Sirpiglenastat (DRP-104), a broad acting glutamine antagonist, metabolically reprograms glutamine addicted cancer cells and significantly remodels the tumor microenvironment leading to anti-tumor immune responses

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Glutamine Addiction of Tumors Provides Novel Treatment Strategy & Precision Medicine Opportunities Across Multiple Cancers

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-020-15136-9 OPEN

A shift in glutamine nitrogen metabolism contributes to the malignant progression of cancer

Manabu Kodama[1], Kiyotaka Oshikawa[1], Hideyuki Shimizu[1], Susumu Yoshioka[1,2], Masatomo Takahashi[3], Yoshihiro Izumi[3], Takeshi Bamba[3], Chisa Tateishi[1], Takeshi Tomonaga[4], Masaki Matsumoto[5]✉ & Keiichi I. Nakayama[1,5]✉

Nat Commun **11,** 1320 (2020).

Oncogene www.nature.com/onc

ARTICLE

Targeting glutamine metabolism network for the treatment of therapy-resistant prostate cancer

Lingfan Xu[1], Bing Zhao[1], William Butler[1], Huan Xu[1,9], Nan Song[1,10], Xufeng Chen[1], J. Spencer Hauck[1], Xia Gao[2,3], Hong Zhang[1], Jeff Groth[1], Qing Yang[4], Yue Zhao[1,5], David Moon[1], Daniel George[6,7], Yinglu Zhou[8], Yiping He[1] and Jiaoti Huang[1,2,7]✉

Oncogene **41,** 1140–1154 (2022).

nature medicine

LETTERS

Keap1 loss promotes *Kras*-driven lung cancer and results in dependence on glutaminolysis

Rodrigo Romero[1,2,15], Volkan I Sayin[3,15], Shawn M Davidson[1,2], Matthew R Bauer[1], Simranjit X Singh[3], Sarah E LeBoeuf[3], Triantafyllia R Karakousi[3], Donald C Ellis[1,2], Arjun Bhutkar[1], Francisco J Sánchez-Rivera[1,2], Lakshmipriya Subbaraj[1,2], Britney Martinez[3], Roderick T Bronson[4,5], Justin R Prigge[6], Edward E Schmidt[6], Craig J Thomas[7], Chandra Goparaju[8], Angela Davies[9], Igor Dolgalev[10], Adriana Heguy[10], Viola Allaj[11,12], John T Poirier[11,12], Andre L Moreira[3], Charles M Rudin[11,12], Harvey I Pass[8], Matthew G Vander Heiden[1,2], Tyler Jacks[1,2,13] & Thales Papagiannakopoulos[3,14]

Nat Med. 2017 Nov;23(11):1362-1368

Broad Glutamine Antagonism Drives Metabolic Reprogramming And Activates Innate & Adaptive Immune Response

The NEW ENGLAND JOURNAL of MEDICINE

RESEARCH

IMMUNOMETABOLISM

Glutamine blockade induces divergent metabolic programs to overcome tumor immune evasion

Robert D. Leone[1], Liang Zhao[1], Judson M. Englert[1], Im-Meng Sun[1], Min-Hee Oh[1], Im-Hong Sun[1], Matthew L. Arwood[1], Ian A. Bettencourt[1], Chirag H. Patel[1], Jiayu Wen[1], Ada Tam[1], Richard L. Blosser[1], Eva Prchalova[2], Jesse Alt[2], Rana Rais[2], Barbara S. Slusher[2], Jonathan D. Powell[1]*

The metabolic characteristics of tumors present considerable hurdles to immune cell function and cancer immunotherapy. Using a glutamine antagonist, we metabolically dismantled the immunosuppressive microenvironment of tumors. We demonstrate that glutamine blockade in tumor-bearing mice suppresses oxidative and glycolytic metabolism of cancer cells, leading to decreased hypoxia, acidosis, and nutrient depletion. By contrast, effector T cells responded to glutamine antagonism by markedly up-regulating oxidative metabolism and adopting a long-lived, highly activated phenotype. These divergent changes in cellular metabolism and programming form the basis for potent antitumor responses. Glutamine antagonism therefore exposes a previously undefined difference in metabolic plasticity between cancer cells and effector T cells that can be exploited as a "metabolic checkpoint" for tumor immunotherapy.

Leone et al., Science 366, 1013–1021 (2019)

