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#NACLC20

Nitric Oxide Lung Cancer Active Vaccination

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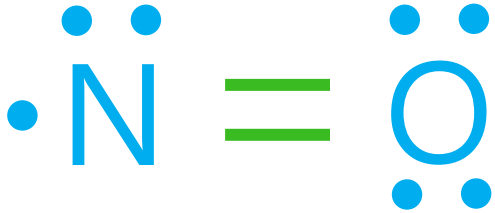

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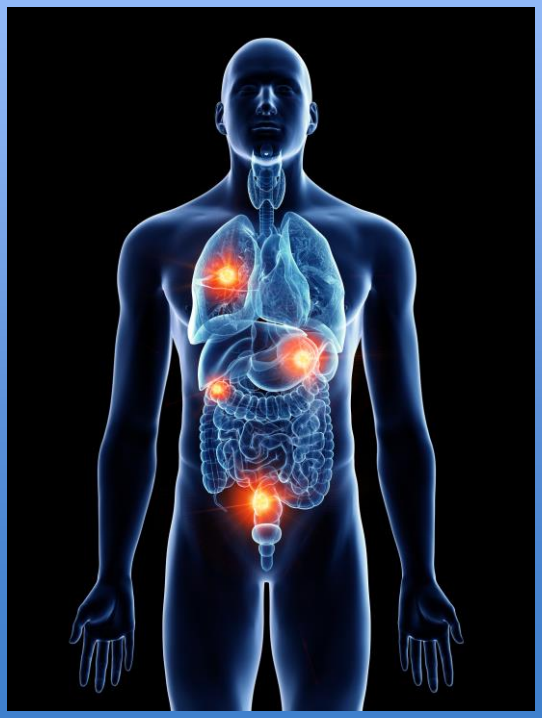
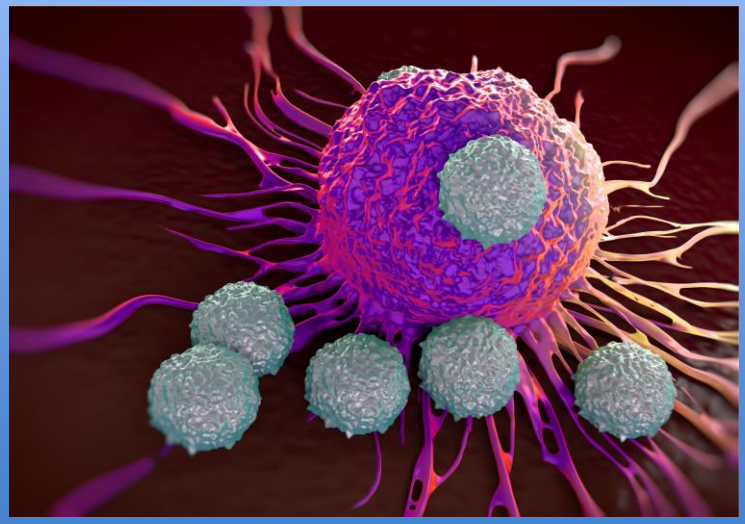
DISCLOSURES – Prof. Ido Wolf

Commercial Interest	Relationship(s)
MSD	Honorarium, research support, lectures
BMS	Honorarium, research support, lectures
Novartis	Honorarium, research support, lectures
Roche	Honorarium, research support, lectures

Metastases are responsible for ~90% of all cancer-related deaths.



Anti-tumor immunity may destroy metastases and prevent new metastatic growth.



