LISBON

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NITRIC OXIDE THERAPEUTICS

IN CANCER AND OTHER DISEASES

BOOK OF ABSTRACTS

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ISNOC

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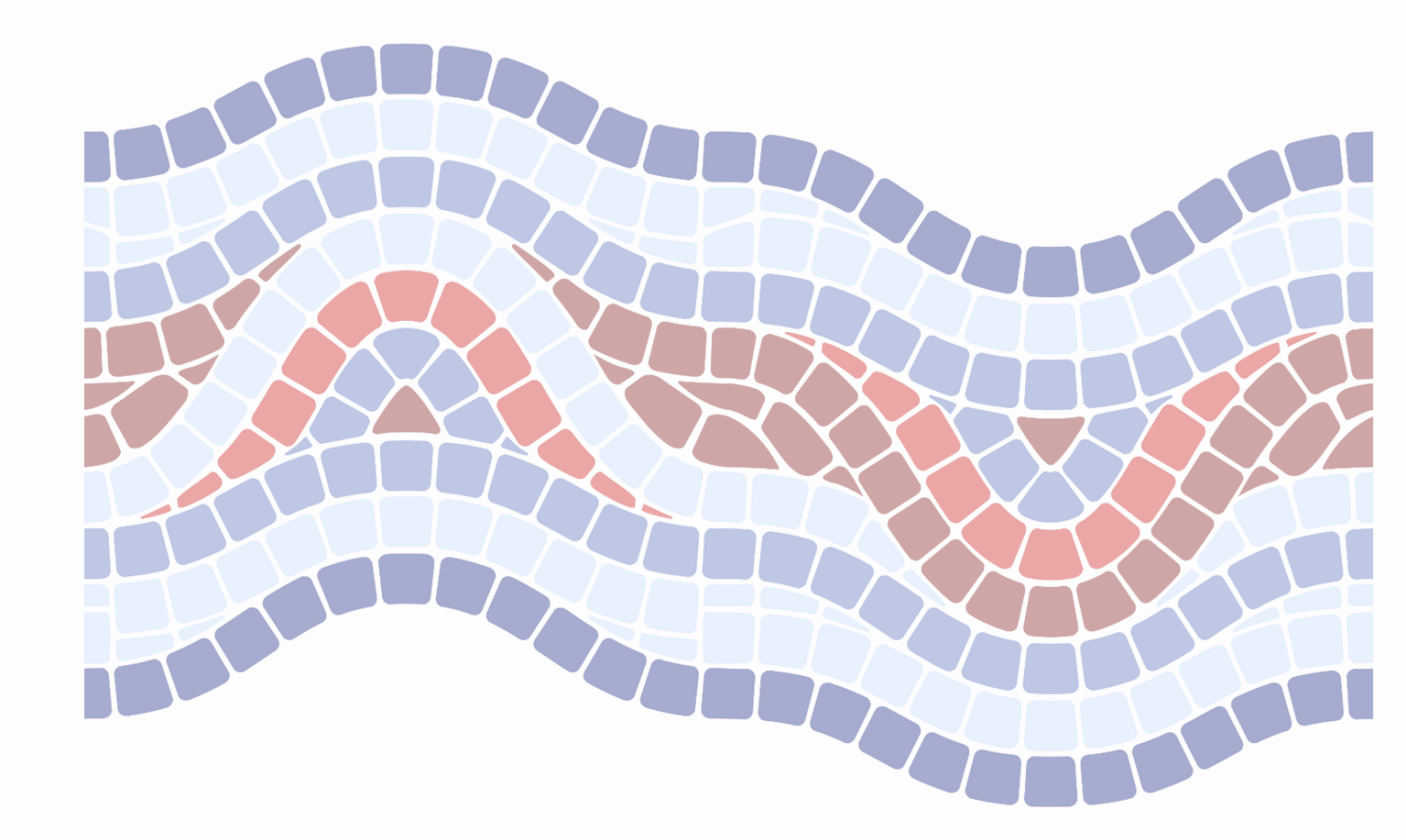


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ABSTRACTS PLENARY PRESENTATIONS



P1 – Plenary lecture 1 – 14.35h DAY 1

Development of NOSH-aspirin and its potential therapeutic applications



Khosrow Kashfi

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Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) have well-established therapeutic activities, but their long-term use can be limited by gastrointestinal, cardiovascular, and other adverse effects. The discovery of the biological signaling functions of the gasotransmitters nitric oxide (NO) and hydrogen sulfide (H_2S) provided a rationale for developing hybrid NSAIDs capable of releasing these mediators. NOSH-aspirin (NBS-1120) was developed as a novel enhanced aspirin derivative that combines NO- and H_2S-releasing moieties within a single molecular framework.

Preclinical studies have demonstrated a broad pharmacological profile for NOSH-aspirin. It retains potent anti-inflammatory activity while exhibiting a favorable gastrointestinal safety profile compared with conventional aspirin. NOSH-aspirin also displays marked anticancer activity against multiple human cancer types at sub-micromolar concentrations and has demonstrated efficacy in several tumor xenograft models. Its actions involve modulation of cellular redox status, inflammatory signaling, proliferation, cell-cycle progression, apoptosis, and posttranslational modifications, reflecting the complex interplay among aspirin, NO, and H_2S signaling.

More recently, the anti-inflammatory and neuroprotective potential of NOSH-aspirin has been extended to neurodegenerative disease. In a 6-hydroxydopamine-induced rat model of Parkinson's disease, oral NOSH-aspirin improved motor deficits, preserved dopaminergic neurons, and attenuated activation of MAPK signaling pathways associated with neuroinflammation and neuronal injury.

This presentation will trace the development of NOSH-aspirin from its original pharmacological rationale through its preclinical evaluation, highlighting its anti-inflammatory, anticancer, and emerging neuroprotective properties. The opportunities and challenges associated with translating this multifunctional gasotransmitter-releasing platform into potential therapeutic applications will also be discussed.

P2 – Plenary lecture 2 – 09.15h DAY 2

The spatial analysis of the tumor microenvironment elucidates mechanisms of immunosuppression by NOS2 and COX2, and their roles in cancer stemness and metastasis, facilitated by the specific architecture of cellular niches.



David Wink

National Cancer Institute, NIH, Frederick (USA)

The spatial analysis of the tumor microenvironment elucidates the mechanisms of immunosuppression by NOS2 and COX2, as well as their roles in cancer stemness and metastasis, facilitated by the specific architecture of cellular niches.

Increased expression of Nitric oxide synthase 2 (NOS2) and cyclooxygenase 2 (COX2) poses substantial challenges for the effective treatment of aggressive malignancies, such as ER-negative breast cancer. This study examines the spatial analysis of NOS2, COX2, and CD8 expression in patient malignancies, discovering mechanisms of treatment inhibition in conventional and immune therapy. NOS2 and COX2 mutually stimulate one another through a feed-forward paracrine mechanism. NOS2 facilitates cancer stemness and the development of metastatic niches, whereas COX2 orchestrates the inhibition of CD8+ T cells. The co-expression of nitric oxide synthase 2 (NOS2) and cyclooxygenase 2 (COX2) establishes an immune-resistant barrier that advances through many stages of immunological desert, ranging from type I inflamed restricted to type II growing immune desert and type III mature immune desert. We examine the influence of these immunological deserts concerning metastasis, cancer stemness, chemoresistance, and immune suppression. This novel insight serves as a model for employing multiplex immunofluorescence imaging as a diagnostic tool and underscores the rationale for combining NOS/COX inhibitors to effectively restore T-cell infiltration and reverse chemoresistance.

- **Core Discovery:** This study details howcthe co-expression of nitric oxide synthase 2 (NOS2) and cyclooxygenase 2 (COX2) creates an immune-resistant barrier in Estrogen Receptor-negative (ER-) and triple-negative breast cancers. High levels of these enzymes orchestrate a spatial landscape that actively suppresses and restricts CD8+ T cells (killer T cells) to the outer stroma, blocking them from entering the tumor core to destroy cancer cells. [1, 2, 3, 4, 5]
- **Clinical Impact**: It provides a blueprint for using multiplex immunofluorescence imaging as a diagnostic tool and reinforces why combining NOS/COX inhibitors could successfully restore T-cell infiltration and reverse chemoresistance

P3 – Plenary lecture 3 – 09.15h DAY 3

Nitric oxide-releasing therapeutics for treating chronic respiratory infections



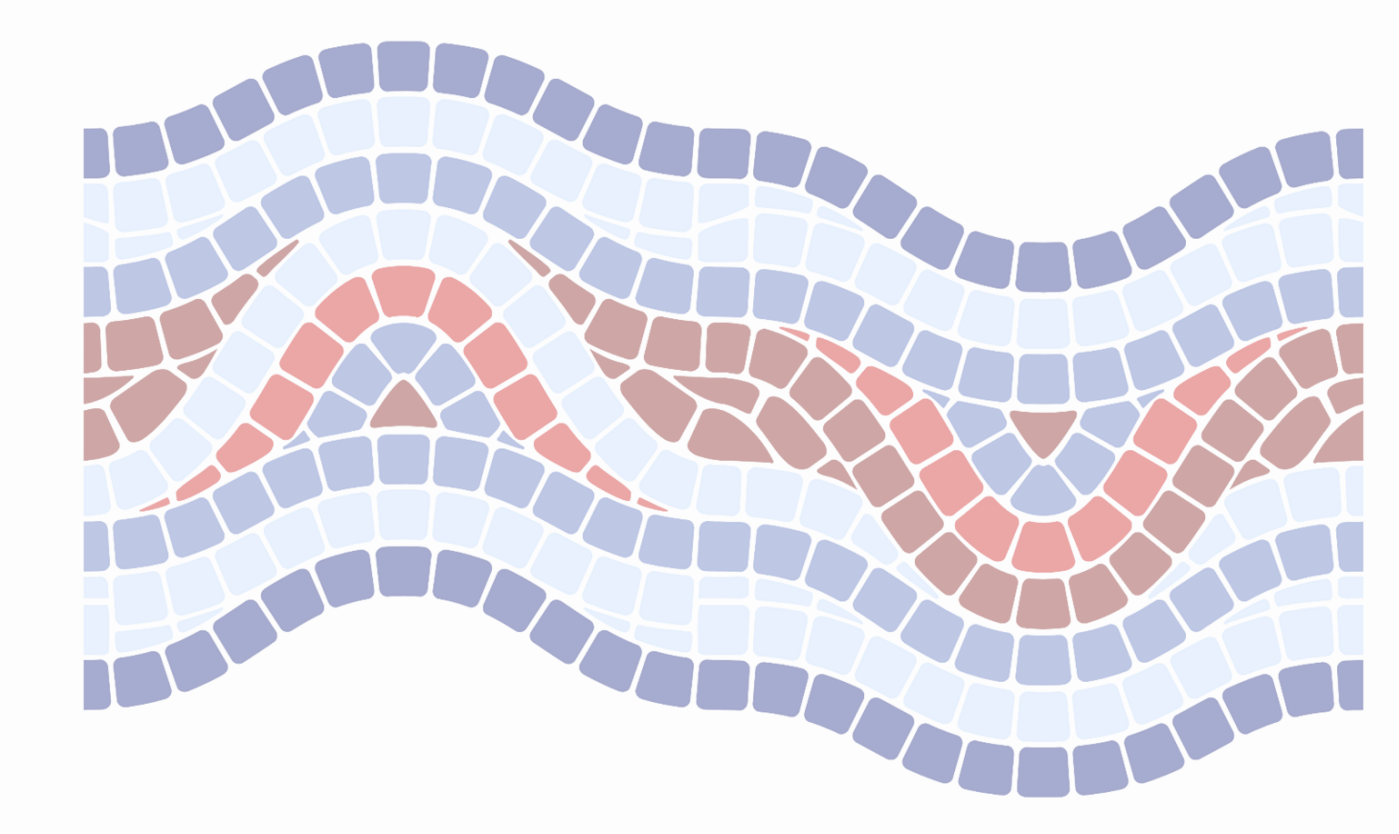
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Bronchiectasis results from dysregulated neutrophilic inflammation, mucus build-up, and bacterial infections, with sustained presence of P. aeruginosa leading to exacerbations. Nitric oxide (NO), an endogenously produced free radical, holds promise as a potential therapeutic due to its broad-spectrum antibacterial activity against planktonic bacteria and biofilms. In this presentation, I will describe the development of an inhaled nitric oxide-releasing prodrug for the treatment of chronic respiratory infections. Results will be presented detailing the therapeutic potential of the inhaled NO-releasing prodrug in a mucus-producing humanized rodent model of chronic infection and neutrophilic inflammation caused by P. aeruginosa. Given NO's activity against other pathogens including non-tuberculosis mycobacteria (NTM) and the ability to elude antimicrobial resistance, inhaled NO prodrugs represent an attractive strategy for treating respiratory and other infections broadly.

ABSTRACTS INVITED TALKS



IT1 – Invited Talk 1 – 15:15h DAY 1

Oxygen Defines Chemistry: How Tumour Hypoxia Redirects Nitric Oxide into Sulfur Biology



Christopher H. Switzer

University of Leicester (UK)

Oxygen availability constrains the chemistry available to cells. As tumours become hypoxic, oxygen-dependent reactive oxygen species (ROS) and reactive nitrogen oxide species (RNOS) chemistry becomes increasingly limited, while sulfur metabolism emerges as an alternative sink for reducing power. In parallel, electrons supplied to nitric oxide synthase (NOS) reductase domains can no longer be efficiently channelled into canonical oxygen-dependent NO production and are redirected into sulfur pathways. This promotes the formation of reactive sulfur species (RSS), including persulfides and elemental sulfur, which accumulate in hypoxic cells and tissues. In this talk, I will present evidence that hypoxia drives a fundamental shift in redox biology—from ROS and RNOS chemistry toward RSS-mediated signalling and oxidation. These findings support a new view of the tumour microenvironment in which oxygen availability dictates the dominant redox network and sulfur biology becomes a central feature of adaptation to hypoxia.

IT2 – Invited Talk 2 – 16:40h DAY1

Mechanistic insights into the regulation of cancer cell death by hydrogen sulfide and related molecules



Moran Benhar

Technion – Israel Institute of Technology (Israel)

Accumulating evidence suggests that hydrogen sulfide (H2S) and related reactive sulfur species (RSS) play a role in regulating cell death responses. However, the molecular interactions between H2S/RSS and cell death pathways remain complex and poorly understood .
We explored the idea that H2S/RSS could regulate apoptotic signaling through RSS-mediated protein persulfidation. We showed that persulfidation inhibits the activity of caspase-3 and caspase-9 during intrinsic apoptosis in HeLa cancer cells. We further demonstrated that thioredoxin-mediated depersulfidation enables caspase activation. These findings indicate that caspase persulfidation serves as a redox checkpoint in apoptosis.
Extending upon these findings, we investigated the effects of H2S on apoptotic and necroptosis signaling in colorectal cancer cells. Necroptosis is a pro-inflammatory form of regulated cell death that can be triggered by death receptors, such as the tumor necrosis factor (TNF) receptor, under conditions where apoptosis is impaired or inhibited. Our results uncover that H2S antagonizes TNF-induced necroptosis in colorectal cancer cells by down-regulating the activation of key necroptotic enzymes, RIPK1, RIPK3 and MLKL. This effect is associated with H2S driving the cellular environment toward a more reduced state. Concurrently, H2S enhances the activation of caspase-8 activation, promoting a transition from necroptosis to apoptosis. This latter effect involves the H2S-dependent regulation of the ubiquitination and stability of the active form of caspase-8. Overall, our findings point to H2S as an important regulator of the interplay between apoptosis and necroptosis. Additional findings revealed that RSS act as inhibitors of other regulated necrosis modalities, in particular, pyroptosis and ferroptosis. In conclusion, RSS emerge as versatile regulators of both apoptotic and necrotic cell death pathways, with implications to inflammation and cancer therapy.

IT3 – Invited Talk 3 – 17:35h DAY 1

Nitric oxide-driven regulation of tumor metabolism and immune resistance in cancer



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Keywords: Nitric oxide, GSNOR, Tumor metabolism, Immune evasion,

Nitric oxide (NO) is a key regulator of multiple cellular processes involved in cancer progression.

Emerging evidence indicates that dysregulated protein S-nitrosylation, resulting f rom impaired activity of the denitrosylase S-nitrosoglutathione reductase (GSNOR), promotes profound metabolic and immunological adaptations in tumors. Recent findings demonstrate that GSNOR deficiency drives metabolic reprogramming toward glycolysis, enhances lactate-dependent signaling, and fosters an immune-resistant tumor microenvironment. These observations highlight the intricate interplay between NO signaling, tumor metabolism, and antitumor immunity, and suggest that a better understanding of GSNOR biology may uncover novel therapeutic vulnerabilities and identify new strategies to enhance the efficacy of cancer immunotherapy.

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IT4 – Invited Talk 4 – 09:55h DAY 2

Methyl-nicotinamide (MNA) and vitamin K — two vitamins improving NO-dependent endothelial function; implications for cancer treatment



Stefan Chlopicki

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Vascular endothelium, covering the huge inner surface of the entire cardiovascular system, is recognized as a multifunctional organ that regulates a number of important functions to maintain cardiovascular homeostasis, while endothelial dysfunction contributes to the pathophysiology of multiple cardiovascular and non-cardiovascular diseases[1]. Altered endothelial function is also present in cancer, locally in primary tumour, in metastatic-organs as well as in systemic vasculature contributing to the pathophysiology of cancer and cardiovascular complications of cancer treatment as well as in many other pathophysiologically distinct diseases. Endothelial dysfunction in most cases is diagnosed as impaired NO-dependent function and as such displays prognostic value. Therefore, it is expected that the reversal of NO-dependent function brings therapeutic benefit.

In an effort to better understand the phenotype of endothelial/vascular dysfunction related to impaired NO-dependent function in various diseases, we developed the panel of methods for assessing endothelial phenotype *in vivo* (functional and biochemical endothelial profiling)[2-4]. Using such a unique *in vivo* approach, complemented with other methods, we were able to uncover heterogenous nature of endothelial dysfunction phenotype in various murine models of cardiovascular diseases[5,6, 7,8] endotoxic shock [9,10] cancer metastasis[11] or accelerated ageing[12] as well as to profile endothelial response to cardiovascular or anti-cancer pharmacotherapy *in vivo*. Based on this experience accumulated over years, we increasingly appreciate the heterogeneous and complex nature of endothelial dysfunction that in some cases can only be experimentally recapitulated *in vivo* and is not limited to impaired NO-dependent function. Therefore, two agents improving NO-dependent function do not uniformly affect a disease progression as exemplified by two vitamins improving NO-dependent function [13].

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IT5 – Invited Talk 5 – 11:35h DAY 2

Nitric Oxide Applications in Critical Care Setting



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Keywords: Nitric oxide; Acute Kidney Injury; Hemolysis; Acute Respiratory Distress Syndrome; Multi-Drug Resistant.

A variety of clinical applications for Nitric oxide (NO) have been proposed in critical care. Three emerging applications include the prevention of hemolysis-associated acute kidney injury (AKI) after cardiac surgery, modulation of pulmonary vascular in acute respiratory distress syndrome (ARDS), and high-dose inhaled NO as an antimicrobial therapy.

Nitric oxide for the prevention of hemolysis-associated AKI after cardiac surgery

Hemolysis during cardiopulmonary bypass may contribute to postoperative AKI through the release of cell-free hemoglobin[1]. Restoring NO bioavailability with inhaled NO (iNO) may counteract these effects. Intraoperative iNO at 80 ppm was associated with a reduced incidence of AKI after cardiac surgery[2]. More recently, a randomized trial in patients with pre-existing endothelial dysfunction showed that perioperative iNO at 80 ppm did not significantly reduce postoperative AKI[3]. These findings highlight hemolysis-induced NO depletion as a potentially modifiable pathway linking cardiopulmonary bypass to renal dysfunction. Future studies are needed to define target populations and optimize dosing strategies.

iNO in ARDS: modulation of pulmonary blood flow and ventilation–perfusion matching

By selectively dilating vessels in ventilated lung regions, iNO may improve V/Q matching without causing systemic vasodilation in ARDS[4]. Although it does not improve major clinical outcomes[5], its physiological effects may provide an important therapeutic window, particularly in severe hypoxemia or right ventricular dysfunction. Recent studies using electrical impedance tomography have demonstrated improved V/Q matching and redistribution of pulmonary perfusion distribution following iNO administration[6,7], supporting the potential role of bedside physiological monitoring to identify responders and guide individualized therapy.

