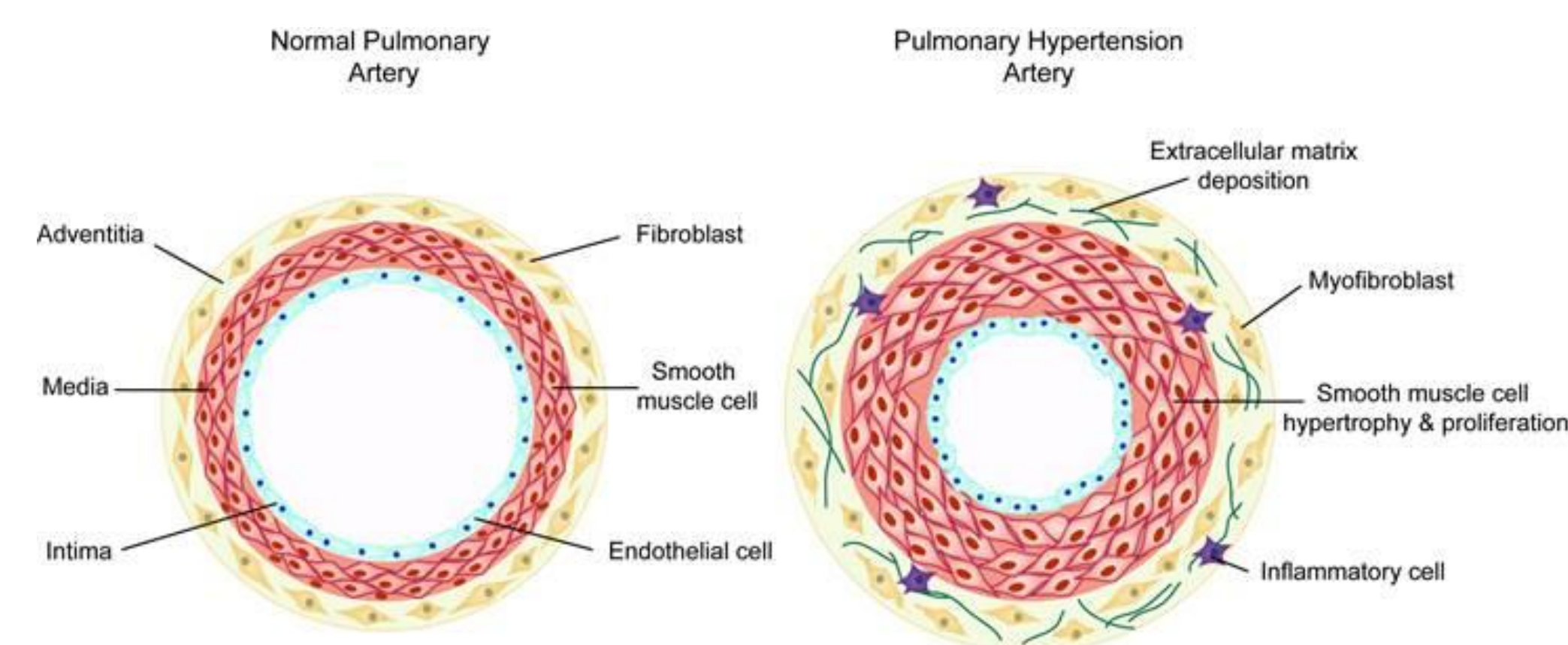


Background

Bardoxolone Methyl (BARD)

- BARD is presently being evaluated in Phase 2 and Phase 3 clinical trials for safety and efficacy in patients with pulmonary hypertension.
 - LARIAT (NCT02036970) Phase 2 trial in pulmonary hypertension (PH).
 - CATALYST (NCT02657356) Phase 3 trial in connective tissue disease-associated pulmonary arterial hypertension (CTD-PAH).
- Initial data from LARIAT, presented at the CHEST Annual Meeting in 2015, suggested that BARD increased 6-minute walk distance (6MWD) in patients with pulmonary arterial hypertension.¹

