AATS 93RD ANNUAL MEETING 2013

AMERICAN ASSOCIATION FOR THORACIC SURGERY
We Model Excellence

Inhibition of the Hedgehog (Hh) Signal Transduction Pathway Significantly Suppresses Survival of Malignant Pleural Mesothelioma Cells in vitro

Dao M. Nguyen, MD[1], Javier Varona-Santos, PhD[1], Min You, PhD[1], David J Robbins, PhD[1], Medhi Wangpaichitr, PhD[1], Niramol Savaraj, MD[2]

[1]Department of Surgery, [2] Department of Medicine
Miller School of Medicine
University of Miami, Miami, FL

No COI to disclose

SYLVESTER COMPREHENSIVE CANCER CENTER
UNIVERSITY OF MIAMI HEALTH SYSTEM

UHealth UNIVERSITY OF MIAMI HEALTH SYSTEM
UNIVERSITY OF MIAMI MILLER SCHOOL of MEDICINE

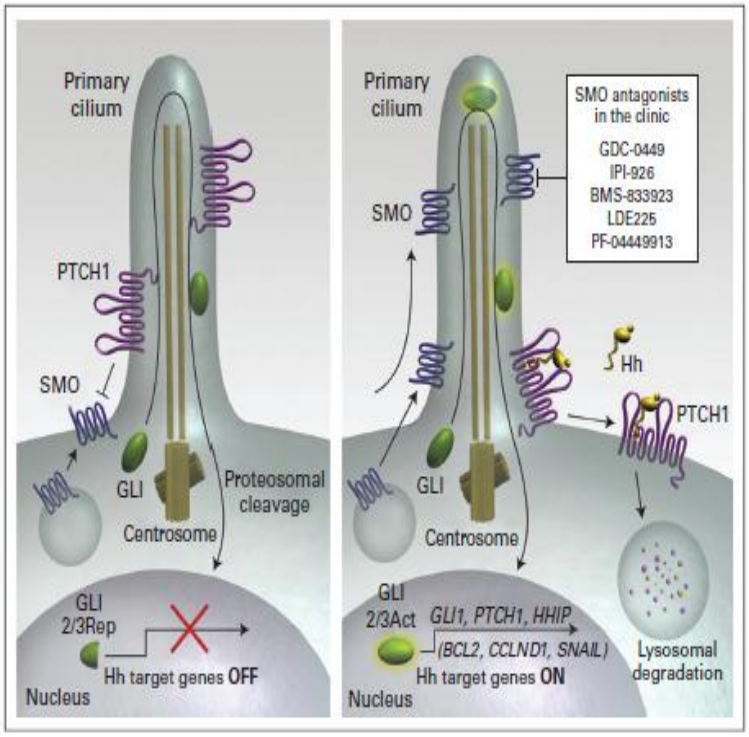
Introduction

Hedgehog (Hh)-mediated signaling network:

- essential for embryonic development and stem cell maintenance
- quiescent in terminally differentiated cells, reactivated in tissue injury/repair

- Pathway activation ⟶

- Oncogenic driver in BCC
- Pathway activation (high Gli1 levels) has been observed in many cancers
- Hh-independent Gli1 overexpression



Targeting of Hh pathway:
Inhibit SMO or Gli function by pharmacologic antagonists

Hedgehog pathway inhibitors (HPIs)

- SMO antagonists: inhibit Hh-mediated SMO activation and suppresses Gli2/3A processing:
  - GDC-0449 (GDC): medicinal chemistry design as selective SMO inhibitor; FDA-approved for metastatic BCC
  - Itraconazole (ITRA): identified by screening of FDA-approved drug library

- Gli antagonists: suppresses Gli transcriptional activity and target gene expression:
  - Specifically designed to block Gli function → GANT61
  - Arsenic Tri-Oxide (ATO): identified by screening of FDA-approved drug library

Repurposing of ITRA or ATO as HPIs for cancer therapy

Abestos → inflammation, tissue injury/repair → activations of many signaling pathways and transcription factors → re-activation of Hh ?? → carcinogenesis of MPM