Endogenous Nitric Oxide (NO) at Physiologic Concentrations

- Endogenous NO *at physiologic concentrations* has been shown to activate innate and adaptive responses of the immune system against tumors[1]
- Endogenous NO affects the immune system by multiple pathways, including selective enhancement of Type 1 T-cell differentiation and induction of CD4+CD25+ Treg cells[2]
- Interaction of endogenous NO with O_2 or O_2^- *in situ* results in reactive oxygen species formation, resulting in nitrosative and oxidative chemical stressors on cells[3,4]
- Exogenous, high-concentration (>10,000 ppm), local delivery of gaseous NO may cause tumor cell death, resulting in release of tumor-specific antigens

1. Vannini F et al., The dual role of iNOS in cancer. Redox Biol 6:334-343, 2015.
2. Niedbala W et al., Role of nitric oxide in the regulation of T cell functions. Ann Rheum Dis 65: iii37-iii40, 2006.
3. Shang ZJ et al., Effects of exogenous nitric oxide on oral squamous cell carcinoma: an in vitro study. J Oral Maxillofac Surg 60: 905-910, 2002.
4. Harada K et al., Overexpression of iNOS gene suppresses the tumorigenicity and metastasis of oral cancer cells. In Vivo 18: 449-455, 2004.

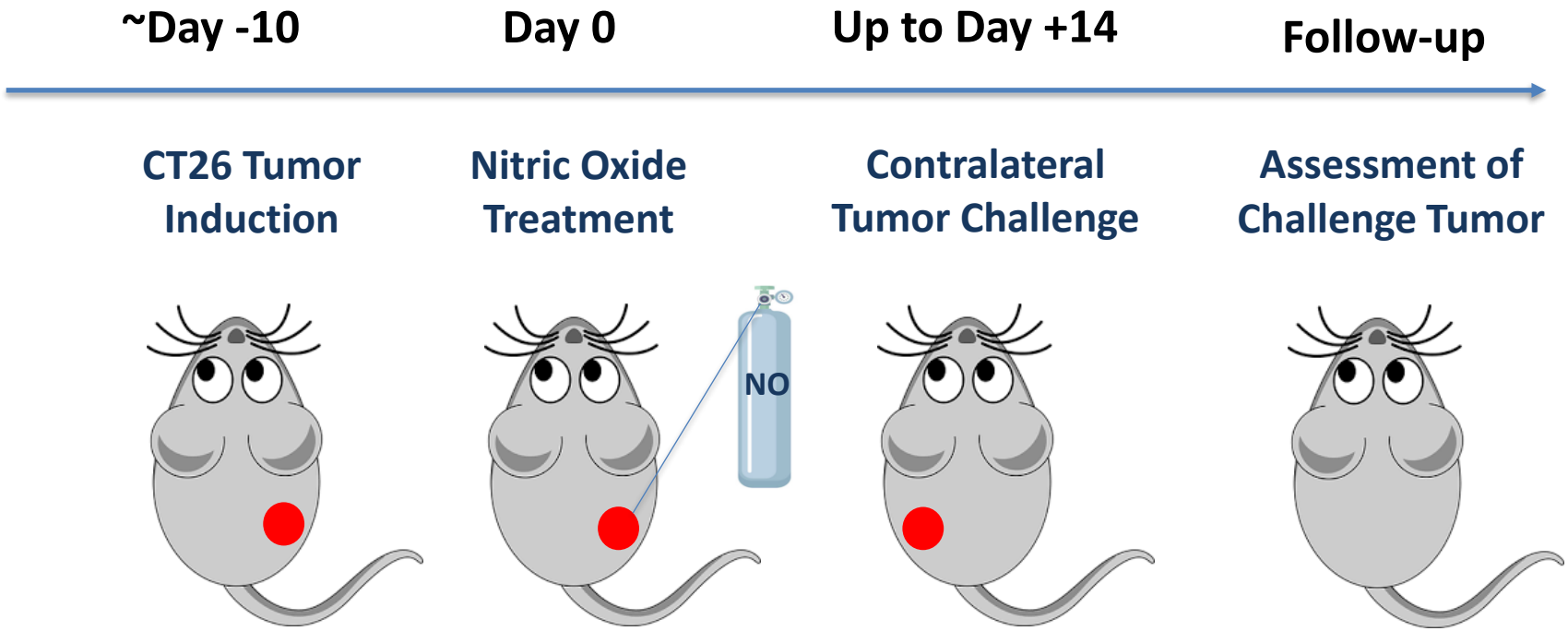
Exogenous High-Concentration NO (>10,000 ppm)

- Here we present a novel treatment paradigm that involves *in situ* tumor destruction with high-concentration gaseous NO.
- Our research group has developed an innovative NO-based tumor ablation method, in which high-concentration NO gas is delivered locally to solid tumors
- To our understanding, this is the first time a concept of injecting gas to a tissue, and specifically high-concentration NO gas to tumors, is reported.

