

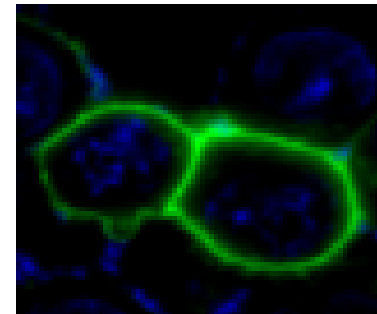
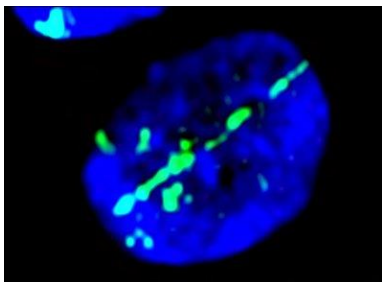


# Targeted and non-targeted effects of radionuclide therapy: role of extracellular vesicles

Jean-Pierre Pouget

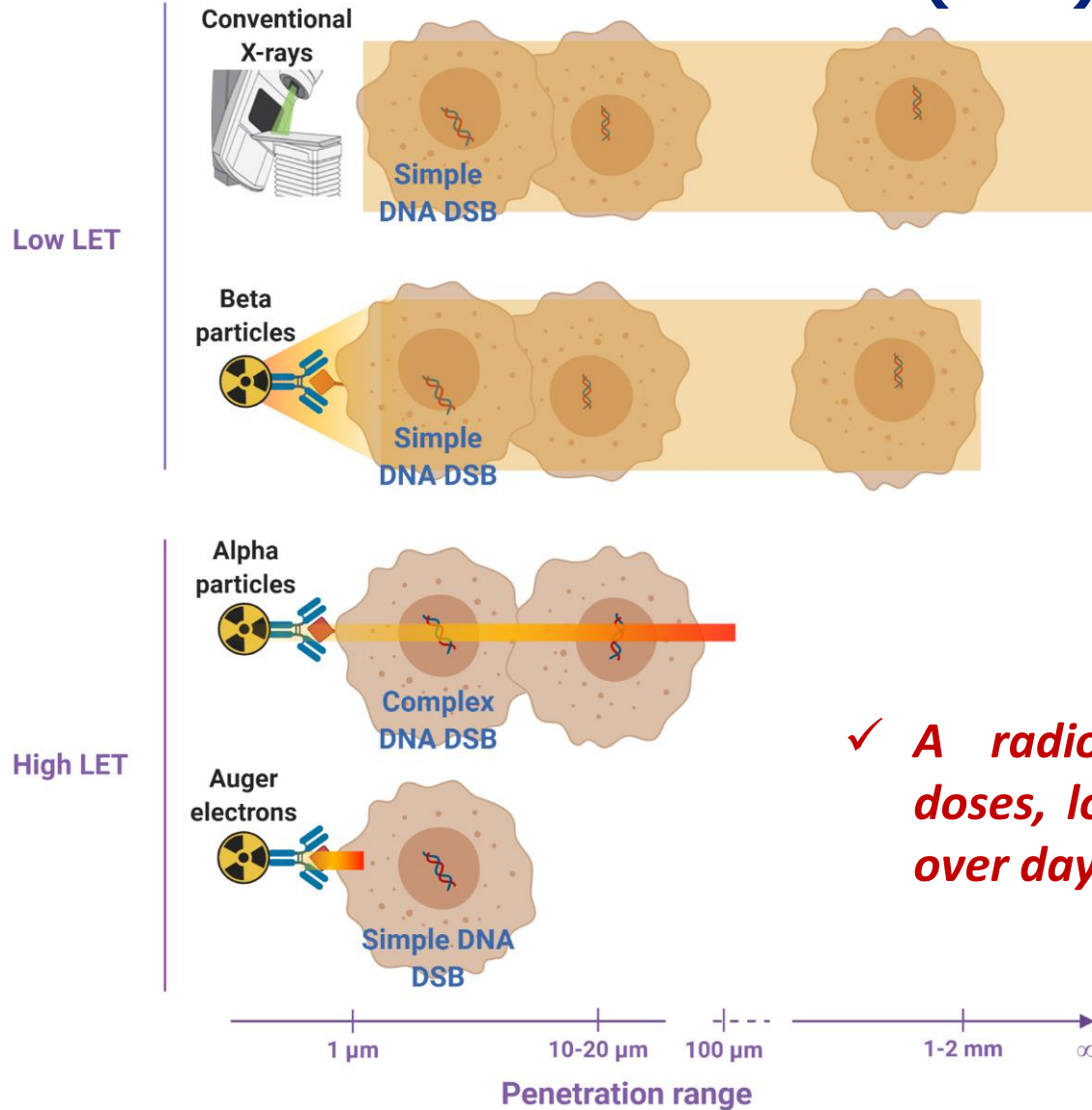
INSERM, Montpellier France

Wednesday 17 November 2021



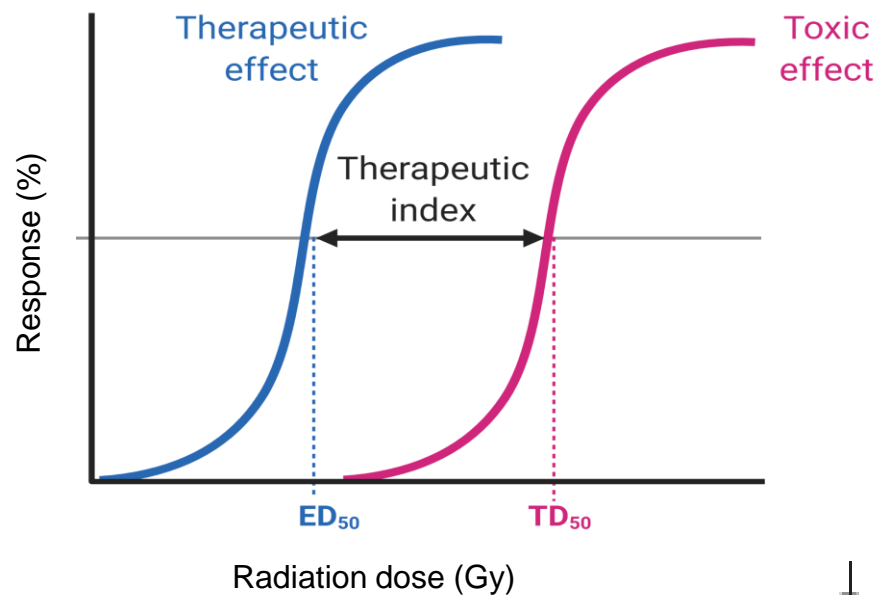


# CONVENTIONAL RADIOTHERAPY VERSUS TARGETED RADIONUCLIDE THERAPY (TRT)

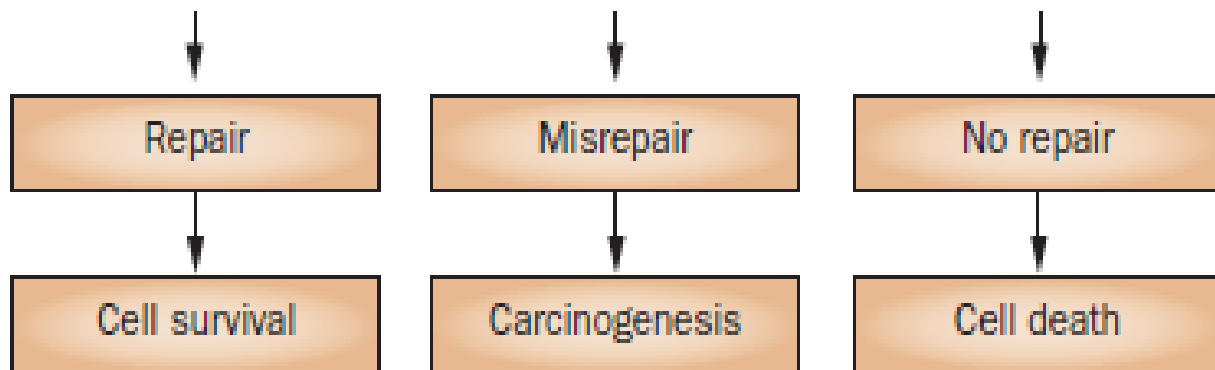


✓ *A radiotherapy modality at low doses, low dose rate and protracted over days.*