High-dose iNO as an antimicrobial therapy

At substantially higher concentrations, NO exerts direct antimicrobial effects through multiple mechanisms[8]. A recent translational study demonstrated that iNO at 300 ppm was safe in healthy volunteers, and reduced multidrug-resistant *Pseudomonas aeruginosa* burden in a swine pneumonia model[9]. This approach is particularly attractive in the context of increasingly multidrug-resistant pathogens. A case report described high-dose iNO as adjunctive therapy for *Burkholderia multivorans* infection in a patient with cystic fibrosis[10], suggesting its potential role as a complementary, non-antibiiotc strategy against difficult-to-treat respiratory infections.

Together, these applications highlight the versatility of NO as a therapeutic option, allowing its biological effects to be exploited according to the underlying pathophysiology.

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IT6 – Invited Talk 6 – 12:00 DAY 2

Nitric oxide: a target molecule for the treatment of type 2 diabetes



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Keywords: Type 2 diabetes; Nitric oxide; Glucose homeostasis.

Type 2 diabetes (T2D) is the most common endocrine disorder, affecting approximately 10% of the global population. Although impaired insulin secretion and insulin resistance constitute the core pathophysiology of T2D, endothelial dysfunction, characterized in part by reduced nitric oxide (NO) bioavailability, also contributes to its development and progression. This provides a rationale for exploring strategies that restore NO signaling as a potential therapeutic approach in T2D. Among these, the inorganic anions nitrate and nitrite are of particular interest because they can serve as dietary precursors for NO generation through the nitrate-nitrite-NO pathway. Evidence from animal models of T2D indicates that nitrate and nitrite can improve glucose homeostasis by enhancing pancreatic β-cell function and peripheral glucose utilization. In pancreatic islets, these anions increase blood flow and insulin content, enhance the expression of genes involved in insulin exocytosis, and reduce oxidative stress. In skeletal muscle and adipose tissue, nitrate and nitrite increase mRNA and protein expression of glucose transporter 4 (GLUT4), improve insulin resistance, reduce adiposity and adipocyte size, and decrease body weight. Collectively, these findings suggest that restoration of NO bioavailability may influence multiple pathways involved in glucose homeostasis. NO-based interventions, particularly nitrate and nitrite supplementation, therefore represent a potential therapeutic strategy for T2D. However, evidence from clinical studies remains limited and does not yet establish therapeutic efficacy in humans. Further investigation is needed to define the clinical utility of these interventions and to clarify unresolved aspects of nitrate and nitrite biology, including their transport across mammalian cell membranes and their NO-independent signaling effects.

IT7 – Invited Talk 7 – 14:00h DAY 2

Nitric Oxide-Mediated Redox Cycles Regulating Neurovascular Coupling to Improve Cognition in Neurodegeneration



João Laranjinha* and Cátia Lourenço

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The non-canonical action of nitric oxide (NO) as intercellular messenger translates into advantages for communication within the brain. This is particularly evident in neurovascular coupling (NVC), the finely tuned spatial and temporal dialogue between active neurons and local microvessels. Through volume signaling, NO is uniquely positioned to synchronize and integrate the activity of neuronal and vascular cells across the three-dimensional space of the neurovascular unit.

The association between NVC impairment and cognitive decline, well documented in older adults (in the context of brain aging, neurodegenerative diseases, and type II diabetes mellitus) as well as in rodent models, supports the premise that restoring NVC functionality should translate into cognitive improvement. To test this hypothesis, we developed a multimodal experimental approach based on custom microarrays stereotaxically implanted in the hippocampus of living rodents. These devices combine microinjection pipettes, laser Doppler probes for cerebral blood flow measurement, and selective microelectrodes, and are complemented by behavioral and biochemical assays. This platform enabled us to probe the dynamics and functional impact of nitrite, ascorbate, and NO on NVC in animal models of aging and neurodegeneration.

In vivo evidence in rodents supports three main conclusions: (1) NVC is impaired in models of cerebral hypoperfusion and type II diabetes as a consequence of vascular dysfunction; (2) the redox interplay between nitrite, ascorbate, and NO, functionally coupled to neuronal activation, sustains competent NVC; and (3) rescuing impaired NVC translates into measurable enhancement of cognitive performance. Taken together, these findings indicate that compromised NVC can be restored through a diet-driven redox mechanism, namely, dietary nitrate supplementation feeding into the nitrite/ascorbate/NO redox cycle, thereby supporting cognitive performance in aging and neurodegeneration.

Supported by FCT Grant DietNObrain - 2022.05454.PTDC

IT8 – Invited Talk 8 – 14:25h DAY 2

Endothelial cell eNOS regulates sodium excretion and glomerular filtration rate in the kidney as determined in endothelial-cell specific eNOS KO mice



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The nitric oxide (NO)/soluble guanylate cyclase pathway regulates renal blood flow, sodium excretion and blood pressure through interactions among endothelial, epithelial and vascular cells. This talk will examine the cell-specific contributions of endothelial (EC) and red blood cell (RBC) eNOS to renal function.Using conditional eNOS knockout and knock-in mouse models, we assessed blood pressure, glomerular filtration rate (GFR), urinary sodium excretion and renal vascular responsiveness under basal conditions and following angiotensin II (AngII) infusion.Global, EC-specific and RBC-specific eNOS deletion caused hypertension. EC eNOS deficiency reduced sodium excretion and prevented the compensatory increase in GFR observed in wild-type mice during AngII infusion, whereas endothelial eNOS reactivation preserved GFR. In contrast, RBC eNOS deletion did not impair GFR or sodium excretion, and RBC-specific eNOS reactivation did not restore renal function in globally eNOS-deficient mice.These findings identify endothelial eNOS as a key regulator of renal sodium handling and GFR during AngII treatement. Its renal actions may contribute to blood pressure regulation beyond the direct control of vascular tone.

Acknowledgements

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IT9 – Invited Talk 9 – 15:35h DAY 2

Carbon Monoxide: A Surprising Strategy Against Obesity



Roberto Motterlini

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Carbon monoxide (CO) is endogenously generated during the degradation of heme by the enzyme heme oxygenase and acts as a ubiquitous gaseous signaling molecule with a wide range of pharmacological effects. The development of CO-releasing molecules (CO-RMs), a class of compounds capable of delivering controlled amounts of CO *in vivo*, has enabled the investigation of the therapeutic potential of CO in a variety of preclinical disease models. CO-RMs have been shown to exert anti-ischemic and anti-inflammatory effects, and emerging evidence indicates that CO modulates mitochondrial function, thereby preventing high-fat diet (HFD)-induced body weight gain and metabolic dysfunction. However, the mechanisms underlying these beneficial effects remain largely unknown. The gut microbiota is a key mediator of the host response to HFD and is profoundly altered during obesity, making it an attractive therapeutic target for metabolic disease. Here, we show that orally administered CORM-401 delivers CO to the gut, where it accumulates in the faeces and promotes the enrichment of multiple bacterial species disrupted by HFD-induced dysbiosis. Notably, the beneficial gut bacteria *Akkermansia muciniphila*, which is severely depleted by HFD, became the dominant bacterial species following CORM-401 treatment. Bacterial transcriptomic analysis revealed restoration of key metabolic pathways, including the tricarboxylic acid (Krebs) cycle, β-oxidation, the respiratory chain, and glycolysis. Furthermore, CORM-401 normalized plasma and fecal metabolite profiles, particularly indoles, amino acids, and tryptophan-derived metabolites, leading to reduced intestinal inflammation and improved metabolic homeostasis. Collectively, these findings demonstrate that CORM-401 reprograms the gut microbiota and identify CO as a promising gas-based therapeutic strategy for the treatment of obesity and associated metabolic disorders.

IT10 – Invited Talk 10 – 16:00h DAY 2

The molecular mechanisms of carbon monoxide-induced neuroprotection: targeting glial cells and red blood cells



Margarida Reis-Pereira[1,2], Sara R. Oliveira[3,4], Cláudia S.F. Queiroga[3], Cláudia Figueiredo-Pereira[1,2,3], Nuno L.Soares[3], Patricia Boya[5], Carlos C. Romão[6] and **Helena L.A. Vieira[1,2,*]**

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Carbon monoxide (CO) is an endogenous gaseous signalling molecule produced by the degradation of haem. Increasing evidence indicates that CO exerts anti-inflammatory, anti-apoptotic, pro-homeostatic, and cytoprotective effects across multiple tissues, including the brain parenchyma. Stroke is the 2nd leading cause of mortality and the major cause of long-term disability, particularly among individuals over 60 years of age (WHO). Ischemic stroke accounts for approximately 85% of all stroke cases and represents a major global health burden. Despite its substantial clinical and socioeconomic impact, currently approved therapies for ischemic stroke are focused on restoring cerebral blood flow through reperfusion and do not directly target brain parenchyma. Our team has been investigating the therapeutic potential of CO as a cytoprotective molecule with the final aim of targeting the ischemic penumbra. Penumbra is the region surrounding the ischemic core in which oxidative stress, metabolic dysfunction, inflammation, and programmed cell death develop over the hours to days following the ischemic event.
Beyond the direct neuroprotective effects of CO [1], we have demonstrated that CO can also target astrocytes and microglia, controlling their metabolism and inflammatory responses, that in turn protect neurons. Low levels of exogenous CO improves mitochondrial homeostasis in astrocytes [2] and increases ATP production which is a key factor in astrocyte-to-neurons communication and neuroprotection against excitotoxicity [3]. Likewise, CO-induced modulation of microglial inflammation has a double target: (i) prevention of neuronal cell death induced by exacerbated microglial inflammation and (ii) improvement of neuronal function by increasing the release of microglial anti-inflammatory and neurotrophic factors [4]. Finally, CO prevents BBB permeabilization in a mouse model of ischemic stroke [5].
More recently, the potential biological role of carboxyhaemoglobin (COHb) has caught our attention. In fact, low levels of COHb exist under physiological conditions, and they increase in response to stress. Moreover, there is no straightforward correlation between COHb levels and CO toxicity, and still COHb seems to scavenge free CO avoiding its cytotoxicity in tissues. Thus, we are now exploring the antioxidant role of COHb in red blood cells (RBC), and how it can impact brain tissue, namely by the characterization of RBC-derived extracellular vesicles (EV).

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IT11 – Invited Talk 11 – 17:00h DAY 2

Gasotransmitters and vascular disease: the inflammatory perspective



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Background: Specialized pro-resolving mediators (SPMs), including lipoxins, resolvins, protectins and maresins, activate specific G-protein-coupled receptors such as formyl peptide receptor-2 (FPR2/ALX), promoting the resolution of inflammation and vascular homeostasis. The FPR2/annexin A1 (AnxA1) pathway represents a major endogenous pro-resolving signaling axis and a potential pharmacological target in vascular inflammation. Hydrogen sulfide (H_2S), an endogenous gasotransmitter generated mainly by cystathionine-γ-lyase (CSE), exerts vasculoprotective and anti-inflammatory effects, but its interaction with pro-resolving GPCR signaling remains poorly characterized. This study investigated the pharmacological modulation of the FPR2/AnxA1 pathway by H_2S in vascular and immune cells and evaluated its impact on endothelial function and lipid mediator biosynthesis under inflammatory conditions.

Methods: Bovine aortic endothelial cells (BAECs) were exposed to TNF-α (10 ng/mL, 6 h) and treated with the FPR2 agonist Ac2-26 (0.1–1 μM) or the H_2S donor AP123 (1–10 nM). Expression of CSE, endothelial nitric oxide synthase (eNOS), AnxA1 and FPR2, together with intracellular H_2S levels, was assessed. In vivo, C57BL/6 mice received TNF-α (500 ng/mouse, 6 h) alone or combined with Ac2-26 (30 μg/mouse) or AP123 (10 μg/mouse). Vascular reactivity was evaluated in isolated aortae by measuring contractile responses to phenylephrine and relaxation to acetylcholine and SPMs generation was measured in plasma samples. In parallel, LPS-stimulated M1 macrophages were treated with the CSE inhibitor D,L-propargylglycine (PAG, 10 mM) and AP123 (1 nM–1 μM), and SPM production was analyzed.

Results: TNF-α downregulated AnxA1 and FPR2 expression in endothelial cells and reduced CSE and eNOS levels, resulting in decreased nitric oxide and H_2S availability. Pharmacological activation of FPR2 with Ac2-26 or supplementation with the H_2S donor AP123 restored AnxA1/FPR2 expression and normalized CSE/eNOS signaling. In vivo, TNF-α induced endothelial dysfunction with impaired contractile and relaxant responses, which were prevented by Ac2-26 and AP123. In plasma from these animals, several SPMs, including RvD2n-3DPA, were found upregulated by AP123. Similarly, in macrophages, AP123 increased the production of the pro-resolving mediator RvD2n-3DPA, while CSE inhibition reduced SPM generation.

Conclusions: Pharmacological modulation of the H_2S pathway regulates FPR2/AnxA1 pro-resolving signaling and preserves endothelial function during inflammation. The H_2S–FPR2 interaction controls vascular reactivity and SPM biosynthesis, identifying this axis as a potential therapeutic target for the treatment of vascular inflammatory disorders.

IT12 – Invited Talk 12 – 09:55h DAY 3

Nitric Oxide releasing nano/biomaterials for biomedical applications



Amedea Seabra

Universidade Federal do ABC, UFABC, Santo André, SP, Brasil

Nitric oxide (NO) is an endogenous gasotransmitter that plays a crucial, concentration-dependent role in pathophysiological processes, particularly in oncology and inflammatory disorders. While physiological nanomolar levels of NO can promote cell proliferation and vascular homeostasis, micromolar fluxes trigger oxidative and nitrosative stress, inducing cell apoptosis, inhibiting tumor cell proliferation, and sensitizing resistant cancer lineages to conventional therapies. Furthermore, NO is a master regulator of biological responses, modulating microvascular tone, balancing macrophage polarization, and accelerating wound healing. Despite its immense therapeutic potential, the clinical translation of free NO is severely hindered by its short half-life, rapid systemic clearance, gaseous nature, and risk of non-targeted toxicity.

To overcome these challenges, our research group at the Federal University of ABC (UFABC) has focused on engineering advanced nanostructured delivery platforms and biomaterials capable of localized, sustained, and spatiotemporally controlled NO release. This presentation provides an overview of our recent developments in developing multifunctional biomaterials designed for cancer nanotherapy and inflammatory applications.

Our engineered platforms incorporate various NO donors *(e.g.*, *S*-nitrosoglutathione [GSNO], and NO-releasing aspirin derivatives [AspNO]) integrated into diverse nanostructured frameworks, including:

- **Polymeric and Biopolymeric Nanoparticles:** Chitosan, alginate, and PLGA nanoparticles tailored via chemometric designs for optimal encapsulation efficiency and prolonged release kinetics.
- **Hybrid and Inorganic Nanostructures:** Core-shell magnetic iron oxide (Fe_3O_4@Ag), zinc oxide (ZnO), biogenic copper oxide (CuO), and molybdenum metal-phenolic networks (Mo-MPN) designed for dual NO donor/chemotherapeutic co-delivery *(e.g.*, Cisplatin, DOX).
- **Polymeric Hydrogels and Solid Lipid Nanoparticles:** Pluronic F-127/alginate hydrogels and lipid nanocarriers engineered for topical application and dual-gasotransmitter therapy (NO and H_2S).

Physicochemical characterizations were extensively performed using DLS, NTA, HRTEM, AFM, FTIR, and real-time electrochemical NO/H_2S sensing. *In vitro* biological evaluations were conducted across multiple tumor lineages (melanoma A375/SK-MEL-28, prostate cancer PC3, breast cancer, and hepatocellular carcinoma HepG2/HUH7) as well as non-tumorigenic cell lines (HUVEC, HaCaT, and dermal models). Our findings demonstrate that nanoencapsulation significantly stabilizes NO donors, extending release kinetics over hours to days. In oncology models, NO-releasing nanocomposites selectively induce apoptosis in tumor cells via elevated reactive oxygen and nitrogen species (ROS/RNS) generation. Notably, the combination of NO release with metallic cores or chemotherapeutic drugs (such as Cisplatin or DOX) successfully reverses drug resistance in cancer cells while downregulating pro-inflammatory markers (IL-6, IL-1β, iNOS). In inflammatory and tissue repair models, localized NO/H_2S delivery protects endothelial and mitochondrial integrity under oxidative stress, suppresses excessive inflammatory responses, and promotes re-epithelialization. In conclusion, the rational combination of nanotechnology, biomaterials, and nitric oxide donor chemistry provides a highly promising, versatile therapeutic strategy to overcome current limitations in cancer treatment and chronic inflammatory management.

Acknowledgements The author acknowledge the financial support from São Paulo Research Foundation (FAPESP) (Grant, 2024/12112-2), Capes, CNPq, FINEP.

reactivity and SPM biosynthesis, identifying this axis as a potential therapeutic target for the treatment of vascular inflammatory disorders.

IT13 – Invited Talk 13 – 11:05h DAY 3

Porous Materials for NO Delivery: From Microporous water to extruded MOF-Polymer composites for mitigation of complications associated with catheterization

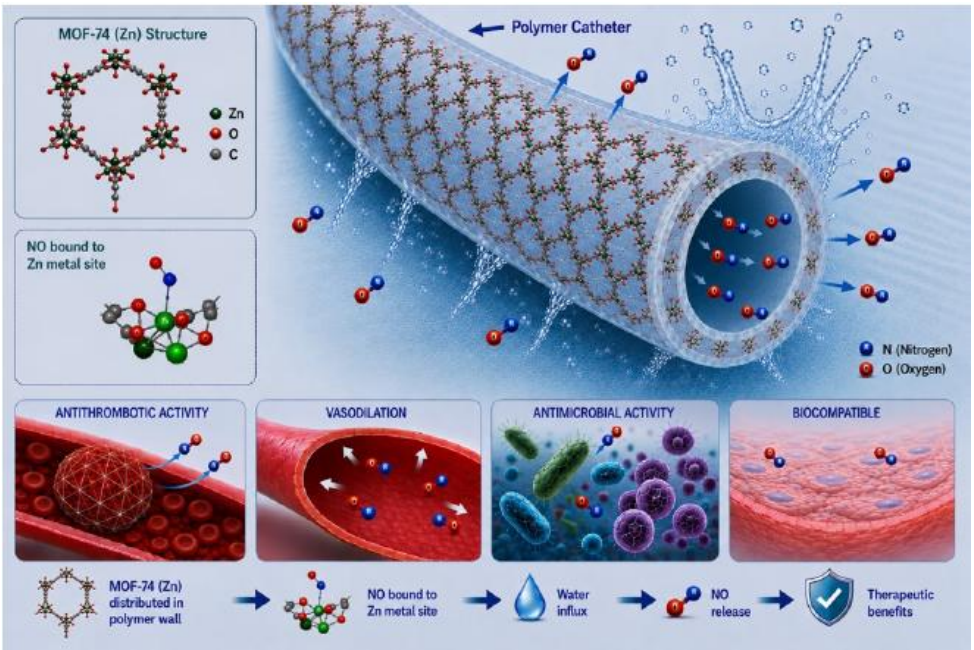


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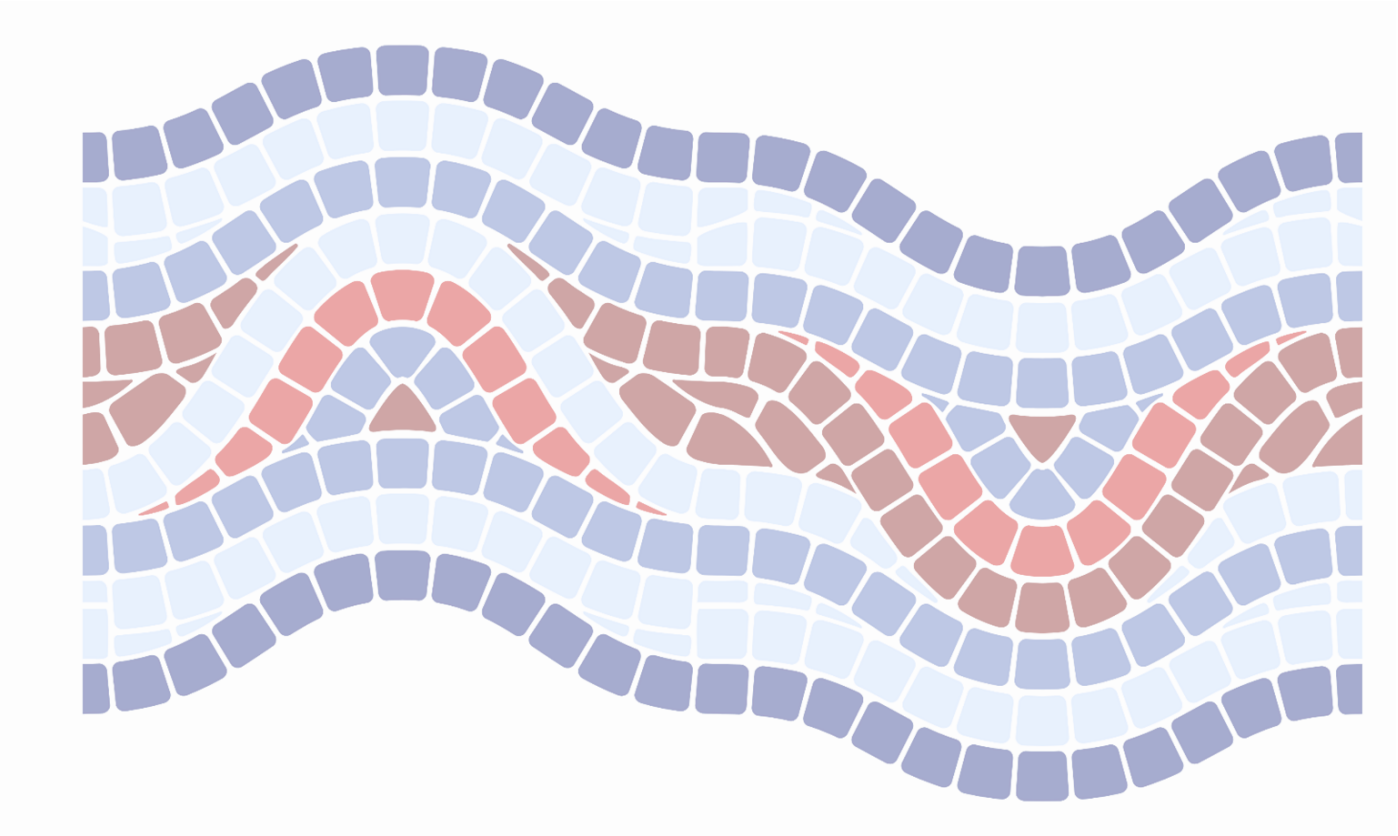
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Keywords: Metal-organic frameworks, polymer composites, cardiovascular catheters, anti-bacterial, anti-thrombosis, vasodilation

The large internal surface areas and porosities of metal-organic frameworks (MOFs) and zeolites promise a wide variety of applications in gas capture, storage and delivery. To realise their potential, MOFs/zeolites need to be processed into suitable forms for the target function. In this presentation I will explain what MOFs and zeolites are and why they are so good for gas handling applications and how they can be adapted to the types of healthcare applications presented here. I will explain what microporous water is and how it can be used as a gas storage medium for medical applications. I will also demonstrate the preparation and functional assessment of extruded MOF-polymer composite catheter tubing comprising incorporated into a medical-grade polyamide/polyether block co-polymer for the delivery of nitric oxide (NO). I will then demonstrate using *in vitro* experiments that gas-loaded tubing is non-toxic and that the MOF-polymer composite delivers NO at amounts relevant for the prevention of three of the most common complications in different types of catheterisation: arterial vasospasm, catheter-related thrombosis and microbial infection. Finally, I will show how *in vivo* experiments in an animal model demonstrates the long-lasting effect of the catheters in reducing issues of thrombosis.