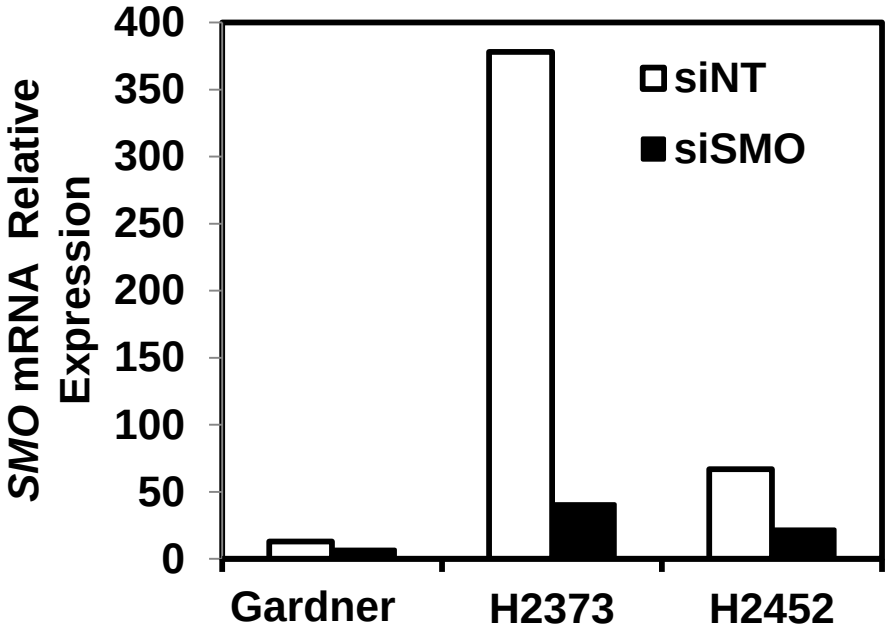
Objectives

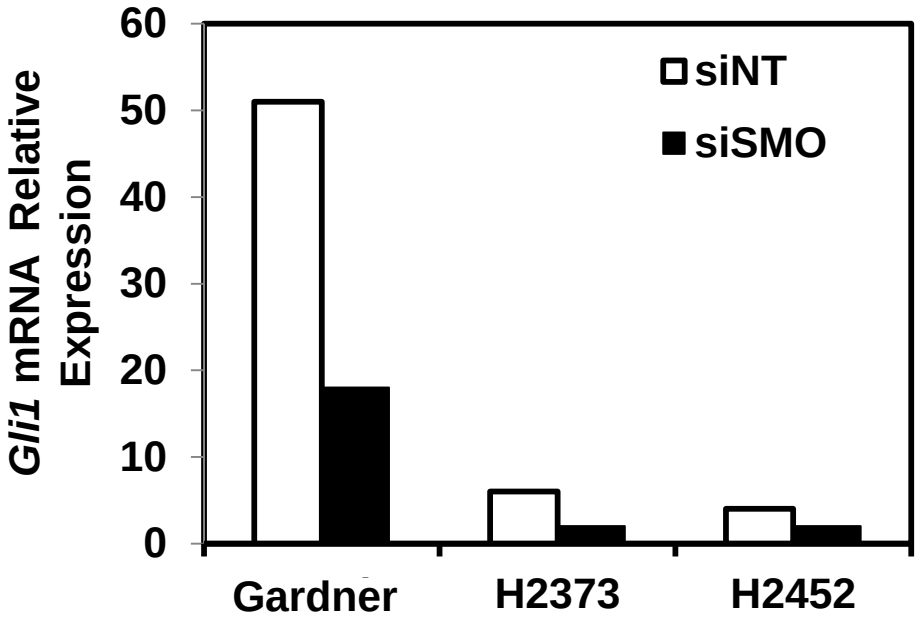
- To determine if Hh pathway is active in MPM cells

- To perform comparative analysis of therapeutic efficacy of selective or repurposed SMO or Gli inhibitors in MPM cells in vitro.

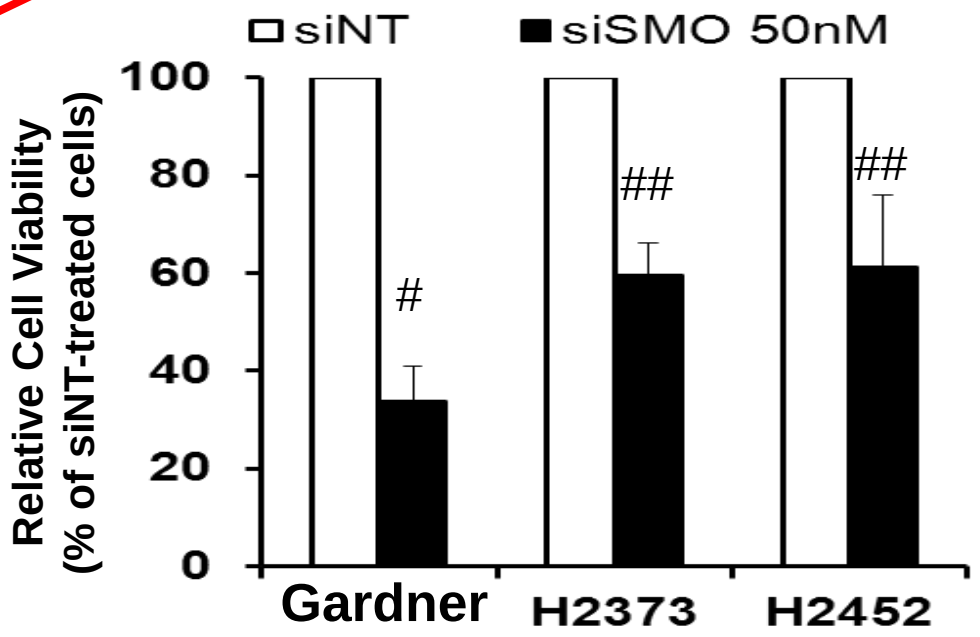
Experimental designs

- Cultured MPM cells
- *Gli1, SMO, GAPDH* gene expression by qRT-PCR
- Evaluation of HPI-mediated Hh pathway inhibition using sHH-Light2 cells /luciferase assay or by qRT-PCR of *Gli* mRNA
- Cell viability/proliferation MTT assay
- Cell death assay with AnnexinV/PI and flow cytometry
- Cell cycle analysis by PI staining and flow cytometry



