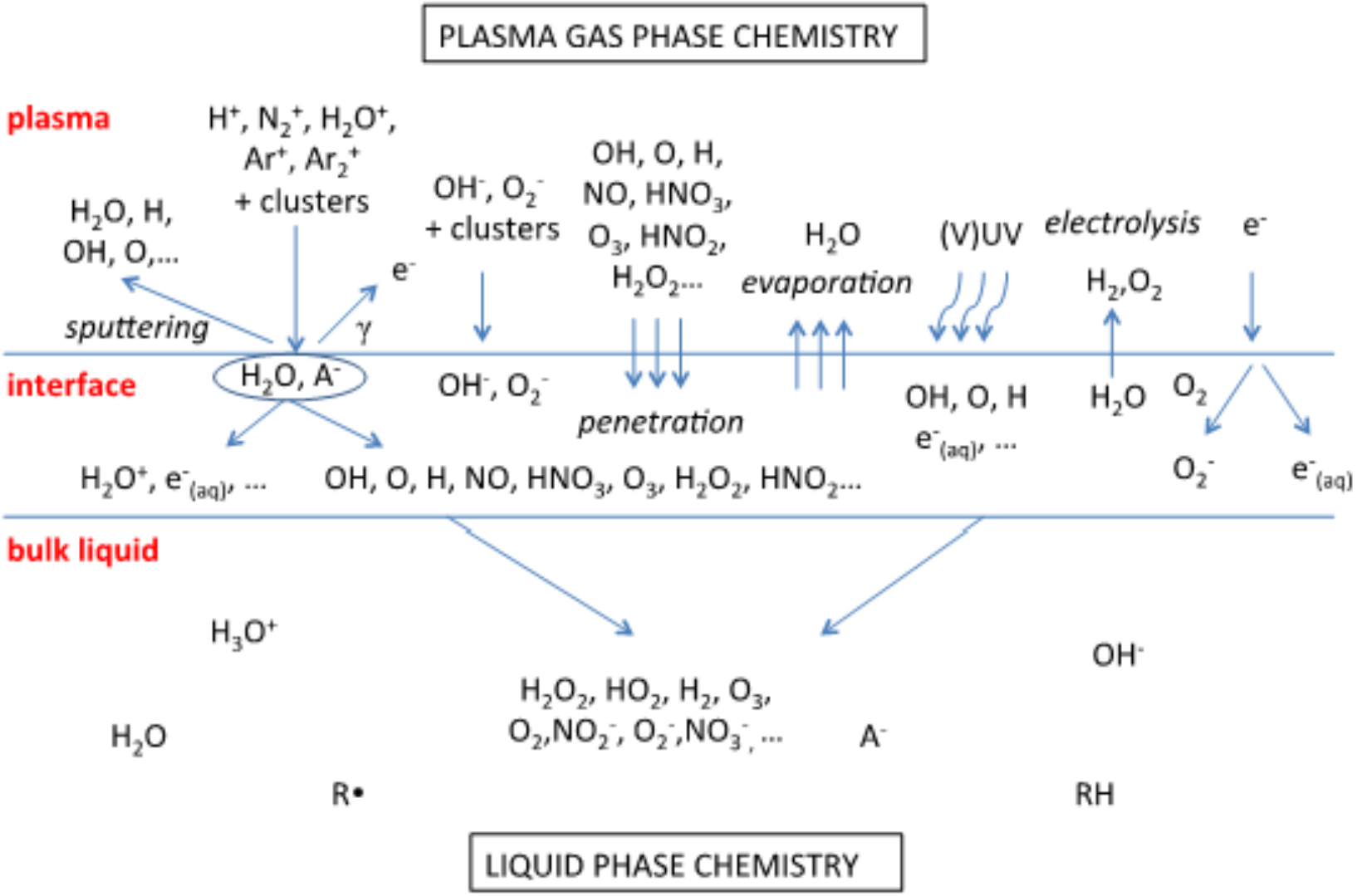
Peak fields ~ 5-10 kV/cm



The promising alliance of anti-cancer electro-chemotherapy with immunotherapy: Calvet & Mir (Cancer Metastasis Rev (2016) 35:165–177)



Vision of Air Plasma-Liquid Interactions



Bruggeman et al., 2016

Reactive Oxygen and Reactive Nitrogen Species (RONS)

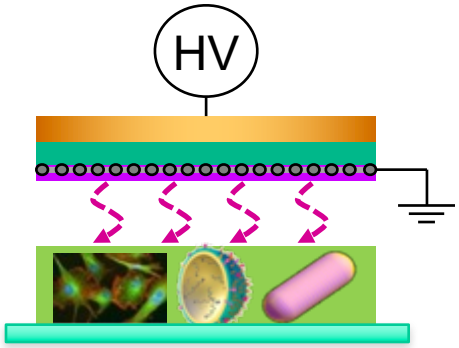
Reactive oxygen and nitrogen species often cited as key species in plasma biomedical applications.

But what is known about these species in biology and medicine?

I now focus on these species and their role in biology and medicine

RONS: Key Immune System Species in Response to Injury, Infection or Tumor Detection