ABSTRACTS SELECTED TALKS



ST1 – Selected Talk 1 – 15:40h DAY 1

Anoikis resistance in human melanoma cells is mediated by Nitric Oxide, Reactive Oxygen Species, and Focal Adhesion Kinase



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Keywords: melanoma; nitric oxide; NO synthases; anoikis resistance; NADPH oxidase 4; hydrogen peroxide

Melanoma, a highly aggressive and invasive form of skin cancer, is characterized by early spread through the lymphatic system and metastasis. The success of metastasis is linked to the ability of melanoma and other cancer cells to resist anoikis, a type of cell death that occurs when cells lose their adhesion to the extracellular matrix. Redox signaling plays an essential role in anoikis resistance. The balance between intracellular levels of nitric oxide (NO) and the reactive oxygen species (ROS) O_2^- and H_2O_2 stimulates signaling pathways related to proliferation and survival, or cell death. A375 and SK-MEL-28 human melanoma cell lines, representing primary melanoma and lymph node metastatic melanoma cells, respectively, were cultured under suspension and adherent conditions to investigate the redox regulation of anoikis resistance. Both cell lines express the three isoforms of nitric oxide synthases (NOS) and NADPH oxidase 4 (NOX4) as endogenous sources of NO and ROS, respectively. When A375 cells in suspension were treated with the pan-NOS inhibitor L-NAME, their viability decreased. The treatment resulted in a decrease in phosphorylation of FAK at Tyr397 and of ERK 1/2. The expression of FAK, ERK 1/2, β-actin, and α-tubulin was significantly reduced. Treatment with L-NAME led to an increase in the expression of the metalloprotease MMP-9. On the other hand, SK-MEL-28 cells in suspension and treated with the NOX4 inhibitor, GKT36901, exhibited reduced viability. This was accompanied by the inhibition of FAK phosphorylation at Tyr397, ERK 1/2 phosphorylation, and a reduction in the expression of FAK, ERK 1/2, β-actin, and α-tubulin, with a slight elevation in the expression of MMP-9. Migration and invasion were strongly inhibited in A375 cells upon treatment with L-NAME, while treatment with GKT36901 had a marginal effect on the migration and invasion capacities of SK-MEL-28 cells. In summary, melanoma cells employ nitrosative and oxidative stress to protect themselves from anoikis. Nitric oxide was essential for melanoma cells at the primary site for resisting anoikis, while H_2O_2 contributed to anoikis resistance in metastatic melanoma cells.

Acknowledgements

FAPESP: Grant numbers: 2018/15038-7 and 2025/10560-0.

ST2 – Selected Talk 2 – 15:55h DAY 1

The NO-Nrf2-Hypoxia Axis: A redox regulatory network driving resistance to photodynamic therapy



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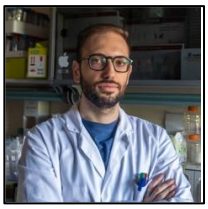
Photodynamic therapy (PDT) is an established anticancer treatment based on the activation of photosensitisers by light, leading to the generation of reactive oxygen species (ROS) and selective tumor cell destruction. Although PDT has demonstrated significant clinical efficacy, its therapeutic outcome is frequently compromised by adaptive cellular responses that enable tumor survival and resistance. Among the major regulators of these responses are nitric oxide (NO), Nrf2-mediated antioxidant signaling, and tumor hypoxia, which form a highly interconnected network controlling cellular redox homeostasis and treatment sensitivity.
NO exerts a dual and concentration-dependent role during PDT. At low concentrations, it promotes cytoprotection by preserving mitochondrial function, maintaining redox balance, and activating pro-survival signaling pathways, thereby enhancing tumor progression and resistance. Conversely, elevated NO levels amplify oxidative and nitrosative stress, potentiating PDT-induced cell death and improving therapeutic efficacy [1].
Another major adaptive mechanism is the activation of Nrf2, the master regulator of the cellular antioxidant response. Upon PDT-induced oxidative stress, Nrf2 induces the expression of antioxidant and detoxifying enzymes that limit ROS accumulation, reduce oxidative damage, and ultimately favor tumor cell survival, representing a major determinant of resistance to treatment [2].
Tumor hypoxia represents an additional critical barrier to PDT. Limited oxygen availability directly impairs ROS generation while simultaneously activating adaptive programs, including angiogenesis, metabolic rewiring, and enhanced resistance to oxidative stress. Importantly, increasing evidence indicates that hypoxia signaling, NO production, and Nrf2 activation are functionally interconnected, creating a dynamic regulatory network that allows cancer cells to adapt to PDT-induced stress [3].
Understanding the molecular interplay between NO signaling, Nrf2 activation, and tumor hypoxia is essential for overcoming PDT resistance. Targeting these interconnected pathways may offer innovative combination strategies capable of disrupting redox adaptation, enhancing ROS-mediated cytotoxicity, and ultimately improving the therapeutic efficacy of photodynamic therapy in cancer.

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Exp Mol Med. **2024**, 56, 501–514.

ST3 – Selected Talk 3 – 17:05h DAY 1

GSNOR Downregulation Enhances Rhabdomyosarcoma Proliferation Through CDK4-Mediated Retinoblastoma Hyperphosphorylation



Gianmarco Matrullo[1, #], Margherita Galanti[1], Paola Giglio[2], Giovanni Larotondo[1], Claudia Palmieri[1], Chiara Pecorari[2], Elena Maresca[1], Salvatore Rizza[2], Costanza Montagna[1,3] and Giuseppe Filomeni[1,2,#]

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Keywords: Nitric oxide, S-nitrosylation, Cell cycle, GSNOR, Cancer, CDK4.

Nitric oxide (NO) and S-nitrosylation (–SNO), are both critical regulators of normal physiology and disease[1,2].S-nitrosoglutathione reductase (GSNOR) mainly functions as a key denitrosylase that maintains protein Snitrosylation balance[3]. Dysregulation of the NO/–SNO/GSNOR pathway has been linked to cancer, but its role in rhabdomyosarcoma (RMS)—the most frequent pediatric soft-tissue sarcoma—has not been investigated yet. In this work, we revealed that GSNOR expression is significantly decreased in RMS samples, with a low GSNOR expression correlating with unfavorable outcomes. Through in-vitro studies, we demonstrate that loss of GSNOR promotes RMS cell growth by driving retinoblastoma (RB) protein hyperphosphorylation and accelerating G1/S transition. Mechanistically, we identify cyclin-dependent kinase 4 (CDK4), the first protein promoting RB inhibition and cell cycle progression[4], as a direct S-nitrosylation target, possibly at Cys135. To further explore CDK4 S-nitrosylation role, we generated RMS cells stably expressing the wild type or the non-nitrosylable form of CDK4 (C135A), highlighting how Cys135 mutation affects CDK4 activity. Therefore, GSNOR deficiency increases CDK4 S-nitrosylation, maintaining RB inactivation and enabling unchecked S-phase entry and DNA synthesis. Collectively, these findings identify a previously unrecognized role for the NO/–SNO/GSNOR axis, showing that GSNOR deficiency drives tumorigenic proliferation through CDK4 S-nitrosylation in RMS. Targeting this pathway could provide a therapeutic opportunity to slow RMS progression and re-establish differentiation potential in tumor cells, while increasing the overall knowledge underlying GSNOR deficiency role in cancer.

Acknowledgements

The authors thank all members of the laboratories for helpful discussions and technical assistance.

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ST4 – Selected Talk 4 – 17:20h DAY 1

Dinitrosyl Iron Complexes as Reactive Sources of Peroxynitrite



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Nitric oxide (NO) is a radical molecule involved in both physiological and pathological processes.[1,2] Under inflammatory conditions, high levels (μM) of NO is produced by inducible nitric oxidase synthase (iNOS) alongside the superoxide anion radicals ($O_2^{\bullet-}$) produced by enzymes such as NADPH oxidase (NOX) in defence cells, yielding peroxynitrite ($ONOO^-$/ONOOH), a strong oxidant.[1] Peroxynitrite can generate even stronger oxidizing species that causes biomolecules damage, such as protein tyrosine nitration. Although effective for pathogen elimination, this oxidative damage can also cause host tissue injury.[3,4] Beyond its reaction with $O_2^{\bullet-}$, NO is also consumed by reacting with Fe^{2+} (primarily from chelatable iron pool) and thiols to yield dinitrosyl iron complexes (DNICs), which represent the major intracellular reservoirs of NO-derived adducts.[5] In this study, we investigated whether these DNICs can further react with $O_2^{\bullet-}$ to produce peroxynitrite. To address this, DNICs bound to glutathione (DNIC-GS) or bovine serum albumin (DNIC-BSA) were synthetized, exposed to $O_2^{\bullet-}$ and monitored by UV-vis and EPR spectroscopic techniques. KO_2 in DMSO and the xanthine/xanthine oxidase system were used as $O_2^{\bullet-}$ sources. Both DNIC-GS and DNIC-BSA underwent decay upon exposure to $O_2^{\bullet-}$ generated by either system. Mass spectrometry analysis employing the boronate-based probe o-mitoPBA confirmed peroxynitrite formation by detecting its specific oxidation products. Under conditions where DNICs were exposed to an excess of $O_2^{\bullet-}$, oxidation of the boronate probe FIBA showed that about 84% of NO fragment in DNIC-GS were converted into peroxynitrite, whereas a slightly higher conversion (90%) was observed for DNIC-BSA. The time-dependent peroxynitrite formation in the reaction between DNIC-GS and $O_2^{\bullet-}$ was subsequently followed by FIBA oxidation. Kinetics data were initially fitted to a direct bimolecular mechanism using Dynafit software, giving rise an apparent rate constant of 10^3 $M^{-1}s^{-1}$. However, when superoxide dismutase (SOD) was added to compete with DNIC-GS for $O_2^{\bullet-}$, peroxynitrite formation was not markedly suppressed, requiring a much higher competitive rate constant. To rationalize these findings, we proposed a two-step mechanism involving the reversible formation of a [DNIC··$O_2^{\bullet-}$] intermediate followed by its conversion to peroxynitrite. Kinetic simulations performed in DynaFit showed agreement with the experimental data, supporting the proposed pathway. Overall, these results demonstrate that DNICs react with $O_2^{\bullet-}$ to generate peroxynitrite, highlighting the relevance of further investigating the participation of DNICs in inflammatory processes.

Acknowledgements

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ST5 – Selected Talk 5 – 10:20h DAY 2

The NO-Macrophage Axis in Triple Negative Breast Cancer: Immunometabolic Reprogramming, EMT, and Pre-Metastatic Niche Formation



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[a]CÚRAM, Research Ireland Centre for Medical Devices, University of Galway, Galway, Ireland; [b]Department of Biophysics, School of Basic Medical Sciences, Health Science Centre, Xi'an Jiaotong University, Xi'an, P. R. China; [c]Discipline of Pathology, Lambe Institute for Translational Research, University of Galway, Galway, Ireland

Keywords: nitric oxide; triple-negative breast cancer; macrophages; tumour microenvironment; immunometabolism; metastasis

Triple-negative breast cancer (TNBC) progression is shaped by redox crosstalk between cancer cells and tumour-associated macrophages (TAMs). Although inducible nitric oxide synthase (iNOS) is associated with aggressive estrogen receptor-negative breast cancer, how tumour-derived nitric oxide (NO) regulates macrophage metabolism and reciprocal tumour behaviour remains unclear.[1,2] We established a human macrophage–TNBC model using primary monocyte-derived M0-, M1- and M2-like macrophages, parental MDA-MB-231 cells, and an iNOS-overexpressing variant **(Fig. 1A)**. Macrophage polarisation was validated by iNOS/arginase-1 staining, live ROS/NO imaging, Seahorse profiling and cytokine arrays. Tumour-derived NO suppressed macrophage oxidative phosphorylation and induced a hyper-inflammatory, TAM-like secretome **(Fig. 1B)**, consistent with NO-dependent macrophage immunometabolism.[3] Reciprocally, macrophage cues acted according to tumour iNOS status. In parental cells, macrophage-conditioned media suppressed mitochondrial respiration, while M0/M1 signals promoted migration and M2 signals restrained invasion **(Fig. 1C)**. In iNOS-expressing cells, M1 and especially M2 secretomes inverted this response, enhancing oxidative phosphorylation, glycolysis, spare respiratory capacity and EMT/stemness markers, while partially rescuing NO-mediated cytostasis **(Fig. 1D)**. In vivo, MDA-MB-231 xenografts showed Arg-1-positive/iNOS-low macrophage-associated populations, while lungs from tumour-bearing mice displayed M2-skewed, EMT-associated niche remodelling. Together, these findings define a reciprocal NO–macrophage feedback loop in which tumour iNOS acts as a redox-metabolic switch that reprogrammes macrophages and licenses pro-metastatic TNBC phenotypes **(Fig. 1E)**. This model supports therapeutic testing of NO–macrophage-axis disruptors, including NO-scavenging materials.[4]

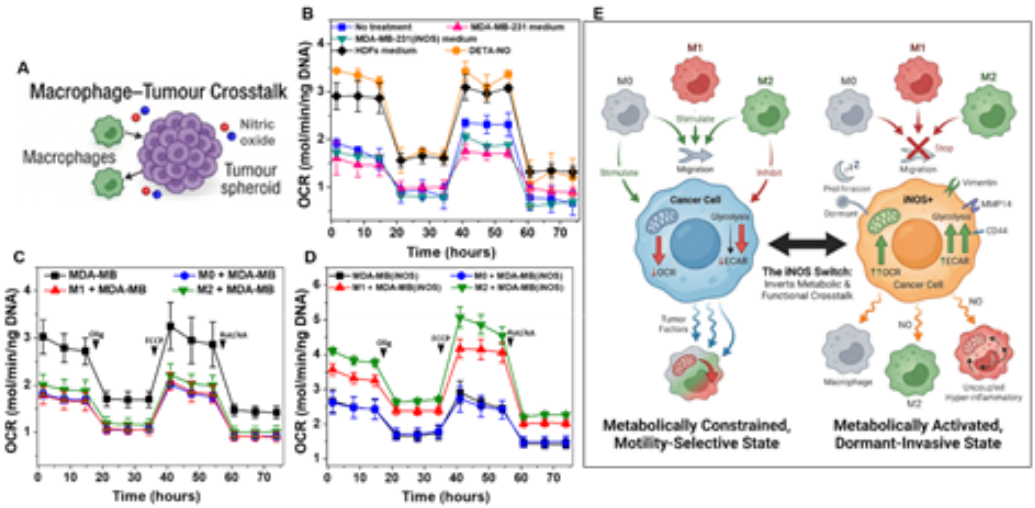


Figure 1: The iNOS switch in macrophage–TNBC metabolic crosstalk. (A) Experimental model of NO-mediated macrophage–TNBC spheroid communication. **(B–D)** Seahorse profiling shows that macrophage-conditioned signals suppress bioenergetic activity in parental MDA-MB-231 cells but enhance OCR/ECAR-associated activity in MDA-MB-231(iNOS) cells. **(E)** Proposed model in which

tumour iNOS acts as a redox-metabolic switch, shifting macrophage–TNBC crosstalk from a metabolically constrained, motility-selective state to a metabolically activated, EMT/stemness-associated, invasion-primed state.

Acknowledgements: This research was supported by Research Ireland co-funded under the European Regional Development Fund (13/RC/2073_P2). A.M.A. acknowledges Taighde Éireann - Research Ireland (GOIPD/2023/1640).

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ST6 – Selected Talk 6 – 11:05h DAY 2

Nitric Oxide regulation of Human Endogenous Retrovirus K in Triple Negative Breast Cancer: Implications for Metastatic Progression and Patient Outcomes



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Keywords: Nitric oxide, Human Endogenous Retrovirus K, Triple negative breast cancer

Triple-negative breast cancer (TNBC) is characterized by a high propensity for metastasis, poor clinical outcomes, and limited targeted therapeutic options. Emerging evidence suggests that both inducible nitric oxide synthase (iNOS) and the human endogenous retrovirus K (HERV-K) contribute to breast cancer progression; however, their functional relationship in TNBC remains poorly understood. This study aimed to investigate the clinical significance of iNOS and HERV-K expression and to elucidate the molecular pathways through which these factors contribute to disease progression.

Elevated expression of both iNOS and HERV-K was associated with increased risk of distant metastasis and poor patient outcome in women diagnosed with TNBC at Galway University Hospital, suggesting their potential utility as prognostic biomarkers. To explore the mechanistic basis of these observations, HERV-K knockdown models were generated in TNBC cell lines using an shRNA approach. RNA seq transcriptomic profiling was performed. Differential gene expression and pathway analyses identified key signalling networks regulated by HERV-K and nitric oxide-related pathways, including those associated with tumour invasion, epithelial-mesenchymal plasticity, inflammatory signalling, and treatment resistance.

Functional studies revealed that suppression of HERV-K significantly altered cellular proliferation and migratory capacity, supporting a role for HERV-K in maintaining the aggressive phenotype of TNBC cells. Integration of transcriptomic and phenotypic analyses identified novel molecular interactions between nitric oxide signalling and HERV-K activity that may promote metastatic dissemination and adverse clinical outcomes.

Collectively, these findings demonstrate a clinically relevant association between iNOS, HERV-K, and TNBC progression, while identifying molecular pathways that may be exploited for therapeutic intervention. Targeting the iNOS-HERV-K axis may represent a novel strategy to improve outcomes in patients with metastatic and treatment-resistant TNBC.