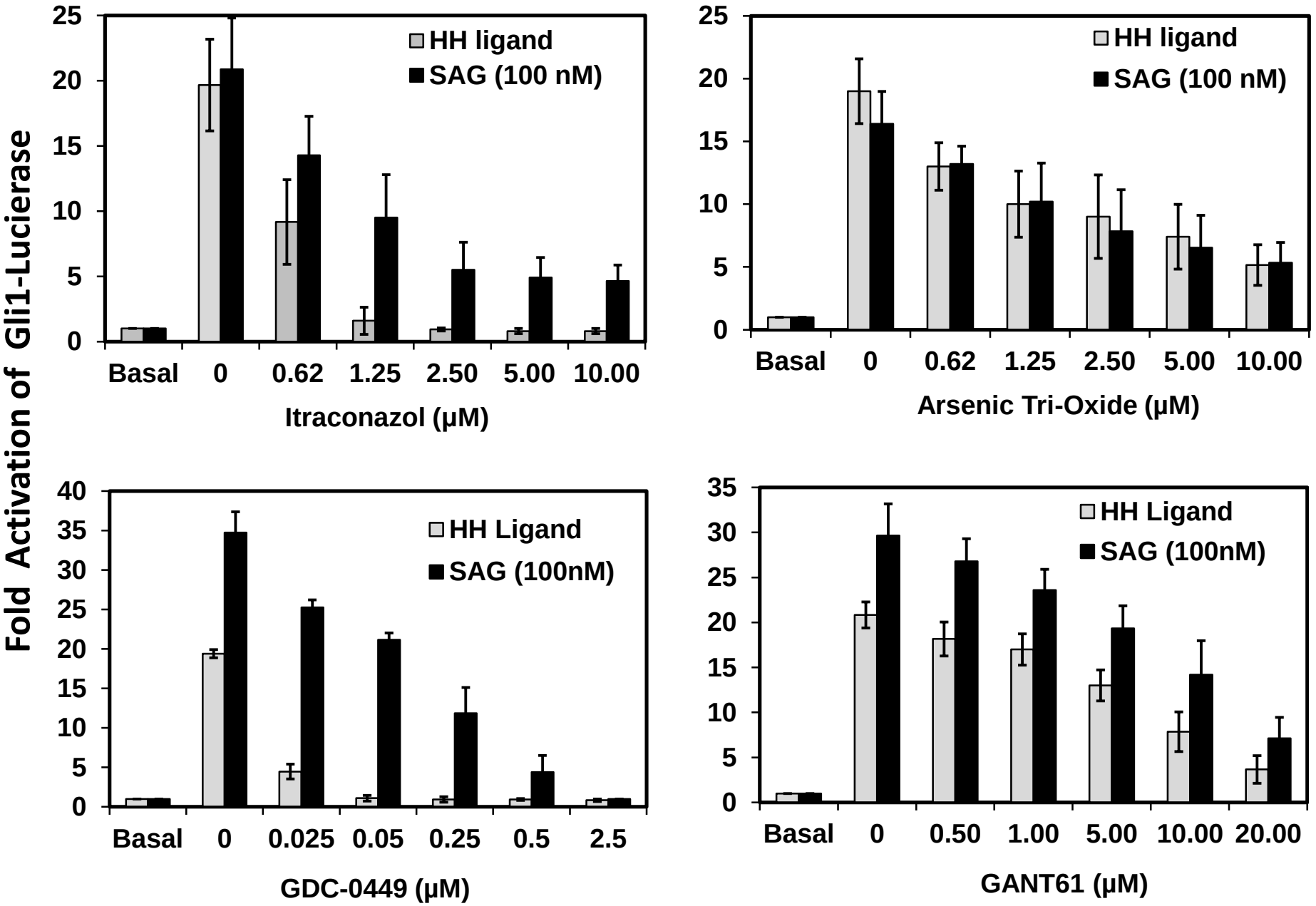


siSMO-mediated depletion of *SMO* mRNA and suppression of *Gli1* mRNA in representative MPM cells
(50 nM siRNA x 72hrs and qRT-PCR)



