Dracen Pharmaceuticals

DRP-104, A Novel Broad Acting Glutamine Antagonist, Induces Distinctive Immune Modulation Mechanisms and Synergistic Efficacy in Combination with Immune Checkpoint Blockade

Yumi Yokoyama, Ph.D., Michael Nedelcovych, Ph.D. and Robert Wild, Ph.D.
Dracen Pharmaceuticals Inc., 780 Third Ave, 45th Floor, New York, NY 10017

Summary

Glutamine is a conditionally essential amino acid for rapidly proliferating cancer cells, thus depriving the same fuel from immune cells and contributing to tumor immune evasion. DRP-104 was designed as a novel prodrug of the broad acting glutamine antagonist 6-Diazo-5-oxo-L-norleucine (DON). DRP-104 is inert in its prodrug form, affords high levels of plasma and gastro-intestinal (GI) tissue stability; has high tumor cell permeability and preferential tumor versus plasma/GI tissue distribution for DON. Here we sought to (1) compare immunological modulation of DRP-104 to anti-PD-1Ab, and (2) evaluate the combination effect of DRP-104 with PD-1/PD-L1 checkpoint inhibitors.
DRP-104 treatment in CT26 mouse colon carcinoma model showed broad immune cell modulation effects including increased T, NK, and macrophages; while anti-PD-1Ab affected mainly CD8⁺T cells. Cytokine modulation in tumor and plasma revealed that DRP-104 decreased pro-tumorigenic cytokines such as VEGF and KC(IL-8) while anti-PD-1Ab showed either no change or slight increase in these cytokines. CT26 bearing mice treated with anti-PD-1Ab alone, DRP-104, and the combination showed tumor growth inhibition at day 12 of 48%, 90%, and 94%, respectively. Median survival days were 31.5, 36, and 56 days, respectively (vehicle; 17.5 days). Notably 9 mice treated with combination of anti-PD-1 with DRP-104 were tumor free at end of the experiment (day 77) and 100% of these mice rejected a CT26 tumor re-challenge. In the H22 HCC cancer model, mice were treated with either anti-PD-L1 Ab, DRP-104, or combination. While anti-PD-L1Ab did not show tumor growth inhibition in this model, DRP-104 significantly inhibited tumor growth and the combination further enhanced efficacy, illustrated by extended survival for both DRP-104 alone (50 days) and combination (96 days) treatment groups compared to vehicle (33 days) and anti-PD-L1 alone (33 days). Combination treatment also resulted in long term durable cures in 50% of the mice.

Background

Dracen's Glutamine Antagonist, DRP-104, Preferentially Delivers DON to Tumors Leading to Broad Glutamine Pathway Inhibition

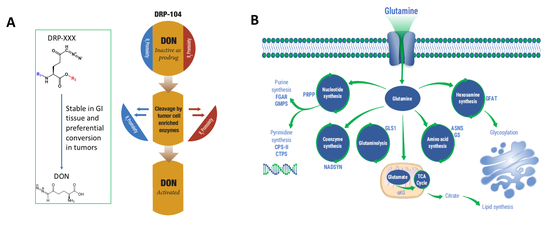


Figure 1. A. DRP-104 is a prodrug of the broad acting glutamine antagonist DON (6-Diazo-5-oxo-L-norleucine). DRP-104 is inactive in its prodrug form with high plasma and GI tissue stability. DRP-104 is preferentially distributed in tumors where it is biotransformed and activated to the active moiety DON. **B.** Glutamine dependent pathways inhibited by DON. Enzymes targeted by DON are shown in blue bold letters.

DRP-104 Single Administration Yields Time-Dependent Changes in Plasma Glutamine Metabolites

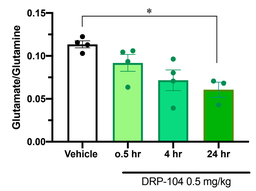
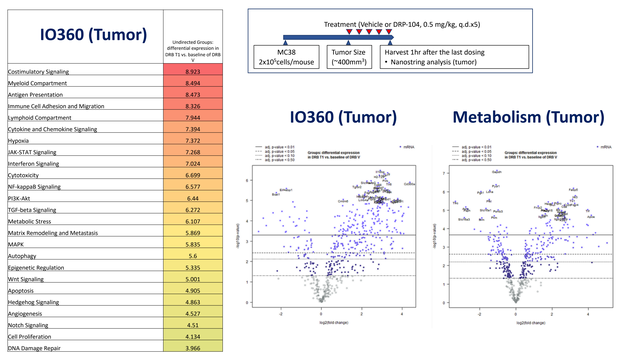


Figure 2. MC38 tumor bearing animals were dosed *s.c.* with DRP-104 0.5 mg/kg, and tumors were obtained at the indicated time after dosing. Glutamate/Glutamine in plasma was measured by Glutamine/Glutamate Glo Assay (Promega).
Significant time-dependent changes in plasma Glutamate/Glutamine levels were observed linking DRP-104 treatment to target engagement. Data indicate mean+/- SEM. *: p<0.05