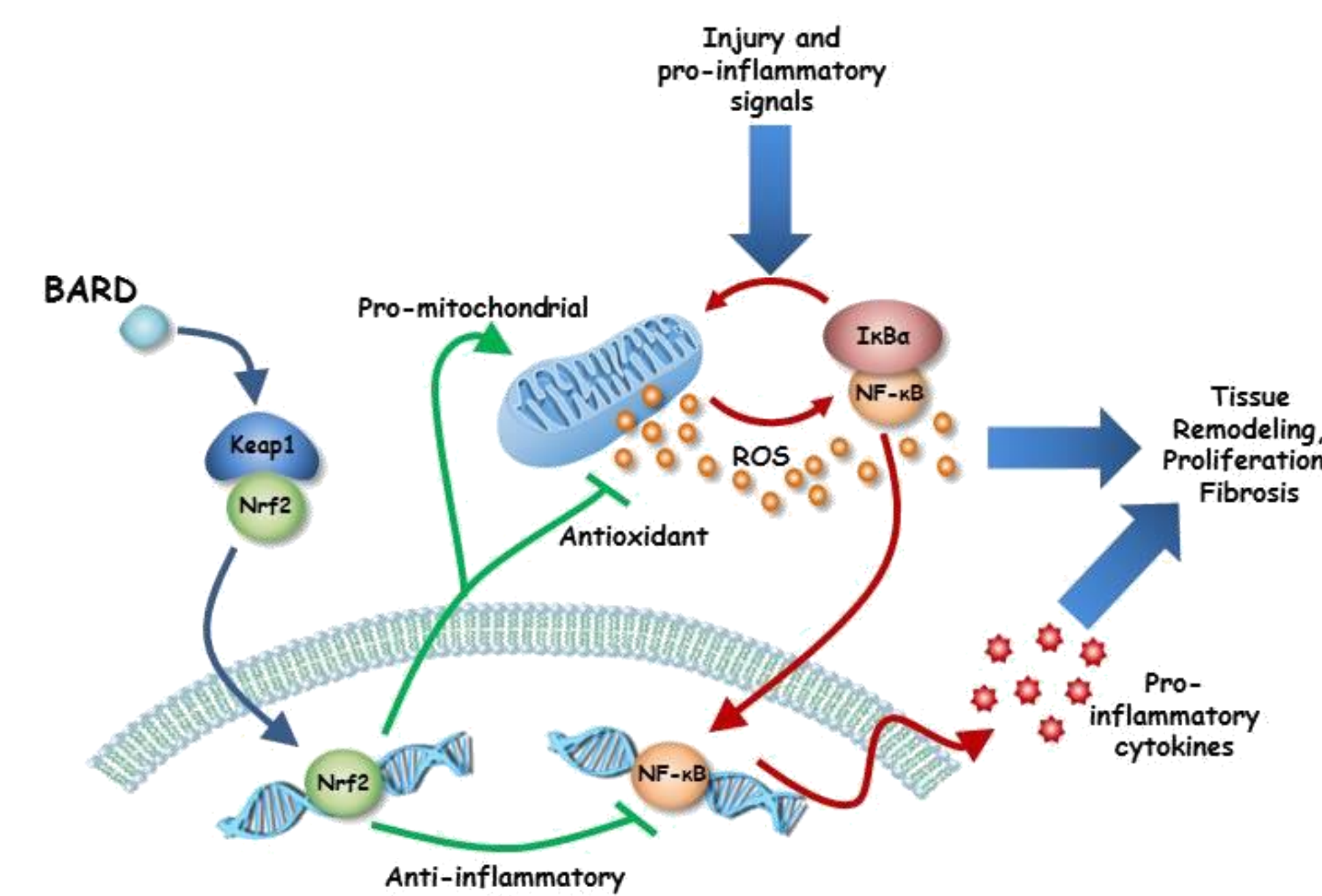
Vascular Remodeling



- Pathogenic remodeling of the pulmonary vasculature in PH and PAH increases vascular resistance.²
- All 3 layers of the arterial wall (adventitia, medial, intima) are involved in the process.²
- A prominent feature of PAH vascular remodeling is medial thickening with smooth muscle cell proliferation, which can be triggered by hypoxia, inflammation, and growth factors.³