Acknowledgements

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ST7 – Selected Talk 7 – 11:20h DAY 2

Liquid biopsy in prostatic disease assessment: potential role for iNOS and HERV-K



Ciara E. O'Neill[a], J. McAuliffe[a], M. Almezen[a], M.E. Siegle[a], X. Yang[a], GUH Urology Registrars[b], A. Altieri[c], S. Barry[a], N. Walters[c], M.S.Stack[c], G. Callagy[a], G. Durkan[b], S.A. Glynn[a,d,e]

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Keywords: Prostate Cancer, Liquid Biopsy, Biomarker, Diagnostic, HERV-K, iNOS

Prostate cancer is the most prevalent cancer in men in the western world. Prostate specific antigen (PSA) is the most widely used biomarker for the detection of prostate cancer. While 40-60% of men with elevated PSA are found to have prostate cancer upon biopsy, PSA elevation in the remaining proportion of men is usually due to a combination of factors including aging, benign prostatic hyperplasia (BPH), inflammation of the prostate, & preneoplastic lesions. We previously found that human endogenous retrovirus K (HERV-K) gag mRNA was elevated in the blood of men diagnosed with prostate cancer compared to healthy controls[1]. This suggests that HERV-K may be a biomarker for the detection of prostate cancer, independent of PSA. We have also found that HERV-K is associated with high levels of inducible nitric oxide synthase (iNOS) in breast cancer. We hypothesised that HERV-K & iNOS expression would be elevated in men with prostate cancer, compared to benign prostatic conditions.

This prospective study followed 100 men who were referred due to elevated PSA to the Rapid Access Prostate Cancer clinic at Galway University Hospital between 2019-2022. Blood samples were collected from each patient prior to biopsy for buffy coat RNA isolation & plasma collection. In a blinded fashion, HERV-K Gag, HERV-K Env type I & II, iNOS, COX2, IDO1, EPCAM, AR, & HER2 mRNA levels were measured in the PBMC fraction by qRT-PCR, while plasma levels of IFNα2, IFNβ, IFNγ, IL-1β, & TNFα were measured by ELISA. Separately, the clinical data related to the patient biopsy results were extracted from the hospital clinical database to assess biomarker association with patient diagnosis. iNOS expression alone had no predictive capacity of prostate cancer diagnosis, but when iNOS was high in a patient, they were more likely to also have high expression of many of the other mRNA markers assessed. This also had a significant impact on the predictive capacity of these biomarkers. High EpCAM alone indicates a significant increase (OR 2.4, p=0.038) in prostate cancer risk across the whole cohort. When combined with high iNOS expression, this further increases (OR 4.5, p=0.025) prostate cancer risk. The combination of high EpCAM & high iNOS has stronger predictive capacity in differentiating between prostate cancer & BPH (OR 6, p=0.021). Similarly, when patients had high iNOS alongside high HERV-K Env type II (OR 4.2, p=0.069) the risk of prostate cancer diagnosis increased. While the risk of prostate cancer compared to BPH was increased in patients with high iNOS alongside high HERV-K Env type I (OR 3.3, p=0.112), HERV-K Env type II (OR 7.5, p=0.019), & HERV-K Gag (OR 3.7, p=0.112).

This further indicates the relationship between HERV-K & iNOS in cancer, both of which are associated with an inflammatory environment & highlights the potential of NO & HERV-K related gene signatures as diagnostic biomarkers for prostate cancer in populations with raised PSA, alongside their known ability to drive tumour progression.

Acknowledgements

This research was funded by a University of Galway Hardiman PhD Scholarship, Research Ireland [17/CDA/4638], Research Ireland and European Regional Development Fund (ERDF) [13/RC/2073], & Horizon Europe MSCA Doctoral Networks programme (Grant Agreement No. 101119873).

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ST8 – Selected Talk 8 – 12:15h DAY 2

ACE2 inhibition elicits distinct pulmonary NO-signaling in healthy and cardiometabolic disease models



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Keywords: ACE2, nitric oxide, hypertension, diabetes, obesity, long COVID

Introduction: Angiotensin-converting enzyme 2 (ACE2) is a key regulator of pulmonary redox homeostasis and nitric oxide (NO) signaling. Although ACE2 deficiency has been implicated in endothelial dysfunction and inflammatory lung injury, it remains unclear whether pulmonary responses to ACE2 inhibition differ between healthy and cardiometabolic disease conditions. We therefore investigated the effects of ACE2 inhibition on pulmonary NO signaling and inflammatory status in normotensive Wistar Kyoto (WKY), spontaneously hypertensive rats (SHR), and obese Zucker diabetic fatty (ZDF) rats, and evaluated whether treatment with S-nitrosoglutathione (GSNO) alone or in combination with captopril could restore pulmonary redox balance.
Methods and Results: ACE2 was inhibited by MLN-4760 administered by miniosmotic pumps at the dose of 2 mg/kg/day for three weeks. During the first week, animals received MLN-4760 alone, followed by two weeks of combined treatment with either GSNO (50 µg/kg/day i.p.), or GSNO plus captopril (50 mg/kg/day in the tap water). Pulmonary total NOS activity, eNOS and iNOS protein expression, NF-κB expression, thiol groups, and S-nitrosothiols were evaluated. ACE2 inhibition induced strikingly different responses depending on the underlying phenotype. In SHR and ZDF rats, MLN-4760 significantly reduced NOS activity and eNOS expression, while increasing iNOS and NF-κB expression, accompanied by depletion of thiol groups and S-nitrosothiols, indicating impaired NO/redox homeostasis and enhanced inflammatory signaling. In contrast, WKY rats responded to ACE2 inhibition with increased NOS activity and eNOS expression without activation of NF-κB or iNOS, suggesting the presence of effective adaptive compensatory mechanisms. GSNO treatment alone only partially improved pulmonary alterations and its efficacy differed between disease models, being less effective in obese ZDF rats. In contrast, combined GSNO and captopril treatment largely normalized NOS signaling, attenuated inflammatory activation, and restored thiol and S-nitrosothiol levels in both SHR and ZDF animals. In WKY rats, combined treatment further increased thiol and S-nitrosothiol availability without inducing inflammatory changes.
Conclusion: Pulmonary responses to ACE2 inhibition are strongly dependent on the underlying cardiometabolic phenotype. Healthy lungs retain the capacity to compensate for ACE2 loss by enhancing endogenous NO signaling, whereas hypertension and diabetes are associated with impaired adaptive responses, inflammatory activation, and disruption of pulmonary redox homeostasis. Restoration of NO bioavailability by GSNO was incomplete under pathological conditions, while its combination with captopril provided substantially greater protection, suggesting that simultaneous modulation of nitrosative signaling and the renin–angiotensin system may represent a promising therapeutic strategy for pulmonary complications associated with ACE2 dysfunction. These findings may contribute to understanding pulmonary mechanisms associated with ACE2 dysregulation in disorders such as long COVID.

Acknowledgements

Supported by research grants VEGA 2/0025/23, 2/0131/24, and 2/0122/24.

ST9 – Selected Talk 9 – 14:50 DAY 2

The role of KEAP1 in nitric oxide biology – focus on ageing

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Keywords: KEAP1, NRF2, ageing, NO, endothelium, oxidative stress

Kelch-like ECH-associated protein 1 (KEAP1) has traditionally been recognized as the cytoplasmic repressor of the transcription factor NRF2, acting as a redox sensor. However, our data has substantially expanded this paradigm by demonstrating that KEAP1 also functions as a central regulator of NO signaling through mechanisms that extend beyond NRF2. KEAP1 directly controls protein S-nitrosation by forming a complex with nitric oxide synthase (NOS) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), thereby facilitating targeted transnitrosation of specific protein substrates. In endothelial cells (ECs), disruption of NRF2 signaling leads to accumulation of unrestrained KEAP1, resulting in widespread protein S-nitrosation that protects cells from oxidative damage while promoting a switch from apoptosis to premature senescence. Increased S-nitrosation is also seen in ageing ECs. Notably, S-nitrosation of NOX4 suppresses excessive reactive oxygen species production, illustrating the cytoprotective consequences of KEAP1-dependent NO signaling. Beyond regulating redox-sensitive transcription, KEAP1 therefore emerges as a scaffold coordinating spatially restricted NO bioactivity and determining cell fate under stress. KEAP1-dependent S-nitrosation is linked to endothelial proteostasis and age-associated protein aggregation, highlighting its broader role in vascular aging. Together with earlier evidence identifying KEAP1 itself as a sensor of nitric oxide through S-nitrosation of Cys151, these findings establish KEAP1 as both a target and an effector of NO signaling. We propose that this revised model positions KEAP1 at the interface of oxidative and nitrosative stress, integrating environmental cues to regulate protein homeostasis, cellular senescence, and vascular function, and identifies KEAP1 as a promising therapeutic target in cardiovascular disease and aging.

Acknowledgements

This study was supported by National Science Centre grants no. 2016/22/E/NZ3/00405 and 2021/43/B/NZ4/02130 to AGP.

ST10 – Selected Talk 10 – 15:05h DAY 2

NO-rules: nitrosylation-driven metabolic reprogramming redefines paradigms in cancer



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Keywords: S-Nitrosylation**;** AKR1A1/S-Nitroso-CoA Reductase (SCoR); Methylglyoxal; Keap1-NRF2; Cancer metabolism**;** Cell migration

Nitric oxide (NO) is increasingly recognized as a key regulator of cancer biology through protein S-nitrosylation, a reversible post-translational modification that dynamically controls protein activity, metabolism and cell fate. Although several metabolic enzymes are known to be regulated by S-nitrosylation, the mechanisms by which NO-dependent metabolic remodeling contributes to tumor progression remain partly understood.

We identify the denitrosylase AKR1A1/S-nitroso-CoA reductase (SCoR) as a critical node linking NO signaling to cancer metabolism control. Loss of AKR1A1 promotes S-nitrosylation of pyruvate kinase M2 (PKM2), impairing terminal glycolytic flux and redirecting glucose metabolism toward alternative pathways. Rather than being a simple metabolic consequence, this rewiring generates signaling metabolites that actively remodel cancer cell behavior. Our work challenges two long-standing paradigms: that methylglyoxal (MGO) is merely a toxic glycolytic by-product, and that Keap1 functions primarily as a dedicated repressor of NRF2.

In particular, we demonstrated that accumulation of MGO emerges as a signaling intermediate that covalently modifies Keap1, promoting its degradation and activation of the NRF2 transcriptional program. This NO-driven metabolic circuit enhances adaptive stress responses and confers resistance to clinically relevant anticancer therapies, establishing a direct mechanistic link between NO signaling, metabolic rewiring and adaptive stress programs in cancer. Moreover, the metabolic dependencies created by AKR1A1 loss are therapeutically targetable: pharmacological inhibition of the glyoxalase pathway —the main route of MGO detoxification— with S-p-bromobenzylglutathione cyclopentyl diester (BBGC) exploits the reliance of AKR1A1-deficient tumors on methylglyoxal detoxification, inducing synthetic lethality and restoring sensitivity to standard-of-care therapies.

Importantly, we further demonstrate that the consequences of Keap1 inactivation extend beyond NRF2-transcriptional adaptation. Whereas NRF2 activation accounts for therapy resistance, only restoration of Keap1—but not genetic silencing of NRF2—rescues the enhanced migratory phenotype induced by AKR1A1 deficiency, restoring focal adhesion organization and actin stress fiber assembly. These findings uncover a previously unrecognized NRF2-independent role for Keap1 in regulating cytoskeletal architecture and cell migration. By integrating AI-based target prediction with quantitative proteomics, we further identify possibly Keap1-targets candidate involved in focal adhesion dynamics and cytoskeletal remodeling that correlate with the migratory phenotype.

Collectively, these studies support a conceptual shift in our understanding of NO biology. Rather than acting solely through modification of individual protein targets, S-nitrosylation appears to function as a master regulator of metabolic plasticity, generating metabolite-dependent signaling networks that determine therapeutic response, cell migration and, ultimately, cancer progression. By expanding the biological roles of both MGO and Keap1 and identifying impaired denitrosylation as a therapeutically targetable state, these findings uncover a new class of metabolic vulnerabilities that can be exploited for precision cancer therapy.

ST11 – Selected Talk 11 – 15:20h DAY 2

Chemodynamic/nitric oxide therapy in chronic wound healing and antibiotic-resistant infection management

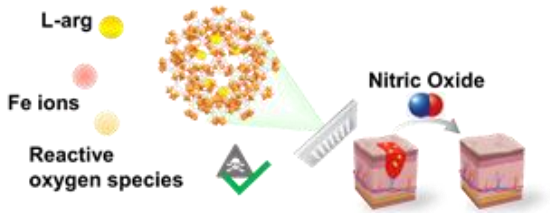


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Keywords: Metal-organic frameworks; nitric oxide; chemodynamic therapy; microneedles; infected wounds; antibacterial therapy

The increasing prevalence of antibiotic-resistant bacteria calls for alternative therapeutic strategies for the treatment of infected wounds and the promotion of wound healing.[1] Metal-organic frameworks (MOFs) have emerged as promising multifunctional platforms for biomedical applications owing to their high porosity, tunable composition, and ability to host and release therapeutic agents.[2,3] Here, we develop a MOF-based microneedle platform combining nitric oxide (NO) delivery with chemodynamic therapy (CDT) for synergistic antibacterial treatment. The mesoporous iron-based MOF MIL-100(Fe), which exhibits peroxidase-like activity, was selected as both a catalytic and NO precursor delivery platform.[4] L-arginine (L-Arg), a biocompatible NO precursor, was physically encapsulated within MIL-100(Fe) particles. The resulting MIL-100(Fe)/L-Arg composite was further combined with lactate oxidase (LOx) and incorporated into a polymeric matrix composed of polyvinylpyrrolidone (PVP) and hyaluronic acid (HA) to fabricate dissolvable microneedle patches using 3D-printed molds (Figure 1). Upon application to an infected wound, the microneedles dissolve in the wound environment, enabling the localized release of the active components. LOx catalyzes the oxidation of excess lactate, a metabolite typically enriched in the wound microenvironment, generating hydrogen peroxide (H_2O_2). The produced H_2O_2 triggers two complementary pathways. First, it reacts with Fe species in MIL-100(Fe), promoting Fenton-like reactions and the generation of highly reactive hydroxyl radicals (•OH), thereby inducing chemodynamic antibacterial activity.[5] Second, H_2O_2 reacts with the released L-Arg, promoting NO generation. The simultaneous production of •OH and NO provides synergistic oxidative and nitrosative stress, resulting in enhanced bacterial killing. This multifunctional microneedle platform therefore integrates enzymatic generation of the therapeutic trigger, MOF-mediated chemodynamic activity, and localized NO delivery within a single dissolvable dressing. By exploiting the biochemical characteristics of the wound microenvironment, the system offers a promising strategy for localized treatment of drug-resistant bacterial infections while supporting wound repair. More broadly, this work highlights the potential of MOF-based responsive microneedles as versatile platforms for precise and localized wound therapy.



Scheme: Strategy: NO gas therapy to kill bacteria

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ST12 – Selected Talk 12 – 17:25h DAY 2

Advanced Hydrogel Platforms for Tuneable NO and/or H2S Delivery: A new frontier in Keratinocyte Modulation



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Keywords: Nitric Oxide Delivery, Gasotransmitters, Alginate Hydrogels, S-nitrosoglutathione, Hydrogen Sulfide, Keratinocytes.

Background: Nitric oxide (NO) is a short-lived gasotransmitter with concentration-dependent effects on skin physiology, tissue repair, inflammation, and keratinocyte behaviour. Low NO levels support physiological signalling, whereas sustained or higher exposure can suppress keratinocyte proliferation and promote cytostatic responses, relevant to hyperproliferative skin disorders. Hydrogen sulfide (H_2S) also regulates cytoprotection, redox balance, inflammation, and repair. However, therapeutic use of NO and H_2S is limited by high reactivity, short lifetime, rapid diffusion, and dependence on the local redox microenvironment. Thus, hydrogel-based biomaterials capable of controlling gasotransmitter availability are promising for topical and regenerative applications. Objective: To develop alginate-based hydrogel platforms for controlled NO delivery and coupled NO/H_2S release, combining GSNO delivery from alginate–chitosan hydrogels and dual gasotransmitter delivery from thiolated alginate hydrogels. Methods: Both systems were prepared by ionotropic gelation. In the first platform, S-nitrosoglutathione (GSNO) was generated in situ by mixing glutathione (GSH)- and nitrite ($NaNO_2$)-loaded alginate hydrogels, using either free GSH or GSH encapsulated into chitosan nanoparticles (CSNPs), generating H_GSNO and H_GSNO-CS. In the second platform, thiolated alginate was synthesized and formulated into single- and dual-donor hydrogels containing NO- and/or H_2S-releasing components, generating NO-Hyd, H_2S-Hyd, and Dual-Hyd. The systems were characterized by DLS, FTIR, SEM, TGA, rheology, UV–Vis spectroscopy, Franz diffusion assays, and electrochemical sensing. Biological performance was assessed in HaCaT keratinocytes using AlamarBlue/resazurin viability assays, scratch/cell expansion assays, Transwell-based exposure, and cell-cycle analysis. Results: CSNPs showed nanometric size around 82 nm, low polydispersity, and positive zeta potential near +17.8 mV, supporting their use as GSNO nanocarriers. Alginate–chitosan hydrogels displayed interconnected porous morphology and pseudoplastic, predominantly elastic behaviour. UV–Vis monitoring of the S–NO band at 336 nm confirmed sustained GSNO decomposition and continuous NO release for at least 72 h. GSNO diffusion followed Higuchi-type kinetics, with H_GSNO-CS showing superior cumulative permeation. Electrochemical quantification confirmed NO release from H_GSNO-CS and H_GSNO, while HaCaT viability remained preserved. GSNO-loaded hydrogels reduced keratinocyte migration, and H_GSNO-CS induced strong G0/G1 cell-cycle arrest, reaching 79.87% at 1 mM of GSNO, indicating a cytostatic effect associated with sustained NO delivery and chitosan nanocarrier activity. For the thiolated alginate platform, chemical characterization supported successful thiol functionalization, while rheological, thermal, and morphological analyses confirmed cohesive, self-supporting, porous hydrogels suitable for localized application. NO-Hyd and Dual-Hyd generated detectable NO signals, whereas H_2S-Hyd and Dual-Hyd produced measurable H_2S-equivalent release. Compared with NO-Hyd, Dual-Hyd increased the NO-equivalent fraction released, while reducing freely detectable H_2S recovery relative to H_2S-Hyd. Real-time electrochemical profiles showed temporally separated release (40 min), with an early H_2S burst followed by decay and a slower, sustained NO signal. HaCaT assays showed cytocompatibility after 24 h. NO-Hyd strongly reduced cellular expansion without acute cytotoxicity, whereas Dual-Hyd partially restored expansion, suggesting that H_2S-derived chemistry modulates NO bioactivity rather than simply adding toxicity. Conclusion: Alginate-based hydrogels can be engineered as sustained NO-releasing systems or chemically coupled NO/H_2S delivery matrices. The alginate–chitosan/GSNO system provides localized, cytocompatible NO delivery with cytostatic activity in keratinocytes, supporting application in hyperproliferative skin disorders. The thiolated alginate Dual-Hyd platform shows that NO and H_2S release is governed by matrix architecture, thiol chemistry, diffusion, and sulfur–nitrogen cross-talk. Overall, these hydrogels are tunable biomaterial platforms for topical redox modulation, keratinocyte regulation, and future regenerative or inflammatory skin applications.

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ST13 – Selected Talk 13 – 17:40h DAY 2

Distinct nitric oxide and carbon monoxide signaling is associated with cardioprotection after myocardial infarction

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Keywords: myocardial infarction, hypertension, nitric oxide, Nicorandil, CORM-3

Endothelial dysfunction, driven by impaired nitric oxide (NO) bioavailability, is a critical contributor to increased myocardial susceptibility to ischemic injury. Whether nitric oxide and carbon monoxide cardioprotection converge on common anti-inflammatory and antioxidant mechanisms under conditions of chronic NO deficiency remains unclear. Therefore, we compared the effects of the NO donor nicorandil and the CO-releasing molecule CORM-3 on cardioprotective signaling in L-NAME-induced hypertensive rats subjected to experimental myocardial infarction.

Chronic hypertension was induced in male Wistar Kyoto rats by administration of L-NAME (20 mg/kg/day) for four weeks. Myocardial infarction was induced by ligation of the left descending coronary artery followed by reperfusion. Immediately prior to reperfusion, animals received a single intramyocardial injection of Nicorandil or CORM-3. Seven days later, systolic blood pressure, plasma inflammatory cytokines (TNF-α and IL-6), myocardial total nitric oxide synthase (NOS) activity, protein expression of eNOS, iNOS, NF-κB, Nrf2, HO-1, superoxide dismutase, and glutathione peroxidase were evaluated.