Background

Gaseous NO stimulates apparent immune response against murine CT26 colon cancer *in vivo*

Assay Scheme

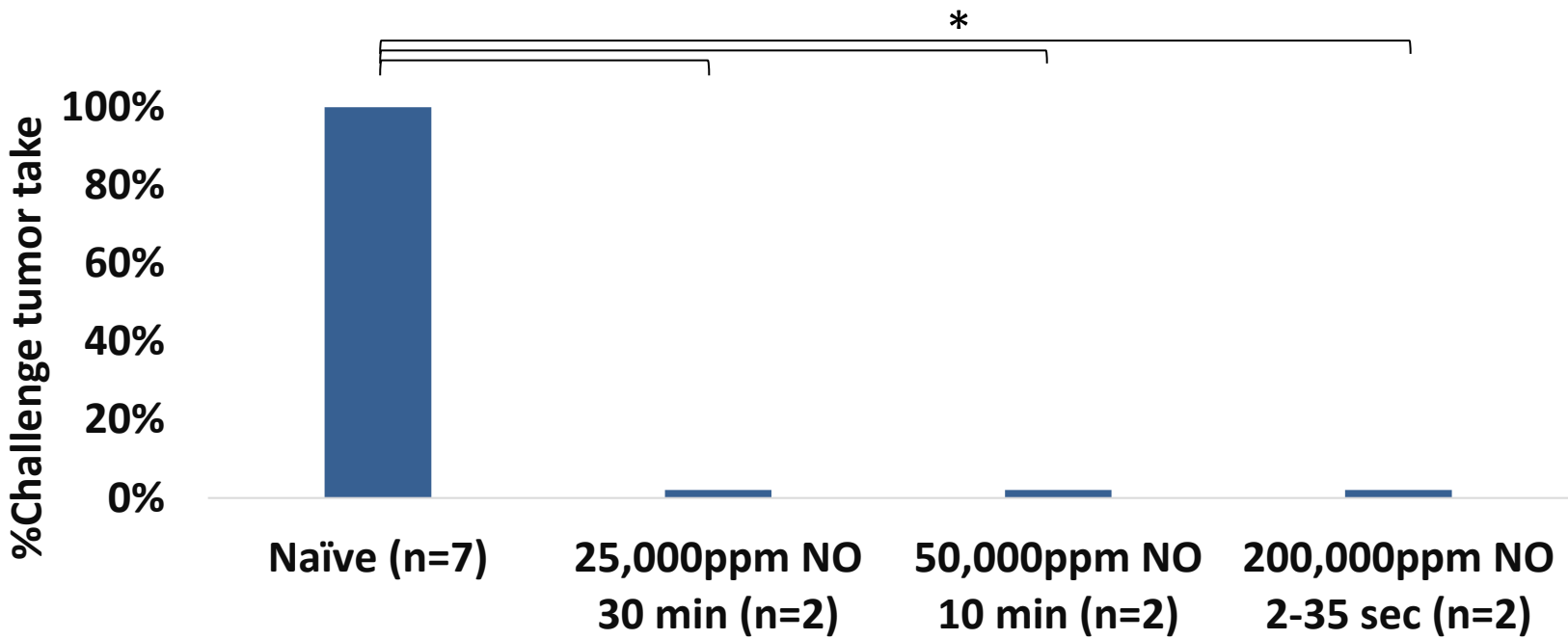


Data presented at the American Association for Cancer Research (AACR) June 22, 2020

Background

Gaseous NO stimulates apparent immune response against murine CT26 colon cancer *in vivo*