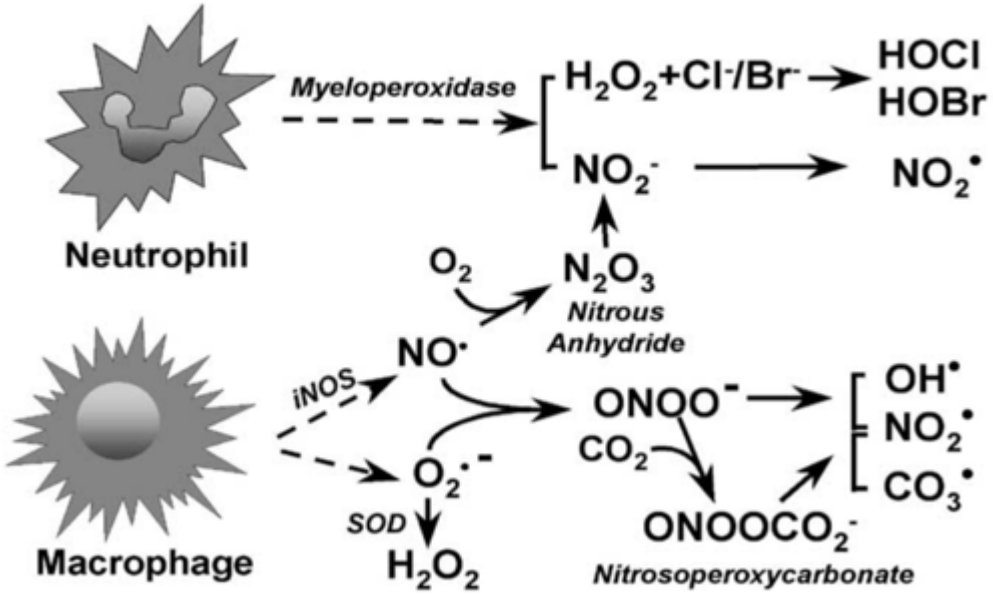
Air/Water Plasma Chemistry



O, OCl^-/HOCl, OH, $^1O_2$, O_2^-/HO_2, O_3
N, NO_3, NO_2, NO, N_xO_y, NO_2^-, NO_3^-,
$ONOO^-$, H, H_2, H_2O_2, HNO_2, HNO_3

Innate Immune System Chemistry

Dedon and Tannenbaum, 2004



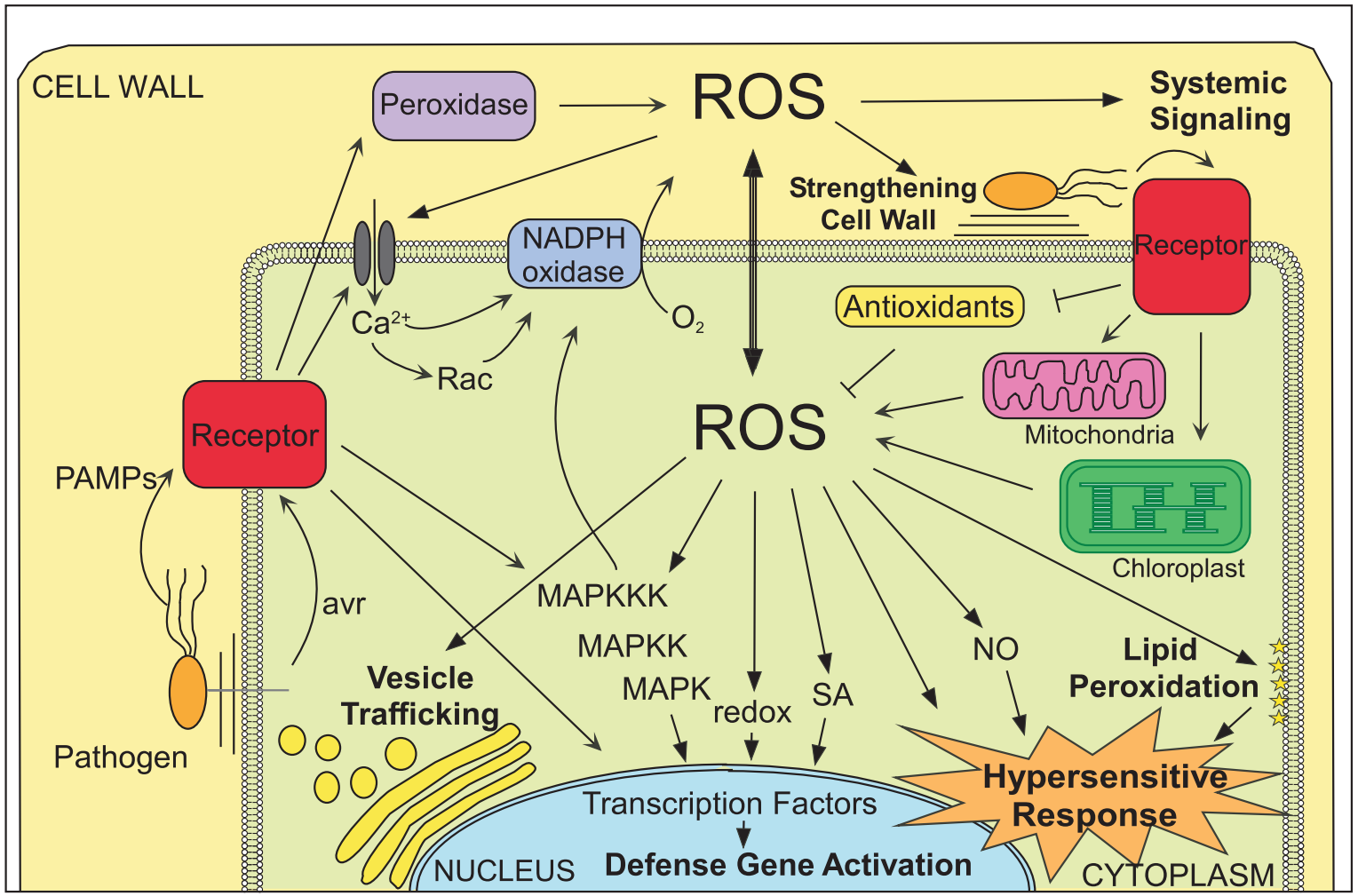
Respiratory/oxidative burst

ROS in biotic interactions

Miguel Angel Torres[a,b,*]

Physiologia Plantarum 138: 414–429. 2010

***Plant* oxidative burst**



ROS in biotic interactions

Miguel Angel Torres[a,b,*]

Physiologia Plantarum 138: 414–429. 2010

“Production of ROS is a hallmark of successful recognition of infection and activation of plant defenses.

ROS play multifaceted signaling functions mediating the establishment of multiple responses and can act as local toxins.”

Key Concepts

Traditional view of RONS as only damaging species, the cause of disease and aging, has now evolved into a more sophisticated understanding.

Wide recognition now of positive role of RONS, especially in cancer therapy.

Reactive oxygen and nitrogen species (RONS) generally acknowledged to be important in plasma therapeutics; E-fields and photons are important in some cases (e.g. gene transfection/transdermal delivery; or photon-induced chemistry).