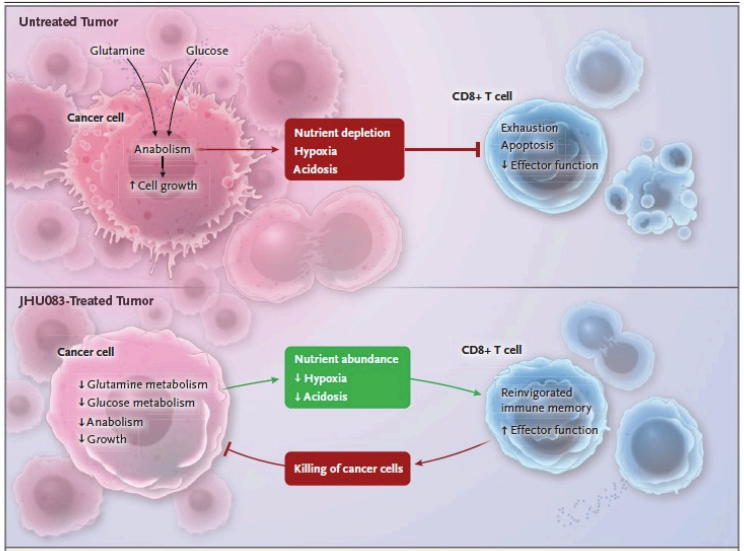


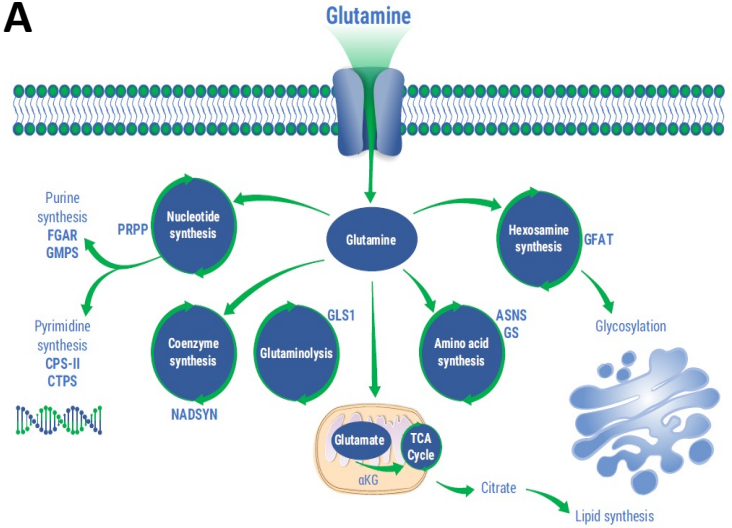
Figure 1. Depleting Tumors while Boosting T-Cell Function.

Leone et al.[2] recently reported that JHU083, an antagonist of glutamine metabolism, suppresses cancer-cell growth and stimulates anti-tumor T-cell function. Cancer cells take up glutamine, glucose, and other nutrients to support anabolism (macromolecular synthesis) and cell growth. Cancer cells acidify the microenvironment and deplete nutrients and oxygen, impairing the function of CD8+ T cells and preventing their cytotoxic effects against cancer cells. JHU083 elicited broad metabolic effects in the cancer cells, preventing their uptake of both glutamine and glucose, limiting cancer-cell growth, and resulting in a microenvironment favorable to T-cell effector function. CD8+ T cells in JHU083-treated tumors (established from mouse cancer-cell lines) proliferate and display hallmarks of immune memory and enhanced cytotoxic effects against cancer cells, which ultimately results in potent antitumor immunity.

N Engl J Med 382;9 nejm.org February 27, 2020

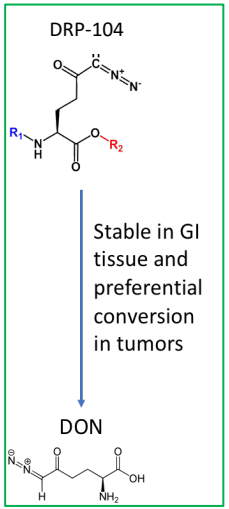
Sirpiglenastat (DRP-104) Preferentially Delivers the Glutamine Antagonist DON to Tumors Providing Broad Glutamine Pathway Inhibition