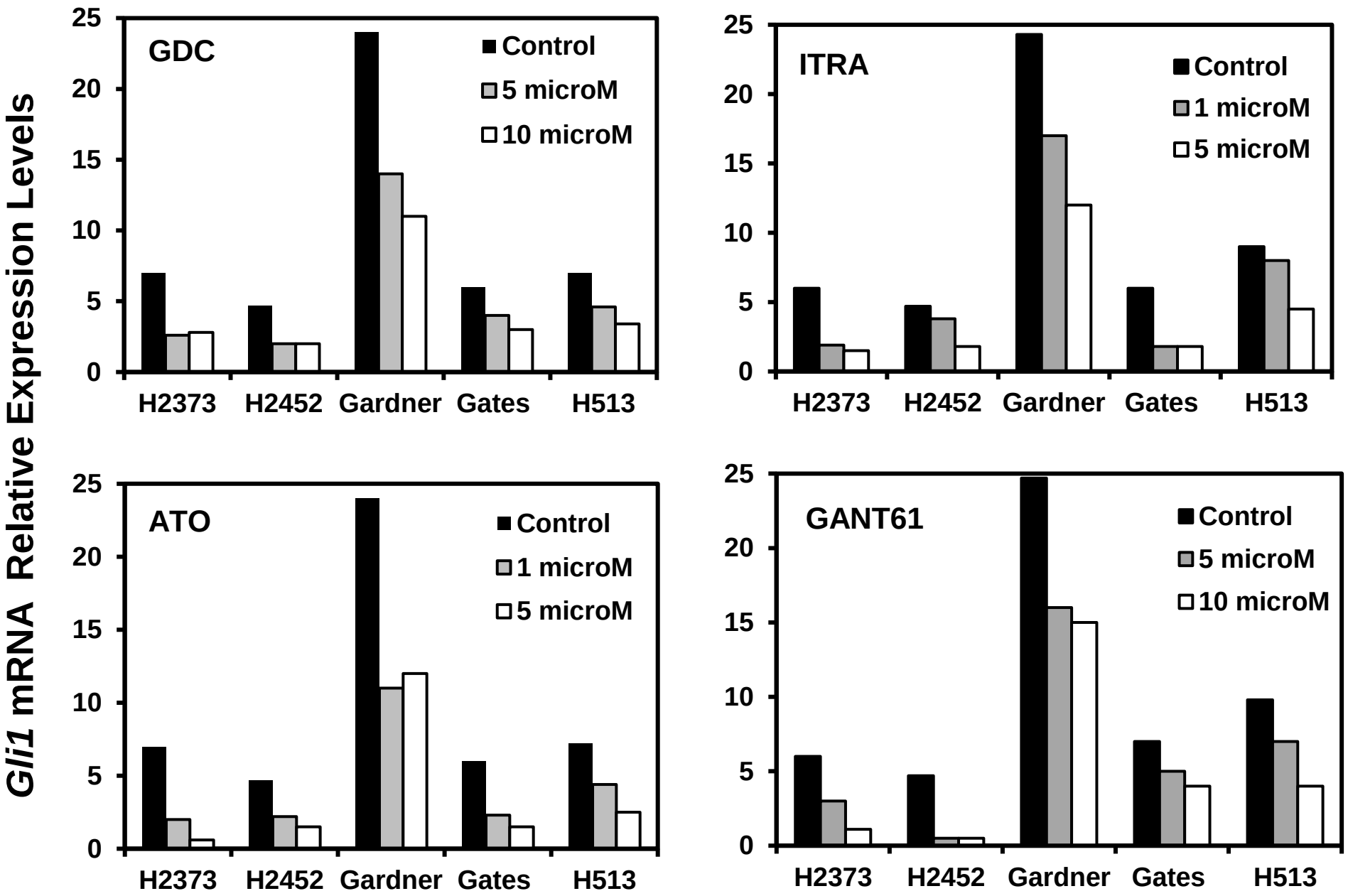
siSMO-mediated significant reduction of cell proliferation/viability
(siRNA transfections and assayed for cell viability by MTT 96 hours later, #: $p<0.001$; ##: $p<0.05$)

HPI-mediated dose-dependent and profound suppression of Hh-mediated signaling in sHH-Light2 reporter cells