Mechanism of Action

- BARD binds to Keap1, activates Nrf2, and inhibits NF-κB.
 - Nrf2 activation increases antioxidant and detoxification proteins.
 - NF-κB inhibition reduces pro-inflammatory mediators.
- The combined effect has the potential to:
 - Inhibit inflammatory signaling⁴
 - Suppress ROS production and signaling⁴
 - Inhibit abnormal proliferation^{5,6}
 - Decrease proteins related to fibrosis and tissue remodeling⁷
 - Improve mitochondrial function^{8,9}



Study Rationale

- BARD's mechanism of action may potentially affect several key characteristics of pulmonary hypertension including mitochondrial dysfunction, inflammation, and abnormal proliferation.
- We investigated the effect of BARD on human pulmonary arterial smooth muscle cell (PASMC) proliferation cultured under hypoxic conditions, an established trigger of vascular remodeling.

Methods

Cell Culture

- Primary human pulmonary arterial smooth muscle cells (PASMCs, Lonza) were cultured under hypoxic (2% O₂, 5% CO₂) or normoxic (21% O₂, 5% CO₂) conditions. After 24h cells were treated with BARD or DMSO (vehicle) and returned to the appropriate hypoxic/normoxic conditions for 72h
- Hypoxia was maintained using a ProOx P110 oxygen controller placed inside a humidified incubator. Control cells grown under normoxic conditions were placed in the same humidified incubator but outside the hypoxia chamber

Proliferation and Viability Assays

- Proliferation was measured by BrdU incorporation (Sigma). Cell density measurements were made using CyQuant assay (Life Technologies). Cell toxicity was determined using CellTox Green Cytotoxicity assay (Promega).

Western blot and qPCR

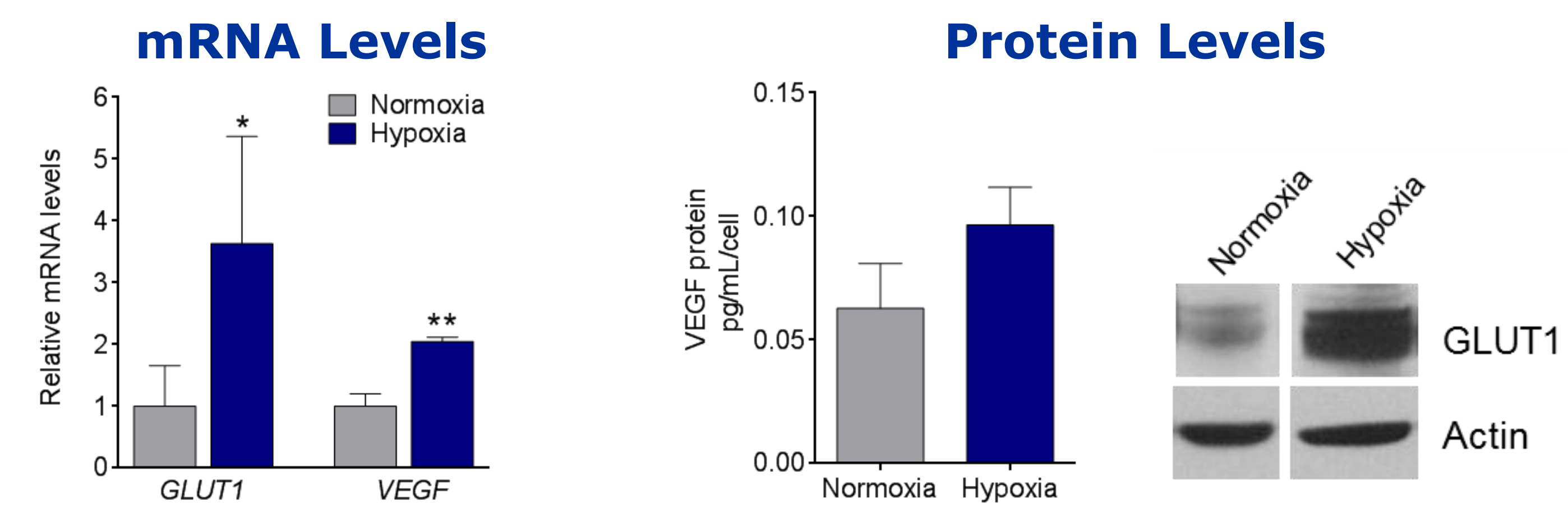
- GLUT1, VEGF, NQO1, GCLM, and CCL2 mRNA levels were measured by reverse transcription and quantitative PCR
- VEGF protein levels were assessed by ELISA (R&D Systems) and GLUT1 protein levels were assessed by western blot
- CCL2 protein levels were determined using homogeneous time-resolved fluorescent (HTRF) assay (Cisbio).