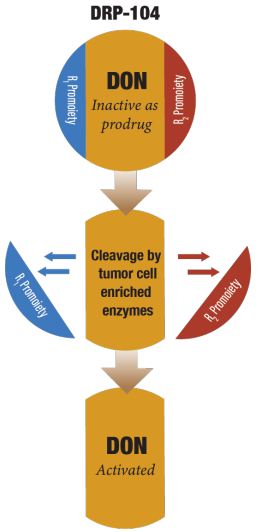
A



Mol Cancer Ther; 17(9) September 2018

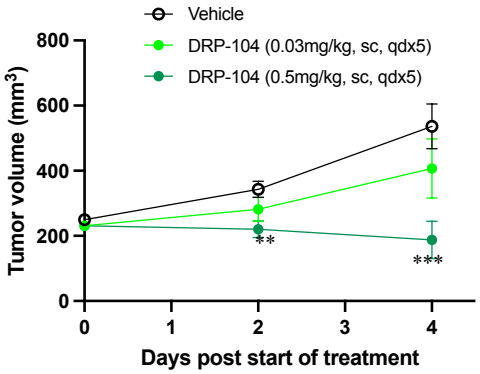
B

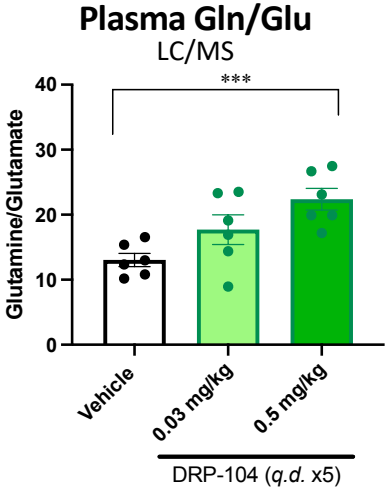


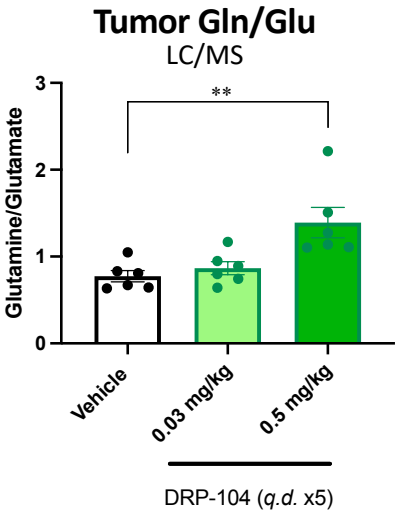


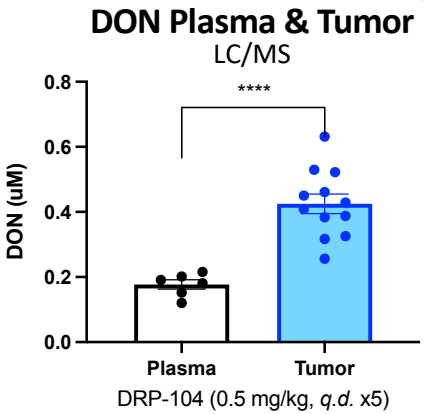
- Glutamine feeds into multiple key pathways required for cancer cell growth and survival including nucleotide synthesis, NAD+ synthesis, lipid, amino acid and hexosamine synthesis. Broadly targeting glutamine metabolism has significant therapeutic potential
- DON (6-Diazo-5-oxo-L-norleucine) is an irreversible broad acting glutamine antagonist of all 10 Glutamine metabolizing enzymes
- DRP-104 (sirpiglenastat) was designed as a prodrug of DON. 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

DRP-104 Demonstrates Dose Dependent Tumor Growth Inhibition and Modulation of Glutamine/Glutamate Ratios in Plasma and Tumor *in vivo*





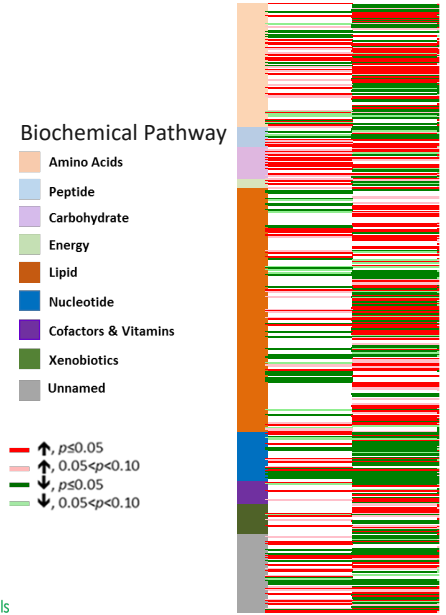


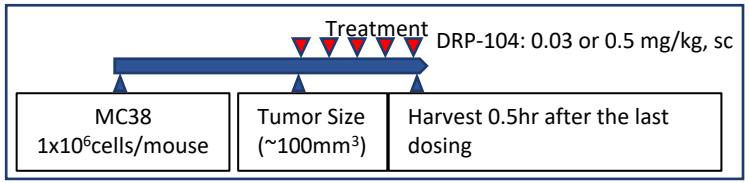


- DRP-104 was administered sc for 5 consecutive days (left) into MC38 tumor bearing mice
- Plasma and tumor samples were collected 30 min after the last dose and analyzed for Glutamine, Glutamate and DON
- Significant changes in Glutamine/Glutamate ratios in plasma correlated with significant changes in tumor at the minimum efficacious dose of 0.5 mg/kg corresponding to DON tumor levels of 430 nM and DON plasma levels of 178 nM (2.4x)
- Data support preferential tumor targeting of DON leading to target engagement and efficacy
- Plasma Glutamine/Glutamate ratio changes may be used as a potential surrogate PD biomarker for DRP-104

DRP-104 Treatment Results in Broad Metabolomic Reprogramming of the Tumor and TME and Providing Favorable Conditions for Immune Cell Function

Summary Counts Welch Two Sample t-Test	Low QD Veh QD	High QD Veh QD
Total number of biochemicals detected	869	
Total number of biochemicals with p≤0.05	157	359
Biochemicals (↑|↓)	91 | 66	188 | 171
%Total biochemicals significantly different	18%	41%





DRP-104 treatment resulted in dose dependent changes in various metabolites in the tumor/TME demonstrating target engagement on several glutamine-utilizing enzymes/pathways in vivo:

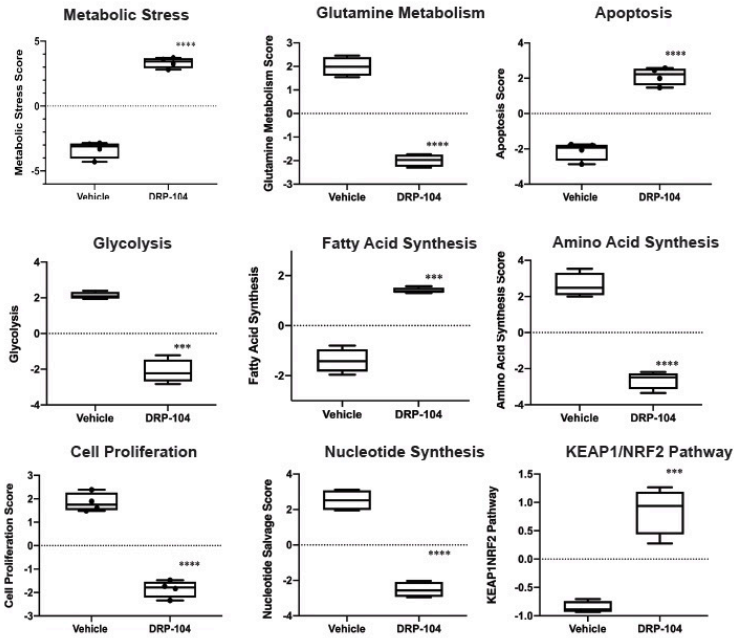
- Inhibition of ***glutaminolysis*** leading to increased glutamine (GLS1)
- Decreased ***nucleotide synthesis*** (PPAT, FGAR, CPS-II, CTPS)
- Increased ***amino acid metabolites*** (ASNS, GS)
- Decreased de novo ***NAD+ synthesis*** and NAD+ salvage pathway (NADSYN)
- Decreased ***Hexosamine Biosynthesis*** and Glycosylation precursors (GFAT)
- Altered ***glutathione metabolism*** (decreased cysteine, glutathione)
- Decreased ***Glycolysis*** (increased glucose and fructose levels)

Several Immunosuppressive oncometabolites were also altered suggesting remodeling of the TME:

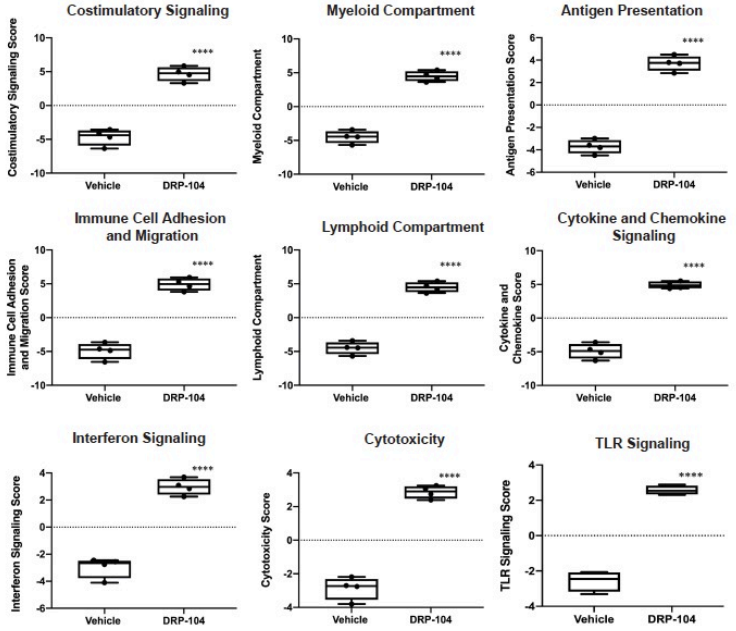
- Decreased ***Kynurenine*** and Tryptophan catabolism
- Decreased levels of COX/LOX metabolites such as ***PGE2***
- Decreased ***adenine/adenosine***

DRP-104 Treatment *In Vivo* Results in Significant Modulation of Multiple Relevant Tumor and Immune Cell Scores as Measured by NanoString

Tumor

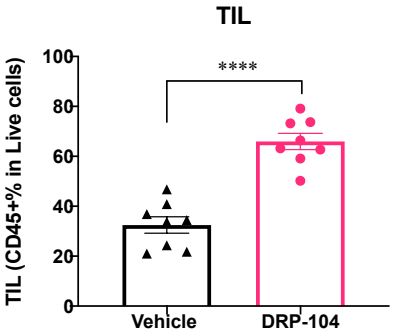


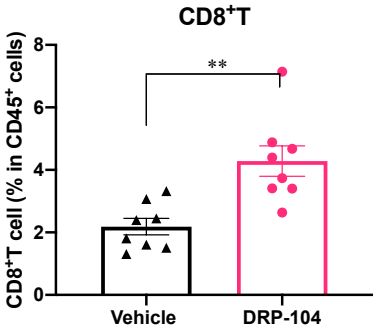
IO

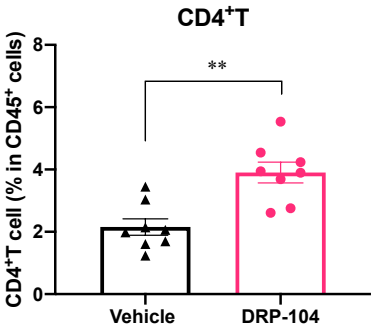


Sirpiglenastat (DRP-104) Increases Proliferation, Activation and Infiltration of Adaptive and Innate Immune Cells into Tumors *In Vivo*