Key Concepts, continued

Plasma-generated RONS effects are confined to *near-surface regions* and are applied on *timescales short compared to biological responses.*

But observed plasma therapeutic effects suggest longer time and length scales are involved. *How can this occur?*

Key Concepts, continued

In order to make sense of this rapidly emerging and complex field, seek insights from current understanding of (a) aerobic biological systems and (b) from established therapies.

(a) Animal and plant immune system responses to infection/damage/tumors.

(b) Radiotherapy, chemotherapy and photodynamic therapy for cancer treatment.

Oxidative Shielding or Oxidative Stress?

Robert K. Naviaux JPET 342:608–618, 2012

Naviaux (2012) suggests the traditional view of reactive oxygen (and nitrogen) in the context of 'oxidative stress' as a cause of disease/aging may be completely wrong.

He suggests 'oxidative shielding' is an evolutionarily conserved way that cells protect themselves.

Oxidative Shielding Rather than *Oxidative Stress*

Naviaux (2012) suggests:

"ROS and oxidative changes in chronic disease are the symptoms of disease and not the cause."

"Oxidative shielding…ultimately increases membrane rigidity, decreases permeability and inhibits cell division."

"The machinery of oxidative shielding evolved from pathways of innate immunity designed to protect the cell from attack and limit the spread of infection."

Does plasma act as an exogenous source of therapeutic oxidative shielding?

Existing Therapies Use RONS

- **Antibiotics**
- **Antifungals**
- **Antiparasiticals**
- **Cancer therapy:**
  - **PDT ($^1O_2$);**
  - **radiation;**
  - **chemotherapies**

Ionizing Radiation Effects Similar to Plasma

Generation of RONS

DNA damage (SSB and DSB)

Apoptosis of tumor cells

Known that direct radiation effects are only part of anti-tumor effects: bystander effects alter unexposed cells

What is currently thought about the role of RONS in radiation bystander effects?

Bio-Radicals Formed by Ionizing Radiation

Int. J. Radiat. Biol., Vol. 85, No. 1, January 2009, pp. 9–25

O'Neil and Wardman

Name	Formula
singlet oxygen (excited state)	$^1O_2$
superoxide/hydroperoxide radical	$O_2^{\bullet -}/HO_2^{\bullet}$
hydroxyl radical	$^{\bullet}OH$
hydrogen peroxide	H_2O_2
hypochlorous acid	HOCl
hypobromous acid	HOBr
hypothiocyanous acid	HOSCN
nitric oxide radical	$NO^{\bullet}$
nitrogen dioxide radical	$NO_2^{\bullet}$
dinitrogen trioxide	N_2O_3
nitroxyl	HNO
peroxynitrite/peroxynitrous acid	$ONOO^-/ONOOH$
nitrosoperoxycarbonate	$ONOOCO_2^-$
carbonate radical	$CO_3^{\bullet -}$
carbon-centred radicals	$RC^{\bullet}(X)R'$
peroxyl radicals on carbon	$RC(OO^{\bullet})(X)R'$
thiyl radicals	$RS^{\bullet}$
disulfide radical-anions	$(RS.\!:\!SR')^-$
thiylperoxyl radicals	$RSOO^{\bullet}$
sulfonyl radicals	$RS(O)(O)^{\bullet}$
sulfonylperoxyl radicals	$RS(O)(O)OO^{\bullet}$
nitrogen-centred indolyl radicals	$-N^{\bullet}-$
phenoxyl radicals, e.g., tyrosine	$TyrO^{\bullet}$

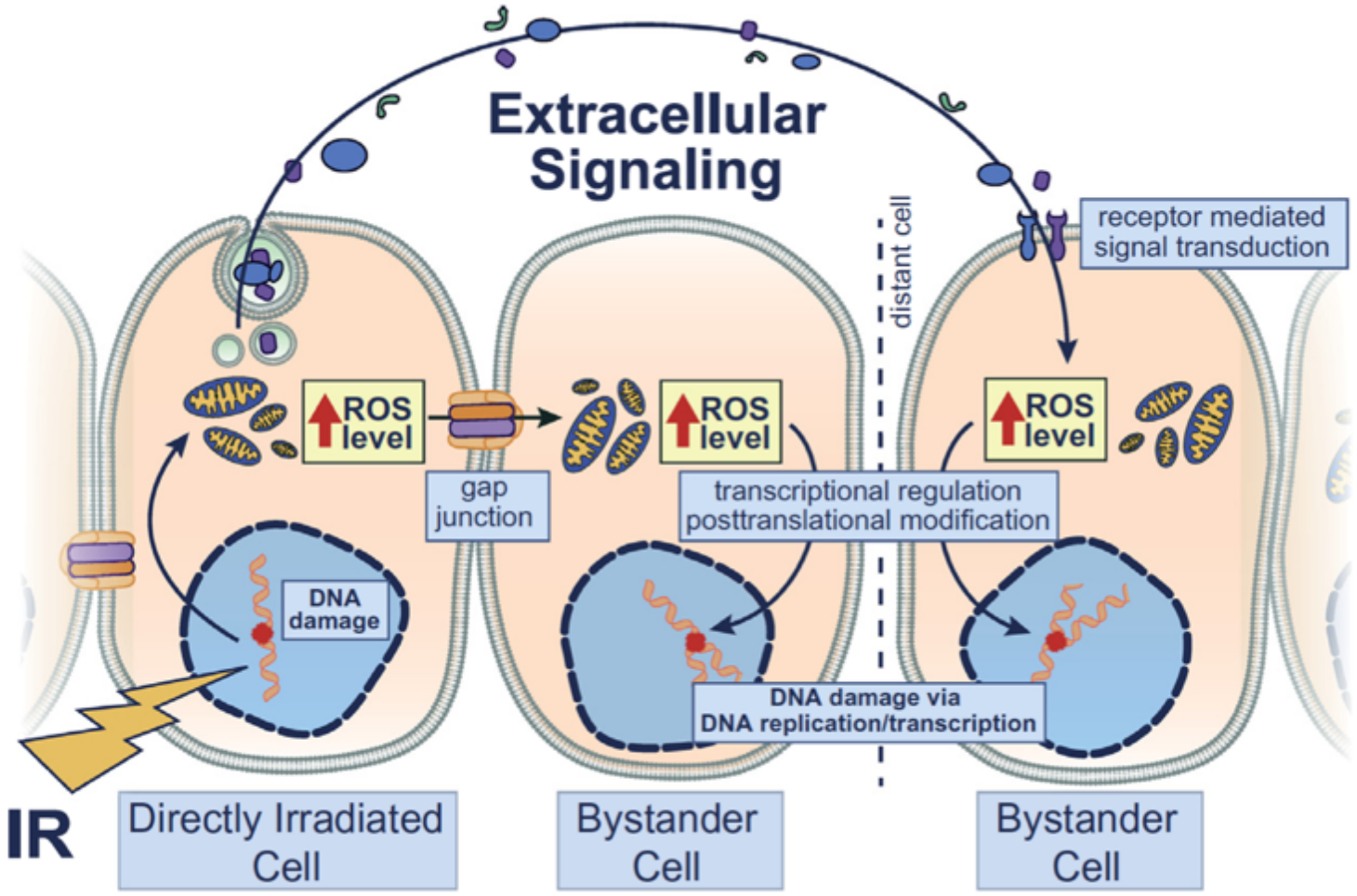
Radiation therapy radical chemistry:

~2/3 therapeutic effect

Bystander effects as manifestation of intercellular communication of DNA damage and of the cellular oxidative status

Holger Klammer, Emil Mladenov, Fanghua Li, George Iliakis *

Cancer Lett, 2014



Ionizing radiation (IR) induces ROS in 'bystander' cells via modulation of ROS/redox. Might plasma do something similar?

Huge Literature on Pro-Oxidant Anti-Tumor Mechanism…..*for example*

The emerging role of reactive oxygen species in cancer therapy

Markus F. Renschler *

European Journal of Cancer 40 (2004) 1934–1940

ROS stress in cancer cells and therapeutic implications

Helene Pelicano [a], Dennis Carney [a,b], Peng Huang [a,*]

Drug Resistance Updates 7 (2004) 97–110

Oxidative stress and apoptosis: a new treatment paradigm in cancer

Ryan H. Engel, and Andrew M. Evens

[Frontiers in Bioscience 11, 300-312, January 1, 2006]

Oxidative Stress and Apoptosis: Impact on Cancer Therapy

TOMRIS OZBEN

JOURNAL OF PHARMACEUTICAL SCIENCES, VOL. 96, NO. 9, SEPTEMBER 2007

Tumor-targeted induction of oxystress for cancer therapy

Journal of Drug Targeting, August–September 2007; 15(7–8): 475–486

J. FANG[1,†], H. NAKAMURA[1] & A. K. IYER[1,2]

Cancer cell killing via ROS

To increase or decrease, that is the question

Jie Wang and Jing Yi*

[Cancer Biology & Therapy 7:12, 1875-1884; December 2008]

Redox-Directed Cancer Therapeutics: Molecular Mechanisms and Opportunities

ANTIOXIDANTS & REDOX SIGNALING
Volume 11, Number 12, 2009

Georg T. Wondrak

The causes of cancer revisited: "Mitochondrial malignancy" and ROS-induced oncogenic transformation – Why mitochondria are targets for cancer therapy

Molecular Aspects of Medicine 31 (2010) 145–170

Stephen J. Ralph [a,*], Sara Rodríguez-Enríquez [b], Jiri Neuzil [c,d], Emma Saavedra [b], Rafael Moreno-Sánchez [b]

Reactive Oxygen Species: The Achilles' Heel of Cancer Cells?

ANTIOXIDANTS & REDOX SIGNALING
Volume 16, Number 11, 2012

Xiaojiang Cui

Upsides and Downsides of Reactive Oxygen Species for Cancer: The Roles of Reactive Oxygen Species in Tumorigenesis, Prevention, and Therapy

ANTIOXIDANTS & REDOX SIGNALING
Volume 16, Number 11, 2012

Subash C. Gupta, David Hevia, Sridevi Patchva, Byoungduck Park, Wonil Koh, and Bharat B. Aggarwal

Oxidative Stress and Lipid Peroxidation Products in Cancer Progression and Therapy

International Scholarly Research Network
ISRN Oncology
Volume 2012, Article ID 137289, 21 pages

Giuseppina Barrera

Mitochondria as a Source of Reactive Oxygen and Nitrogen Species: From Molecular Mechanisms to Human Health

ANTIOXIDANTS & REDOX SIGNALING
Volume 18, Number 16, 2013

Tiago R. Figueira,[1] Mario H. Barros,[2,*] Anamaria A. Camargo,[3,4,*] Roger F. Castilho,[1,*] Julio C.B. Ferreira,[5,*] Alicia J. Kowaltowski,[6,*] Francis E. Sluse,[7,*] Nadja C. Souza-Pinto,[6,*] and Anibal E. Vercesi[1]

Oxidative stress and cancer: An overview

Venus Sosa[a], Teresa Moliné[a], Rosa Somoza[a], Rosanna Paciucci[b], Hiroshi Kondoh[c], Matilde E. LLeonart[a,*]

Ageing Research Reviews 12 (2013) 376–390

Oxidative Stress in Cancer

U. Jakob and D. Reichmann (eds.), *Oxidative Stress and Redox Regulation*,
DOI 10.1007/978-94-007-5787-5_15,

Peter Storz

Oxidants, antioxidants and the current incurability of metastatic cancers

Jim Watson

Open Biol. 2013 **3**, 120144, published 9 January 2013

Overcoming Drug Resistance Through Elevation of ROS in Cancer

B. Bonavida (ed.), *Molecular Mechanisms of Tumor Cell Resistance to Chemotherapy*, Resistance to Targeted Anti-Cancer Therapeutics 1, DOI: 10.1007/978-1-4614-7070-0_7,