Statistical Analysis

- Statistical significance was determined using Student's t-test or one-way ANOVA, as appropriate
- * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001.

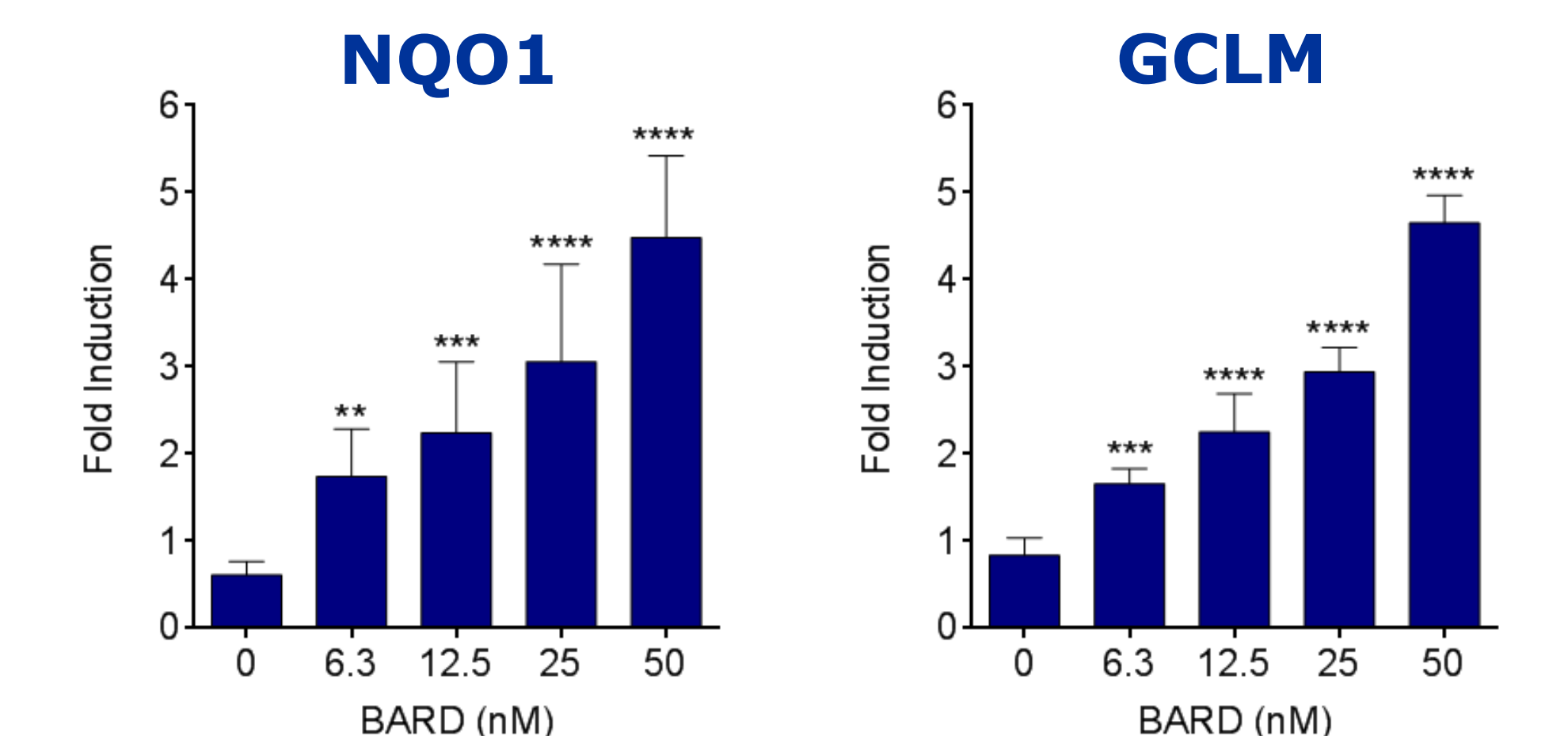
Results

Hypoxia Increases HIF1 Activity in PASMCs



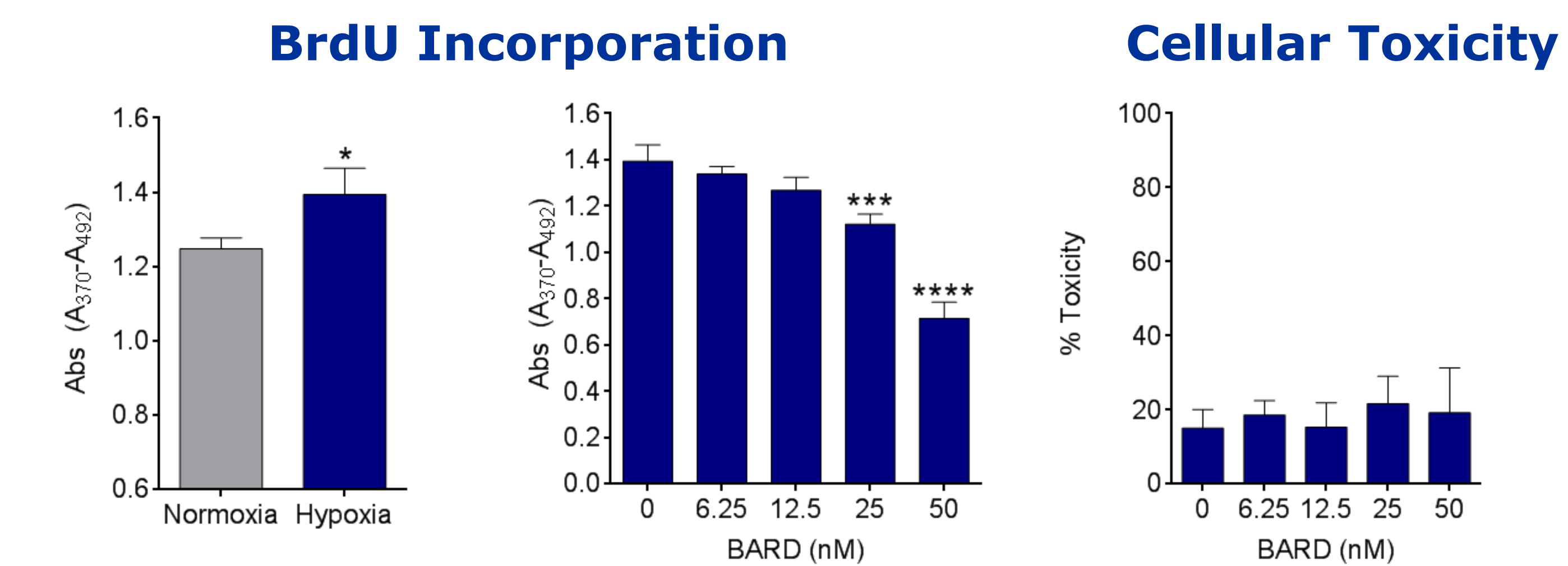
- Expression of GLUT1 and VEGF (HIF1 target genes) is increased by hypoxia.