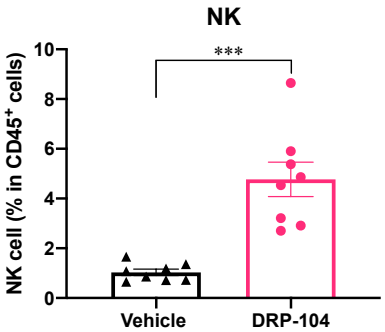
Adaptive Immune System

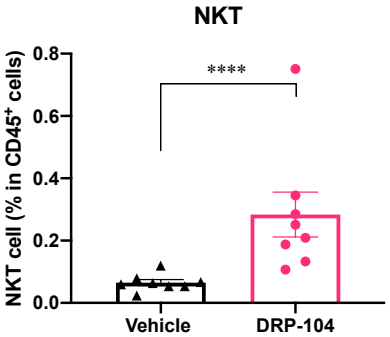


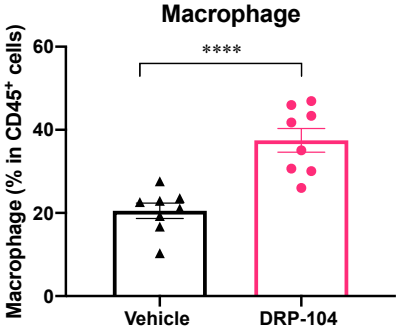




Innate Immune System

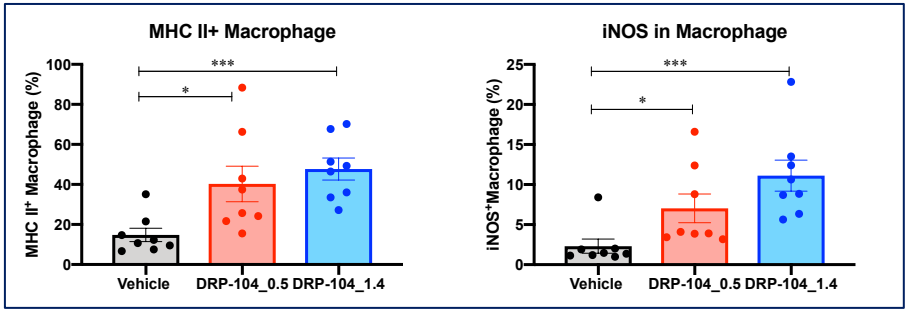




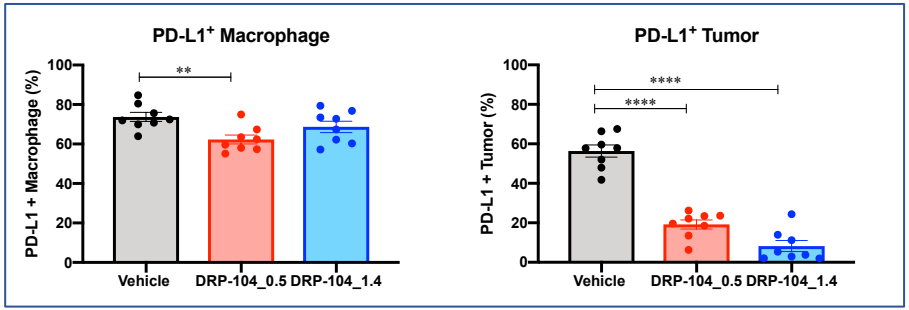


DRP-104 Treatment Increases M1 Polarization of Macrophages and Reduces Expression of PD-L1 in Tumors