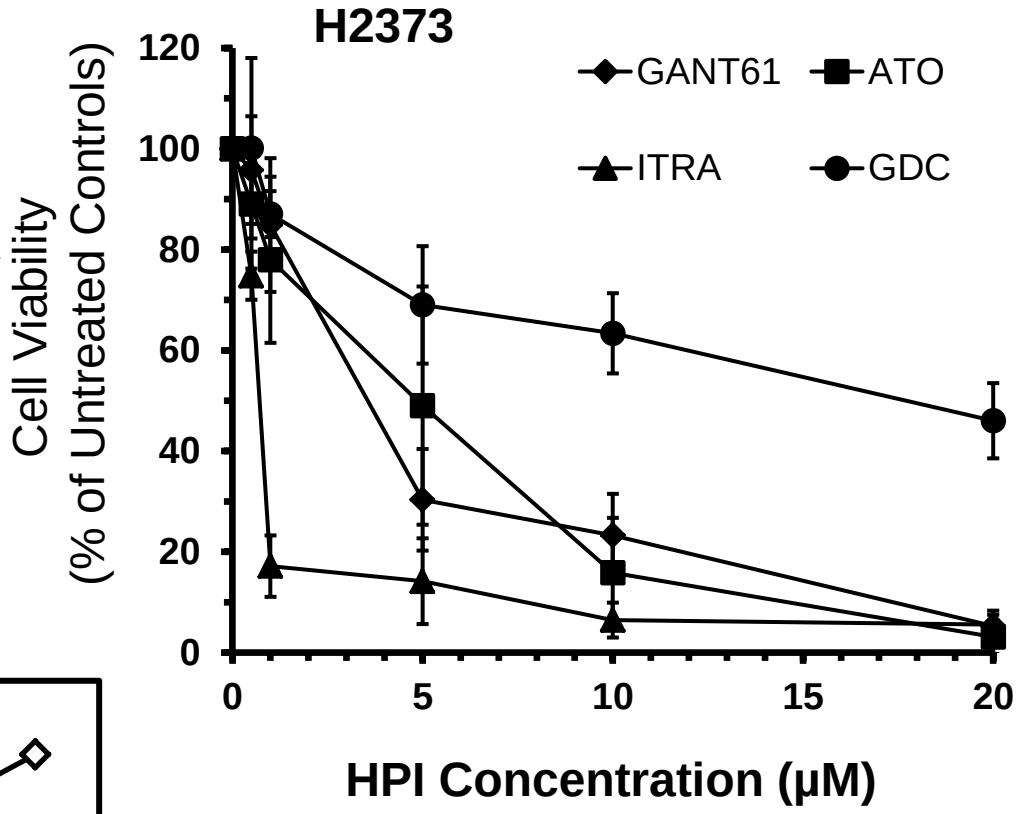


Suppression of Gli mRNA expression by HPIs in MPM cells

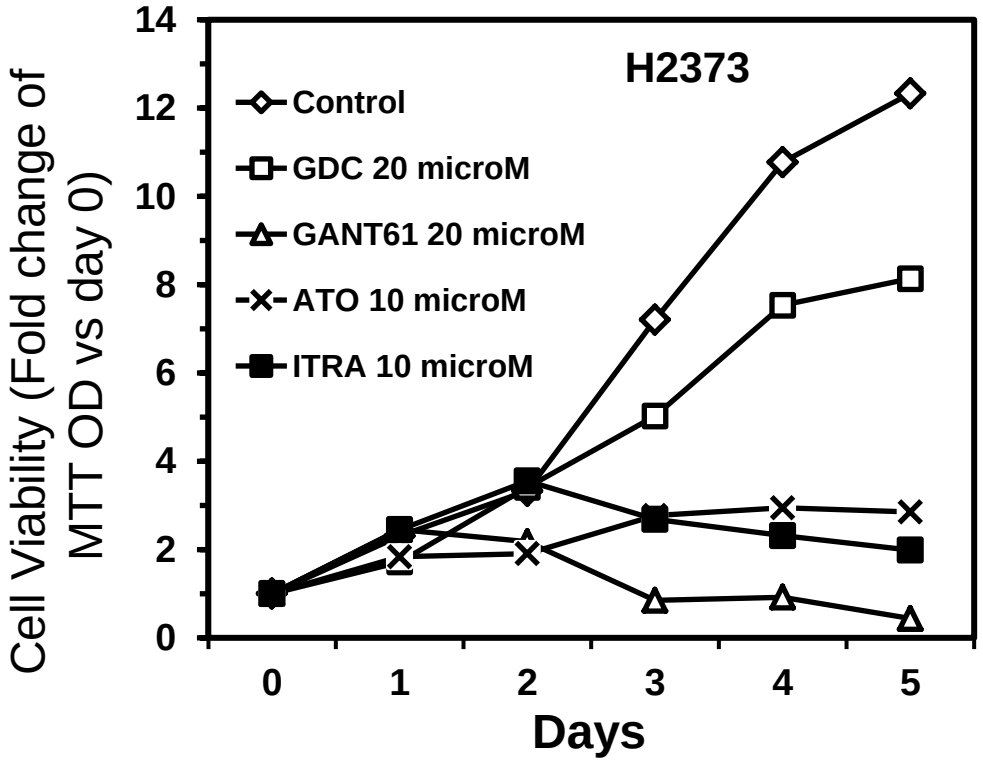
(HPI treatments at indicated concentrations x 24 hours and *Gli* mRNA expression by qRT-PCR)



HPI-mediated dose-dependent reduction of cell viability of representative H2373 MPM cells (dose-response viability curves)