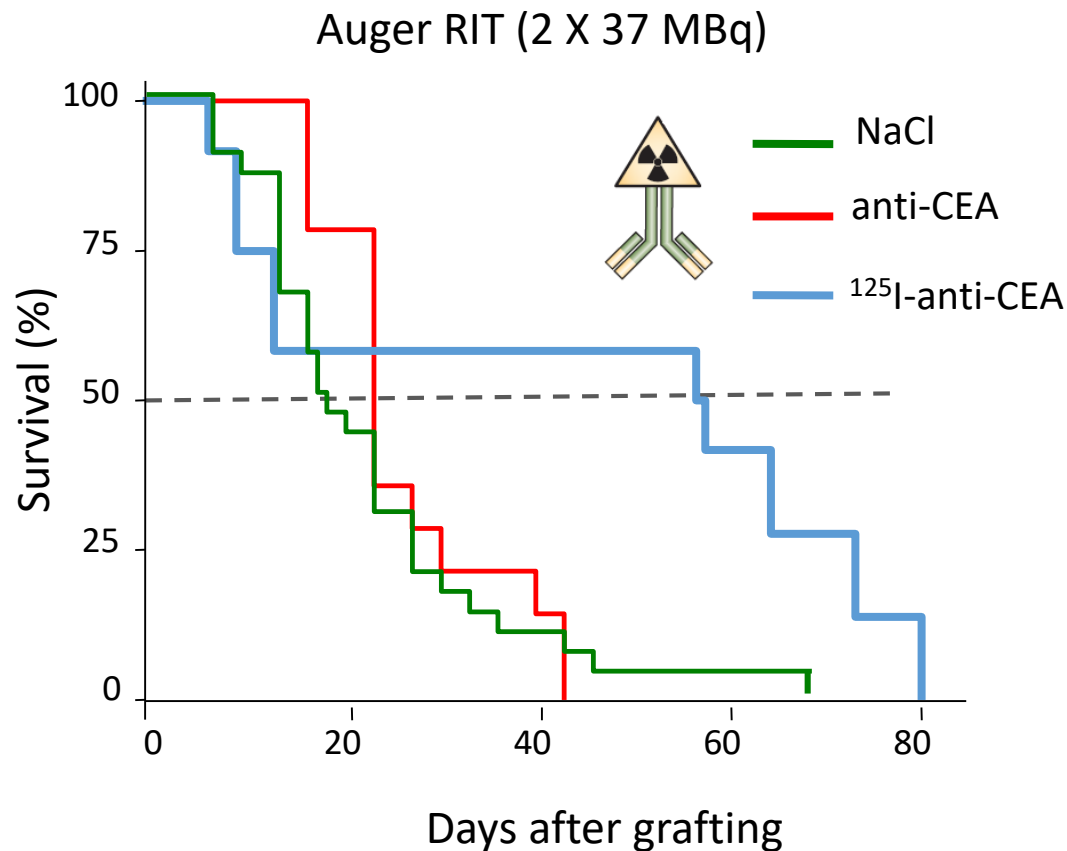
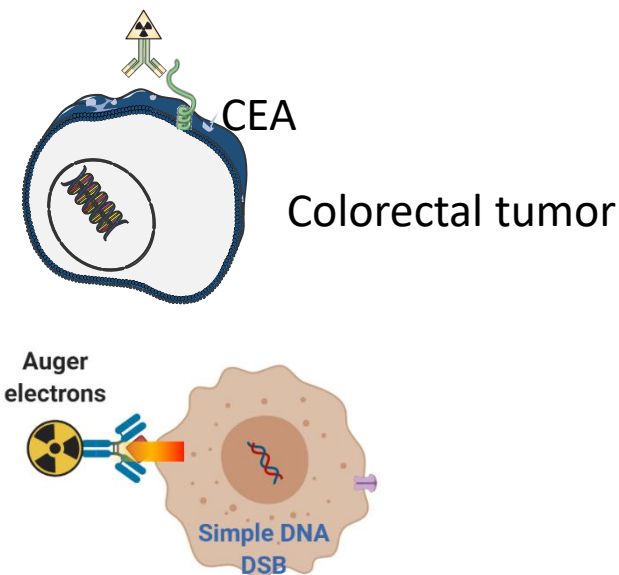
# THE PARADIGM OF RADIOBIOLOGY: THE TARGET CELL THEORY



✓ **Target theory: only cells traversed by particles can be killed.**

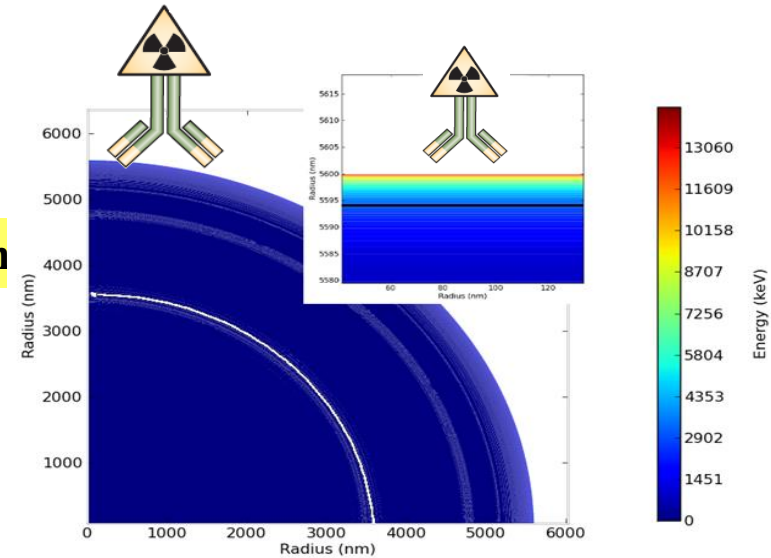
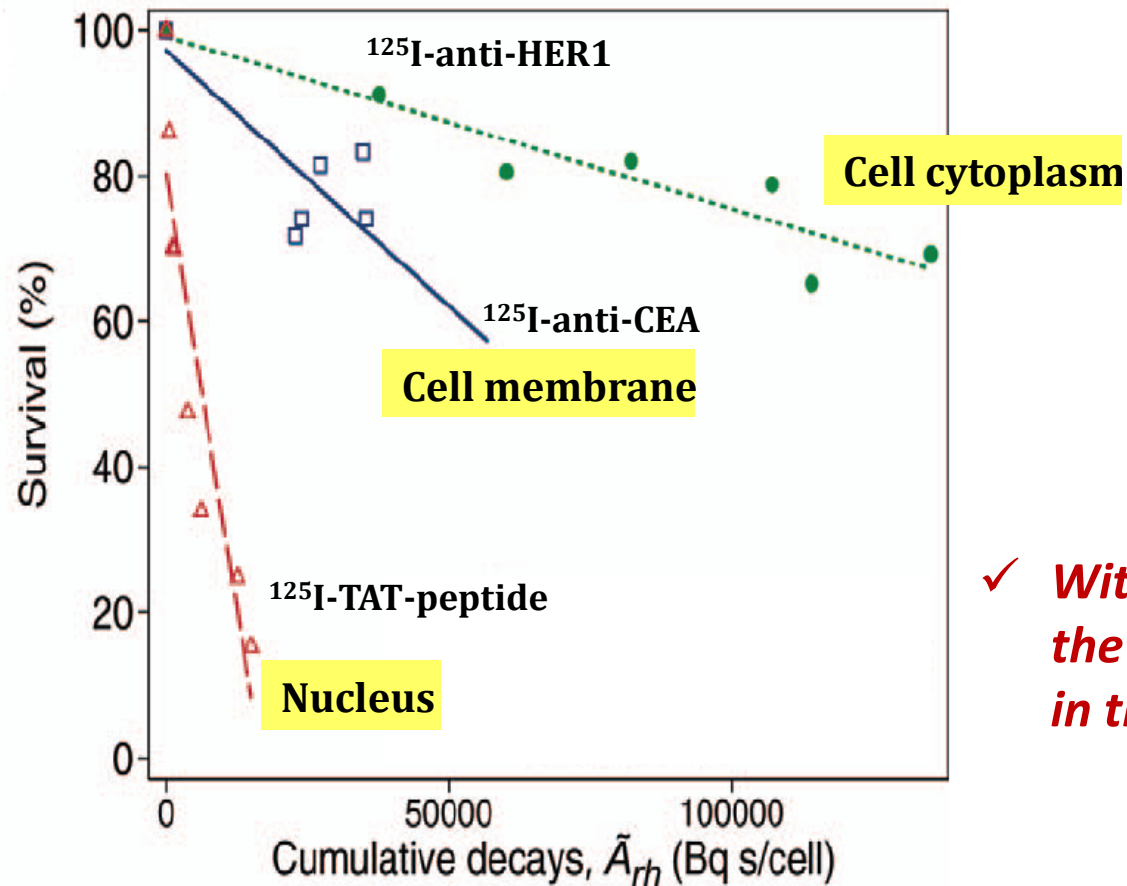