DRP-104 Treatment *In Vivo* Results in Dramatic Modulation of Multiple Relevant Tumor and Immune Cell Scores as Measured by Nanostring IO360 and Metabolism Panels



IO360 (Tumor)	Undirected Groups: differential expression in DRB T1 vs. baseline of DRB
Costimulatory Signaling	8.923
Myeloid Compartment	8.494
Antigen Presentation	8.473
Immune Cell Adhesion and Migration	8.326
Lymphoid Compartment	7.944
Cytokine and Chemokine Signaling	7.394
Hypoxia	7.372
JAK-STAT Signaling	7.268
Interferon Signaling	7.024
Cytotoxicity	6.699
NF-kappaB Signaling	6.577
PI3K-Akt	6.44
TGF-beta Signaling	6.272
Metabolic Stress	6.107
Matrix Remodeling and Metastasis	5.869
MAPK	5.835
Autophagy	5.6
Epigenetic Regulation	5.335
Wnt Signaling	5.001
Apoptosis	4.905
Hedgehog Signaling	4.863
Angiogenesis	4.527
Notch Signaling	4.51
Cell Proliferation	4.134
DNA Damage Repair	3.966

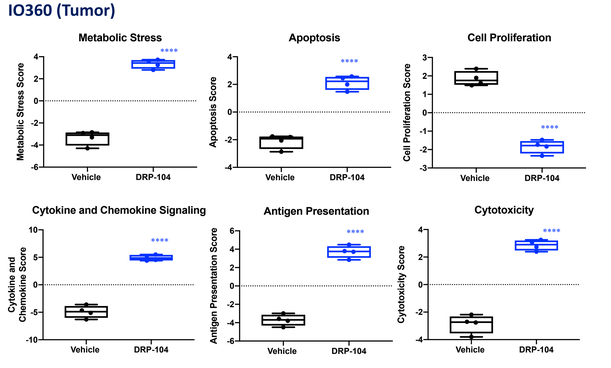


Figure 3. MC38 cells were inoculated *s.c.* in C57BL/6 mice. When tumor size reached ~400 mm³, mice were treated with DRP-104 (0.5 mg/kg) or vehicle *s.c., q.d.* for 5 days. mRNA was extracted from tumors and analyzed by Nanostring IO360 Panel. ****: p<0.0001
DRP-104 treatment was associated with dramatic modulation of multiple relevant tumor and immune cell scores suggesting direct anti-tumor effects while stimulating immune cell function.

DRP-104 Treatment Shows Distinct Immuno-Phenotypic Changes Compared to Anti-PD-1 Ab in the CT26 Syngeneic Model (TIL, Lymphocyte)

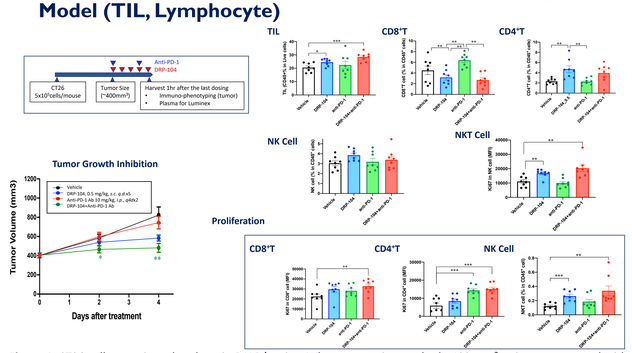


Figure 4. CT26 cells were inoculated *s.c.* in BALB/c mice. When tumor size reached ~400 mm³, mice were treated with DRP-104 (0.5 mg/kg) or vehicle *s.c., q.d.* for 5 days and/or anti-PD-1 Ab (10mg/kg) at day1 and 4. Phenotypic analyses of tumor infiltrating immune cells and tumor cells were performed by flow cytometry. *: p<0.05; ***: p<0.001; ***: p<0.0001. Distinct immunological modulation by DRP-104 and anti-PD-1 Ab was observed.

DRP-104 Treatment Shows Distinct Immuno-Phenotypic Changes Compared to Anti-PD-1 Ab in the CT26 Syngeneic Model (Macrophage and Tumor)

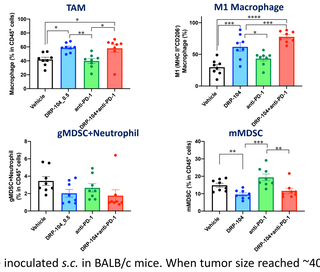


Figure 5. CT26 cells were inoculated *s.c.* in BALB/c mice. When tumor size reached ~400 mm³, mice were treated with DRP-104 (0.5 mg/kg) or vehicle *s.c., q.d.* for *5 days* and/or anti-PD-1 Ab (10 mg/kg) at day1 and 4. Phenotypic analyses of tumor infiltrating immune cells and tumor cells were performed by flow cytometry. Data indicate mean+/- SEM. *: p<0.05; ***: p<0.001; ***: p<0.0001.
M1 polarized TAM and decreased MDSCs were only observed in DRP-104 treated tumors.