M1 macrophage markers

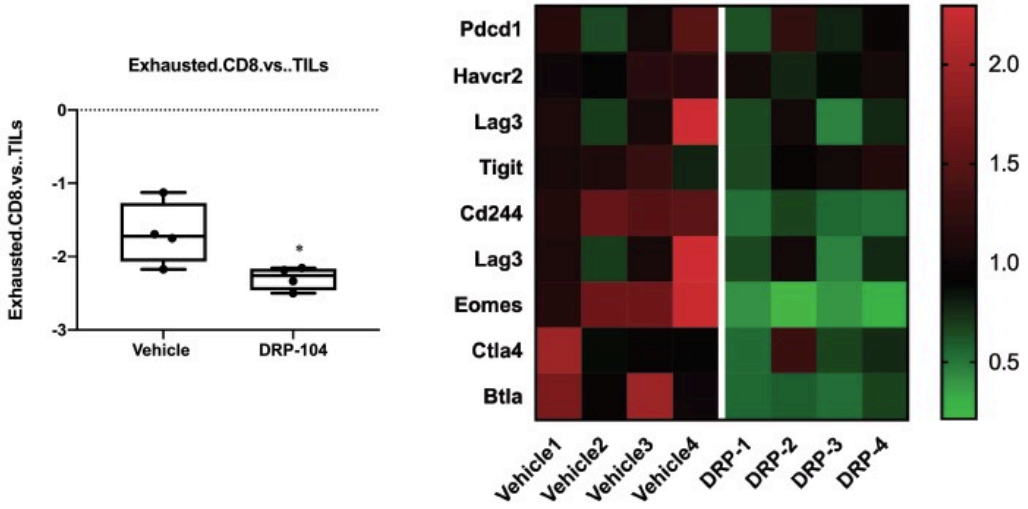


PD-L1 Expression



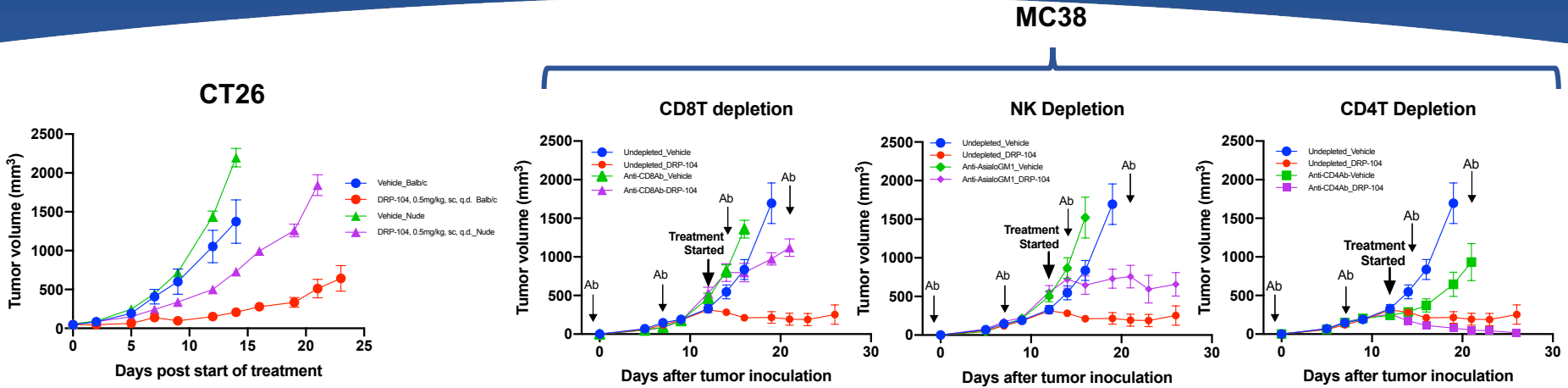
- DRP-104 treatment results in dose dependent increase in M1 macrophage (anti-tumor) polarization
- PD-L1 expression was prominently reduced by DRP-104 treatment in tumors in vivo
- PD-L1 expression has been linked to GFAT inhibition (*Carcinogenesis*, Volume 42, Issue 9, September 2021)

DRP-104 Treatment *In Vivo* Results in Significant Reduction of Exhausted CD8+ T Cells



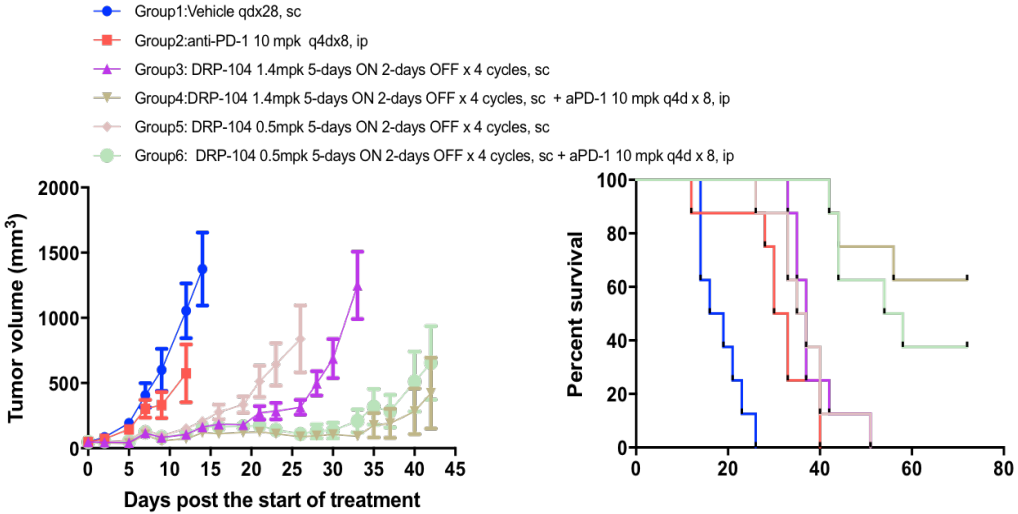
- Gene expression changes were analyzed by NanoString IO360 panel in vehicle vs DRP-104 treated tumors
- NanoString cell type analysis revealed that among increased TILs, proportionally exhausted CD8 related genes relative to TIL (*Ptprc*) were downregulated by DRP-104 treatment

Efficacy in Immunocompromised Mice Showcases the Important Contribution of the Immuno-Oncology MoA of DRP-104



- CT26-bearing Balb/C wild type mice or nude mice were treated with DRP-104. Nude mice lack mature T cells
- MC38 bearing C57BL/6 mice were treated with anti-CD8, anti-Asialo GM1, anti-CD4 (or isotype control) to deplete CD8 cells, NK cells or CD4 cells, respectively. On day 12, when tumors were ~330 mm^3 treatment with DRP-104 was started
- DRP-104 treatment was 0.5 mg/kg, sc (5-day ON, 2-day OFF)
- **DRP-104 showed maximum efficacy in mice with an intact immune system as compared to nude mice or CD8/NK depleted mice**
- **CD4 (Treg) depleted mice show enhanced efficacy with DRP-104**
- Data suggest MoA for DON-based glutamine antagonists involves innate and adaptive immune cells
- Combination with Treg depleting approaches (e.g. CTLA4, TIGIT) may have therapeutic synergy