Treatment with either nicorandil or CORM-3 effectively limited the inflammatory response to myocardial infarction, as demonstrated by lower circulating TNF-α and IL-6 concentrations, reduced NF-κB activation, diminished iNOS expression, and recovery of antioxidant defense. Despite these shared biological effects, the mechanisms underlying cardioprotection differed. Nicorandil primarily restored NO signaling by increasing total NOS activity and eNOS expression, whereas CORM-3 preferentially activated the Nrf2/HO-1 cytoprotective pathway without compromising its anti-inflammatory efficacy. In addition, both interventions produced a modest reduction in systolic blood pressure despite sustained inhibition of endogenous NOS.

Our findings demonstrate that nicorandil and CORM-3 elicit cardioprotection through distinct primary signaling pathways but ultimately promote a common anti-inflammatory and antioxidant response. These results indicate that effective myocardial protection in chronic NO-deficient hypertension can be achieved not only by restoring NO signaling but also by stimulating endogenous cytoprotective mechanisms, highlighting the Nrf2/HO-1 axis as a potential therapeutic target for ischemic heart disease associated with endothelial dysfunction.

Acknowledgements

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ST14 – Selected Talk 14 – 10:20h DAY 3

Long-term controlled release of Nitric Oxide from stabilized liquid formulations of S-Nitrosothiols

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Keywords: S-Nitrosothiol, Cyclodextrin, Nanocrystal, Controlled release

The physiological and pathological functions of nitric oxide (NO) are highly dependent on both its local concentration and exposure duration. Precisely controlling NO release profiles remains a major challenge for therapeutic and prophylactic applications. We have developed several formulation strategies that markedly prolong the stability and release duration of S-nitrosothiol type NO donors, including S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP). We investigated host–guest complexation between RSNOs and cyclodextrin derivatives. Using α-cyclodextrin derivatives, NO release from aqueous GSNO formulations was obtained from a few days to approximately two weeks.[1-3] Acetylated β-cyclodextrin with an average substitution degree of approximately seven stabilized SNAP sufficiently to sustain NO release from aqueous solutions for over one month at physiological temperature.[4] We further developed GSNO nanosuspensions in alkane diols, including propylene glycol and low-molecular-weight liquid polyethylene glycol.[5] In the absence of an aqueous medium, dispersed rather than dissolved GSNO exhibited exceptional stability for more than two months at physiological temperature. Compared with previously reported formulations based on dissolved NO donors or NO donor-loaded nanoparticles, these formulations provide exceptionally high drug payloads, minimal initial burst release, sustained and relatively constant NO release, and favorable storage stability. Collectively, they enable NO delivery over timescales ranging from days to months, matching the requirements of diverse biomedical applications. For example, shorter-duration formulations have been applied as antimicrobial lock solutions for central venous catheters, whereas month-long formulations are well suited as antimicrobial inflation solutions for urinary catheters. These formulations may also be applicable to a broad range of localized NO delivery applications, including topical, pulmonary, ocular, intravesical, and intraperitoneal therapies, where prolonged and controlled NO release is desirable. One limitation is that the stabilization effect is expected to diminish upon extensive dilution.

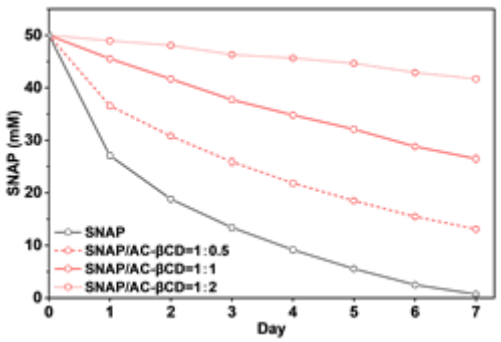


Figure 1: Decomposition kinetics of 50 mM SNAP at 37°C in the presence and absence of acetyl-β-cyclodextrin (AC-βCD) as the stabilizing agent.

Acknowledgements

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ST15 – Selected Talk 15 – 11:30h DAY 3

Stimuli-responsive graphene nanoplatforms for targeted nitric oxide delivery in cardiovascular therapeutics



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Keywords: Nitric oxide delivery; graphene; Stimuli-responsive; cardiovascular therapeutics; vascular regeneration

Nitric oxide (NO) is a key endogenous signalling molecule released by vascular endothelial cells that is essential for maintaining vascular homeostasis. Dysregulation of NO is a common feature in cardiovascular diseases, including hypertension, angina, heart failure and vascular injury. For this reason, the exogenous administration of systemic NO donors, such as organic nitrates and nitrites, has remained a cornerstone in the treatment of cardiovascular diseases.1 However, these donors primarily generate NO in the circulation and lack spatial and temporal control, resulting in off-target effects and limited therapeutic precision. Ideally, NO delivery should be localised, controlled and responsive to the underlying disease microenvironment. To address these limitations, we have developed a suite of graphene-based nanomaterial platforms for controlled and targeted NO delivery (Figure 1). Porous graphene oxide nanosheets functionalised with cysteamine generate S-nitrosated carriers capable of sustained NO release at physiologically relevant rates, mimicking endothelial function and selectively promoting endothelial proliferation while inhibiting smooth muscle cell growth via activation of canonical NO–cGMP signalling.2 Building on this, pH-responsive dipeptide-graphene hybrid nanosheets facilitate controlled NO storage and release, suppressing burst release under physiological conditions while improving delivery in acidic microenvironments. Extending this concept towards translational applications, we developed an amine-functionalised graphene coating for bioresorbable vascular stents that catalytically generates NO from endogenous S-nitrosothiols at physiologically relevant fluxes, providing a durable strategy to locally mimic endothelial NO production.3 Collectively, these findings highlight the versatility of graphene-based nanomaterials as platforms for precise, localised, and sustained NO delivery. These systems offer a promising strategy for combining NO delivery into next generation cardiovascular therapeutics.

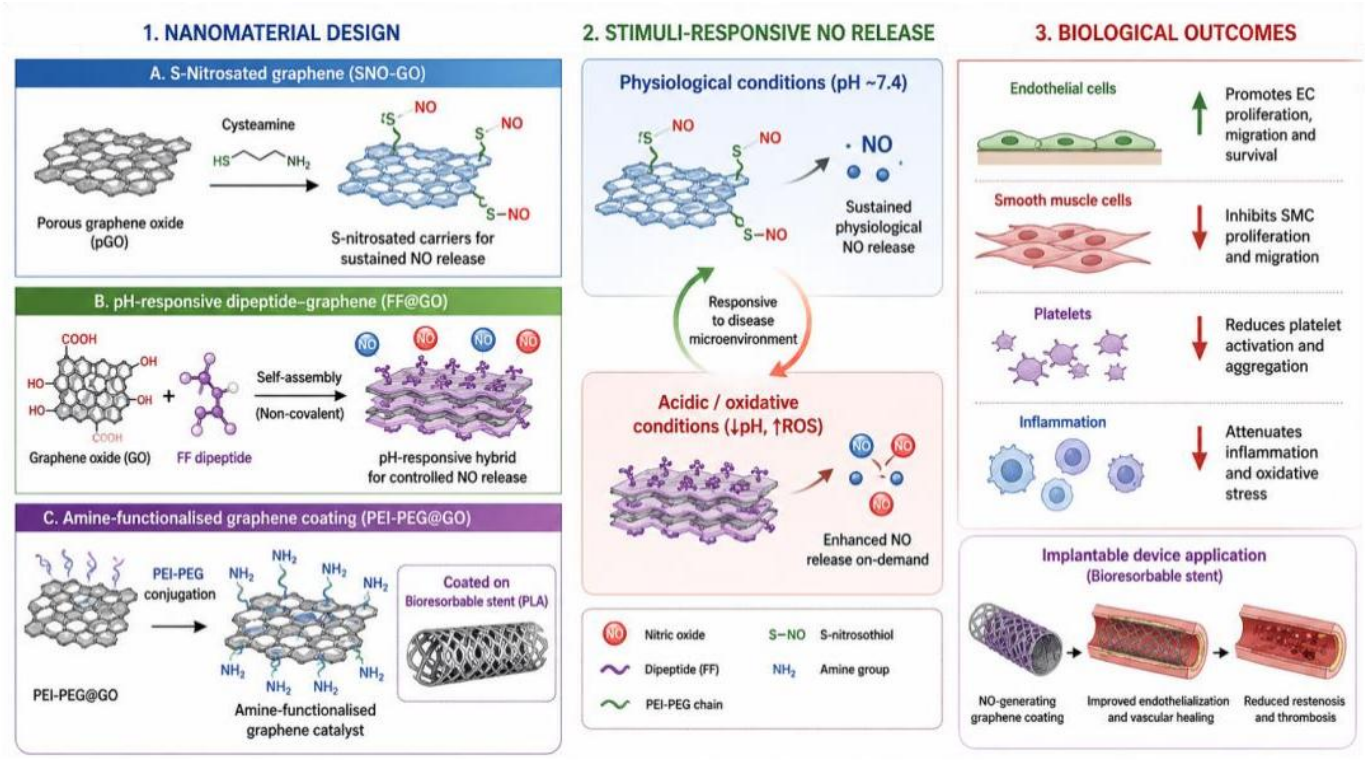


Figure 1. Schematic illustration of graphene-based NO delivery for cardiovascular applications, showing nanomaterial design, stimuli-responsive NO release and downstream effects on vascular function.

Acknowledgements

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ST16 – Selected Talk 16 – 11:45h DAY 3

Nanoencapsulation strategies for redox modulation and nitric oxide delivery: selective cytotoxicity in melanoma models



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Keywords: Nitric Oxide Delivery, Melanoma, Redox Modulation, Reactive Oxygen Species.

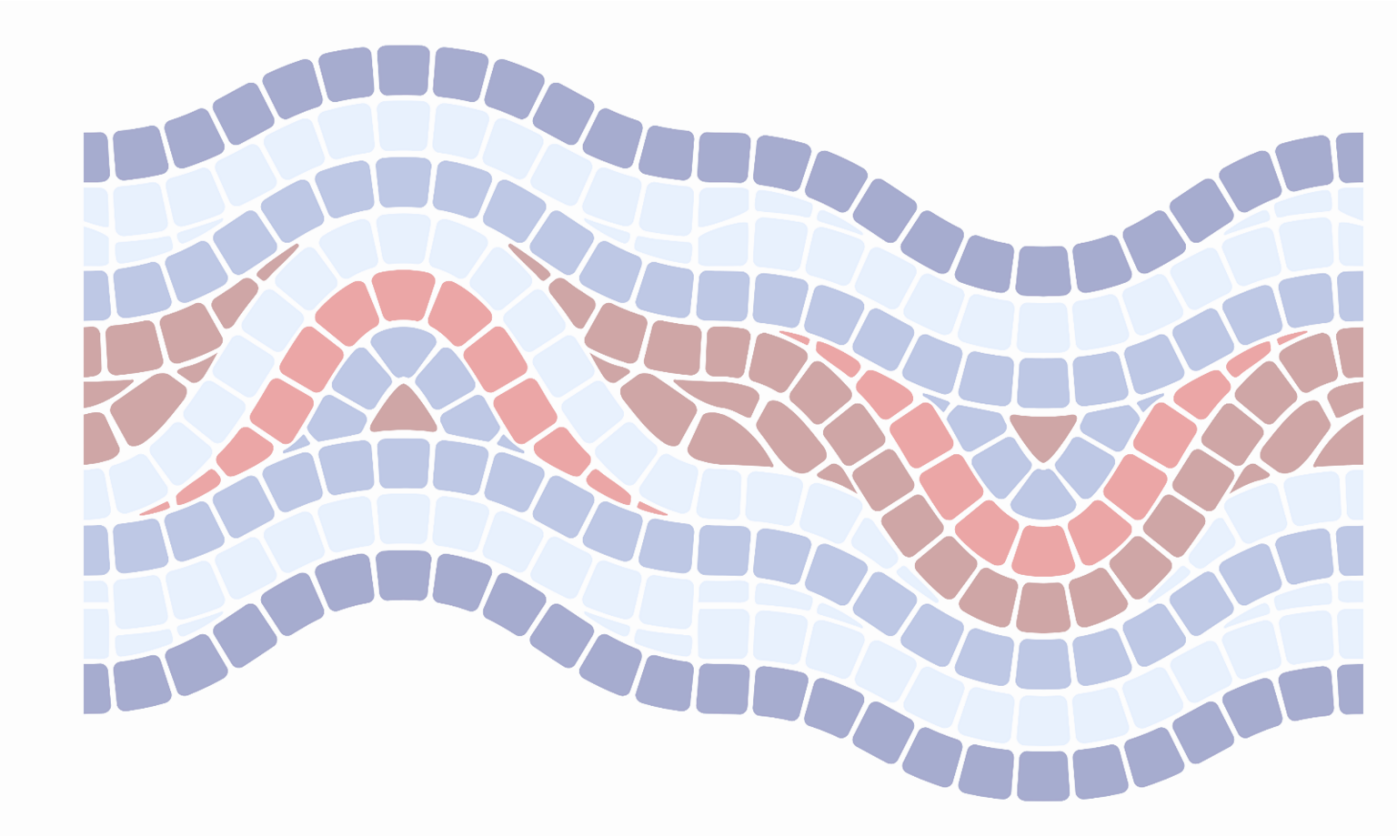
Background The tumor microenvironment is characterized by oxidative stress and redox imbalance, which contribute to melanoma progression and therapy resistance. Nitric oxide (NO) displays concentration-dependent effects in cancer, with high intracellular levels promoting oxidative/nitrosative stress and cell death. However, free NO donors are limited by rapid diffusion and systemic toxicity, highlighting the need for controlled delivery systems. In parallel, redox-active transition metal nanoparticles, particularly molybdenum-based systems, offer an underexplored strategy to modulate altered tumor redox homeostasis through enzyme-mimetic activity. Objective To evaluate the selective in vitro antitumor potential of NO-releasing solid lipid nanoparticles (LNP AA-NO) and redox-modulating molybdenum metal–phenolic nanoparticles (Mo-MPN) in melanoma models. Methods Mo-MPN were synthesized from ammonium heptamolybdate, ascorbic acid, quercetin, and gallic acid, with chitosan stabilization. LNP AA-NO were prepared by hot homogenization using tripalmitin and poloxamer 188 to encapsulate an NO-releasing aspirin derivative. Both platforms were characterized by DLS, NTA, HRTEM, AFM, UV–Vis, FTIR, Micro-Raman, XRD, and TGA. Melanoma cells (A375 and SK-MEL-28) and non-tumorigenic cells (HUVEC and HaCaT) were treated for 24–48 h. Cell viability was assessed by Alamar Blue/resazurin assays, and intracellular H_2O_2 levels, under basal and oxidative stress conditions, were quantified using Amplex® Red. Results Mo-MPN displayed favorable physicochemical properties, including quasi-spherical nanometric morphology, hydrodynamic diameter of approximately 170 nm, low PDI (0.242 ± 0.055), and NTA-confirmed size of 161 ± 12 nm. The chitosan coating provided a positive zeta potential (+15.8 ± 3.3 mV) and sustained colloidal stability for up to 150 days. UV–Vis and FTIR confirmed metal–ligand coordination through LMCT transitions and phenolic/carbonyl band shifts, while XRD revealed an amorphous profile. Mo-MPN also showed high phenolic content and strong radical scavenging activity in DPPH and Amplex Red H_2O_2 assays. In HUVEC cells, Mo-MPN preserved viability and markedly reduced intracellular H_2O_2 after 24 h and 48 h. Conversely, melanoma cells showed reduced metabolic activity after exposure to Mo-MPN at 1.34×10^{10} and 2.67×10^{10} particles/mL, especially after 48 h and under H_2O_2-induced oxidative stress. LNP AA-NO showed suitable parameters for drug delivery, with hydrodynamic diameter of 146.4 ± 8.28 nm and narrow size distribution (PDI = 0.132). The aspirin-to-NO-donor modification prevented the external crystallization observed with free aspirin and favored incorporation into the lipid core. HRTEM and STEM-HAADF mapping confirmed spherical core–shell nanoparticles with a dry core size of 67.64 ± 2.75 nm. TGA and FTIR supported structural integrity and thermal stability, while DLS assays in 10% BSA indicated resistance to protein-induced aggregation. Biologically, LNP AA-NO reduced A375 melanoma viability at concentrations above 600 μM, expressed as equivalent AspNO concentration, while remaining non-cytotoxic to HaCaT cells. Conclusion Both nano-strategies demonstrated cell-type-selective and redox-context-dependent activity. Mo-MPN acted as antioxidant modulators in endothelial cells while impairing melanoma metabolism under oxidative stress, whereas LNP AA-NO enabled controlled NO-donor delivery with selective cytotoxicity toward melanoma cells. Together, these platforms integrate materials engineering and tumor redox biology to target melanoma-associated redox vulnerability and adaptive antioxidant defenses while preserving non-tumor cell viability, supporting their potential as next-generation redox-based nanotherapeutic systems.

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ABSTRACTS FLASH PRESENTATIONS



FP1 – Flash Presentation 1 – 18:00h DAY 1

Nitric Oxide as a context dependent modulator of therapeutic responses in TNBC and HCC



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Keywords: TNBC, HCC, nitric oxide, tumor microenvironment, therapeutic resistance, 3D spheroids

Introduction: Triple-negative breast cancer (TNBC) and hepatocellular carcinoma (HCC) are biologically distinct yet clinically aggressive malignancies with limited therapeutic options and frequent treatment resistance.[1,2] Nitric oxide (NO), a key component of the tumor microenvironment (TME), regulates tumor progression and therapeutic responses in a highly context-dependent manner.[3] Previous studies have reported both cytostatic and cytotoxic effects of NO.
Aim: This study investigated how NO availability shapes tumor cell adaptation to therapeutic stress and influences treatment responses in TNBC and HCC models.
Materials and Methods: Fluorescently labelled TNBC and HCC 3D spheroid models were treated with standard-of-care therapies (Atezolizumab + Paclitaxel for TNBC; Atezolizumab + Bevacizumab, Sorafenib, and Lenvatinib for HCC) in combination with the NO donor NONOate or the NOS inhibitor L-Name. Treatment responses were assessed by spheroid growth, GFP intensity, caspase-3 activity, LDH release, β-gal activity and cytokine secretion. Ongoing studies investigate the contribution of stromal cells in co-culture models.
Results: In TNBC models, treatments reduced MDA-MB-231 spheroid growth and GFP intensity, with decreased caspase-3 activity and increased LDH release, suggesting predominantly apoptosis-independent stress responses. β-Galactosidase activity slightly increased, while the pro-inflammatory cytokines IL-6 and IL-1β were elevated. NONOate further reduced spheroid growth and GFP intensity, decreased caspase-3 activity, LDH release and β-galactosidase activity, increased IL-6 secretion, and abolished detectable IL-1β release. In contrast, L-NAME similarly reduced spheroid growth and viability but was associated with increased LDH release, IL-1β secretion and a slight increase in β-galactosidase activity, indicating a more inflammatory phenotype than NONOate-treated spheroids.
In HCC models, Sorafenib and Lenvatinib exerted the strongest treatment effects, reducing SNU449 spheroid growth and GFP intensity while increasing LDH release and decreasing caspase-3 activity and β-galactosidase levels. In contrast to TNBC, modulation of NO produced only modest additional effects and mainly influenced the response to Sorafenib and Lenvatinib, indicating that the cellular response was primarily driven by the therapeutic agents rather than by NO signaling.
Conclusion: The two tumor models exhibit fundamentally distinct responses to NO modulation. MDA-MB-231 cells display a strongly NO-dependent, stress- and inflammation-associated phenotype, indicating that NO acts as a central regulator of the cellular response. In contrast, SNU449 cells exhibit a predominantly *therapy*-driven phenotype, whereas NO primarily modulates treatment responses. Collectively, these findings highlight the context-dependent role of NO in tumor biology and demonstrate that its impact on treatment response is highly tumor-type specific.