DRP-104 Showed Distinctive Modulation of *In Vivo* Cytokines in TME Compared to Anti-PD-1 Ab

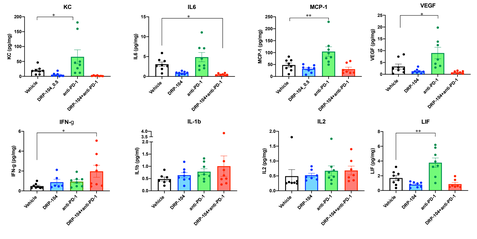


Figure 6. Cytokines in tumor lysates from CT26 study were measured by Luminex. Data indicate mean+/- SEM. *: p<0.05; **: p<0.01.
Distinct immunological modulation by DRP-104 and anti-PD-1 Ab and enhanced IFN-γ production in combination was observed.

Combination of DRP-104 with Anti-PD-1 Ab Enhanced Anti-Tumor Efficacy and Survival in CT26 Colon Cancer Model

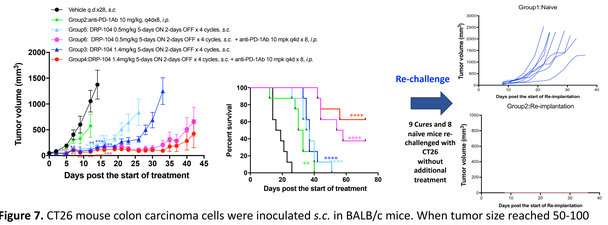


Figure 7. CT26 mouse colon carcinoma cells were inoculated *s.c.* in BALB/c mice. When tumor size reached 50-100 mm³, mice were randomized and treated with DRP-104, anti-PD-1Ab, or DRP-104/anti-PD-1Ab combination.
Anti-PD-1Ab showed marginal effect, while DRP-104 alone showed potent single agent efficacy and combination with anti-PD-1 Ab further inhibit tumor growth and significantly improved OS and long term durable cures (p<0.001 vs monotherapy).(Mean+/- SEM. n=10, **: p<0.01; ***: p<0.001; ****: p<0.0001). Cured animals from the combination group were re-challenged with CT26 tumor cells. 100% of the mice rejected the tumor implant suggesting potential long term immune memory response elicited by DRP-104 and PD-1 combination treatment.

Combination of DRP-104 with Anti-PD-L1 Ab Enhanced Anti-Tumor Efficacy and Survival in the H22 HCC Cancer Model

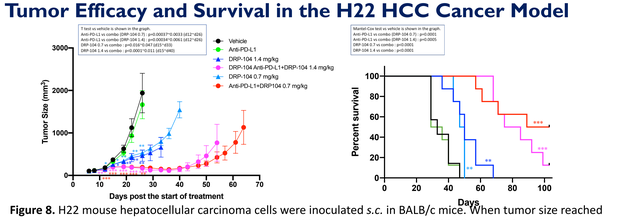
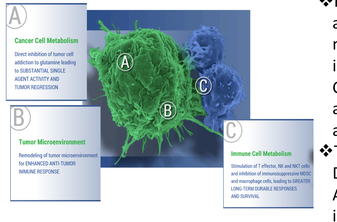


Figure 8. H22 mouse hepatocellular carcinoma cells were inoculated *s.c.* in BALB/c mice. When tumor size reached 50-100 mm³, mice were randomized and treated with DRP-104, anti-PD-L1Ab, or DRP-104/anti-PD-L1Ab combination.
Anti-PD-1Ab did not show any effect in this model, while DRP-104 alone showed potent single agent efficacy and combination with anti-PD-L1 Ab further inhibited tumor growth and significantly improved OS and long term durable cures.(Mean+/- SEM. n=10, *: p<0.05, **: p<0.01, ***: p<0.001)

Conclusions

- ❖Both DRP-104 and the active form DON showed Glutamine dependent inhibition of *in vitro* cancer cell growth (poster P816: Sat, Nov 9, 7:00am – 8:30pm).
- ❖DRP-104 achieved 6x tumor/plasma ratio for DON in CES-1 ko mice (poster P816: Sat, Nov 9, 7:00am – 8:30pm).
- ❖DRP-104 treatment showed dramatic immuno-oncology and metabolism related gene modulations.
- ❖Immuno-phenotypical analysis showed that DRP-104 induced substantial and broad changes in various immune cell infiltrates, such as increased TIL, T, NK and NKT cells, whereas an effect of anti-PD-1 Ab was limited to CD8⁺T cells. DRP-104 also polarized TAMs to M1 phenotype, and decreased immunosuppressive mMDSC in the TME.
- ❖Pro-tumorigenic proteins, such as VEGF, KC (IL-8), MCP-1, LIF and IL-6 were decreased by DRP-104. Combination showed significant increase in IFN-γ in TME.



- ❖Finally DRP-104 inhibited tumor growth as a single agent in CT26 and H22 tumor models which are non-responsive to immune checkpoint inhibitors. Combination with either anti-PD-1 Ab or anti-PD-L1 Ab enhanced efficacy and achieved significantly extended survival.
- ❖This data support clinical development of DRP-104 in combination with anti-PD-1 Ab or anti-PD-L1 Ab immune checkpoint inhibitors including settings of non-response to IO therapies.