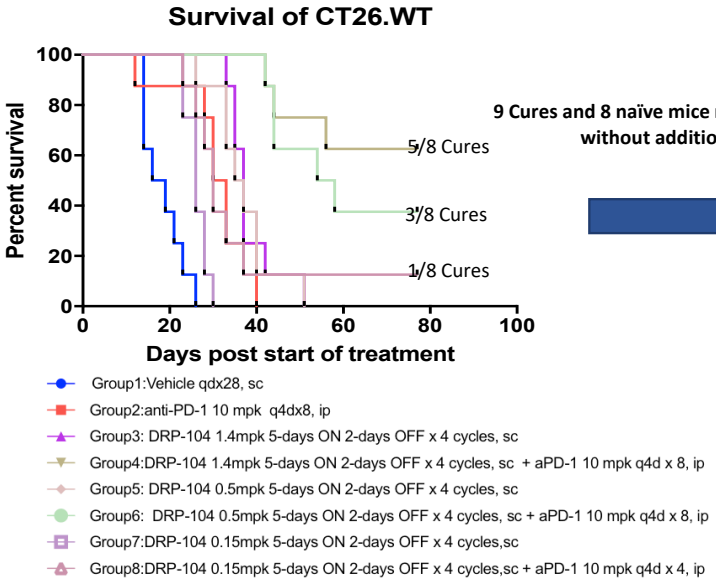
DRP-104 Synergizes with anti-PD-1 Checkpoint Inhibitors in the CT26 Colon Cancer Model



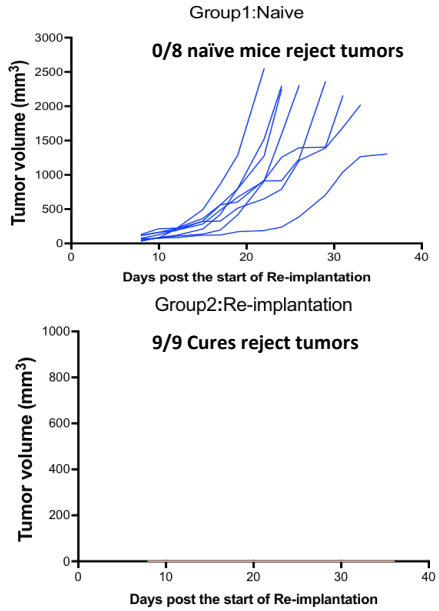
Group	Median Survival (days)	P-value	Cures (%)
Vehicle	17.5	--	0
Anti-PD-1 (10mg/kg) q4dx8	31.5	<0.01	0
DRP-104 (1.4 mg/kg, 5d ON, 2d OFF) x 4 cycles	37	<0.001	0
Anti-PD-1 (10mg/kg)+ DRP-104(1.4 mpk)	**>77**	**<0.001**	**62.5**
DRP-104 (0.5 mg/kg, 5d ON, 2d OFF) x 4 cycles	36	<0.001	0
Anti-PD-1 (10mg/kg)+ DRP-104(0.5 mpk)	**56**	**<0.001**	**37.5**

- Anti-PD-1 is partially active as single agent (increased OS vs control)
- DRP-104 alone showed potent single agent efficacy with tumor stasis during treatment
- DRP-104 + anti-PD-1 treatment shows synergistic tumor growth inhibition
- Combination treatment leads to significant improved OS and long-term durable cures (p<0.001 vs monotherapy)

DRP-104 + Anti-PD-1 Combination is Curative and Elicits Long Term Immune Memory Response in the CT26 Colon Cancer Model



9 Cures and 8 naïve mice re-challenged with CT26 without additional treatment

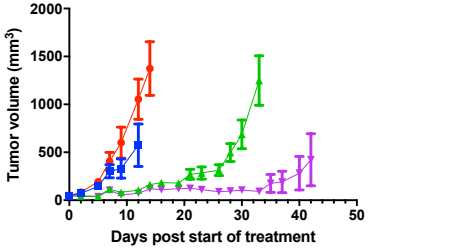


- DRP-104 + anti-PD-1 achieves long term durable cures ($p<0.001$ vs monotherapies)
- 9 Cured mice from Combo group were re-challenged with CT26 tumors versus naïve mice
- 100% (9/9) cured mice rejected re-implant versus 0/8 naïve mice ***suggesting long-term immune memory response*** elicited by DRP-104/anti-PD-1 combination treatment

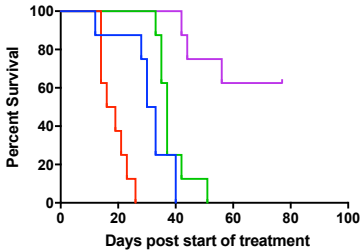
DRP-104 Demonstrates Therapeutic Synergy with Multiple Immune Checkpoint Inhibitors