Amit K. Maiti

Oxidants, antioxidants and the current incurability of metastatic cancers

Jim Watson

Open Biol. 2013 **3**, 120144, published 9 January 2013

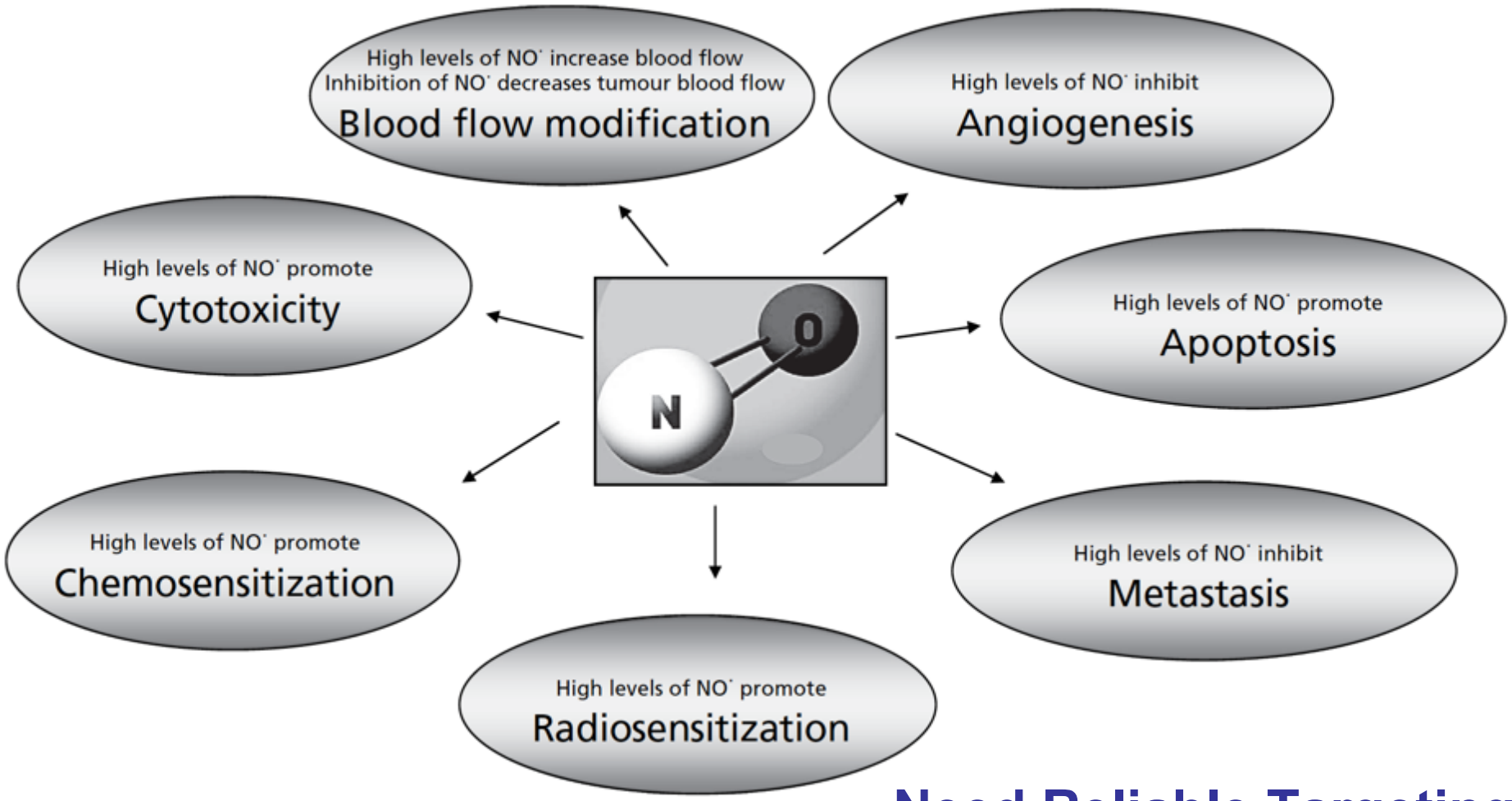
"The vast majority of all agents used to directly kill cancer cells (ionizing radiation, most chemotherapeutic agents and some targeted therapies) work either through directly or indirectly generating ROS that block key steps in the cell cycle…."

"A common ROS-mediated way through which almost all anti-cancer agents induce apoptosis explains why cancers that become resistant to chemotherapeutic control become equally resistant to ionizing radiotherapy."

Plasma RONS-based therapy must offer advantages over existing therapies!

Can plasma therapy overcome tumor resistance??

NO_x Cancer Therapy: *A Possible Mechanism for Plasma to Overcome Resistance?*



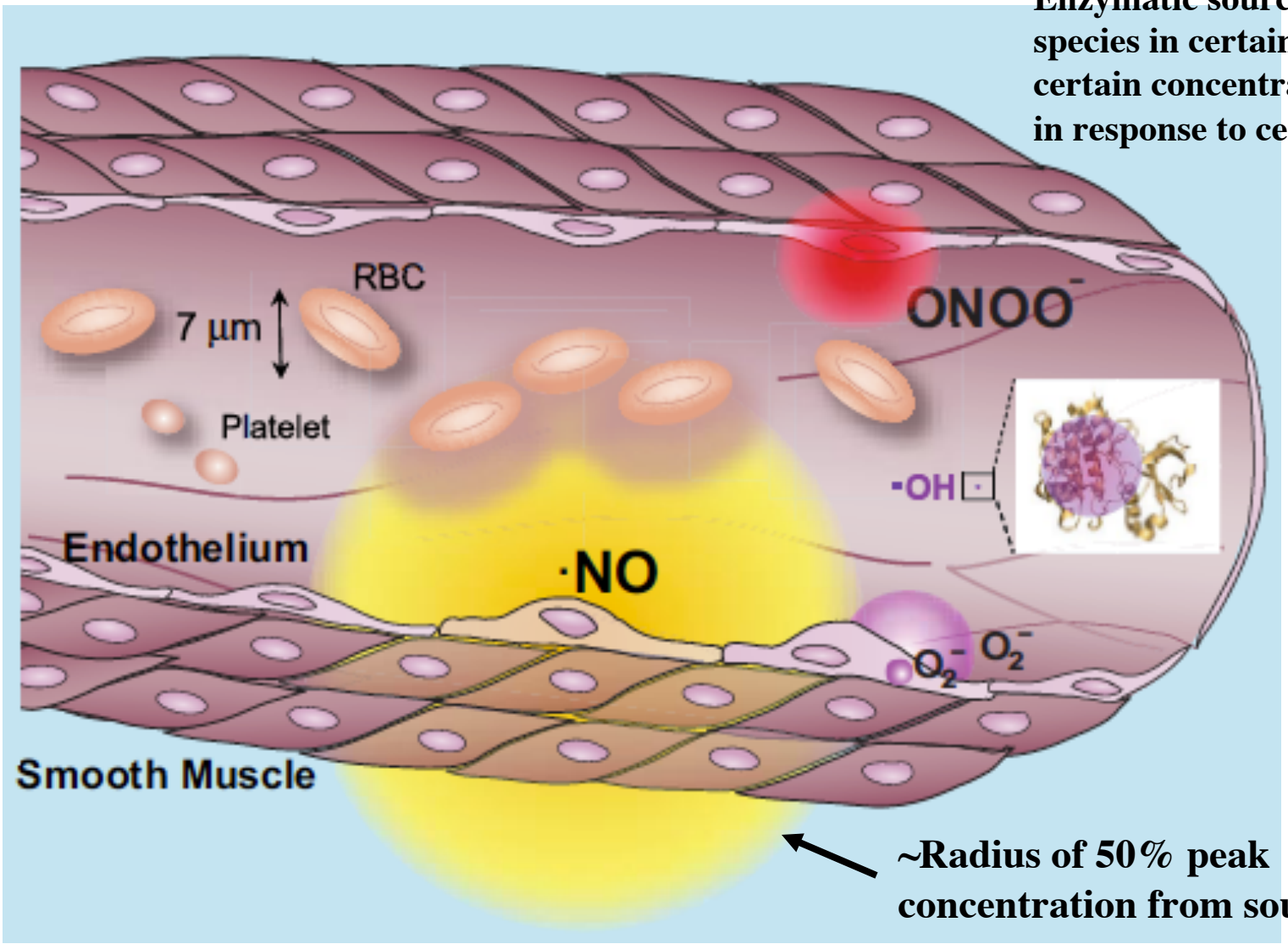
Need Reliable Targeting in Tumors at High Concentration*