Acknowledgements
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FP2 – Flash Presentation 2 – 18:08h DAY 1

GSNOR deficiency promotes DNA damage and replication stress in rhabdomyosarcoma



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Keywords: S-nitrosylation, GSNOR, cancer, cell cycle, CDK4, DNA damage response

S-nitrosylation is a reversible post-translational modification that regulates protein function via the attachment of a nitric oxide (NO) moiety to cysteine residues. This redox-based signaling mechanism is tightly controlled by enzymes like S-nitroso glutathione reductase (GSNOR), which remove SNO from low molecular weight thiols to restore S-nitrosylation homeostasis1. Altered levels of S-nitrosylation have been implicated in several pathological states, including cancer2; however, its impact in rhabdomyosarcoma, the most common pediatric soft tissue sarcoma, has not been explored yet. Here, we demonstrate that in rhabdomyosarcoma cell lines, GSNOR depletion is correlated with a hyper-proliferative phenotype and increased S-phase entry. We identify cyclin-dependent kinase 4 (CDK4) as a key driver of cell cycle deregulation in RMS and a novel target for S-nitrosylation. This proliferative phenotype is accompanied by elevated DNA damage and replication stress, leading to the activation of the DNA damage response pathway and the G2/M checkpoint. More specifically, GSNOR-ablated cells showed increased number of γH2AX foci, a marker for DNA damage3, which was rescued by CDK4 inhibition. Loss of GSNOR correlated with increased phosphorylation of ataxia telangiectasia and Rad3-related protein (ATR), a master regulator of the cellular response to replication stress and single-stranded DNA damage that coordinates the recruitment of the DNA repair machinery4. Finally, overexpressing CDK4WT, but not the non-nitrosylable mutant CDK4C135A, was associated with increased ATR phosphorylation and DNA damage. Future plans include exploiting replication stress as a potential vulnerability of GSNOR depletion, broadening the therapeutic options for rhabdomyosarcoma patients.

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FP3 – Flash Presentation 3 – 18:16h DAY 1

A next generation sequencing transcriptomic signature associated with iNOS Expression in Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, accounting for approximately 10–20% of all cases. Its poor prognosis is driven by rapid proliferation, early disease onset, and the absence of effective targeted therapies. Among the biomarkers associated with clinical outcome, inducible nitric oxide synthase (iNOS) protein expression has emerged as a predictor of survival, with patients exhibiting low iNOS protein levels demonstrating improved overall survival. However, while iNOS has established prognostic value at the protein level, its transcript is expressed at very low levels in bulk RNA sequencing datasets and is particularly scarce in single-cell RNA sequencing data, where expression is restricted to only a very small subset of cells. This limits the use of the vast amount of publicly available next-generation sequencing transcriptomic datasets for investigating iNOS biology in TNBC. To address this challenge, we identified a novel four-gene transcriptomic signature that co-locates with iNOS expression in an in-vivo single-cell transcriptomics dataset entailing 12 patients. In an independent patient cohort, this signature was also significantly associated with overall survival in TNBC. These findings demonstrate the potential of this four-gene signature to facilitate the investigation of iNOS related biology across large next-generation sequencing transcriptomic datasets where direct assessment of iNOS expression is not feasible. Two important questions remain: first, whether the signature spatially maps to the rare in-vivo cells expressing iNOS, thereby confirming their spatial co-localization, and second, whether a causal mechanism links iNOS to the signature genes. Ongoing work integrating spatial transcriptomics, proteomics, and single-cell analyses aims to address this question and further validate the biological relevance of the proposed signature.

FP4 – Flash Presentation 4 – 18:24h DAY 1

Regulation and impact of GSNOR loss in breast cancer progression



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Keywords: S-nitrosylation, GSNOR, Breast cancer, microRNA, cancer metabolism.

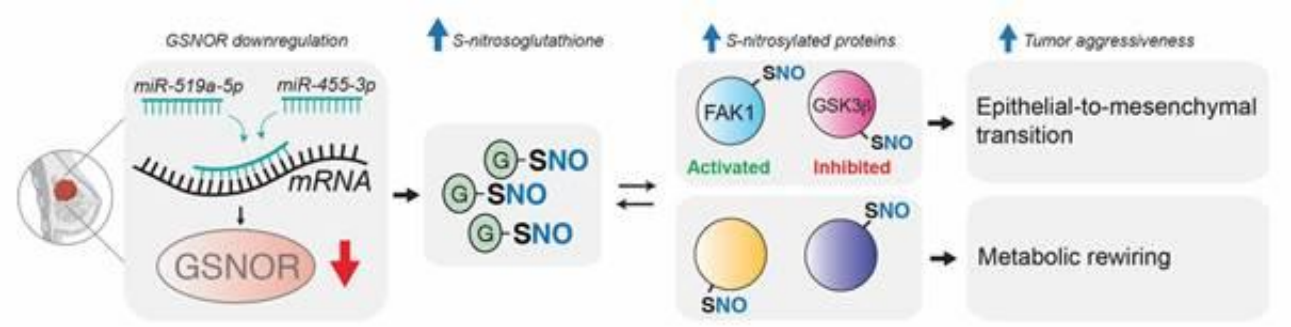
Nitric oxide (NO) signaling is largely mediated by protein S-nitrosylation, a reversible post-translational modification whose steady state is determined by the balance between nitrosylation and denitrosylation[1,2]. While increased NO production has been extensively described in breast cancer, less is known about how denitrosylation is regulated and how its dysregulation impacts tumor biology[3]. A prototypical denitrosylase controlling global S-nitrosylation levels is S-nitrosoglutathione reductase (GSNOR), which has recently reported to be hypoexpressed in breast cancer[4]. Here, we investigated the mechanisms regulating GSNOR expression and the consequences of GSNOR loss on breast cancer progression, with a specific focus on triple-negative breast cancer (TNBC).

Analysis of patient datasets and tumor specimens revealed a marked reduction of GSNOR expression and enzymatic activity in breast cancer compared to normal mammary tissue. Low GSNOR expression significantly correlated with poorer overall survival, supporting a tumor-suppressive role for denitrosylation. We identified a microRNA (miR) signature selectively enriched in GSNOR-low tumors and demonstrated that miR-455-3p and miR-519a directly target GSNOR mRNA, providing a mechanistic explanation for its downregulation.

At the functional level, GSNOR loss increased S-nitrosylation of key signaling proteins involved in cell adhesion, migration, and epithelial-to-mesenchymal transition, including focal adhesion kinase 1 (FAK1)[4] and glycogen synthase kinase-3 beta (GSK3β). Using both 2-dimensional (2D) and 3-dimensional (3D) models of TNBC, we show that GSNOR depletion promotes spheroid growth, invasiveness, and survival, whereas restoration of GSNOR by anti-miRs restrains these aggressive phenotypes.

Given the emerging link between NO signaling and cancer metabolism[5–7], we next examined the metabolic consequences of altered denitrosylation. Preliminary data show that GSNOR^{low} cells are characterized by accumulation of proline. In TNBC, more metastatic cells displayed an upregulation of the proline biosynthetic pathway, consistent with a metabolic state supporting 3D growth and adaptation to anchorage-independent conditions[8]. Modulation of S-nitrosylation levels altered the expression of key enzymes involved in proline metabolism, indicating a direct connection between denitrosylation and metabolic regulation.

Together, these findings identify GSNOR as a critical regulator of S-nitrosylation-dependent signaling and metabolic adaptation in breast cancer. Our work supports a model in which microRNA-driven loss of denitrosylation promotes pro-tumorigenic signaling and metabolic rewiring (Scheme 1), highlighting denitrosylation pathways as potential vulnerabilities in aggressive breast cancer, particularly TNBC.



Scheme 1: Graphical representation of the mechanism of GSNOR loss in breast cancer, mediated by miRs, and its effect on the levels of S-nitrosoglutathione (GSNO) and S-nitrosylated proteins. The subsequent increase of S-nitrosylation of key signalling proteins impacts tumor aggressiveness, promoting epithelial-to-mesenchymal transition and metabolic rewiring.

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FP5 – Flash Presentation 5 – 17:55h DAY 2

Biotin-switch technique/proximity ligation assay-based method for *in situ* analysis of S-nitrosylated proteins



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Keywords: S-nitrosylation, Biotin-switch technique, proximity ligation assay, flow cytometry, fluorescence microscopy

Nitric oxide (NO) is a gaseous free radical that acts as a major signalling molecule involved in the regulation of numerous physiological processes including for example vascular homeostasis, neurotransmission, immune responses, cell proliferation and apoptosis. Conversely, alterations in NO production contribute to the development of various pathological conditions such as cancer, cardiovascular diseases and inflammatory diseases. A major mechanism underlying NO-mediated signaling is S-nitrosylation. This reversible post-translational modification consists of the non-covalent attachment of an NO group to the thiol of a cysteine residue, leading to the formation of an S-nitrosothiol. By modulating protein conformation, stability, localisation and protein-protein interactions, S-nitrosylation plays a central role in cellular homeostasis. However, the intrinsic instability of the S-NO bond, sensitive to both redox conditions and light, considerably complicates the detection and characterization of S-nitrosylated proteins.
The Biotin-switch technique (BST) is the reference method for detecting protein S-nitrosylation from cell lysates. Because BST requires cell lysis, a complementary approach allowing *in situ* detection and spatial localisation of specific S-nitrosylated proteins was still lacking. To address this limitation, we developed a novel method termed "SNO-Biotin-PLA" based on the combination of BST and a Proximity Ligation Assay (PLA) followed by flow cytometry or fluorescence microscopy analysis.[1]
The method was developed and validated to detect S-nitrosylation of a membrane receptor of the Tumor Necrosis Factor family (TNFR1) and a cytoplasmic protein (cIAP1) both previously identified by the BST as target of this post-translational modification[2] (and unpublished data). The versatility of SNO-Biotin-PLA was demonstrated across different protein targets and cell lines. Importantly, for transmembrane proteins, the method enables the selective analysis of S-nitrosylation occurring on either extracellular or intracellular domains.
Overall, “SNO-Biotin-PLA” offers several advantages. It constitutes a sensitive method for the *in situ* analysis of selective protein S-nitrosylation while requiring only a small number of cells and preserving cellular architecture. Compared with conventional BST, this approach is less time-consuming, does not require cell lysis and enables analysis at endogenous protein levels. By enabling the *in situ* detection of protein S-nitrosylation, “SNO-Biotin-PLA” represents a novel methodological approach for investigating NO-dependent signaling pathways and may support future fundamental and translational studies.

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FP6 – Flash Presentation 6 – 18:03h DAY 2

Development of a sequential therapy – docetaxel/cisplatin, then glyceryl trinitrate – for the treatment of triple-negative breast cancer: Role of the tumour necrosis factor superfamily



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Keywords: Triple-negative breast cancer, chemotherapy, NO donors, Tumour Necrosis Factor

Triple-negative breast cancer (TNBC) accounts for 15% of breast cancer cases and is associated with a poor prognosis due to its aggressiveness and high metastatic potential. Currently, post-surgery treatment for TNBC relies on chemotherapy. However, the high recurrence rate and the development of chemoresistance remain major concerns. To achieve the best benefit for the patient, developing new treatment strategies is crucial in TNBC.
Some chemotherapeutic agents are known to increase tumour necrosis factor (TNF) ligand levels within the tumour, which may contribute to chemoresistance. Ultimately, TNFs can promote tumour development and metastasis by sustaining inflammation and activating cell survival pathways. However, TNF/TNF receptor (TNFR) signalling is known to have a dual activity as it can either promote tumour survival or trigger cancer cell death. This dual activity makes TNF signalling pathways attractive therapeutic targets. We are developing new strategies aimed at harnessing TNF/TNFR activity to enhance cancer cell death. Previous studies from our team and another group contributed to demonstrating that nitric oxide (NO) released by NO donors can modulate TNFa/TNFR1, FasL/Fas and TRAIL/DR4 signalling pathways through S-nitrosylation in favour of cancer cell death.
In light of these observations, we hypothesised that if chemotherapy increases levels of specific TNF ligands and/or their receptors (TNFα/TNFR1, Fas/FasL, and TRAIL/DR4-5) within the tumour microenvironment (TME), then administrating NO donor may enhance chemotherapy efficacy.
Here, we further investigated the mechanisms by which NO can regulate the TNFa/TNFR1, FasL/Fas and TRAIL/DR4-5 signalling pathways and their ability to trigger TNBC cell death. Through *in vitro* studies using two-dimensional (2D) and three-dimensional (3D) homotypic/heterotypic spheroid cell models based on TNBC cell lines from murine (4T1) or human (MDA-MB-231, MDA-MB-468) origin, we assessed 1) the dose- dependent effect of NO released from the NO donor glyceryl trinitrate (GTN) and recombinant TNF ligands on cell death and viability; 2) the modulation of endogenous expression of TNFα/TNFR1, FasL/Fas, and TRAIL/DR4-5 by chemotherapy (docetaxel/cisplatin) or GTN at the mRNA and/or protein levels; 3) a sequential combination strategy based on chemotherapy-induced TNF ligands followed by GTN treatment.
Our results show that TNF ligands and GTN sensitize TNBC to cell death and loss of viability in a time- and dose-dependent manner, while subtoxic doses of docetaxel/cisplatin and GTN can modulate TNF ligands (FasL and TRAIL) and TNFR expression respectively. Based on these findings, a sequential combination docetaxel/cisplatin followed by GTN treatment significantly decreased TNBC viability compared with chemotherapeutic agents or GTN alone.
These findings support further investigation of sequential therapy as a strategy to harness chemotherapy-induced TNF ligands to decide whether to add GTN to improve therapeutic efficacy in TNBC.

Acknowledgements

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FP7 – Flash Presentation 7 – 18:11h DAY 2

The effect of nitric oxide on TNBC proliferation, senescence and apoptosis in 2D and 3D



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Keywords: inducible Nitric Oxide Synthase, spheroids, proliferation, apoptosis, senescence

In triple negative breast cancer (TNBC) patients, the overexpression of the enzyme iNOS (inducible Nitrix Oxide Synthase) is significantly associated with decreased survival and contributes to the invasiveness, metastasis and aggressiveness of TNBC[1]. Additionally, iNOS and its product NO are known to modulate senescence-associated secretory phenotype (SASP) factors, thereby initiating cellular senescence programmes[2]. At high concentrations of NO, oxidative and nitrosative stress and consequent DNA damage can lead to the promotion of apoptosis[3]. The aim of this project is to generate 3D models of stably overexpressing TNBC cell lines and characterise the influence of TNBC subtype and iNOS overexpression on various tumour properties.

3D spheroid models of TNBC cell lines including metaplastics (HCC1806, MDA-MB-231, BT549, Hs578T) were generated in the presence or absence of the scaffolding matrix Matrigel®. iNOS was overexpressed by stable lentiviral transduction. The spheroid area and diameter were analysed with ImageJ. Proliferation was assessed with MTS (2D) or CellTiter-Glo (3D) and by analysis of gene expression of key proliferation markers via qPCR (2D) and immunofluorescence (IF, 3D). Apoptosis and senescence were assessed by β-galactosidase staining, protein expression analysis (Western Blot, 2D) gene expression analysis (qPCR, 2D) and staining (IF, 3D).

Spheroids generated from cell lines representing different TNBC subtypes possess diverse properties. The addition of Matrigel® leads to the formation of denser spheroids and increases the growth of the 3D structures. Addition of Matrigel® and iNOS overexpression increase proliferation in 2D, less so in 3D. iNOS overexpression modulates the proliferative capacity of TNBC cells in 2D and 3D as per gene expression analysis and influences the apoptosis and senescence pathways.

3D models better represent the *in vivo* tumour microenvironment. Addition of Matrigel® mimics the extracellular matrix found at the tumour site and provides the cancer cells with biochemical cues, altering their proliferation capacity. iNOS overexpression increases proliferation in 3D, thus representing the pro-tumorigenic effect along with changes in apoptosis and senescence seen in iNOS-overexpressing cancers. Further experiments modelling important pathological mechanisms observed in the *in vivo* tumour setting will provide researchers and clinicians with valuable insights as regards patient outcomes and treatments.

Acknowledgements

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FP8 – Flash Presentation 8 – 18:19h DAY 2

SNAP-loaded poly-lactic-acid honeycomb membranes as nitric oxide releasing materials under light stimulus



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Keywords: honeycomb, barrier membrane, nitric oxide, controlled release, cytocompatibility

Bone reconstruction using bone grafts can be jeopardized by uncontrolled proliferation of soft tissue, which may occupy the defect site and prevent proper bone regeneration. To counter this, in the guided bone regeneration (GBR) approach, physical barriers membranes are proposed to prevent soft-tissue infiltration[1]. Especially in the orofacial area, an additional challenge arises from frequent bacterial exposure, that leads to critical situations for individuals with weakened immune defenses and/or specific pathophysiological conditions. Effective regeneration strategies must therefore not only support bone regeneration and structural integrity but also address the risk of infection. S-nitroso-n-acetyl-DL-penicillamine (SNAP) is a nitric oxide (NO) donor. Owing to its free-radical based antimicrobial activity, NO exhibits broad-spectrum efficacy against numerous pathogens[2].

In this context, the present study explores the use of a SNAP-loaded poly-L-D-lactic acid honeycomb membrane as a platform for on-demand NO delivery using white light.

Honeycomb membranes were generated using the breath figure method[3]. Their structure and pore size were examined using scanning electron microscopy analysis (SEM) and compared with unloaded (blank) membranes. Nitric oxide (NO) release under white-light irradiation was quantified using a NO analyzer. Cytocompatibility of SNAP-loaded membranes, in contact with hADSCs, under white-light exposure and in dark conditions, will be presented.

SEM analysis revealed that SNAP incorporation resulted in larger pore size compared with blank membranes (Figure 1a), likely due to a change in solution viscosity and surface tension.

Under white-light irradiation, SNAP-loaded membranes released NO in a controllable manner: NO levels rose when the light was switched on and returned to the baseline once the light was turned off (Figure 1b).

In conclusion, we demonstrated that the SNAP-loaded honeycomb membranes are a promising material for light-controlled nitric oxide delivery.

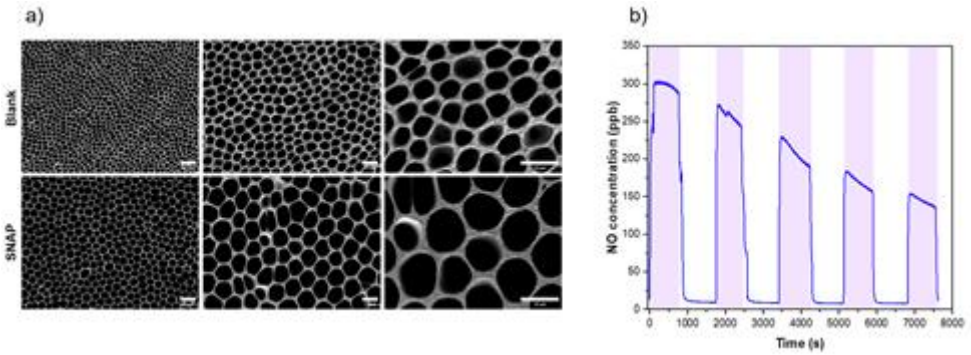


Figure 1: SEM images of blank and SNAP-loaded membranes (a). White light modulated NO release from SNAP-loaded

Acknowledgements

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FP9 – Flash Presentation 9 – 18:27h DAY 2

Antiproliferative and antimigratory activity of S-nitrosoglutathione (GSNO) in skin cancer cell lines



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Keywords: S-nitrosoglutathione; nitric oxide; melanoma; squamous cell carcinoma; antiproliferative activity; selectivity index

Introduction: Skin cancer is one of the most common malignancies worldwide. Although melanoma is less prevalent, it causes the highest mortality because of its aggressiveness and treatment resistance, while squamous cell carcinoma represents a major proportion of skin and head-and-neck cancers. Nitric oxide exerts concentration-dependent effects: low levels may support tumor progression, whereas high levels induce nitrosative stress, mitochondrial dysfunction, and cell death. This dual action supports the therapeutic use of nitric oxide donors. S-nitrosoglutathione, a physiological nitric oxide reservoir, may regulate survival and migration pathways, but its selectivity remains unclear.
Objective: To evaluate the antiproliferative activity and selectivity of GSNO against melanoma (A375) and squamous cell carcinoma (SCC-25 and SCC-4), using normal human keratinocytes (HaCaT) as a non-tumoral control, and to characterize its effect on cell migration.
Methods. GSNO was synthesized by S-nitrosation of glutathione and tested in A375 melanoma, SCC-9, SCC-4, and non-tumoral HaCaT keratinocytes. Cells were treated with GSNO (0.7-100 μM) for 24 h, viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, half-maximal inhibitory concentration (IC_{50}) values were calculated by non-linear regression, and selectivity index (SI) was calculated as IC_{50}(HaCaT)/IC_{50}(tumor cells). Migratory capacity was evaluated by wound healing assay at 25 and 50 μM.
Results: GSNO reduced viability in all cell lines in a concentration-dependent manner. A375 melanoma cells were most sensitive (IC_{50} ≈ 5.6 μM), followed by SCC-25 (≈ 9.9 μM) and SCC-4 (≈ 23 μM). Compared with non-tumoral HaCaT cells (IC_{50} ≈ 23 μM), A375 and SCC-25 showed favorable selectivity, whereas SCC-4 did not. GSNO at 25 and 50 μM also inhibited tumor-cell migration dose-dependently. Overall, GSNO demonstrated antiproliferative and antimigratory effects, particularly against A375 and SCC-25, supporting further investigation as a promising nitric oxide donor for skin cancer therapy and mechanistic studies.

