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Halting head & neck squamous cell carcinoma progression by broadly targeting glutamine metabolic pathways

Michael Allevato[1], Sally Trinh[1],Yumi Yokoyama[2], Robert Wild[2], J. Silvio Gutkind[1]

[1]University of California, San Diego, San Diego, CA [2]Dracen Pharmaceuticals Inc., New York, NY

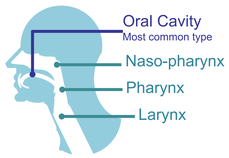
PRESENTER
Michael Allevato
mallevat@ucsd.edu

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 enhancing the anti-tumor immune response of immune checkpoint blockade.

RESULTS

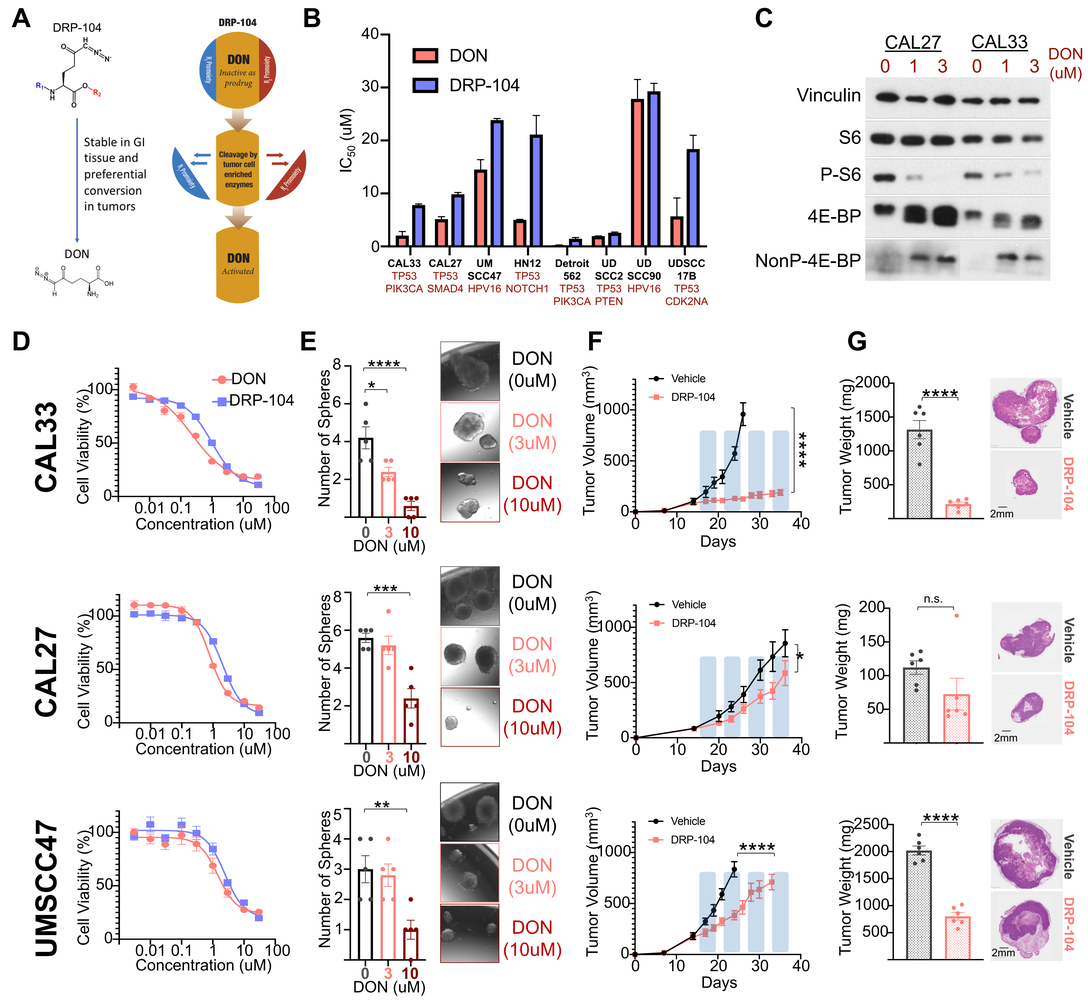


Figure 1. A. DRP-104 is a prodrug of the broad acting glutamine antagonist DON (6-Diazo-5-oxo-L-norleucine). DRP-104 is inactive in its prodrug form with high plasma and GI tissue stability. DRP-104 is preferentially distributed in tumors where it is bio-transformed and activated to the active moiety DON. **B.** IC_{50} 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.** The inhibitory effect of glutamine antagonism on the PI3K/mTOR pathway (↓P-S6 and ↑NonP-4E-BP) is depicted by immunoblotting in CAL27 and CAL33 (*PIK3CA*mut) cell lines with or without DON (1uM and 3uM) treated for 12 hours. Head and neck cancers driven by the hyperactivity of the PI3K-PTEN axis depend on the downstream target mTOR to promote tumor cell growth and survival. This dependence on mTOR can explain why cells bearing mutations in this axis are more sensitive to glutamine antagonism due to its suppression of mTOR signaling. **D.** Dose response experiment for cell viability determined by CellTiter-Fluor Cell Viability Assay in CAL33, CAL27, and UMSCC47 subjected to 72 hours of treatment with DON and DRP-104. **E.** Spheroid formation assay of the cell were treated with DON for 10 days. Quantification of colonies and representative pictures are shown. **F.** In-vivo allograft assay by flank subcutaneous injections in SCID/NOD mice. One million cells were injected in 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 4 cycles. **G.** Tumor weight and representative hematoxylin and eosin stained cross sections of each tumor lesions at collect time are shown.