*Targeting nitric oxide for cancer therapy

David Hirst and Tracy Robson JPP 2007, 59: 3–13

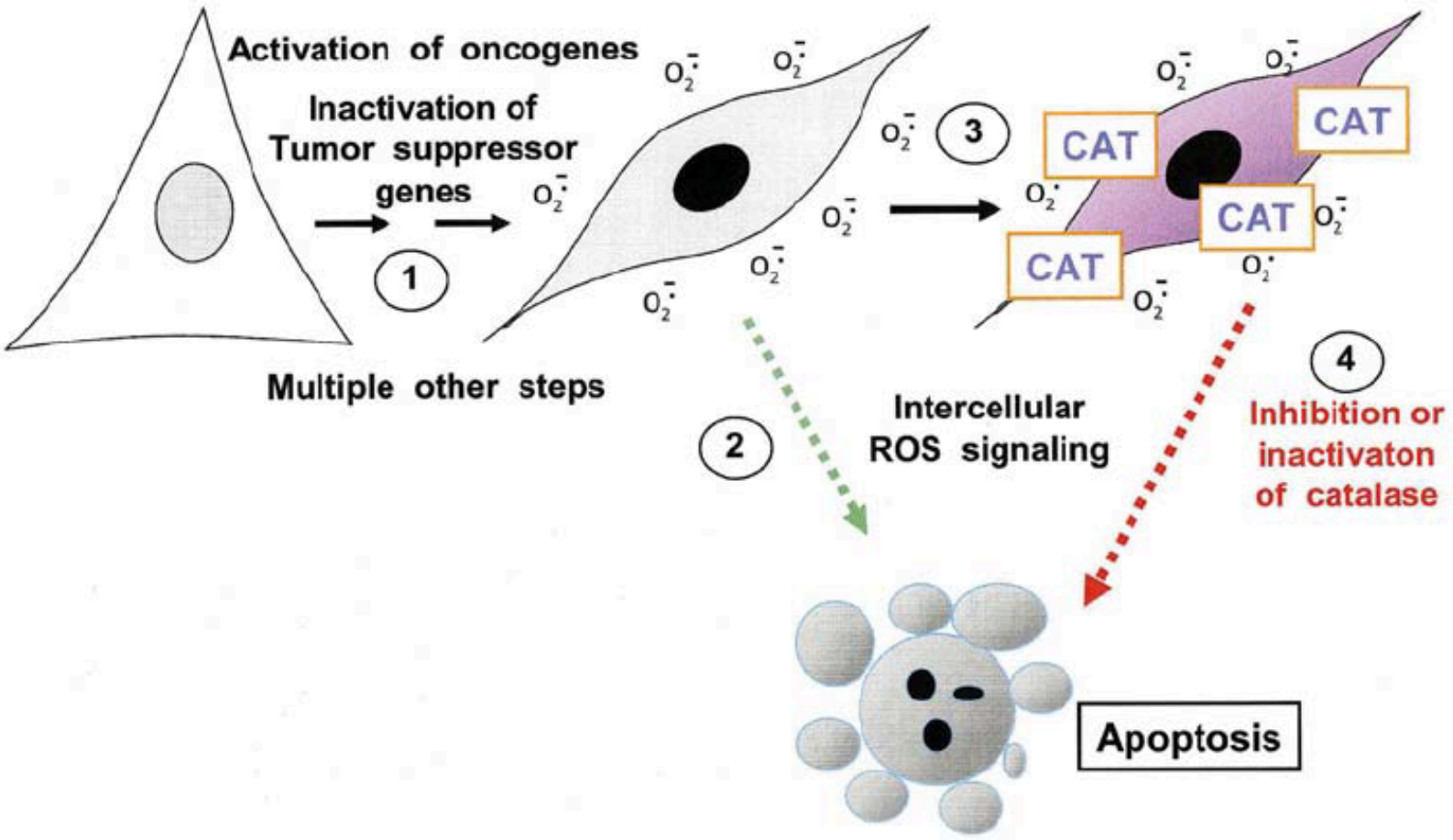
In Vivo RONS Lifetimes/Diffusion Distances: Short!