BARD Activates Nrf2 in PASMCs

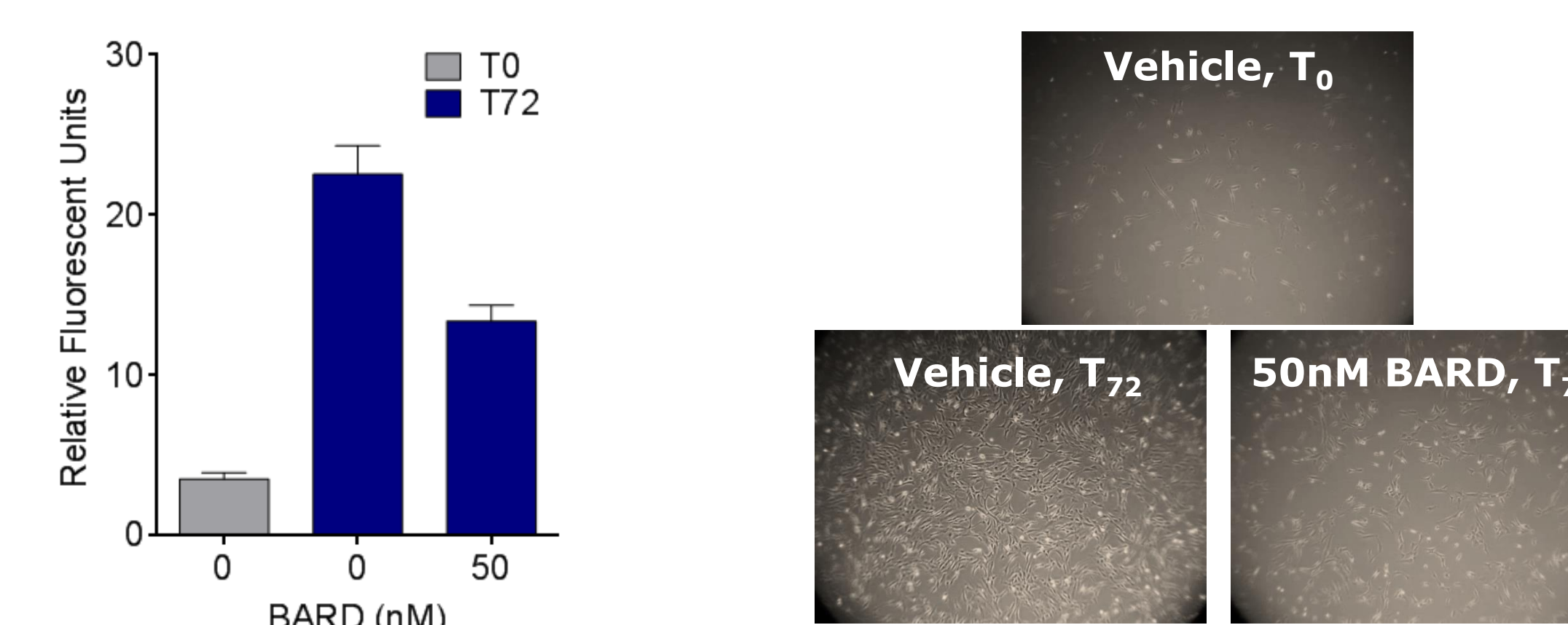


- BARD increased expression of NQO1 and GCLM (Nrf2 target genes).