- *In vivo* results showed that all treated colon tumor-bearing mice were resistant to a second ("challenge") CT26 cancer cell inoculation



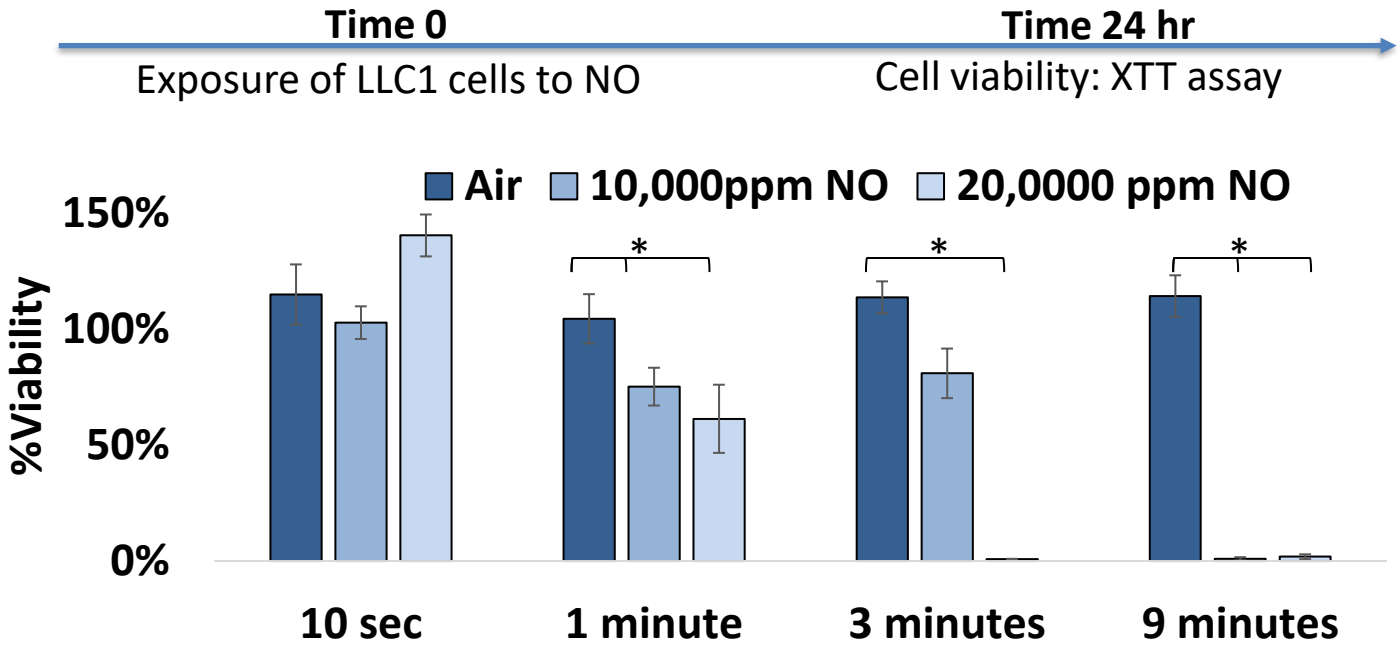
* P-value (Chi-square) <0.05

Data presented at the American Association for Cancer Research (AACR) June 22, 2020

Hypothesis

- **Endogenous** NO at physiologic concentrations has a known role in increasing immune response.
- **Exogenous** high-concentration gaseous NO administered directly to a solid tumor may result in local cell death resulting in systemic exposure to tumor antigens
- Tumor antigens may trigger a systemic immune response, thereby creating a memory immune bank that will recognize and attack subsequent primary tumor regrowth as well as distal metastases.