# AUGER TARGETED RADIONUCLIDE THERAPY (TRT) USING NON-INTERNALISING ANTIBODY (ANTI-CEA)



✓ **Auger RIT using non-internalising antibody (e.g. anti-CEA) targeting the cell membrane is efficient in delaying tumor growth.**

# *IN VITRO* AUGER RIT TARGETING THE CELL MEMBRANE IS ALSO TOXIC



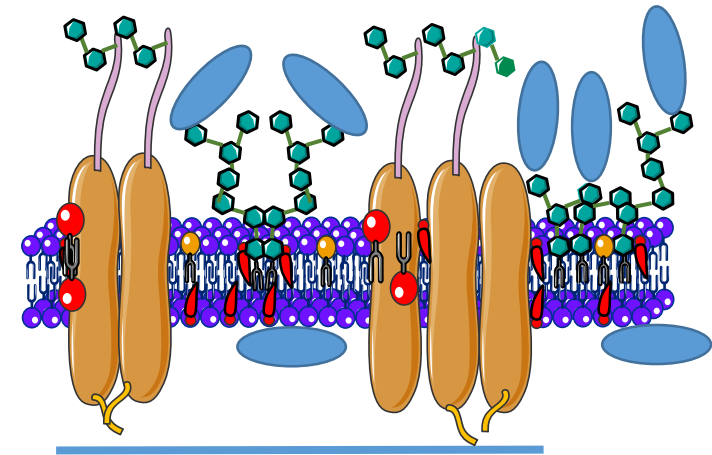
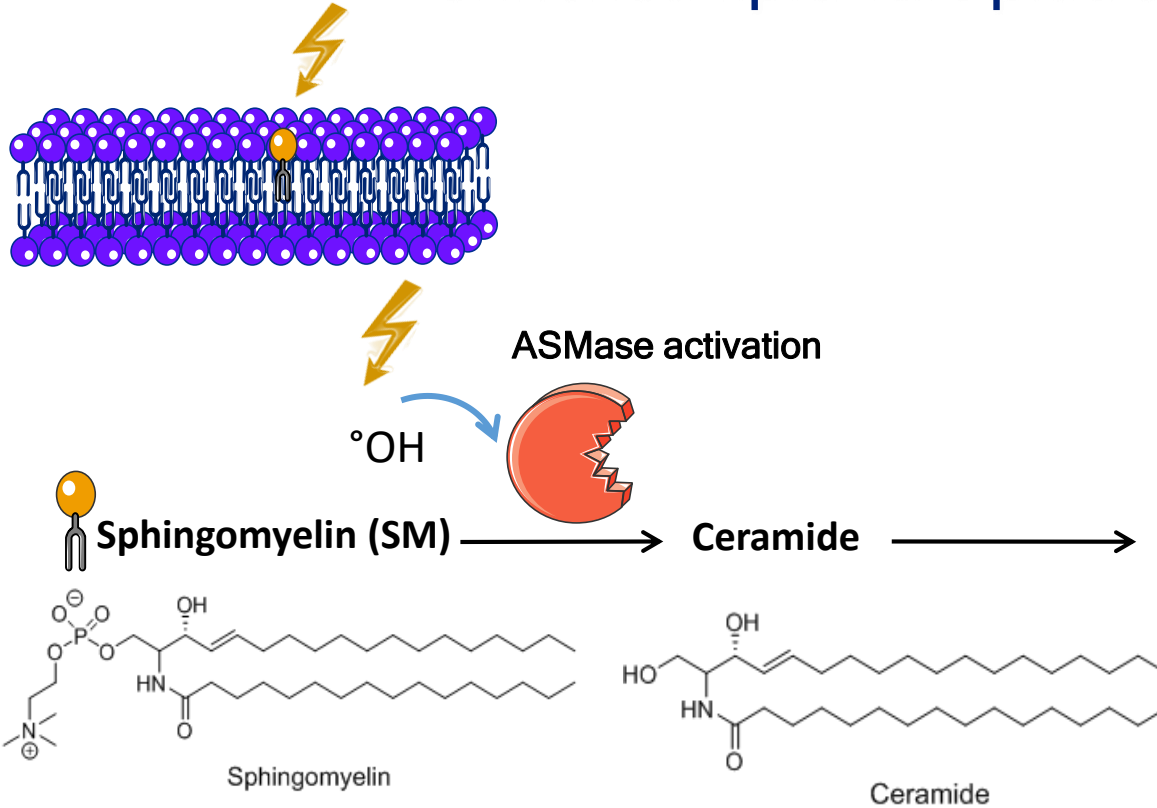
✓ *With non internalising mAb, most of the Auger electron dose is deposited in the cell membrane.*

*Pouget et al. Rad Res 2008; Paillas et al. Nucl Med Biol 2013*

✓ *A localization of  $^{125}\text{I}$  closer to the nucleus (cytoplasm) was unexpectedly less cytotoxic than a localization at the cell membrane.*



# Irradiation leads to the formation of large ceramide-enriched lipid raft platforms



**RAFT PLATFORM**

**Clustering/ activation**

Death/ Growth factor receptors

NOX

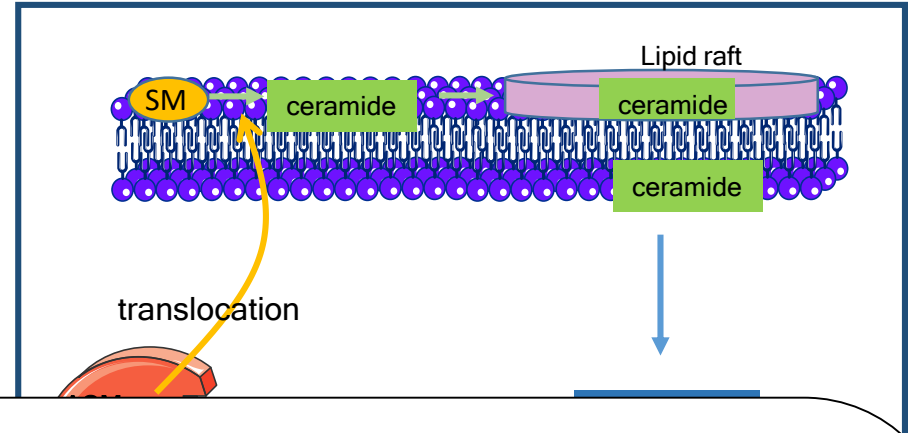
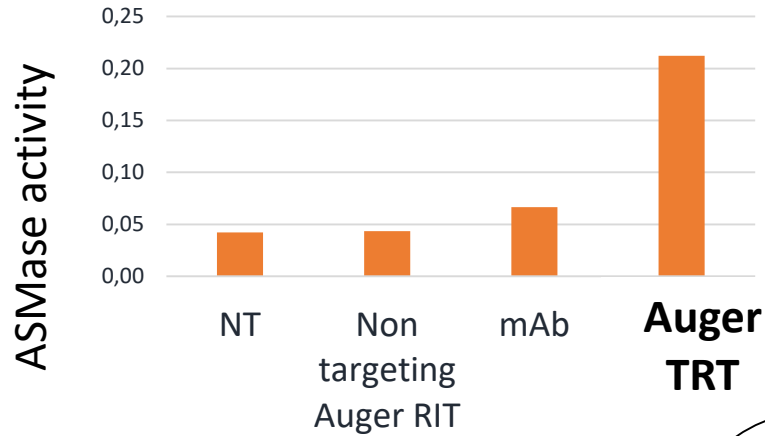
Ca<sup>2+</sup> channels

**Cell death**

*Santana et al. Cell 2016 Reynolds et al. Cancer letters 2004*

*Scheel-Toellner D et al. Blood 2004*

# LIPID RAFTS ARE ALSO FORMED DURING AUGER AND ALPHA TRT

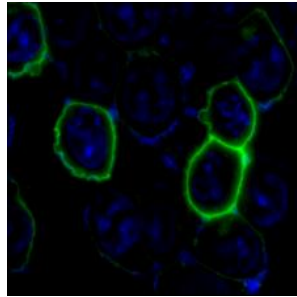
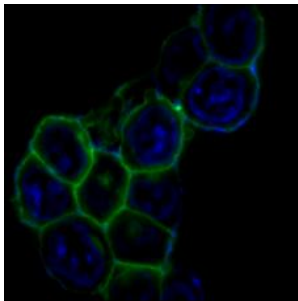