BARD Inhibits PASMC Proliferation in Hypoxia

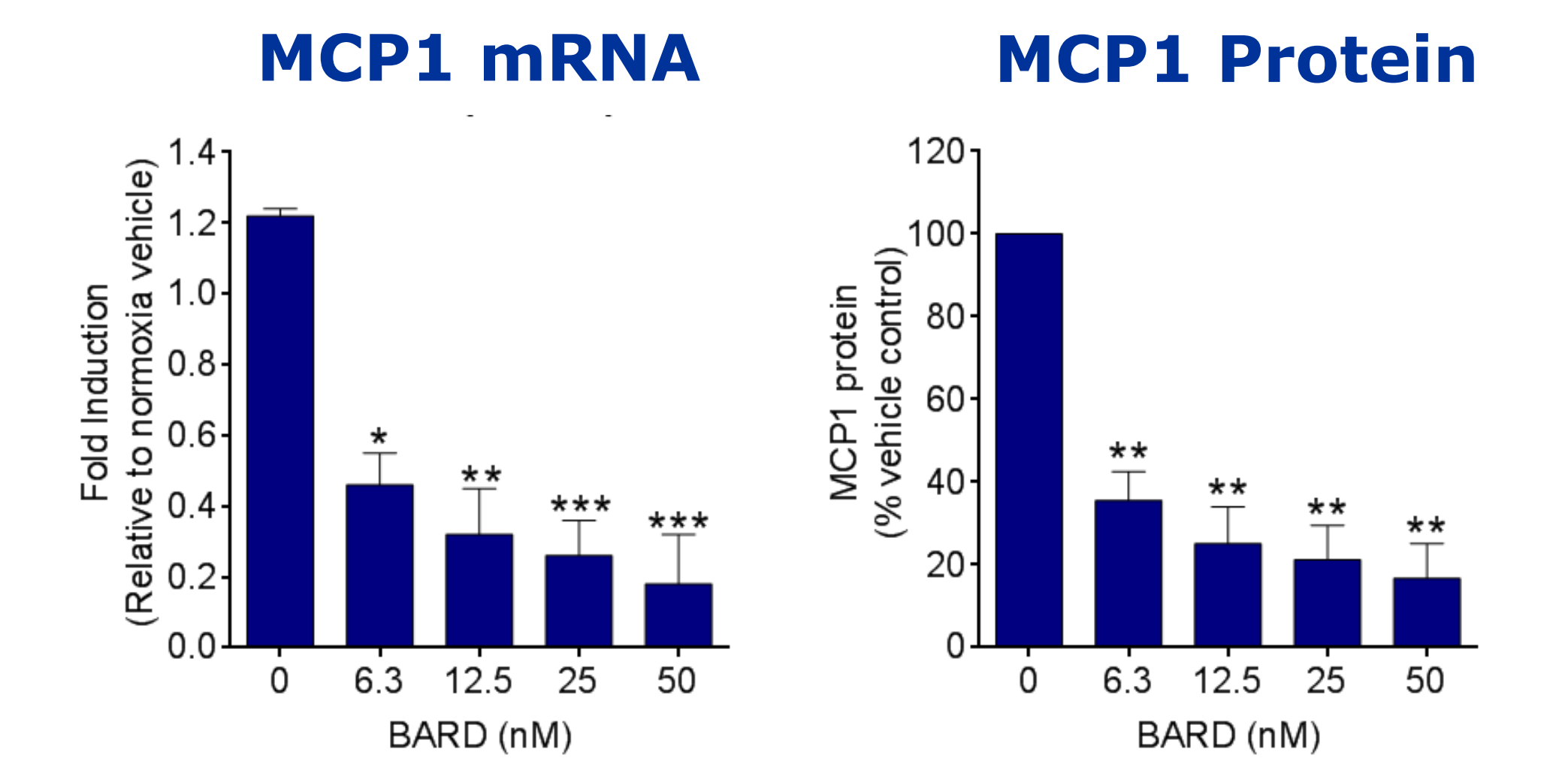


Cell Number



- Hypoxia increased PASMC proliferation (BrdU incorporation).
- BARD reduced PASMC proliferation under hypoxic conditions.
- BARD treatment did not increase cellular toxicity.
- BARD treatment (72h; hypoxia) reduces PASMC numbers relative to control

BARD Suppresses MCP1 (CCL2)



- BARD reduced MCP1 (CCL2) mRNA and protein levels.
- MCP1 is a potent activator of monocytes/macrophages and is elevated in PASMCs from patients with IPAH.^{10,11}
- MCP1 produced by PASMCs could potentially cause autocrine stimulated proliferation.¹¹

Conclusions

- Hypoxia significantly increased PASMC proliferation (P < 0.05).
- BARD significantly reduced PASMC proliferation under hypoxic conditions (P < 0.001).
- BARD increased expression of Nrf2 target genes and suppressed expression and secretion of MCP1 (P < 0.001).
- BARD has the potential to improve vascular remodeling associated with pulmonary hypertension.

Disclosures

- W. Christian Wigley, Gregory A. Miller, and Lyndsey McCauley are employed by and have a financial interest in Reata.
- Deborah A. Ferguson is a consultant to and has a financial interest in Reata.

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