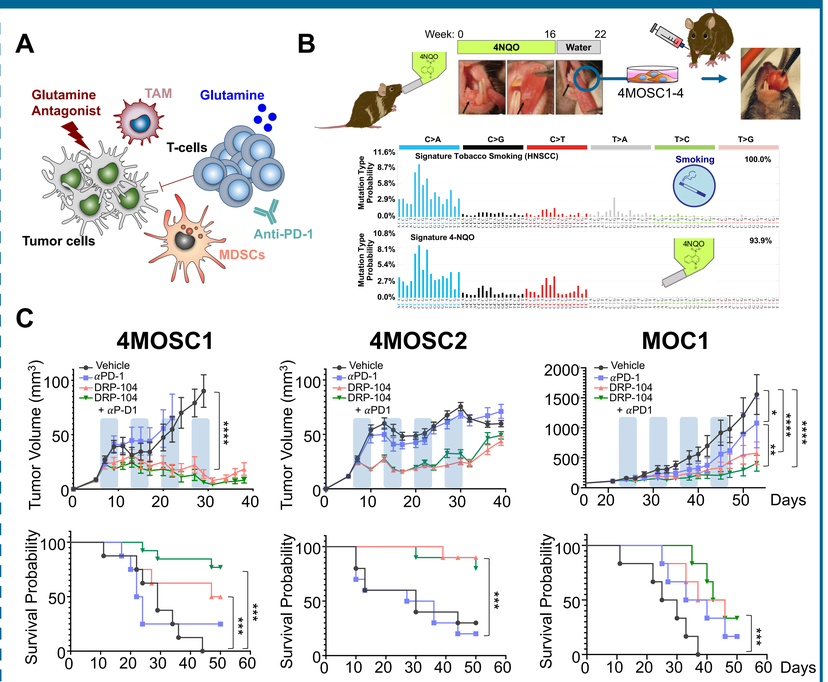


Figure 2. A. Model of mechanism of action of glutamine antagonist (DRP-104) on tumor cells, immune cells and tumor immune microenvironment. **B.** Experimental scheme of 4NQO syngeneic model. C57Bl/6 mice were given 4NQO (50 µg/mL) in the drinking water for 16 weeks and then regular water until week 22. Cells were isolated from the lesions, cultured, and then implanted into the tongue of wild-type C57Bl/6 mice. **C.** C57IBL/6 were implanted with $5x10^5$ 4MOSC1/2 cells into the tongue or $1x10^6$ MOC1 cells into the flank. Once tumors were palpable, tumors were treated three times a week intraperitoneally with 10mg/kg of isotype control or anti-PD-1 and/or with subcutaneous injections of the vehicle or 1.4mg/kg of DRP-104 (5 days ON/2 days OFF) for 4 cycles. Kaplan-Meier curves depicting the survival of mice from tumor growth assay are shown below

CONCLUSIONS

- Both the prodrug DRP-104 (sirpiglenastat) and the active form DON demonstrated glutamine dependent inhibition of *in-vitro* cell growth in a large panel of cell lines (n=8) representing the spectra of HPV- and HPV+ HNSCC, with an IC50 of 0.2-25uM.
- Interestingly, cell lines bearing alterations in the *PIK3CA/PTEN* pathway (hyperactive in over 90% of HNSCC) were more sensitive to glutamine antagonism in comparison to unaltered HNSCC cell lines.
- The dependence of glutamine in HNSCC growth and the increased sensitivity of *PIK3CA/PTEN* aberrant cells was also observed in orosphere assays and distinct HNSCC tumor xenografts in NOD SCID mice.
- Finally, DRP-104 (sirpiglenastat) inhibited tumor growth as a single agent in the novel orthotopic syngeneic tobacco associated oral cancer model 4MOSC1/2 and MOC1. Combination with anti-PD1 Ab enhanced long term survival.
- These data support clinical development of DRP-104 (sirpiglenastat) as a monotherapy and in combination with immune checkpoint inhibitors such as anti-PD-1 in HNSCC.
- A first in-human clinical trial is currently ongoing.