✓ *ASMase is activated during Auger TRT.*

Staining of lipid rafts with

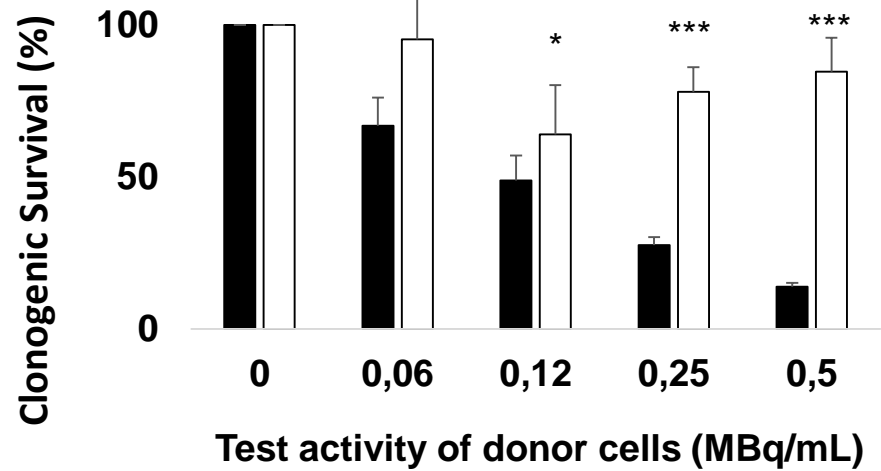
NT

Non Targeting Auger TRT



✓ *Ceramide and Lipid rafts are formed during Auger TRT.*

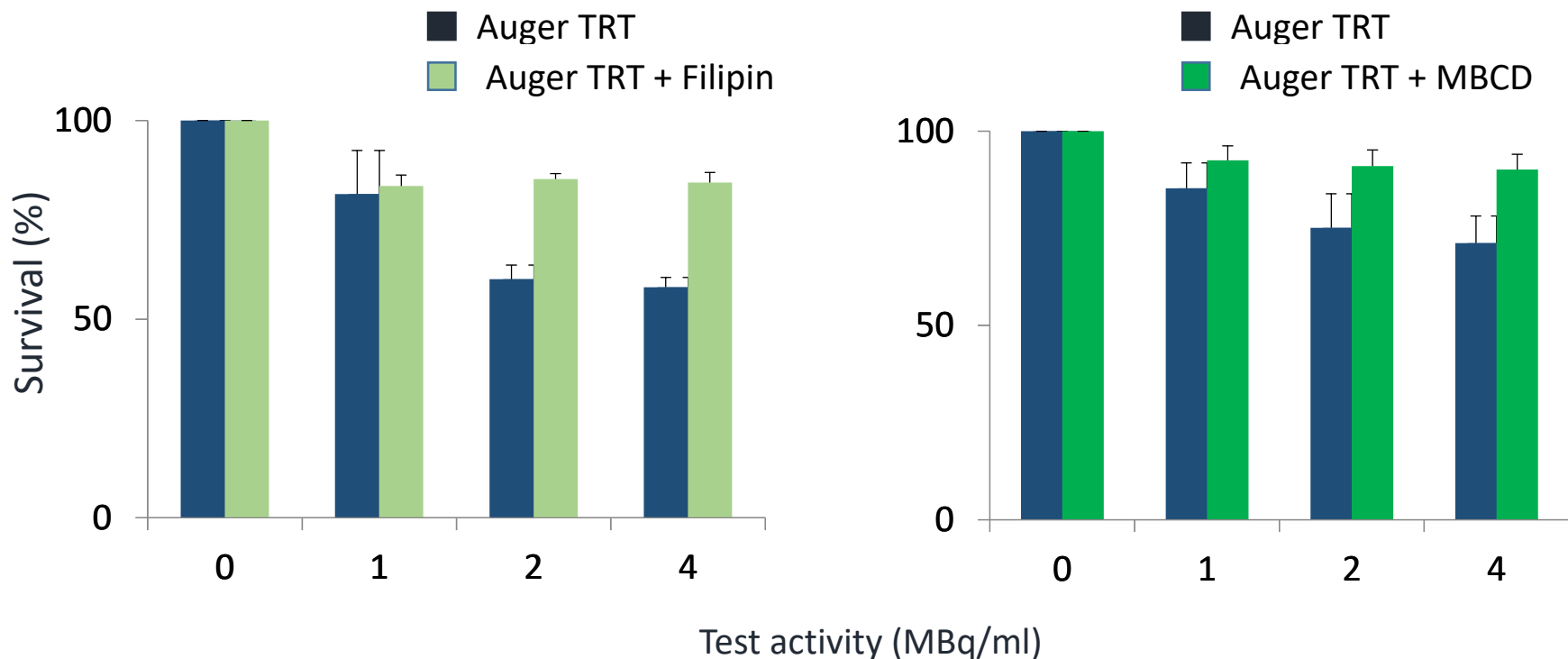
■ TRT    □ TRT + Imipramine



✓ *Inhibition of ASMase restores survival.*



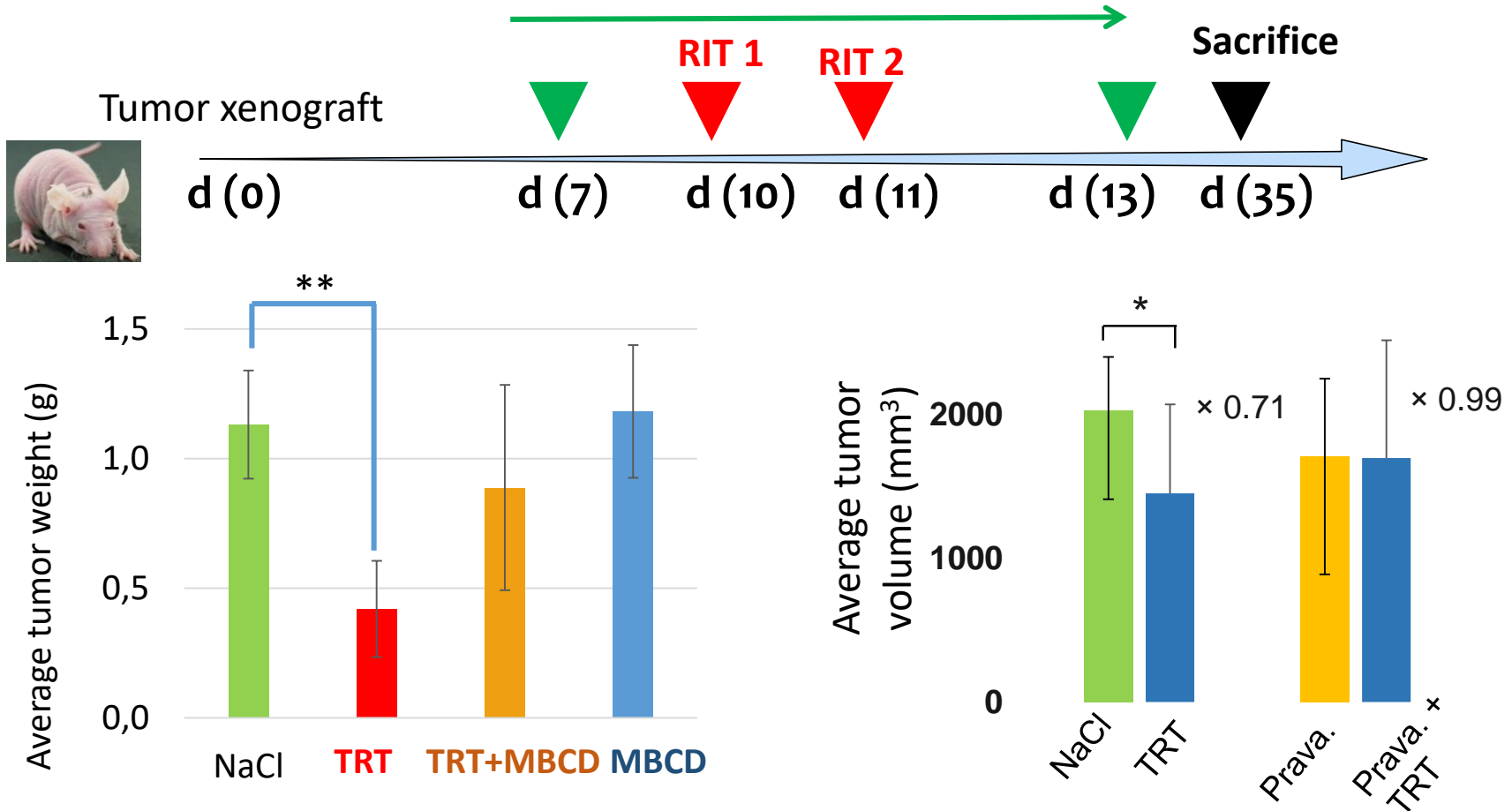
# LIPID RAFT DISRUPTION RESTORES SURVIVAL



