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UC San Diego
Moores Cancer Center

Dracen Pharmaceuticals

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Elucidating the role of glutamine metabolism in head & neck squamous cell carcinoma

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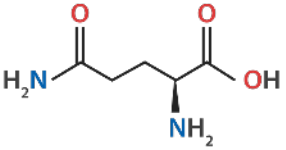

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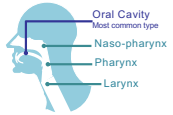
BACKGROUND

Head and Neck Cancer

560,000
New Head and Neck Cancer Cases Annually

300,000
Head and Neck Cancer Deaths Annually



Risk Factors:

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, resulting in over 14,600 deaths each year in the United States alone. HNSCC is associated with human papillomavirus (HPV) infection, and tobacco use and abusive alcohol intake. Recent revolutionary immunotherapy approaches have changed the landscape of treatment options in HNSCC. However, less than 20% of HNSCC patients respond to FDA-approved anti-PD-1 immune checkpoint blockade (ICB) (pembrolizumab and nivolumab), often not leading to durable responses. This highlights the unmet need to identify novel therapeutic options and biomarkers predicting more favorable response to maximize the efficacy of immune-oncology (IO) strategies for HNSCC treatment.

OBJECTIVE

Glutamine is a conditionally essential amino acid for rapidly proliferating cancer cells making glutamine pathway inhibition an attractive approach for anti-cancer therapy. Treatment with the glutamine antagonist DRP-104 (sirpiglenastat), which irreversibly inhibits all known enzymes involved in glutamine metabolism within the tumor, resulted in metabolically halted cell growth in vitro and in vitro in a large panel of allogenic and syngeneic cell lines (n=8) representing the spectra of HPV- and HPV+ HNSCC.

The focus of this study was to evaluate whether broad glutamine antagonism, using DRP-104 (sirpiglenastat), has therapeutic potential in HNSCC by both dismantling cancer metabolism and to further explore the mechanism of glutamine suppression in HNSCC by integrating the results from genome-wide CRISPR-Cas9 knockout library screens and broad-spectrum metabolomics analysis.