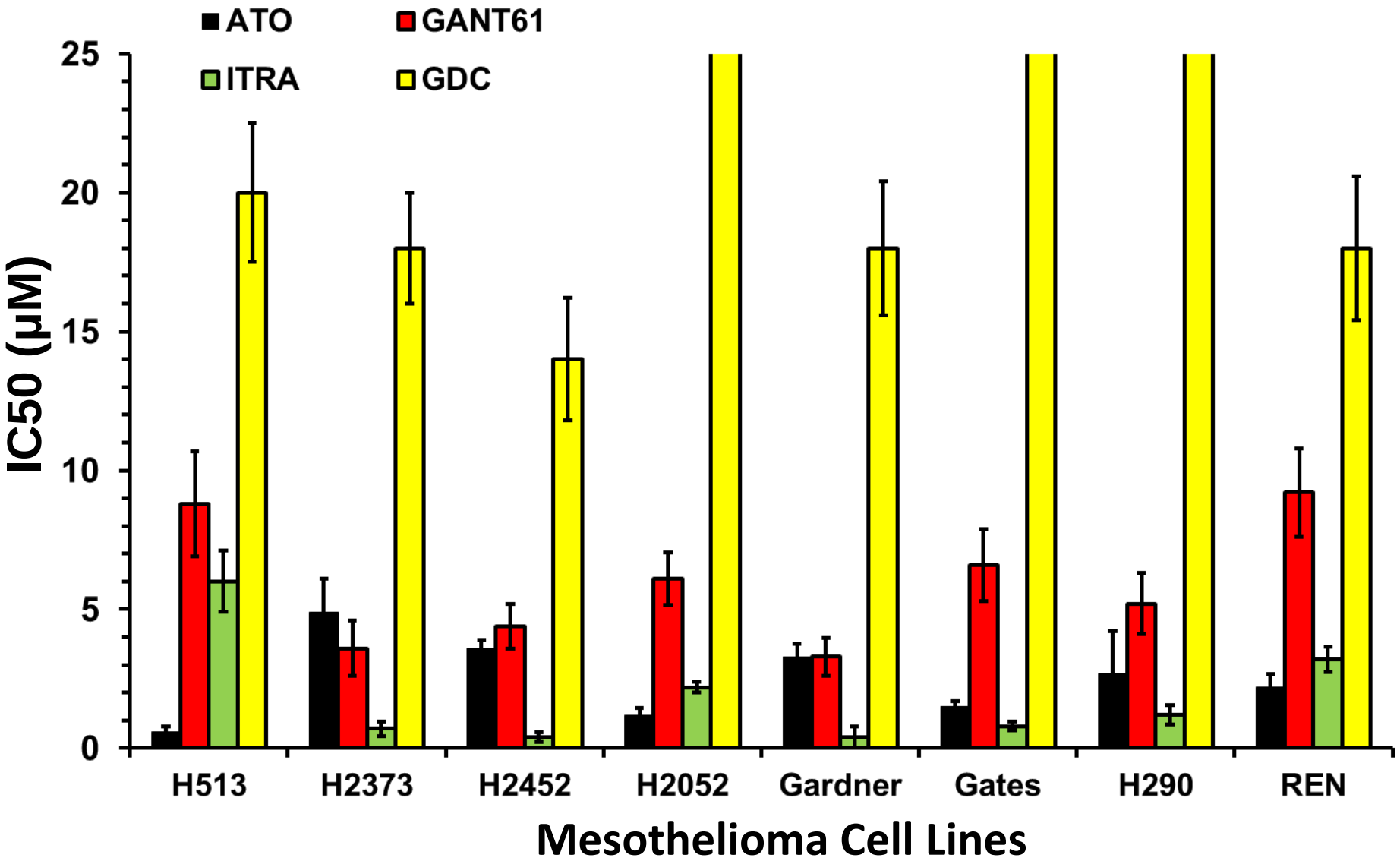


HPI-mediated suppression of H2373 cell growth in vitro (Time-course cell proliferation assay)