Anti-PD-1 Combination

CT26

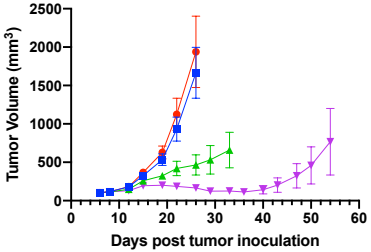


- Vehicle control
- Anti-PD-1 (10 mg/kg, q4dx8, ip)
- DRP-104 (1.4 mg/kg, 5d ON, 2d OFF x 4 weeks, sc)
- DRP-104 + Anti-PD-1

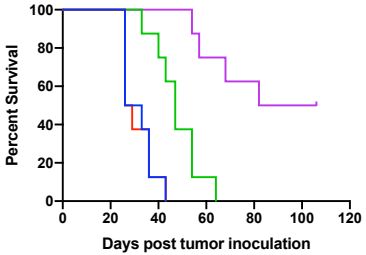


Anti-PD-L1 Combination

H22

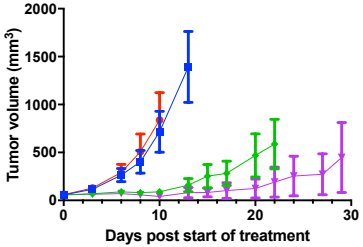


- Vehicle
- Anti-PD-L1 (5 mg/kg, biw x 3 weeks, ip)
- DRP-104 (1.4 mg/kg, 5d ON, 2d OFF x 3 weeks, sc)
- Anti-PD-L1+DRP-104

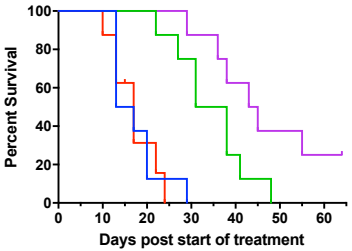


Anti-TIGIT Combination

MC38

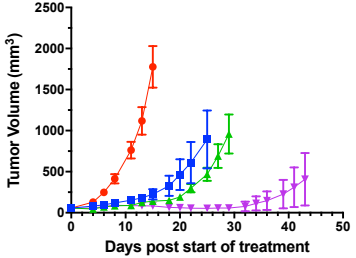


- Vehicle control
- Anti-TIGIT (10 mg/kg, BIW x 4 weeks, ip)
- DRP-104 (0.5 mg/kg, 5d ON, 2d OFF x 4 weeks, sc)
- Anti-TIGIT + DRP-104

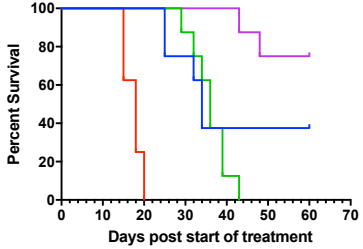


Anti-CTLA-4 Combination

CT26



- Vehicle control
- Anti-CTLA-4 (2.5 mg/kg, q4dx3, ip)
- DRP-104 (1.4 mg/kg, 5d ON, 2d OFF x 3 weeks, sc)
- Anti-CTLA-4 + DRP-104



DRP-104 (Sirpiglenastat) Seamless Phase I/II Clinical Development Program

Design	Rationale	Patient number
Phase 1a: Dose escalation, all comers (IV & SC)	Identify MTD/ DLT for both IV and SC formulations and advance one formulation	~60 patients for dose escalation
Phase 1a: Safety expansion in advanced solid tumors	Safety expansion	14 additional patients
NSCLC (Phase 2a): • Subcohort a: KEAP1 mutations • Subcohort b: STK11 mutations • Subcohort c: NFE2L2 mutations • Subcohort d: KEAP1 and STK11 mutations	Strong preclinical data in an area of unmet medical need and FTD by FDA	Up to 15 patients per cohort, capped at 55 patients total ***FDA Fast Track Designation***
SCCHN (Phase 2a):	Strong preclinical data	15 patients initially, if 2 responders expand to total of 25 patients
Phase 1b: First Line NSCLC KEAP1/NFE2L2/STK11 mutant Combo with CPI	Strong preclinical data	26 patients
CRPC and Neuroendocrine Prostate	Clinical & preclinical data and literature	TBD. In development

Summary and Conclusions

- **Broad Glutamine Metabolism Inhibition Demonstrates Highly Promising Pharmacology**
  - Glutamine antagonism has **differential effects on tumor cells versus immune cells**
  - DRP-104 broadly **remodels the tumor micro-environment leading to stimulation of anti-tumor immune responses of both adaptive and innate immune cells**
  - Broad glutamine antagonism demonstrates **therapeutic synergy with multiple different immuno-oncology therapies** (e.g. anti-PD-1/PD-L1 checkpoint inhibitor therapy)
  - **Many oncogenes and tumor suppressor genes** (e.g. KRAS, MYC, STK11, Keap1/NFE2LE) drive tumor cells into **glutamine addiction** providing a **precision medicine approach**

- **DRP-104 (sirpiglenastat) Clinical Development**
  - Currently completing Phase 1 dose-escalation – (IV and SC formulations)
  - ***Phase 2 Plan***
    - ***Fast Track Designation*** for KEAP1/NFE2L2/STK11 mutations in ***NSCLC (monotherapy)***
    - ***Monotherapy in glutamine addicted CRPC and SCCHN***
    - ***Combination with PD-(L)1 checkpoint inhibitors in NSCLC***

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