Enzymatic sources create species in certain places at certain concentrations in response to certain events



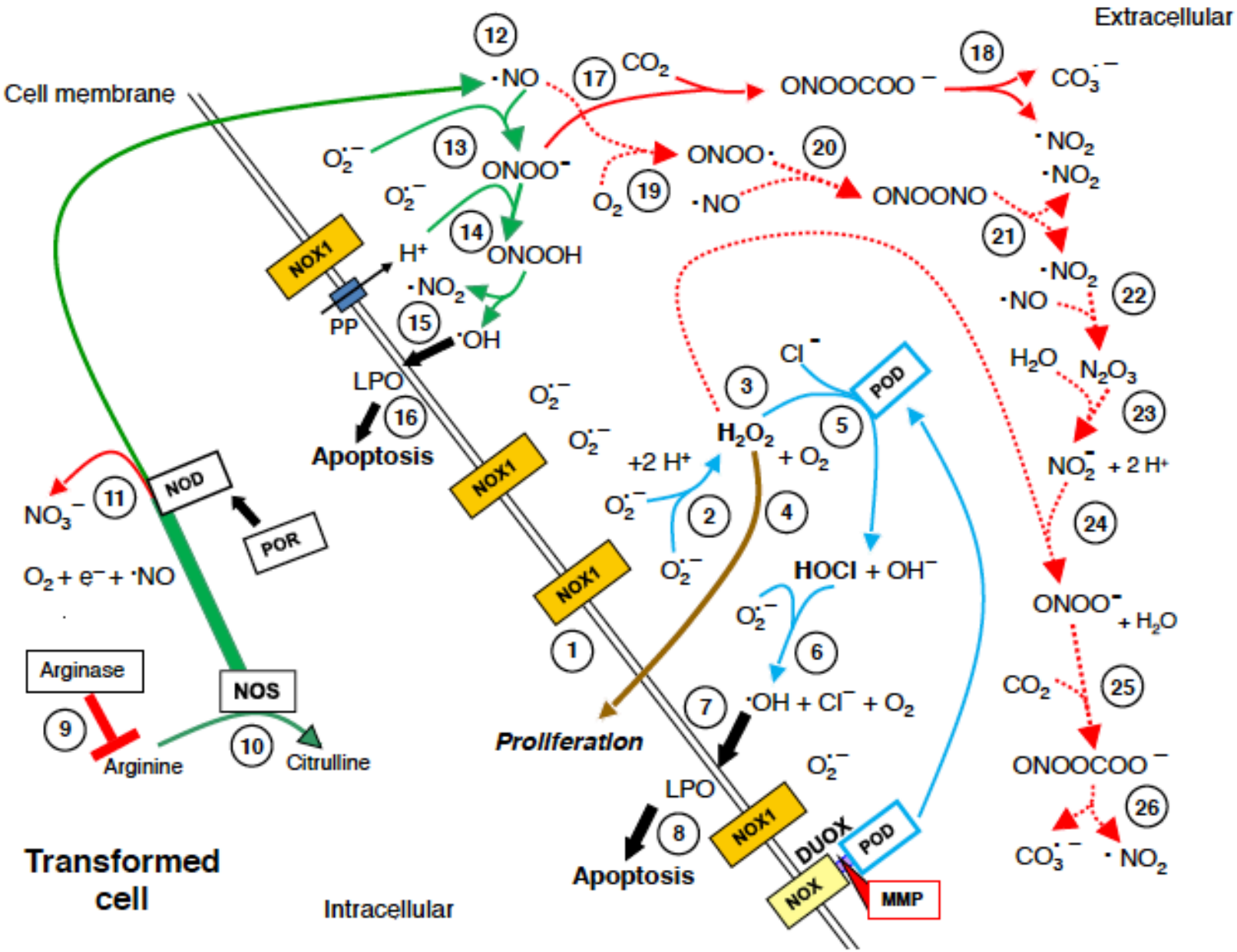
Pacher et al., Physiol. Rev., 87, 315, 2007

Normal Cells Detect and Try to Kill Pre-Cancerous 'Transformed Cells'….



Courtesy Prof. Georg Bauer

…Via Complex Biochemical Mechanisms

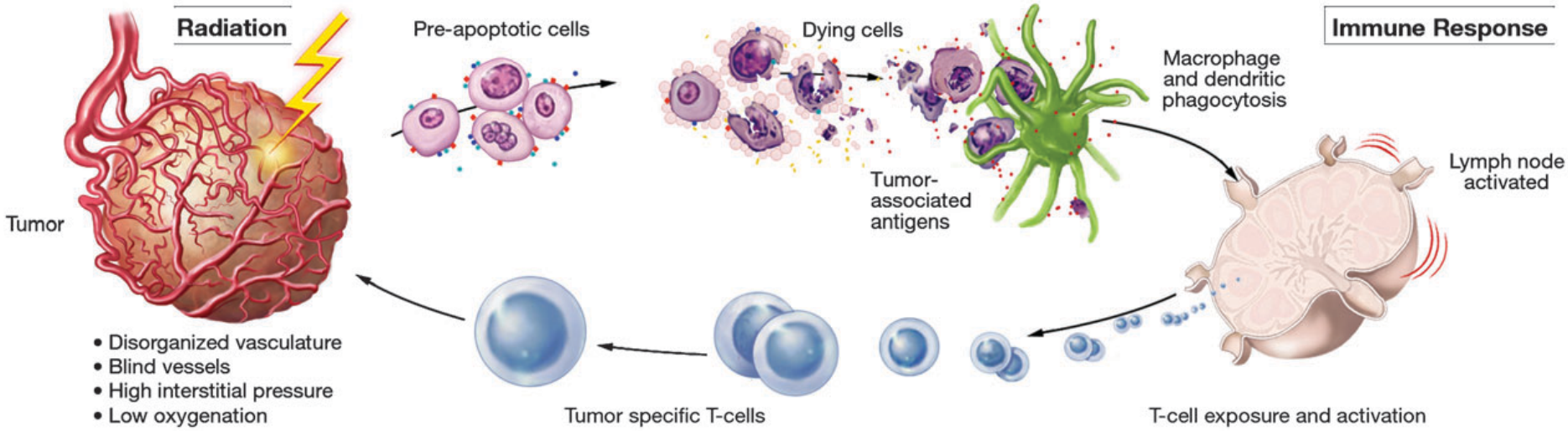


Could Plasma Stimulate the *Adaptive* Immune System*?*

Two other therapies that create radicals (radiation and PDT) both have shown this effect

This suggests that plasma has the potential to do something similar

Radiation - Adaptive Immunity Interactions



Combining radiation, immunotherapy, and antiangiogenesis agents in the management of cancer: the Three Musketeers or just another quixotic combination?