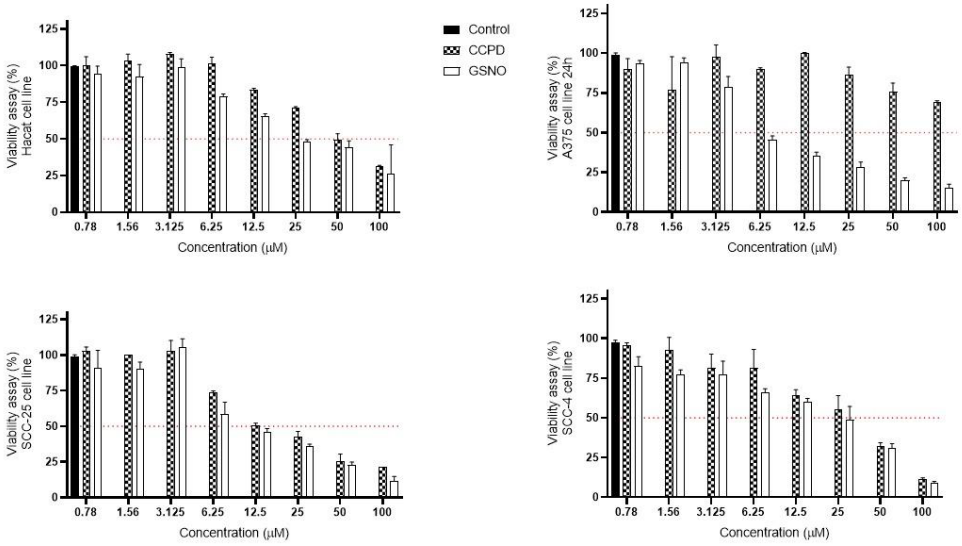
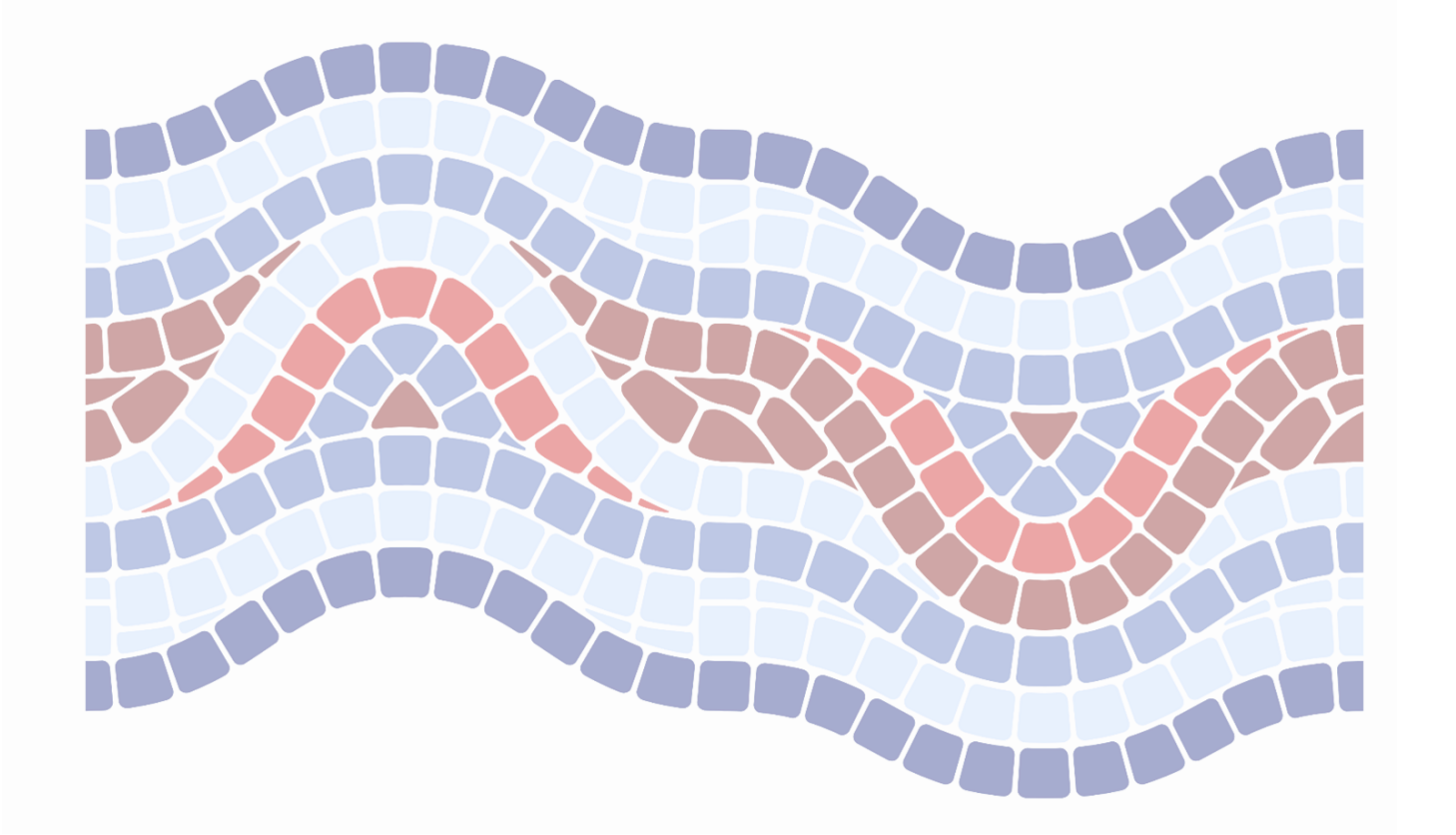


Figure 1: Dose-response curves of GSNO evaluated by MTT assay after 24 h in A375, SCC-9, SCC-4, and HaCaT cells.

Acknowledgements

The authors acknowledge the São Paulo Research Foundation (FAPESP) through the Thematic Project (process 22/14645-2), Cepid CancerThera (grant 2021/10265-8), and the University of Campinas for institutional support.

ABSTRACTS POSTERS



P1 – Poster 1

The complex NOS2-NOX4-GSNOR regulates metastasis and angiogenesis in colon cancer cells



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Keywords: colorectal cancer; NOX4; Nitric Oxide; NOS2; S-nitrosylation; GSNOR

Colorectal cancer (CRC) is the third leading cause of cancer-related mortality worldwide, with metastasis being the primary cause of death. CRC development is particularly dependent on angiogenesis, a step that accelerates the metastatic process. Reactive oxygen species (ROS) and the signaling free radical nitric oxide (NO) are present at elevated levels in tumors and play a role in angiogenesis and in the metastatic cascade. Increased generation of ROS (O_2^-/H_2O_2) and NO by the enzymes NOX4 and NOS2, respectively, correlates with a poorer prognosis in CRC[1,2]. The redox status of the tumor cells and their microenvironment is maintained through the spatiotemporal regulation of ROS and NO production[3]. NO reacts with the amino acid cysteine, introducing a post-translational modification known as S-nitrosylation into proteins and enzymes. S-nitrosylation and its reversal—denitrosylation, mediated by S-nitrosoglutathione reductase (GSNOR), potentially regulate ROS generation in tumor cells by acting on the enzymes responsible for producing them. Alterations in protein S-nitrosylation levels affect processes such as angiogenesis and metastasis promotion. In this context, a regulatory mechanism for the generation of reactive species involving NOS2, GSNOR, and NOX4 was studied in colon cancer cell lines derived from the primary site (SW480) and the lymph node metastasis (SW620). Using immunoprecipitation assays and confocal microscopy, we demonstrated an interaction between NOX4, NOS2, and GSNOR, forming a nitrosylase/denitrosylase complex. Formation of this complex was more evident in SW480 cells, suggesting that the tumor's developmental stage determines the complex's formation. In cells under serum restriction, NO levels were higher in SW620 cells, and ROS levels were higher in SW480 cells. S-nitrosylation of NOX4 was detected in both cell lines, but it was greater in SW620 cells. Two highly conserved cysteine residues, Cys343 and Cys359, located in NOX4, have been predicted to undergo S-nitrosylation. Site-directed mutagenesis was used to obtain SW480 and SW620 cells harboring C343S and C359S mutations. S-nitrosylation of NOX4 occurs at both residues, inhibiting enzyme activity. These cells exhibit differential H_2O_2 and NO production and regulate angiogenesis depending on the specific residue where S-nitrosylation occurs. SW480 and SW620 cells display differential expression patterns of GSNOR, with high expression in SW480 cells and low expression in SW620 cells. CRISPR-Cas9 technology was employed to generate cells with differential GSNOR expression: SW480 GSNOR-KD (GSNOR-deficient (knockdown [KD]) and SW620 GSNOR-OE (GSNOR overexpression) cells. These cells exhibit differential H_2O_2 and NO production. Changes in GSNOR activity in both cell lines regulate angiogenesis. In conclusion, our findings revealed that NOX4 physically interacts with NOS2 and GSNOR, suggesting that NO derived from NOS2 can regulate NOX4 activity, thereby controlling intracellular levels of reactive species across different stages of colon cancer development and influencing angiogenesis and metastasis.

Acknowledgements: FAPESP: Grant numbers: 2018/15038-7 and 2025/10560-0. CAPES: 88887.684923/2022-000 (PhD Fellowship)

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P2 – Poster 2

Investigating NO-mediated activation of HERV-K in gastrointestinal cancers



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Keywords: gastrointestinal cancers, NO donors, HERV-K

Human endogenous retroviruses (HERVs) are remnants of ancient viral infections integrated into the human genome, with HERV-K being one of the most transcriptionally active subfamilies. Emerging evidence suggests that HERV-K expression is dysregulated in various cancers, including gastrointestinal (GI) malignancies, where it may contribute to tumorigenesis through mechanisms such as immune modulation and oncogenic signalling[1,2].

Previous work from our group suggests a link between nitric oxide (NO) signalling and HERV-K in cancer. In breast cancer, nitrosative stress induces HERV-K expression, while HERV-K contributes to migration, invasion and poor patient outcomes. In prostate cancer, chronic NO exposure promotes aggressive behaviour and is associated with elevated HERV-K.

Despite these findings, the relationship between inducible nitric oxide synthase (iNOS), NO and HERV-K activation in GI cancers remains poorly understood. We hypothesise that HERVs activation occurs during the development of GI cancers as a consequence of increased oxidative and nitrosative stress under the regulation of iNOS, and contributes to transition to a metastatic phenotype.

This project investigates HERV-K expression in colorectal and gastric cancer using bioinformatic datasets and *in vitro* cell models. The effect of the NO donor DETA/NO on HERV-K expression was assessed at the mRNA and protein levels. Downstream signalling pathways and functional effects on proliferation, migration, and invasion also were examined.

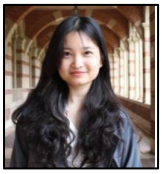
Defining the relationship between iNOS-mediated nitrosative stress and HERV-K activation may identify novel mechanisms driving GI cancer progression and establish HERV-K and iNOS as potential prognostic biomarkers or therapeutic targets.

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P3 – Poster 3

Nitric Oxide-based Nano-immunotherapy for overcoming tumor resistance



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Keywords: Nitric oxide, Nanomedicine, Immunotherapy, Drug resistance

Objective: Tumor immune resistance results from a complex network of mechanisms that include hypoxia, aberrant angiogenesis, metabolic reprogramming, impaired immune cell infiltration, expansion of immunosuppressive cell subpopulations, polarization of tumor-associated macrophages (TAMS) and the upregulation of immune checkpoint molecules[1–3]. We have recently witnessed significant milestones of immune-therapeutics in the treatment of a variety of resistant cancers. However, many solid tumors have *de novo* or acquired resistance to immunotherapy. Hence, our objective is to evaluate the therapeutic potential of nitric oxide (NO)-based nanomedicine as a novel therapeutic strategy to resensitize tumor cells to respond to immunotherapy.
Rationale: NO is a pleiotropic signaling molecule with opposing effects on tumor behavior, and its therapeutic application has been limited by its extremely short half-life, rapid systemic degradation, and dose-dependent biological effects[4,5]. Recent advances in nanotechnology have enabled the development of NO-releasing nanocarriers capable of delivering controlled and site-specific NO concentrations within tumors, thereby maximizing therapeutic efficacy while minimizing systemic toxicity[3,6,7].
Hypothesis: We hypothesize that targeted NO-based nanomedicine can remodel the immunosuppressive tumor microenvironment (TME) by promoting immunogenic tumor cell death and enhancing the infiltration and cytotoxic activity of $CD8^+$ T lymphocytes and NK cells. Furthermore, the combination of NO nanomedicine with immune-therapeutics (e.g. ICIs, CAR-T cell therapy, cancer vaccines) will synergistically overcome both intrinsic and acquired mechanisms of tumor immune resistance.
Findings: Accumulating experimental evidence demonstrates that NO-releasing nanoparticles effectively enhanced penetration of immune effector cells and therapeutic agents [7,8]. NO has been shown, amongst other anti-tumor effects, to reverse T-cell exhaustion and diminish the suppressive activities of Tregs and MDSCs [4,9–11]. We propose that multifunctional nanoplatforms integrating NO donors with checkpoint therapy can have superior antitumor activity compared to monotherapies.
Conclusion: NO-based nano-immunotherapy represents a promising next-generation immunotherapeutic platform capable of simultaneously targeting multiple hallmarks of tumor immune resistance. NO delivery has the potential to substantially improve therapeutic outcomes across a broad spectrum of resistant solid malignancies. The integration of nanotechnology with NO biology provides a versatile and highly adaptable strategy for precision cancer immunotherapy.
Future Perspectives Future investigations should focus on optimizing the pharmacokinetics, dosage, and spatiotemporal control of NO release, while identifying predictive biomarkers that guide patient selection and therapeutic monitoring. Translation into clinical practice will require rigorous evaluation of safety, biodistribution, and long-term immunological outcomes in early-phase clinical trials.

Acknowledgements
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P4 – Poster 4

NO Donors as Modulators of Tumor Hypoxia and Immunotherapy resistance



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Keywords: Nitric Oxide, Tumor Hypoxia, HIF-1α, immunotherapy Resistance

Objective: We have recently reported the pivotal role of the dysregulated RKIP-hypoxia axis in cancer immune evasion[1].Herein, we have investigated the NO-hypoxia axis in immune resistance. Nitric Oxide (NO), synthesized by the inducible nitric oxide synthase (iNOS), holds a paradoxical role in cancer biology and immunotherapy. NO both stabilizes and degrades the hypoxia-responsive transcription factor hypoxia-inducible factor-1α (HIF-1α), and both promotes and reverses tumor immune evasion[2-5]. Herein, we suggest that these opposing findings are not contradictory, but rather, are reflections of one principle: NO behaves as a rheostat whose net effect is dependent on cellular source, local oxygen tension, and flux. We have examined the evidence supporting NO as a flux-gated modulator of HIF-mediated immune evasion.

Findings: The duality of NO's effect on HIF-1α is governed by the local oxygen tension. Under normoxia, NO inhibits prolyl hydroxylases (PHDs) and S-nitrosylates its oxygen-dependent degradation domain (ODDD), blocking von Hippel-Lindau recruitment and stabilizing HIF-1α and NO, thereby, mimics hypoxia[6,7]. Within the hypoxic tumor microenvironment (TME), however, high-flux NO inhibits mitochondrial cytochrome c-oxidase, redistributing oxygen towards PHDs, and promotes HIF-1α degradation[2]. Thus, NO may stabilize or suppress HIF-1α depending on the ratio of O_2:NO balance. This distinction is consequential because HIF-1α facilitates a variety of immune evasion mechanisms. Namely, HIF-1α promotes immune checkpoint expression, myeloid-derived suppressor cell (MDSC) recruitment, regulatory T-cell (Treg) activity, M2 tumor-associated macrophage (TAM) polarization and impairs antigen presentation, thereby inhibiting $CD8^+$ T-cell activation. Therefore, high-flux NO-mediated degradation of HIF-1α within hypoxic tumor regions may attenuate multiple immune evasion pathways rather than only suppressing a single checkpoint molecule.

Discussion: The flux-dependent polarity governs tumor immune evasion. Endogenously, low-flux NO generated by MDSCs and other cells may sustain T-cell dysfunction through T-cell receptor (TCR) nitration and depleted arginine metabolism[8]. In contrast, exogenously, tumor-localized, high-flux NO donors may suppress HIF-1α and reverse HIF-mediated immune evasion and sensitizes tumors to immunotherapy and checkpoint inhibition[4]. Our findings suggest that the therapeutic value of NO donors depends on achieving sufficient intratumor NO flux while avoiding systemic toxicity and counterproductive dose effects.

Future perspectives: We propose that HIF-mediated immune evasion is likely to be reversed when NO is delivered above a defined, therapeutically relevant, flux threshold and spatially confined to the tumor. This positions NO donors, such as glutathione-S-transferase-activated prodrugs, arginine-based nanocarriers, and in situ NO generators as precision modulators of HIF-induced immune evasion. The combination of NO donors and immunotherapy will inhibit resistant tumor growth. Baseline HIF-1α expression, when evaluated alongside immune evasion markers such as PD-L1, may serve as a candidate biomarker of response.

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P5 – Poster 5

The cross talk between Nitric Oxide and Hydrogen Sulfide in nutrient deprivation-induced quiescence and 5-Fluorouracil-induced chemoresistance in CRC



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Keywords: hydrogen sulfide, nitric oxide, quiescent, CRC, starvation.

Colorectal cancer (CRC) remains a leading cause of cancer-related mortality worldwide. Chemoresistance to 5-fluorouracil (5-FU), the most widely used chemotherapeutic agent for CRC, represents a major obstacle to therapeutic success. In addition, acquired resistance may be linked to a state of cellular quiescence, allowing tumor subpopulations to survive the genotoxic stress induced by 5-FU [1,2].

We characterized quiescence using the SW480 and SW620 cell lines as models for primary and metastatic tumors, respectively. Quiescence was induced in SW480 cells by withdrawal of fetal bovine serum (FBS) for four days, followed by a recovery of the proliferative capacity upon replenishing the culture medium with FBS. During FBS restriction, we observed increased nitric oxide (NO) production and increased cystathionine β-synthase expression levels, but reduced hydrogen sulfide (H_2S) production. In contrast, upon FBS restriction, the metastatic SW620 cell line did not enter a quiescent state and produced less H_2S compared to quiescent SW480 cells. Our findings strongly suggested that a balance between NO and H_2S production is a determinant of cellular plasticity and survival during nutritional stress-induced quiescence. Recent findings suggested that the gasotransmitters H_2S and NO are critical regulators of the metabolism and survival of CRC cells [3, 4].

To relate nutritional deprivation-induced quiescence to acquired chemoresistance, we expanded the studies to a heterogeneous panel of CRC cell lines, including SW1116 (primary tumor, MSS), HT29 (BRAF mutation, MSS), and HCT116 (highly aggressive, MSI). A protocol of chronic, escalating 5-FU exposure successfully established chemoresistant cell models in all three lines. We validated the results by determining IC50 and clonogenic capacity after 5-FU treatments.

The transcriptional and enzymatic levels of the enzymes responsible for synthesizing respectively H_2S: cystathionine β-synthase, cystathionine γ-lyase, and 3-mercaptopyruvate sulfur transferase; and NO: NO synthase 2, respectively, were determined in 5-FU-resistant and non-resistant CRC cell lines. It is proposed that resistant cells utilize H_2S and NO to develop a "redox shield". H_2S provides mitochondrial bioenergetics support and, in synergy with NO, activates antioxidant pathways inhibiting apoptosis, and potentially driving chemoresistant tumor cells into a quiescent state.

Acknowledgements
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P6 – Poster 6

A compartmentalised electrochemical platform for quantitative assessment of NO-scavenging biomaterials and therapeutics

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Keywords: Nitric oxide; nitric oxide-scavenging; electrochemical detection; hemin; hyaluronic acid hydrogel

Introduction: Nitric oxide (NO) is a pleiotropic signalling molecule that regulates diverse pathophysiological processes, including cancer.[1] NO modulation, using NO-releasing[2] and NO-scavenging compounds and materials[3], has emerged as promising strategies against cancer. Accurate real-time NO detection is therefore essential for these materials. Electrochemical sensing techniques provide continuous, high-temporal-resolution measurement of NO levels.[4] However, the use of conventional direct-contact ("one-pot") electrochemical assays in the quantitative evaluation of NO-modulating materials remains challenged by analytical artefacts associated with electrode fouling and biomaterial interferences. We hypothesised that physically separating the NO-sensing and biomaterial compartments with a NO-permeable dialysis membrane would eliminate these fouling- and interference-related artefacts, enabling accurate, real-time quantification of both NO release and biomaterial-mediated NO scavenging.