NO Blocks Lung Cancer Cell Proliferation *In Vitro*



* P-value (T-test) <0.05

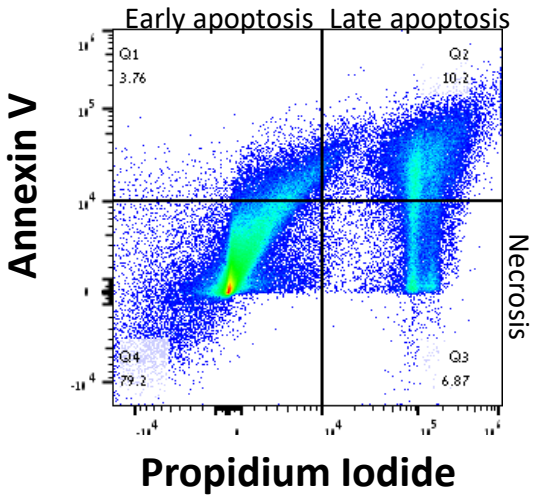
Most Lung Cancer Cells are at Late Apoptosis after Exposure to NO

Time 0 → **Time 24 hr**

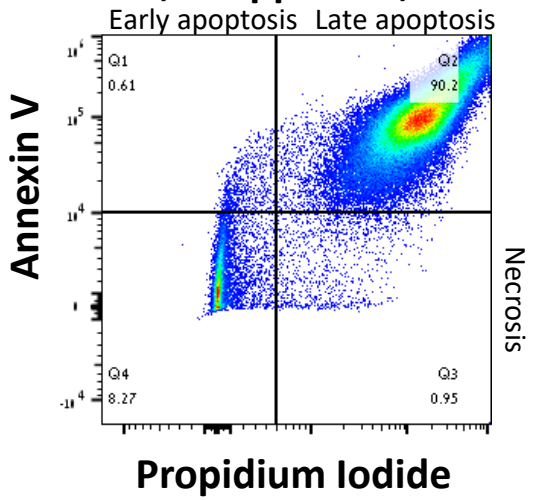
Exposure of LLC1 cells to NO

Cell viability: Annexin V-PI assay

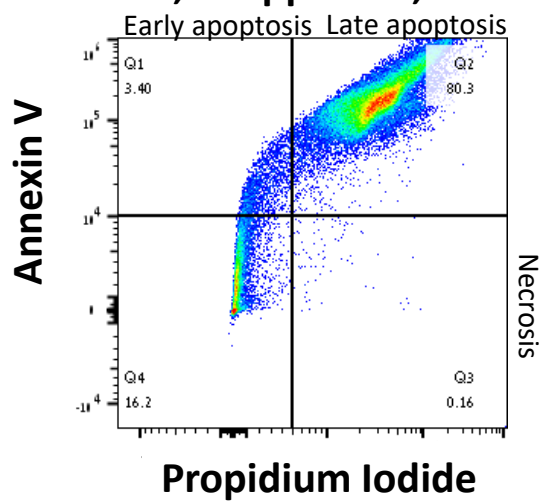
Air, 3 min



20,000 ppm NO, 3 min



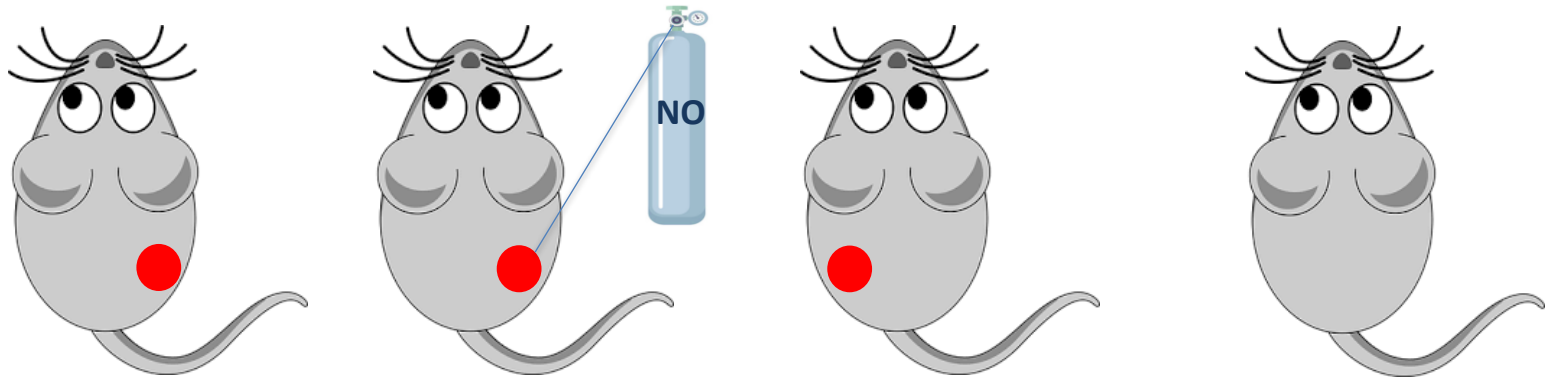
50,000 ppm NO, 3 min



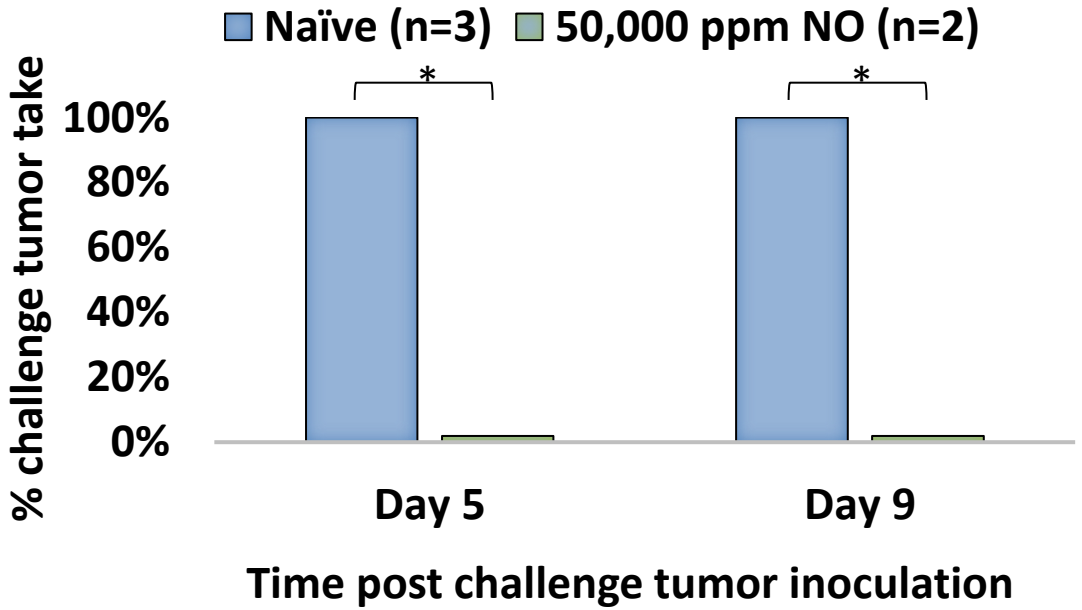
Gaseous Nitric Oxide Vaccinates Against Lung Cancer *In Vivo*

Assay Scheme

~Day -10	Day 0	Up to Day +14	Follow-up
Tumor Induction	NO Treatment	Contralateral Tumor Challenge	Assessment of Challenge Tumor



Gaseous Nitric Oxide Vaccinates Against Lung Cancer *In Vivo*



* P-value (chi-square) <0.05

Treatment: 50,000 ppm NO for 10 minutes

Conclusions

- Gaseous NO treatment results in dose- and time-dependent inhibition of lung cancer cell proliferation and reduced viability *in vitro*.
- Treatment of primary LLC1 lung tumors in mice with gaseous NO at a dose of 50,000 ppm for 10 minutes results in no uptake of a challenge tumor implanted up to 14 days later.
- No unanticipated mortality or signs indicating distress were noted in the animals.
- These preliminary data suggest that our innovative gaseous NO-based treatment may treat lung tumors locally and their distant metastases systemically, potentially via stimulation of an anti-tumor immune response.