- ✓ *Combining RIT with methyl beta cyclodextrin (MBCD) or Filipin, two lipid raft disruptors, reduces RIT efficacy, thereby indicating the role of lipid rafts in Auger-mediated therapeutic efficacy.*

# COMBINING TRT WITH MBCD OR PRAVASTATIN

MBCD 300mg/kg or 40 mg/kg pravastatin, daily IP injection

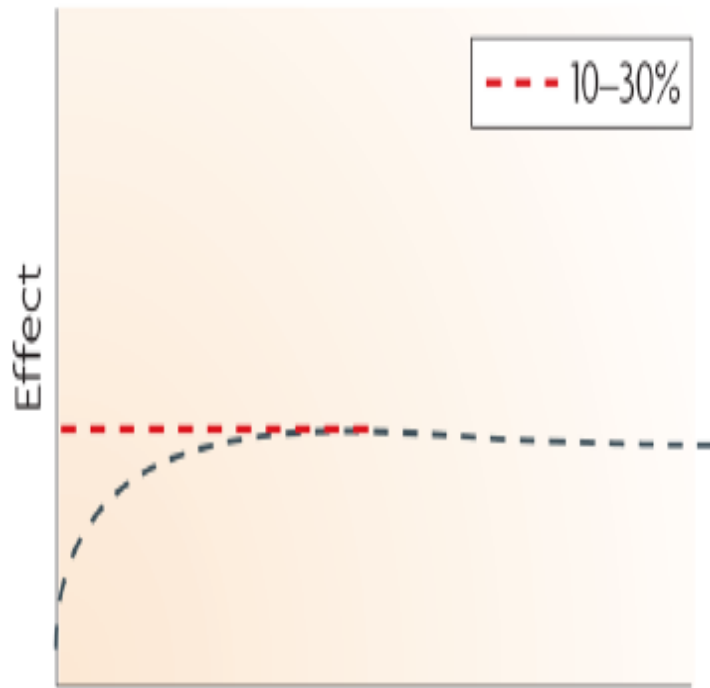


✓ *MBCD or Pravastatin combined with Auger TRT diminishes the therapeutic efficacy of Auger TRT in vivo.*

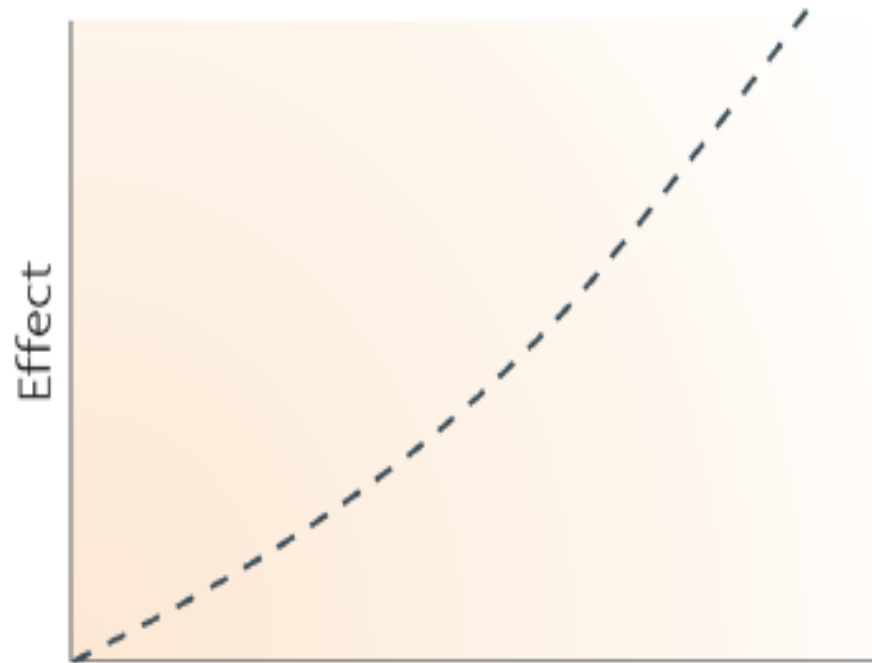
# TARGETED (DIRECT) VERSUS BYSTANDER CYTOTOXIC EFFECTS

- The term “**Targeted effects**” refers to biological effects measured in cells “**hit**” by ionizing particles.
- Bystander effects are observed in non-irradiated cells that are neighbors of irradiated cells.

**Bystander  
Cytotoxicity**

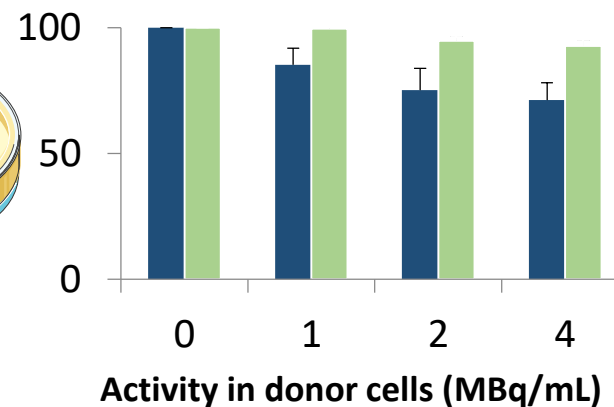
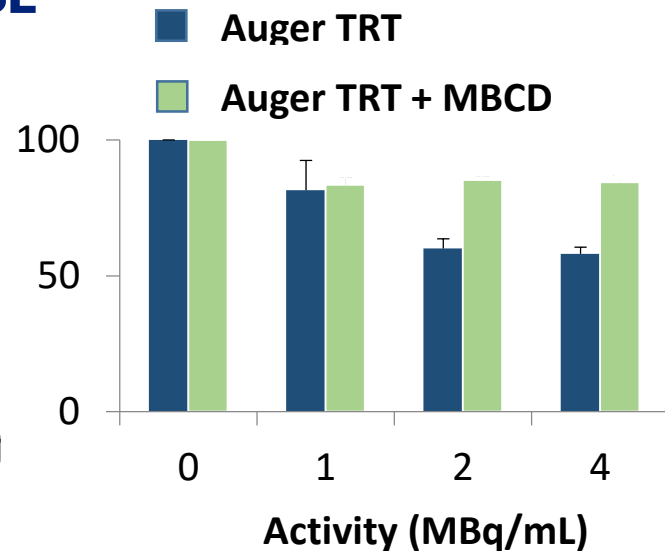
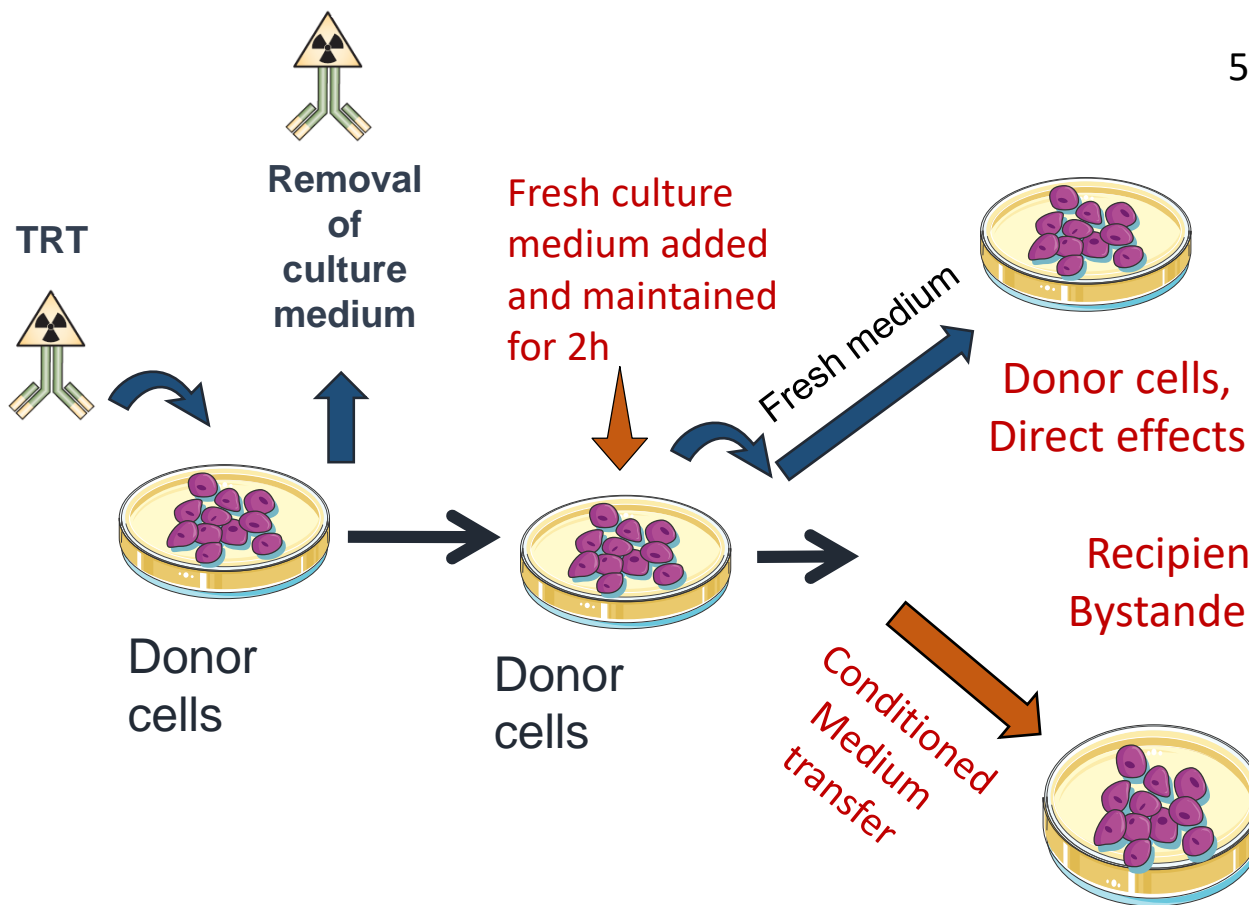