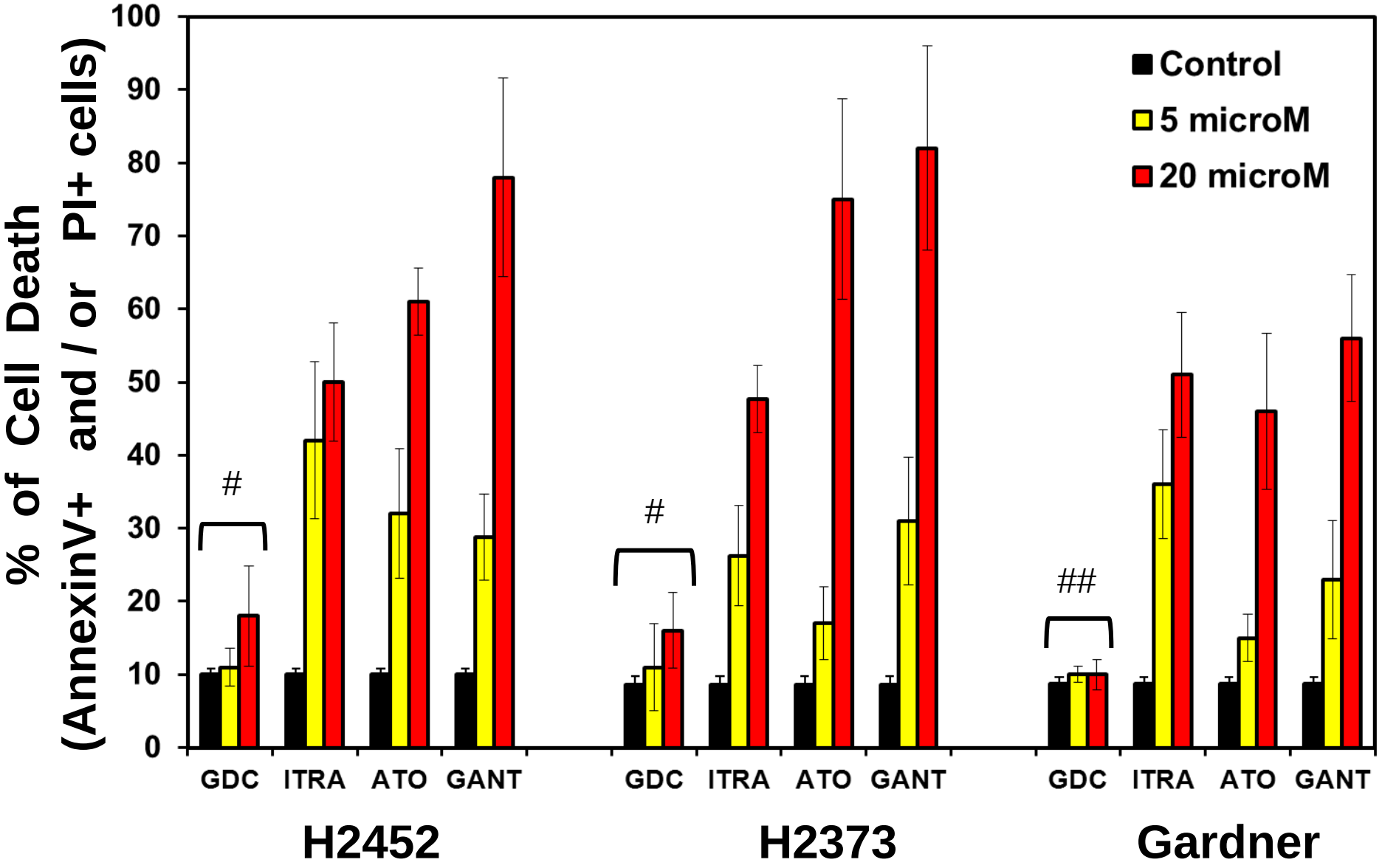
Comparative analysis of growth-inhibitory activities of selective and repurposed HPIs on MPM cells in vitro

(Mean ± SD, n= 4-6)

