Background Identifying therapeutic approaches to treat cancer is laborious, expensive, and often inefficient. Drug repurposing or repositioning in oncology refers to the application of drugs, which are already approved for other medical applications, in treating cancer.\(^1\) Moreover, in order to enhance therapeutic benefits, repurposed drugs are often combined with frequent administrations of low-dose chemotherapy/immunotherapy. Recent advancements in artificial intelligence (AI) technology have led to the development of in silico drug discovery approaches. Therefore, therapeutic discovery through a drug repurposing strategy aided by these technological advancements can potentially accelerate studies into clinical trials more rapidly compared to that using newly developed drugs.

Methods In this study, we employed an AI driven structure-based model (ERSTE-Explorer) to identify an mTOR inhibitor candidate. Using in vitro biochemical and cellular experiments as well as transcriptome sequencing analyses, we identified lomitapide, an inhibitor of hepatic microsomal triglyceride transfer protein (MTTP) approved for the homozygous familial hypercholesterolemia (HoFH) as an mTOR inhibitor.\(^2\) To validate anticaner effect of lomitapide in vivo, we used tumor xenograft model and patient-derived colorectal cancer organoids for clinical relevance. Furthermore, potential synergistic effects were confirmed by combining of lomitapide and immune checkpoint blocking antibodies to inhibit tumor growth in murine MC38 or B16-F10 preclinical syngeneic tumor models.\(^3\)

Results Autophagy is a biological process that maintains cellular homeostasis and regulates the internal cellular environment. Hyperactivating autophagy to trigger cell death has been a suggested therapeutic strategy for cancer treatment.\(^4\) Mechanistic target of rapamycin (mTOR) is a crucial protein kinase that regulates autophagy; therefore, we identified lomitapide, a cholesterol-lowering drug, as a potential mTOR complex 1 (mTORC1) inhibitor (figure 1). Our results showed that lomitapide directly inhibits mTORC1 in vitro (figure 1) and induces autophagy-dependent cancer cell death by decreasing mTOR signaling (figure 2), thereby inhibiting the downstream events associated with increased LC3 conversion in various cancer cells (e.g., HCT116 colorectal cancer cells) (figure 3) and tumor xenografts (figure 4). Lomitapide also significantly suppresses the growth and viability along with elevated autophagy in patient-derived colorectal cancer organoids (figure 5). Furthermore, a combination of lomitapide and immune checkpoint blocking antibodies synergistically inhibits tumor growth in murine MC38 or B16-F10 preclinical syngeneic tumor models (figure 6).

Conclusions Our results elucidate the direct, tumor-relevant immune-potentiating benefits of mTORC1 inhibition by lomitapide, which complement the current immune checkpoint blockade. This study highlights that the U.S. FDA-approved drug, lomitapide, can be potentially repurposed for the treatment of cancer.

REFERENCES

Ethics Approval All mice were housed in a pathogen-free animal facility at KAIST Laboratory Animal Resource Center. The animals were maintained in a temperature/humidity-controlled room on a 12 h light/12 h dark cycle and fed a standard chow diet. All experiments involving animals were conducted according to the ethical policies and procedures approved by the Committee for Animal Care at KAIST.
Abstract 804 Figure 2  Lomitapide inhibits cancer cell viability and reduce
a Cancer-specific growth inhibition of lomitapide on HCT116 colorectal
cancer cell line. b Colony formation of vehicle or lomitapide-treated
were measured in HT29, HCT116, and SW480 CRC cells. c mTOR
downstream signaling defects in CRC cells were analyzed by
immunoblotting treated with vehicle or lomitapide for 24 h at the
indicated concentration. The mTOR inhibitor Torin1 was used as a
control. d S757 phosphorylation of ULK1 and LC3 levels were measured
by immunoblotting to assess autophagy induction.