Method: A custom membrane-compartmentalised electrochemical platform was developed to separate the NO-donor/sensing compartment and a hydrogel/biomaterial (NO-scavenging) compartment while permitting NO diffusion across a dialysis membrane. Using an electrochemical microsensor, platform calibration and analytical performance were established using S-Nitroso-N-acetylpenicillamine (SNAP) and Diethylenetriamine NONOate (DETA-NO). Subsequently, the platform was employed to quantify NO-scavenging by unloaded (***u***) and hemin-loaded (***h***) hyaluronic acid (HA)-hydrogels, prepared as previously described[5], under identical experimental conditions.

Result/Discussion: The platform established two physically distinct but diffusively communicating compartments, preventing direct biomaterial-electrode contact (***Fig.1a***). SNAP generated a linear NO concentration–voltage response in deionised water (R^2 = 0.995) and phosphate buffer, pH 7.4 (R^2 = 0.992) (***Fig.1b,c***). DETA-NO produced concentration-dependent peak and quasi-steady-state NO responses (***Fig.1d***). Building on a previous study[5] by our group, which established the NO-scavenging capacity of *h*HA-hydrogels using electrochemical detection, the compartmentalised platform enabled quantitative assessment of NO-scavenging, with *u*HA and *h*HA hydrogels reducing the area under the response curve, corresponding to apparent NO-scavenging efficiencies of approximately 41 – 43 % and 50 – 62 %, respectively (***Fig.1e–h***).

Conclusion and Future Perspective: The platform enabled quantitative, real-time assessment of nitric oxide release and biomaterial-mediated NO scavenging. Future work will extend its application to biologically relevant media, NO-releasing materials, and biomaterial matrices to investigate how microenvironment and material architecture influence NO transport and scavenging.

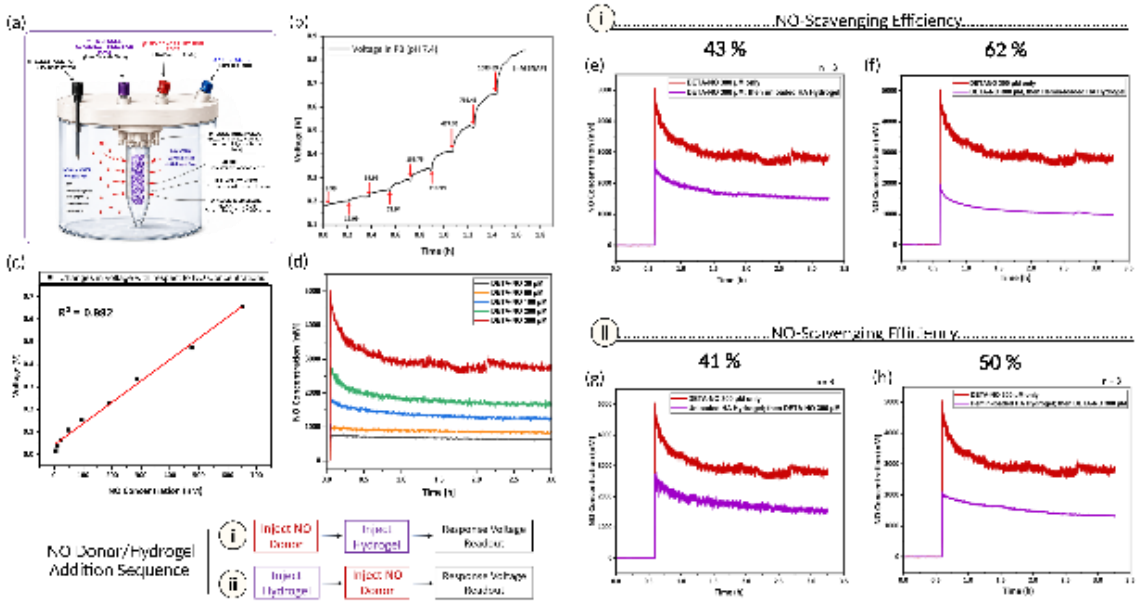


Figure 1. Development and validation of a membrane-compartmentalised electrochemical platform for real-time NO quantification and biomaterial-mediated NO scavenging. (a) Platform schematic. (b,c) Electrochemical calibration of the NO microsensor in phosphate buffer (pH 7.4). (d) Concentration-dependent NO release from DETA-NONOate (30–300 µM). (i,ii) Experimental workflows for sequential NO donor/hydrogel addition. (e–h) Comparison of NO-scavenging by unloaded and hemin-loaded HA hydrogels, demonstrating enhanced apparent NO-scavenging by hemin-loaded hydrogels under both experimental configurations.

Acknowledgements
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P7 – Poster 7

Recovery of vascular function by zofenopril in obese Zucker diabetic fatty rats

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Keywords: hydrogen sulfide; nitric oxide; obesity; type 2 diabetes; ZDF rats

Zucker diabetic obese (ZDF) rats, which develop severe obesity associated with insulin resistance, hyperinsulinemia, and dyslipidemia, represent a genetic model of type 2 diabetes. Hydrogen sulfide (H_2S)-releasing compounds and activation of the endogenous sulfide signaling pathway appear to represent a novel approach in the treatment of cardiovascular and metabolic disorders. H_2S may also trigger compensatory vasoactive effects to balance pathologically increased vascular tone, as we confirmed in a female non-obese hypertriglyceridemic rats, where increased H_2S production balanced nitric oxide (NO) deficiency to maintain vascular function[1]. This study investigated the involvement of H_2S and NO pathways in vascular dysfunction in ZDF rats and evaluated the effects of treatment with zofenopril, a sulfhydryl-containing angiotensin converting enzyme inhibitor.
The experiments were carried out using 16 weeks old obese ZDF (fa/fa) rats and their lean controls (fa/+). Obese ZDF rats (n=16) were randomly divided into two experimental groups (n = 8 in each group): obese control and obese treated with zofenopril. Zofenopril was administered in food at the final dose of 15 mg/kg/day for 4 weeks. For the intestinal glucose tolerance test (IGTT), blood glucose was determined after *i.p.* administration of a 50 % glucose solution (2 ml/1000 g of b.wt.) following overnight fasting. Cardiometabolic parameters, vascular reactivity of isolated thoracic aorta, NO-synthase (NOS) activity, and expression of H_2S-producing enzymes were assessed.
Compared to lean ZDF controls, 20-week-old obese ZDF rats had mild hypertension associated with cardiac hypertrophy, glucose intolerance, and increased adiposity, accompanied by hypercholesterolemia, hypertriglyceridemia, and decreased plasma H_2S levels. Moreover, in obese diabetic ZDFs, endothelial dysfunction was noted, which was related to the anti-relaxant effect of endogenously produced H_2S which was associated with dysregulation of NO signaling. Zofenopril administration reduced systolic blood pressure, attenuated cardiac and renal hypertrophy, but did not alter adiposity, which was consistent with a further increase in plasma triglycerides. In addition, administration of zofenopril normalized NOS activity, predominantly the endothelial isoform and eliminated endothelial dysfunction. This was also related to the restoration of the pro-relaxant effect of H_2S and corresponded to the restoration of plasma H_2S levels.
In conclusion, despite the insufficient effect of zofenopril on metabolic changes, dyslipidemia and adiposity, its administration to diabetic obese rats had beneficial effects on the cardiovascular system - it alleviated hypertension, cardiac remodeling and endothelial dysfunction. The protective vasoactive effects of zofenopril were associated, in addition to its action as angiotensin-converting enzyme inhibitor, also to a significant extent with the activation of the sulfide signaling pathway.

The study was supported by APVV-24-0150, VEGA 2/0131/26.

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P8 – Poster 8

Unlocking neuroprotective effect of carboxyhemoglobin (COHb) in Parkinson's disease: impact on erythrocytes



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Keywords: Parkinson's Disease (PD); Red Blood Cells (RBC); Carboxyhemoglobin (COHb); α-synuclein (α-Syn); Redox Homeostasis; Neuroprotection.

Parkinson's disease (PD), the second most prevalent neurodegenerative disorder, affects over 8.5 million individuals globally[1]. It is characterized by the pathological accumulation of α-synuclein (α-Syn) and the progressive loss of dopaminergic neurons[2].
While α-Syn pathology is well studied in the brain, red blood cells (RBCs) have emerged as a peripheral source of α-Syn, where it is among the 20 most abundant proteins[3]. PD patients exhibit elevated oxidative stress and higher levels of oligomeric α-Syn in RBCs and in RBC-derived extracellular vesicles (EVs), suggesting a possible role for peripheral α-Syn in disease propagation[4,5].
Carbon monoxide (CO), an endogenously produced gas via heme degradation, exerts cytoprotective effects through anti-inflammatory, antioxidant, and anti-apoptotic pathways in several tissues[6]. Importantly, endogenous CO binds to hemoglobin (Hb) in RBCs to form carboxyhemoglobin (COHb), with COHb levels rising under pathophysiological stress[7]. Moreover, experimental and epidemiological evidence suggests a neuroprotective role of CO in PD, including CO-mediated preservation of dopaminergic neurons and reduced disease incidence among smokers, who typically exhibit elevated basal COHb levels[8,9]. However, the precise mechanisms underlying these effects remain poorly understood.
We sought to investigate the potential protective role of carboxyhemoglobin (COHb) in PD by assessing its impact on RBCs, namely on redox homeostasis and α-Syn post-translational modifications (PTMs). By elucidating these pathways, we aim to identify novel peripheral mechanisms involved in PD pathogenesis and evaluate the therapeutic potential of COHb.
RBC were treated with exogenous CO for promoting COHb) formation followed by exposure to a prooxidant stimulus in order to assess the potential antioxidant role of COHb. Actually, COHb revealed to limit the generation of reactive oxygen species (ROS) and lipid peroxidation in RBCs and increased protein glutathionylation, a reversible redox-PTM that has been described to confer protection against oxidative stress damage10. Given the chronic exposure of smokers to CO, RBCs isolated from smokers and non-smokers were compared to determine whether long-term CO exposure influenced antioxidant responses. However, no significant differences were observed between the two groups under the experimental conditions tested. RBC secretome, containing RBC-derived EVs, was used in BV-2 microglial cell model to assess its influence on inflammatory response, but no significant differences were seen.
Future studies should focus on isolation and characterization of the molecular cargo and biological functions of RBC-derived EVs and investigate the metabolic effects of COHb formation in RBCs metabolism to determine whether these changes contribute to protective responses or disease-related pathways.

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P9 – Poster 9

Preventing cardiovascular intervention complications with Metal-Organic Framework Nitric Oxide Releasing Catheters



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Keywords: metal-organic framework (MOF), extrusion, cardiac catheter, thrombosis, PET-CT scanning

Globally, ~46 million cardiac catheterization procedures are performed annually. Unfortunately, even routinely performed invasive procedures carry associated risks and complications[1], the most common being vasospasm, catheter induced thrombosis and infection. Endogenous nitric oxide (NO) normally mitigates these responses, but patients with cardiovascular disease often exhibit endothelial dysfunction resulting in a reduced capacity to generate NO. Herein, we demonstrate that our metal-organic framework (MOF) containing catheter can mimic the body's natural mechanism by releasing NO directly from the device surface into the blood vessel, thereby alleviating these complications.

The CPO-27 (Zn, Mg, Ni) family of MOFs can act as a reservoir for nitric oxide (NO), storing large amounts of NO in the microporous structure and releasing it in a controlled manner when exposed to water[2]. Despite the challenges encountered in processing powdered MOFs, we successfully extruded them into medical-grade polymer tubing, producing catheter-sized devices. Advanced materials characterisation confirmed that our extrusion process achieved a homogeneous distribution of MOF particles throughout the polymer matrix. Given that incorporating MOFs in polymers affects gas diffusion, we describe the methods used to quantify NO storage and release and to optimise processing parameters to achieve clinically relevant performance.

Biocompatibility of the NO-MOF-polymer formulations was confirmed using a selection of toxicological assays. To evaluate biological activity of the NO-eluting tubing, we designed holders for a wire myography system that enabled direct assessment of vasodilation in porcine coronary and radial arteries. Inhibition of platelet activation and adhesion was measured via aggregometry with human platelet-rich plasma. Antimicrobial efficacy was evaluated using standard ISO methods and using a bespoke catheter flow model. A rat jugular vein model of catheter-associated thrombosis was developed to assess the thromboprotective effects of the NO-eluting tubing. *Ex vivo* histology demonstrated a reduction in thrombus after 10 days, with blood analysis showing lower systemic levels of thrombotic and inflammatory biomarkers. Preliminary *in vivo* PET-CT findings support these observations. Our prototype device will be available for demonstration[3].

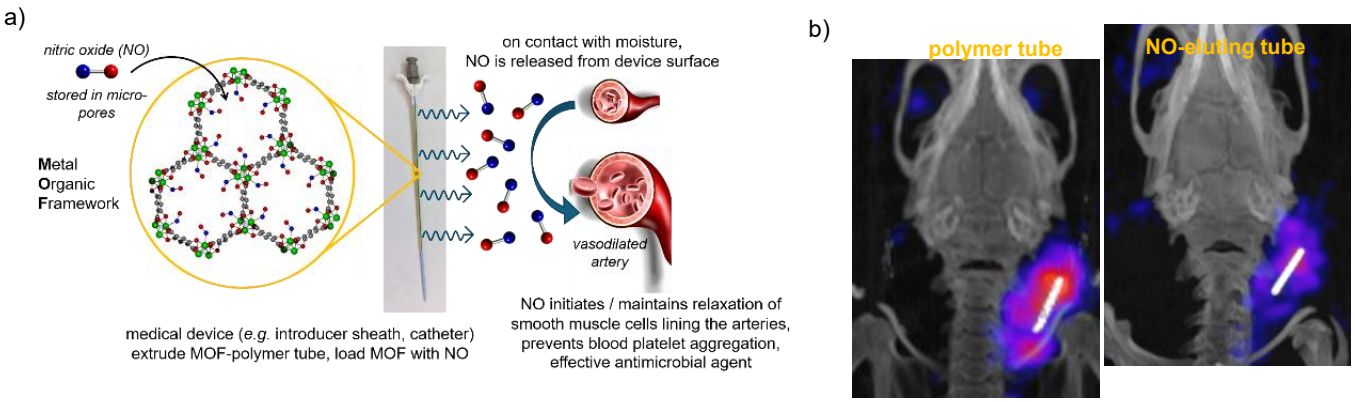


Figure 1: a) Schematic illustrating the functional mechanism of the medical device, b) reconstructed images from PET-CT scans of inserted tubing in jugular vein, highest levels of thrombus (red) in polymer only tubing.

Acknowledgements

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P10 – Poster 10

Dual Gasotransmitter Delivery from a Ti-MOF: First Report of Combined NO and H_2S Adsorption and Release

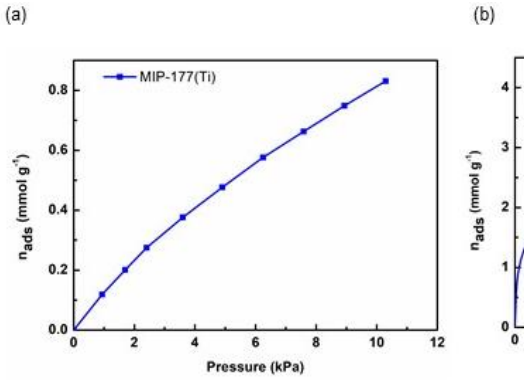


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Keywords: MIP-177(Ti); Metal-organic frameworks (MOFs); Hydrogen Sulfide; Nitric Oxide; gasotransmitters.

Hydrogen sulfide (H_2S) and nitric oxide (NO) are endogenous gasotransmitters with recognized therapeutic potential across a wide range of biomedical applications. Both molecules share overlapping physiological roles, and emerging studies suggest that they can act synergistically, broadening their therapeutic relevance. Metal-organic frameworks (MOFs) have been previously explored as platforms for the delivery and controlled release of individual gasotransmitters, owing to their high porosity and tunable structural and chemical properties, which allow the accommodation of guest molecules within their structure.[1] In this study, we propose, for the first time, a MOF capable of the simultaneous loading and controlled release of two gasotransmitters, H_2S and NO, using the titanium-carboxylate framework MIP-177(Ti). This MOF was selected as a carrier due to its robustness and the presence of active sites capable of accommodating gas molecules.[2] Adsorption studies demonstrated that MIP-177(Ti) can successfully load both H_2S and NO (Figure 1), including the co-delivery of both gases, and their release in buffer was successfully quantified. Ongoing studies include *in situ* FTIR analysis to elucidate the loading mechanism of both gases within the framework, as well as *in vitro* assays with cells and bacteria to investigate the synergistic effects and therapeutic potential of both gases in combination.



(c)

Material	NO mmol/g	H_2S mmol/g
MIP-177	1.15	0.57

Figure 1- Gas adsorption behaviour of the investigated material at 25 °C. (a) H_2S adsorption measured volumetrically at 25 °C, showing the adsorption capacity of the material towards H_2S as a function of pressure; (b) NO adsorption kinetics measured gravimetrically at 25 °C, illustrating the uptake of NO as a function of time; and (c) dual-gas adsorption capacity.

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P11 – Poster 11

A Fe-MOF-Based Dual-Delivery Platform for Combined Nitric Oxide Gas Therapy and 5-Fluorouracil Chemotherapy



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Keywords: Cancer, chemotherapy, gas therapy, metal-organic frameworks, nitric oxide

Despite having several conventional therapies at disposal, cancer is still considered one of the deadliest diseases. This is due to the unique complexity of cancer, which can affect treatment; for example, tumour mutations can reduce the effectiveness of chemotherapeutic drugs such as 5-fluorouracil (5-FU) [1]. Gas therapy has emerged as a promising strategy to overcome these limitations, as endogenous gasotransmitters like nitric oxide (NO) have been shown to sensitise cancer cells to chemotherapy, thereby enhancing drug efficiency [2]. Building on this synergy, this research aims to develop an innovative framework for cancer therapy using iron-based metal-organic frameworks (Fe-MOFs), specifically MIL-100(Fe), as a single platform capable of the controlled co-delivery of NO gas and 5-FU directly at the tumour site [3]. To improve dispersion and enhance gas retention, the MOF surface was further coated with polyethylene glycol (PEG). Two loading strategies were used to incorporate 5-FU into the framework: in situ and post-synthetic loading. Results show that the release of both NO and 5-FU can be quantified simultaneously, with both molecules exhibiting sustained release over several hours. These findings support the potential of Fe-MOFs as a dual-delivery system capable of combining gas therapy and chemotherapy to improve cancer treatment outcomes. Future work will focus on in vitro validation using cancer cell lines relevant to 5-FU-based treatment to assess the therapeutic performance of the combined system.

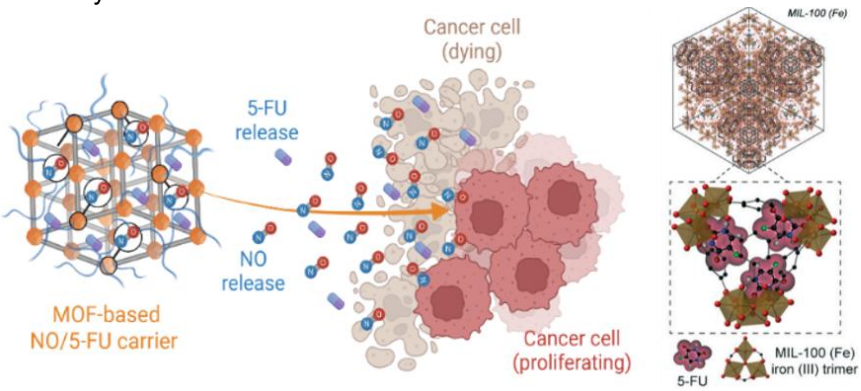


Figure 1. Proposed mechanism for gas delivery using gas-loaded MOFs, highlighting gas encapsulation within the MOF structure, controlled release, and localised cytotoxic effects on tumour cells (left); Representation of the MIL-100(Fe) structure, and how the 5-FU drug can be stored within the MOF's structure (right).

Acknowledgements
This research was developed in the scope of the Projects FCT-UIDB/04028/2025 and FCT-UIDP/04028/2025 (CERENA) and was financed by Portuguese funds through FCT/MCTES. Centro de Química Estrutural was funded by Fundação para a Ciência e a Tecnologia, I.P./MCTES through national projects UID/00100/2025 (10.54499/UID/00100/2025) and UID/PRR/00100/2025 (10.54499/UID/PRR/00100/2025). Institute of Molecular Sciences is an Associate Laboratory funded by Fundação para a Ciência e a Tecnologia through project LA/P/0056/2020 (10.54499/LA/P/0056/2020).

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