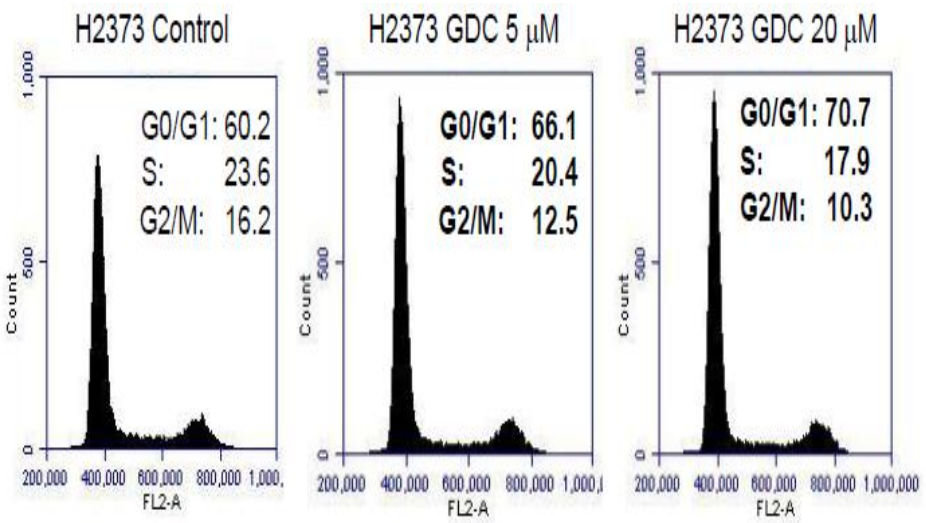


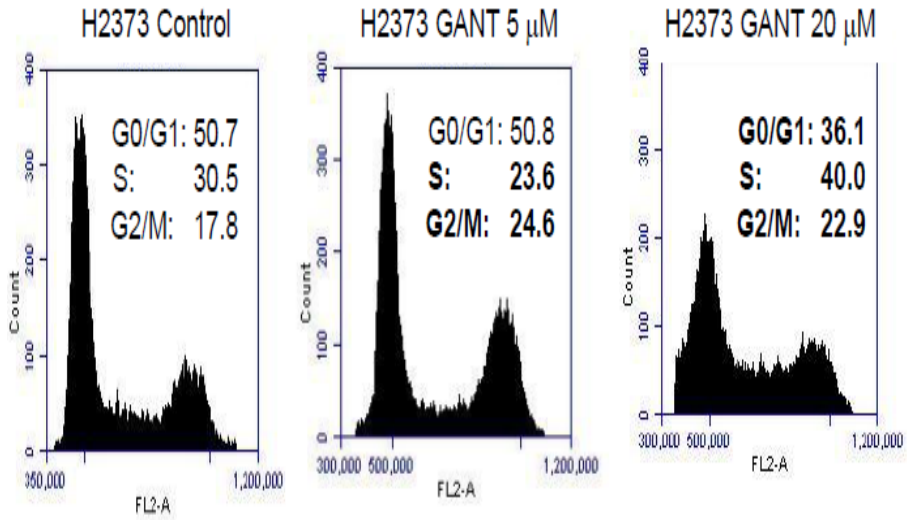
Significant Induction of Cell Death by ITRA, ATO and GANT61 in Representative MPM Cell Lines

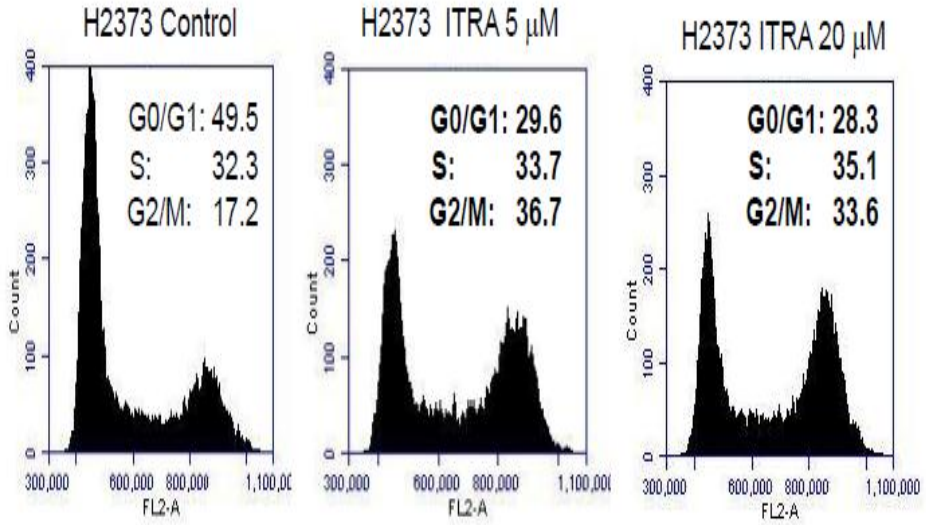
(#: p<0.05 GDC 20 µM vs control; ##: NS)

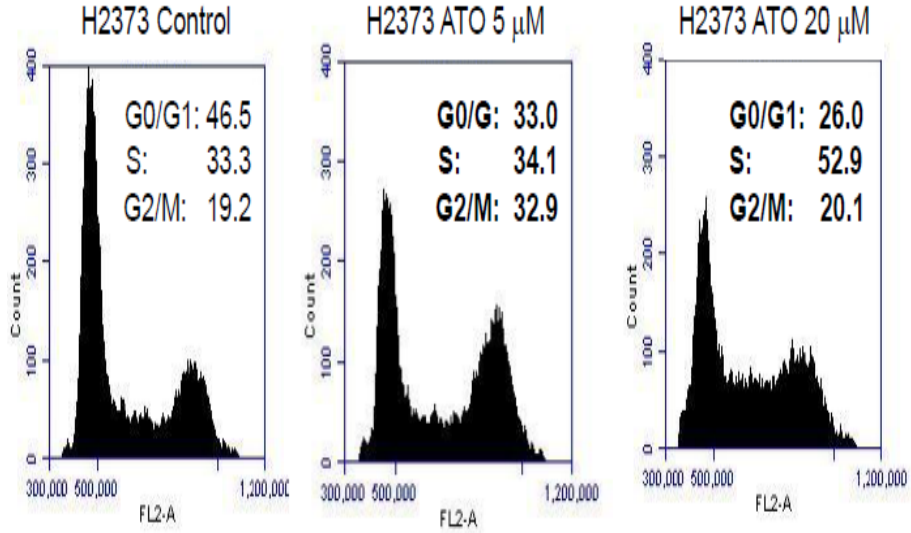


HPI-induced cell cycle arrests at G1/S, S/G2 and G2/M checkpoints









Conclusion

- Hh pathway is active (siSMO-mediated repression of *Gli*) and regulates cell proliferation (siSMO-mediated reduction cell viability)

- HPIs reduce *Gli* mRNA in MPM cells

- HPIs suppress cell proliferation in vitro with GANT61, ATO, ITRA being most potent cytotoxic agents via both induction of cell death and inhibition of cell cycling