**Targeted cytotoxicity**

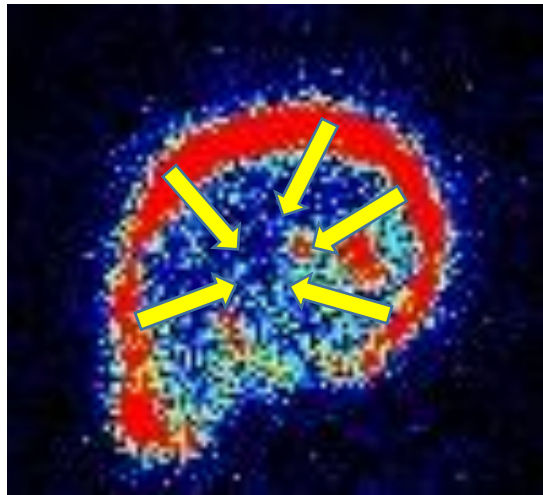
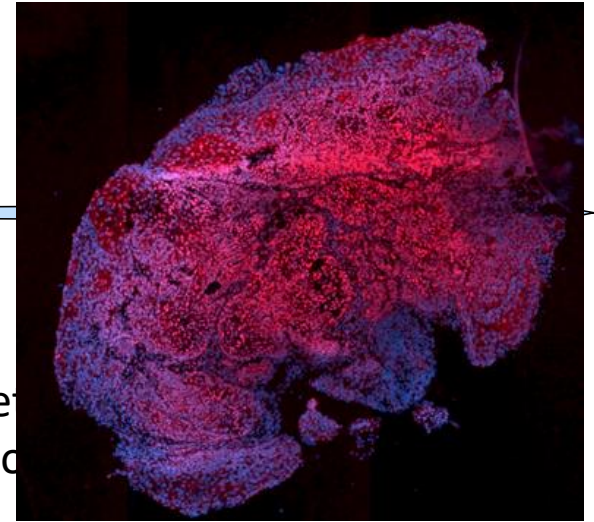
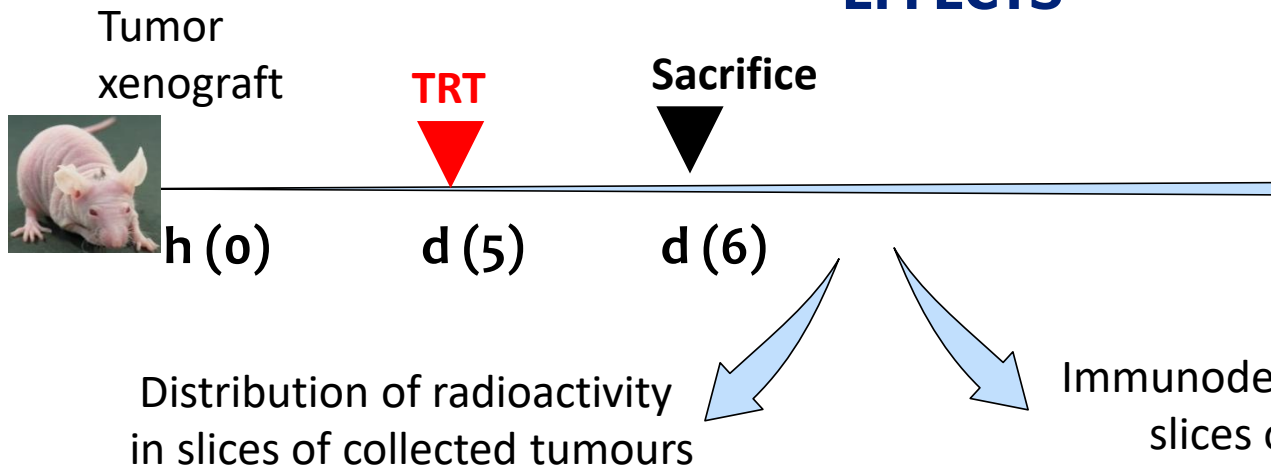


- ✓ *Do bystander effects contribute to TRT cytotoxicity?*
- ✓ *What is the role of lipid rafts in bystander effects*