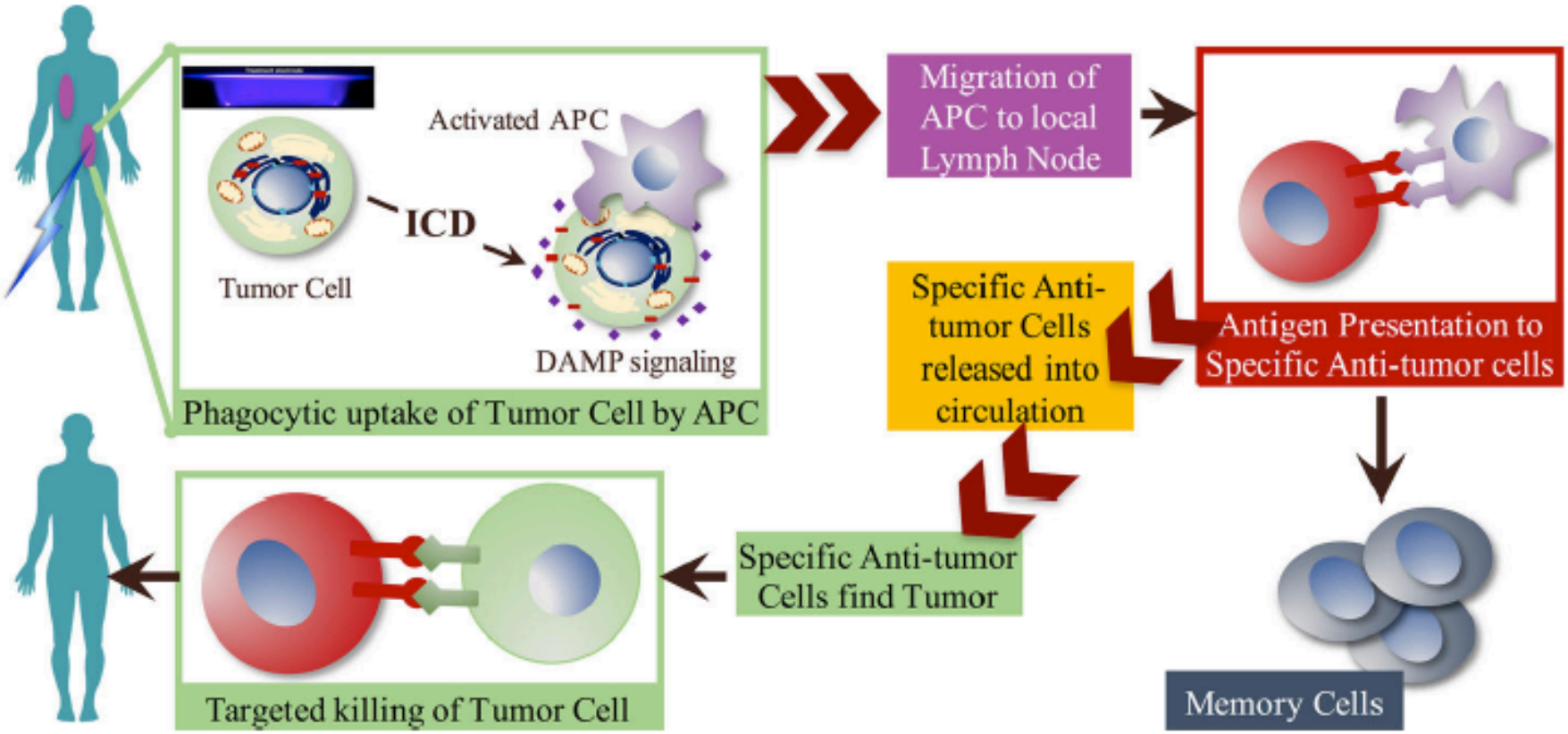
Mitchell Kamrava,†[a] Michael B. Bernstein,†[b] Kevin Camphausen[a] and James W. Hodge*[b]

Mol. BioSyst., 2009, **5**, 1262–1270

Plasma Chem Plasma Process (2016) 36:259–268

Why Target Immune Cells for Plasma Treatment of Cancer

Vandana Miller[1] · Abraham Lin[1] · Alexander Fridman[1]



Concluding Remarks

Central idea is that plasma/RONS applied at tissue surfaces, over a short period of time ('burst-like exposure'), create longer-lived species that interact with cells.

This interaction triggers a series of adaptive biological responses, generally involving cellular RONS creation, and release of cytokines, that ultimately are therapeutic.

Plasma-generated RONS both *simulate* and *stimulate* natural healing responses.

Concluding Remarks

These biological responses take place over much longer time scales and length scales than the original plasma exposure.

Intracellular and intercellular responses are centered around mitochondrial processes and can involve innate and perhaps adaptive immune responses as cells communicate with each other.

Current Status of 'Cold' Atmospheric Plasma Therapeutics

Considerable evidence to date of plasma devices promoting healing of infected tissue; wound healing; wound pain reduction; tumor shrinkage; and others…*it really works!*

However, the field is still exploratory: there is currently no evidence (*yet!*) that plasma is MORE effective than conventional therapies; clinical trials are long and expensive.

Lots of active research – there are ~ 100 groups currently working around the world in the field and results and understanding is rapidly growing.

Some references:

The emerging role of reactive oxygen and nitrogen species in redox biology and some implications for plasma applications to medicine and biology
***J. Phys. D*, 45, 26300, 2012.**

Reactive Species from Cold Atmospheric Plasma: Implications for Cancer Therapy
***Plasma Proc. Polymers*, 11, 1120–1127, 2014.**

Low temperature plasma biomedicine: A tutorial review
***Phys. Plasmas* 21, 080901, 2014.**

Oxy-nitroso shielding burst model of cold atmospheric plasma therapeutics, *Clinical Plasma Medicine* 2, 38-49, 2014.