RESULTS

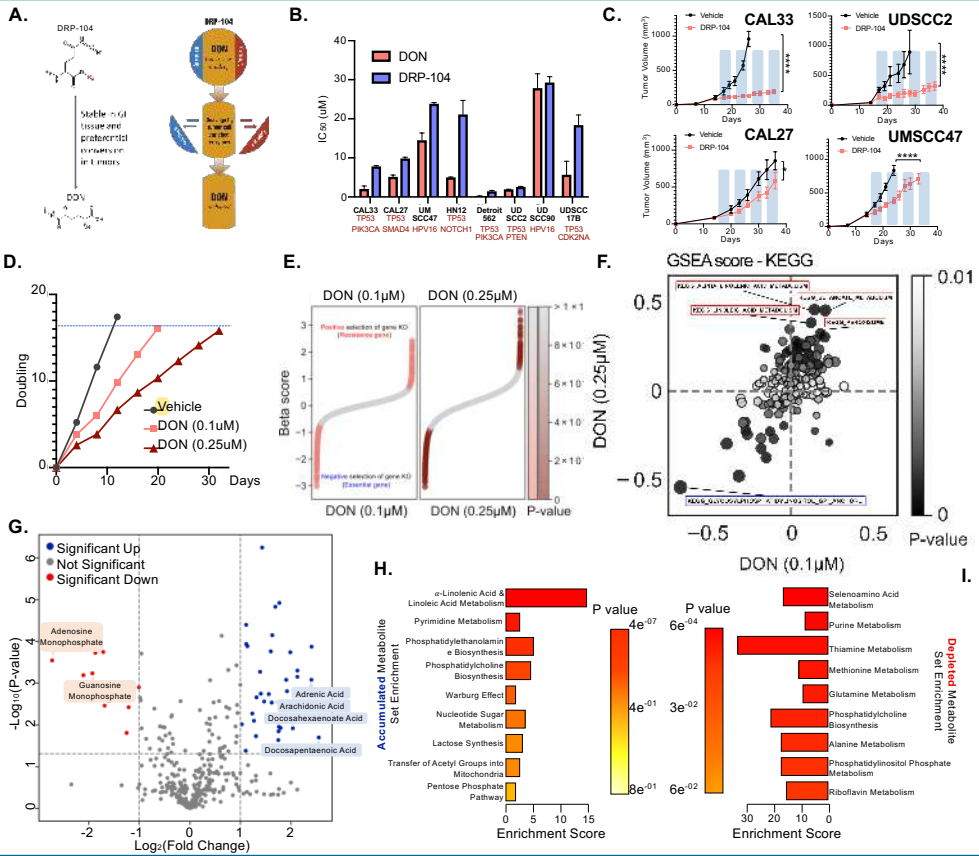


Figure. A. DRP-104 is a prodrug of the broad-acting glutamine antagonist DON (6-Diazo-5-oxo-L-norleucine). DRP-104 is inactive in its prodrug form with high plasma and GI tissue stability. DRP-104 is preferentially distributed in tumors where it is bio-transformed and activated to the active moiety DON. **B.** IC50 of DON and DRP-104 compared in a panel of human HNSCC cells lines. HNSCC cells carrying genetic alterations in PIK3CA/PTEN were more sensitive to glutamine antagonism in comparison to unaltered HNSCC cells. **C.** In-vivo allograft assay of CAL33, CAL27, UMSCC47, and UDSCC2 by flank subcutaneous injections in SCID/NOD mice. One million cells were injected into the flank. After 14 days, mice were treated with DRP-104 (3mg/kg) subcutaneously on the opposite flank (5 days ON/2 days OFF) for four cycles. **D.** CAL33-Cas9 cells were infected with Human Genome CRISPR pooled library (Brunello) at a multiplicity of infection (MOI) of 0.3. CAL33-Cas9-kinome library cells were treated with three different groups; vehicle-treated, DON (0.1μM), or DON (0.25μM) treated group, with triplicate. For population doubling of 16 samples, the barcode was PCR-recovered from genomic samples, and samples were sequenced to calculate the abundance of the different sgRNA probes. **E.** The change in the relative abundance of each sgRNA in the library versus vehicle was measured using PinAPL-Py software: significant essential or resistant genes highlighted in shades of red. **F.** Correlation analysis of two doses of DON identified essential and resistant KEGG gene sets. **G.** Metabolomics analysis, EIC (eicosanoids and other bioactive lipid species), and fPHILIC (polar molecules) were performed on CAL33 cells treated for 16 hours with 3μM of DON. Accumulated and depleted metabolites depicted via Volcano plot. **H.** MetaboAnalyst 5.0 Enrichment Analysis tool was used to highlight enriched metabolite sets. **I.** Accumulation of neutral lipids after glutamine antagonism or glutamine depletion was confirmed using HCS LipidTOXTM assay. Not observed with GLS1 inhibitor BPTES.

CONCLUSIONS

Both the prodrug DRP-104 (sirpiglenastat) and the active form DON demonstrated glutamine dependent inhibition of *in-vitro* cell growth with an IC50 of 0.2-25μM and *in-vivo* in a large panel of cell lines (n=8) representing the spectra of HPV- and HPV+ HNSCC,

Interestingly, a whole-genome CRISPR screen identified the metabolism of alpha linolenic acid and linoleic acid as key resistance pathways while GPI anchor biosynthesis as a pathway of essentiality under two doses of DON treatment (0.1μM and 0.25μM).

Metabolomic analysis of HNSCC cell line CAL33 treated with DON (3μM) confirmed dysregulation of alpha linolenic acid and linoleic acid with an accumulation of its metabolic byproducts.

Our data suggest that broad glutamine antagonism using sirpiglenastat (DRP-104) has therapeutic potential in HNSCC by dismantling cancer metabolism and sensitizing cells to additional perturbations leading to specific cell death. A clinical trial of sirpiglenastat (DRP-104) is currently ongoing (NCT04471415).