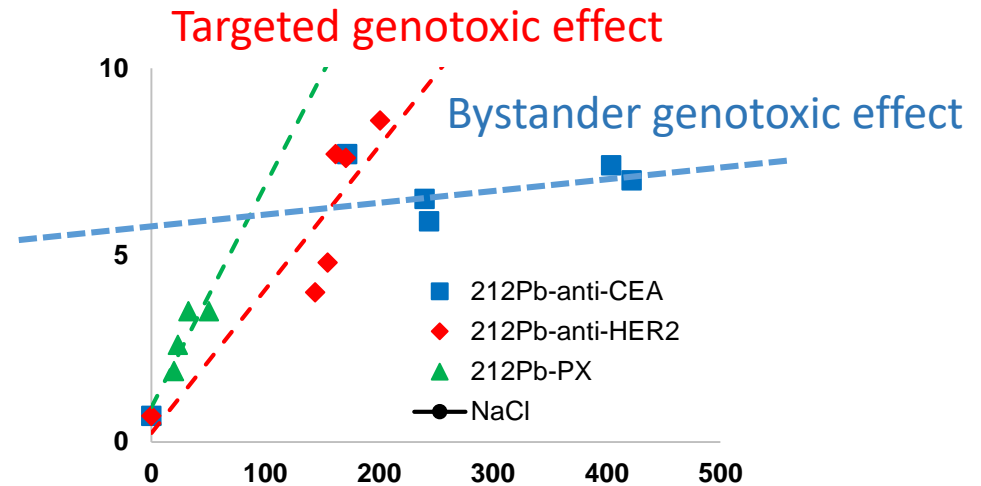
# CONDITIONED MEDIUM (CM) EXPERIMENTS FOR MEASURING BYSTANDER RESPONSE



# IN VIVO EVIDENCE SUPPORTING THE OCCURRENCE OF BYSTANDER EFFECTS



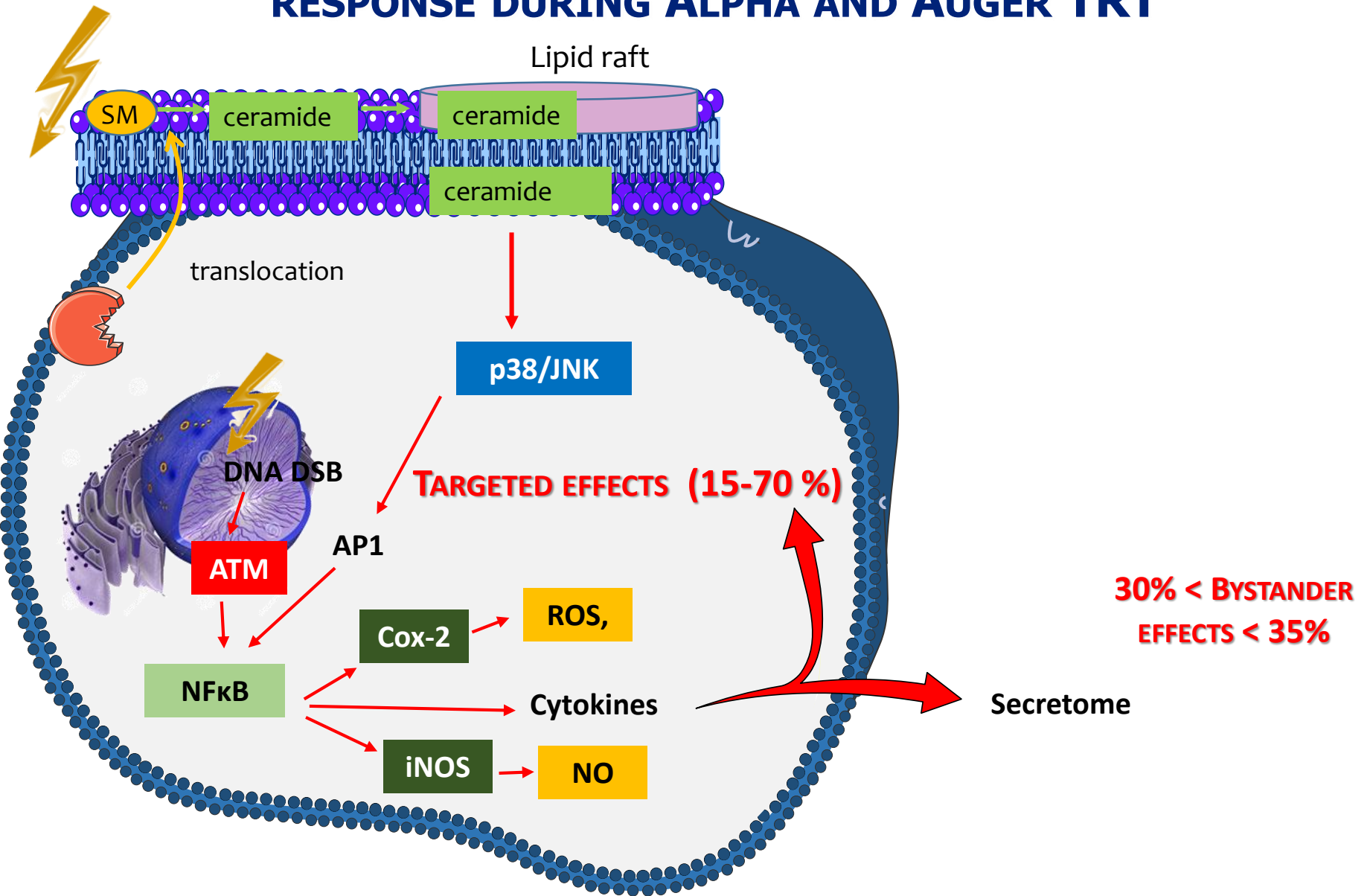
← 1-2mm →



Ladjohounlou et al. Clin Cancer Research 2019

✓ DNA lesions are produced beyond the range of particles in non-irradiated areas. This is indicative of in vivo bystander effects over mm range.

# LIPID RAFT FORMATION TRIGGERS BOTH DIRECT AND BYSTANDER RESPONSE DURING ALPHA AND AUGER TRT

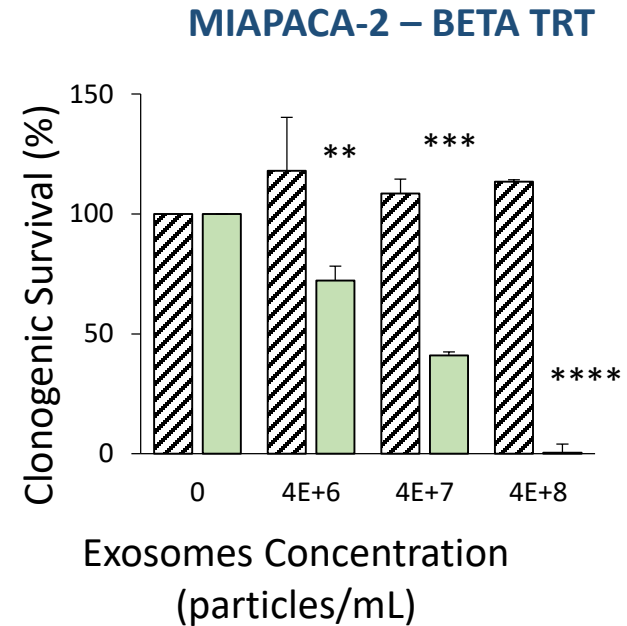
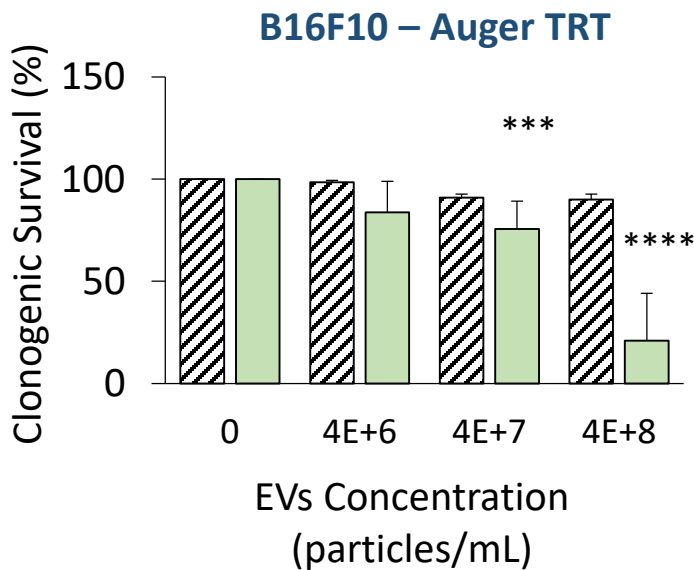
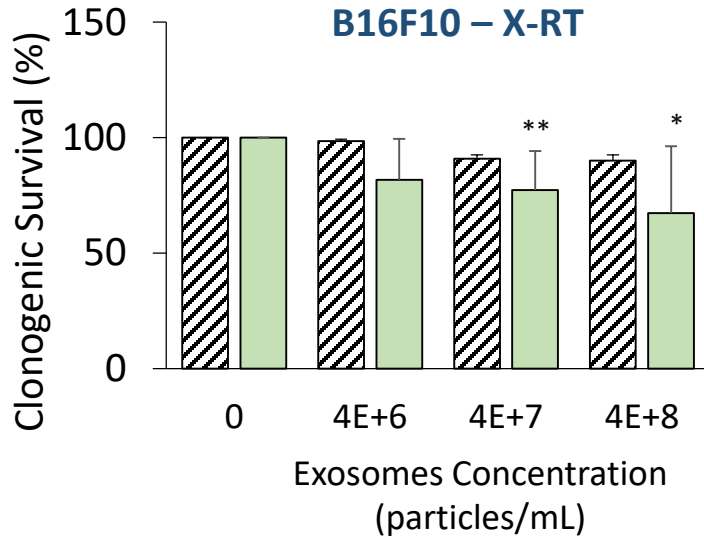