Abstract 804 Figure 3  Lomitapide leads to autophagic cancer cell
death
a Significantly enriched pathways in lomitapide-treated HCT116 cells
compared with vehicle-treated cells identified through KEGG analysis. b
Volcano plot showing significant gene expression changes in response
to lomitapide treatment in HCT116 cells. c HT29 cells were transfected
with GFP-LC3 plasmid for 24 h, and treated with 5 μM lomitapide
for another 24 h. GFP-LC3 puncta was visualized by a confocal microscope.
Scale bar: 20 μm. d Cell viability was measured in HT29 cells treated
with 5 μM lomitapide in the absence or presence of 100 nM
bafilomycin for 24 h. e HT29 cells were treated with 5 μM lomitapide
in the absence or presence of 1 mM 3-MA for 24 h. LC3 levels were
measured by immunoblotting to assess autophagy induction. f si-control
and siATG7–transfected HT29 cells were treated with 5 μM lomitapide
for 24 h. LC3 levels were measured by immunoblotting to assess
autophagy induction.

Abstract 804 Figure 4  Lomitapide suppresses the growth of tumor
xenografts
a HT29 cells were inoculated into flanks of nude mice and tumor volumes were measured for 10 days after intraperitoneal injection into xenograft tumors every 2 days. b Representative images of xenograft tumors at the day of sacrifice. c Body weight of xenograft mice bearing HT29 tumors during the in vivo experiment. Data were expressed as means ± SEM (*P < 0.05; **P < 0.01; ***P < 0.001, Student’s t-test). (n = 6 per group). d Hematoxylin and eosin (H&E) staining of tumor tissues collected from either vehicle or lomitapide-treated mice. e Tumor tissues were subjected to immunohistochemistry staining with Ki67 antibodies. Scale bar: 20 μm.

Abstract 804 Figure 5
Lomitapide suppresses the growth of patient-derived CRC organoids CRC-01 (KRASWT; APC and TP53 mutant) and CRC-02 (KRASG12V; APC and TP53 mutant) treated with 10 μM 5-FU or 10 μM lomitapide. The organoid size was measured and quantified at 48 h of either 5-FU or lomitapide treatment relative to vehicle control. b Dose-response images of patient-derived CRC organoids CRC-01 and CRC-02 treated with DMSO, lomitapide, or 5-FU for 72 h at indicated concentrations. Organoids were stained with CFSE as an organoid marker (blue) and PI as a dead cell marker (red). Scale bars: 2 mm for CRC-01 and 1 mm for CRC-02. c H&E staining of the original matrigel CRC-01 organoid culture. Scale bar: 1 mm. d LC3 levels were measured by immunoblotting to assess autophagy induction. Lysates were prepared from organoids treated with vehicle, 10 μM lomitapide, or 10 μM 5-FU for 48 h.

Abstract 804 Figure 6
Treatment of tumor-bearing mice with lomitapide
a Tumor growth curves of MC38 mouse colon cancer cells in mice treated with control (vehicle) or lomitapide combined with either control isotype or anti-PD-1. Lomitapide at 20 mg/kg every day, anti-PD-1 or isotype IgG 10 mg/kg every other day. Statistically significant differences (indicated by asterisks) are calculated using an unpaired two-tailed Student’s t-test (*P < 0.05, **P < 0.005, and ***P < 0.0005). b Body weight trace during the experiment. c Representative images of tumor tissues at 18 days following inoculation of MC38 cells. d Representative H&E images of tissues (liver, kidney, and lung) collected from mice. Scale bar: 100 μM. e Tumor growth curves of B16F10 mouse melanoma cells in mice treated with control (vehicle) or lomitapide combined with either control isotype or anti-PD-1. Lomitapide at 20 mg/kg every day, anti-PD-1 or isotype IgG 10 mg/kg every other day. Statistically significant differences (indicated by asterisks) are calculated using an unpaired two-tailed Student’s t-test (*P < 0.05, **P < 0.005, and ***P < 0.0005). f Representative images of tumor tissues at 16 days following inoculation of B16F10 cells.