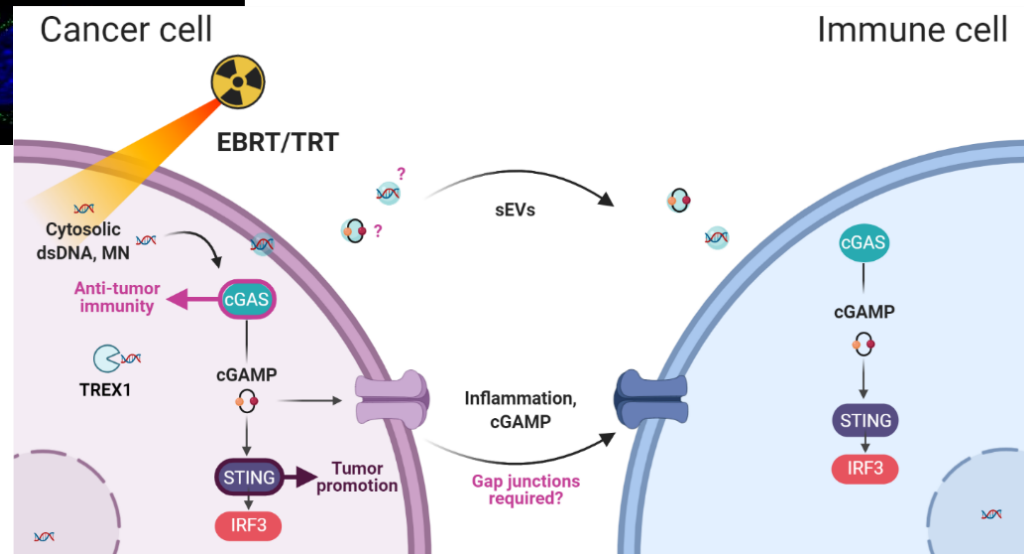
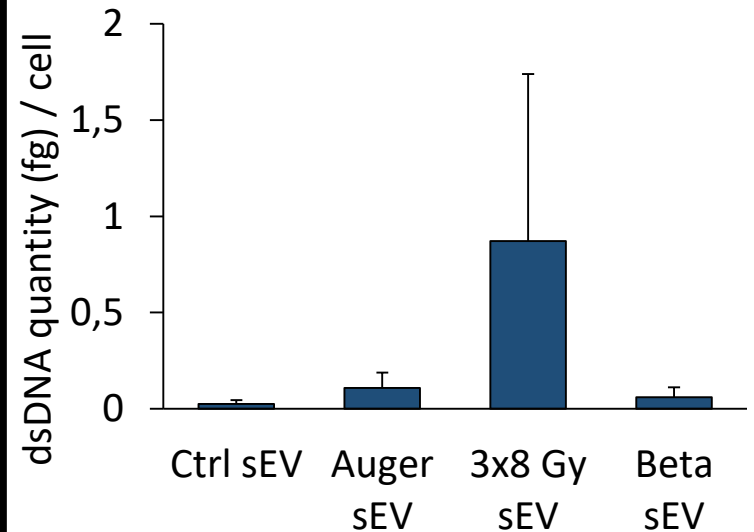
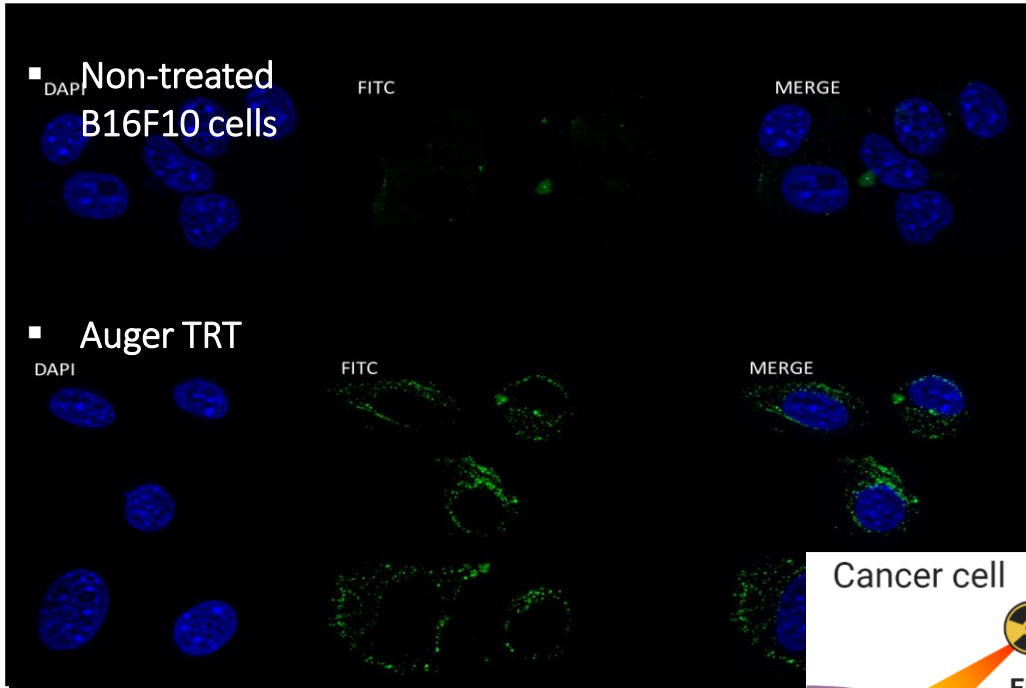
# EVs CONTRIBUTE TO BYSTANDER CYTOTOXICITY OF TRT

*Karam et al. Int J Radiat Biol, 2021*



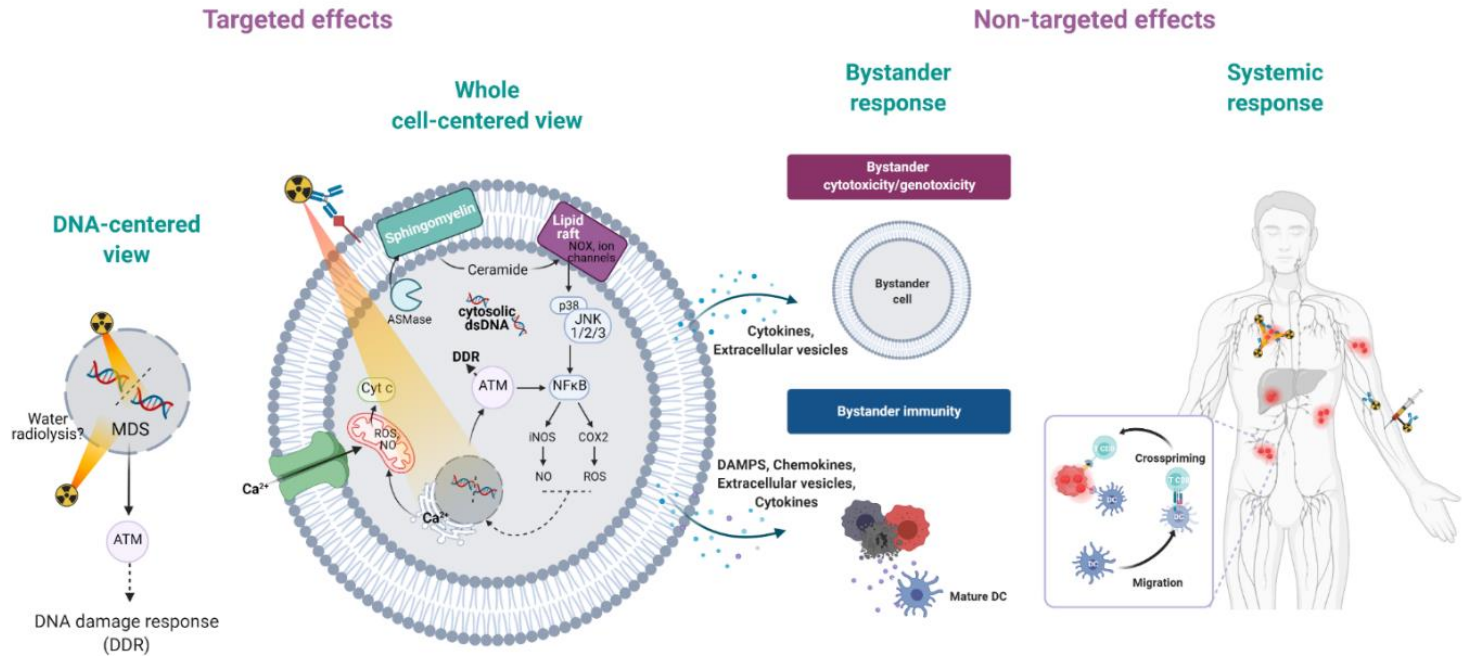
✓ *Strong cytotoxicity of EVs in vitro*

# AUGER TRT INDUCE CYTOSOLIC dsDNA ACCUMULATION



Constanzo, Faget, Ursino, Badie, Pouget, *Frontiers in Immunology*, 2021

# TAKE HOME MESSAGE: LOW DOSE RATE TRT



*Pouget and Constanzo, Frontiers in Medicine, 2021*

- Bystander cells and systemic response are involved in TRT efficacy
- Ceramide-enriched domain are involved in bystander effects
- Extracellular vesicles participate to bystander cytotoxicity
- How do extracellular vesicles participate to bystander immunity ?

# Thank you for your attention

**aviesan**

alliance nationale  
pour les sciences de la vie et de la santé

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J. Faget

C. Ursino

L. Gros

## Genetic and phenotypic plasticity of cancer

S. Dumanoir

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