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DRP-104, a Broad Acting Glutamine Antagonist, Synergizes with Immune Checkpoint Blockade *In Vivo*

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Summary

We previously reported that DRP-104 (sirpiglenastat) has significant therapeutic potential in cancer by directly targeting tumor metabolism and simultaneously inducing a potent antitumor immune response. We also reported the immunomodulatory effect of DRP-104 on tumor growth inhibition (TGI) as a single agent and in combination with PD-1/PD-L1 checkpoint inhibitors in the MC38 and CT26 colon cancer syngeneic models (1). DRP-104 mediated TGI is associated with increased tumor-infiltrating leukocytes (TIL) including T, NKT, and NK cells; M1-polarized tumor associated macrophages; and decreased immune-suppressive cells such as MDSCs.

Here we further elucidated the immunomodulatory effect of DRP-104 on tumor growth inhibition. In the CT26 mouse model, better efficacy was observed in immunocompetent mice compared to in immunodeficient mice. In the MC38 mouse model, $CD8^+$, $CD4^+$, or NK cells were depleted by anti-$CD8^+$Ab, anti-$CD4^+$Ab, or anti-asialoGM1 Ab respectively to assess the contribution of these immune cells towards DRP-104 efficacy. $CD8^+$T cell depletion significantly reduced anti-tumor efficacy for DRP-104. NK cell depletion delayed early anti-tumor response to DRP-104 treatment, while in undepleted mice DRP-104 treatment showed TGI as early as 2 days after dose initiation. Interestingly, $CD4^+$depleted mice showed enhanced efficacy to DRP-104. As such, we tested the combination of DRP-104 with checkpoint inhibitors; anti-CTLA-4, or anti-TIGIT antibody which has been reported to deplete Treg cells in the TME (2, 3). DRP-104 showed significant single agent TGI, and combination of DRP-104 with anti-CTLA-4 Ab, or anti-TIGIT Ab as well as anti-PD-1 Ab demonstrated enhanced tumor growth inhibition resulting in improvement in survival. Furthermore DRP-104 showed enhanced efficacy in combination with anti-PD-1 and anti-TIGIT Ab leading to long-term durable cures.

This unique and non-overlapping mechanism of action supports clinical development of DRP-104 (sirpiglenastat) alone and in combination with immune checkpoint inhibitors. The first in human clinical study of DRP-104 is ongoing.

Background

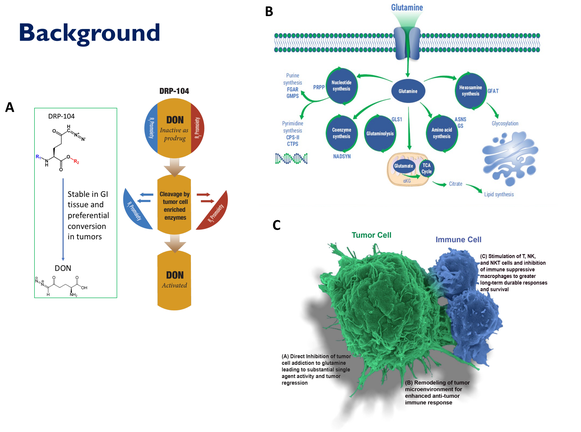


Figure 1. A. DRP-104 is a prodrug of the broad acting glutamine antagonist DON (6-Diazo-5-oxo-L-norleucine). DRP-104 is inactive in its prodrug form with high plasma and GI tissue stability. DRP-104 is preferentially distributed in tumors where it is bio-transformed and activated to the active moiety DON. **B.** Multiple glutamine dependent pathways are inhibited by DON (4). **C.** Model of MOA of DRP-104.

DRP-104 Increases Activation and Infiltration of Immune Cells into Tumors

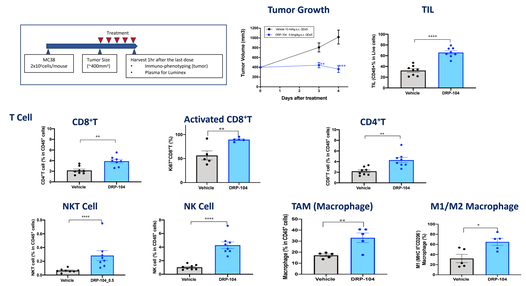
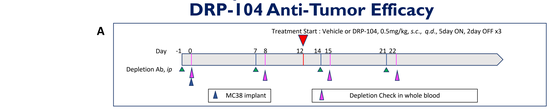
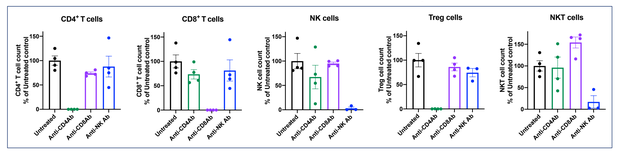


Figure 2. MC38 cells were inoculated *s.c.* in C57BL/6 mice. When tumor size reached ~400 mm^3, mice were treated with DRP-104 (0.5 mg/kg) or vehicle *s.c., q.d.* for 5 days. Phenotypic analyses of tumor infiltrating immune cells were performed by flow cytometry. n=8, *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001.
Increased tumor infiltration of activated T, NK and NKT cells, and M1 polarized TAM was associated with anti-tumor activity suggesting DRP-104's MOA includes immuno-oncology effects.

Innate and Adaptive Immune Cells Contribute to DRP-104 Anti-Tumor Efficacy



Confirmation of Immune Cell Depletion



Efficacy

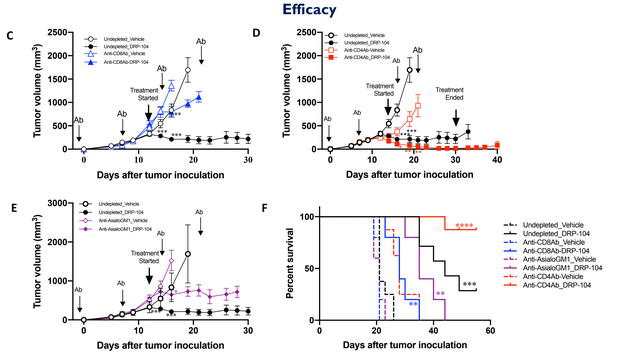


Figure 4. MC38 tumor bearing C57BL/6 mice were treated with anti-$CD8^+$Ab, anti-$CD4^+$Ab or anti-asialoGM1 Ab to deplete immune cells according to diagram **(A)**. Specific immune cell depletion was confirmed by whole blood analysis in 3-4 mice/depletion one day after every depletion Abs injection. Representative data at day 8 by flow cytometer shows confirmed immune cell depletion **(B)**. Mice were treated with DRP-104 0.5 mg/kg *s.c., qdx*5 days ON+2days OFFx3cycles or vehicle starting on day 12 after tumor implantation **(A)**. **(E)** Survival. n=5-8, **: p<0.01; ***: p<0.001.
$CD8^+$ cell depletion significantly reduced TGI (C), and NK cell depletion lacked the early response of DRP-104 leading to partial reduction of TGI (E) resulting in reduced survival effect of DRP-104 in $CD8^+$ T cell and NK depleted mice compared to undepleted mice (F). Interestingly $CD4^+$ cell depleted mice showed enhanced efficacy to DRP-104 with 6/8 tumor free mice at 29 days after the end of treatment (D & F) vs 1/8 mice tumor free in the undepleted group with DRP-104 treatment. These results indicate contribution of both innate and adaptive immune cells to the anti-tumor efficacy of DRP-104.

Immune Cells Contribute to DRP-104 Anti-Tumor Efficacy

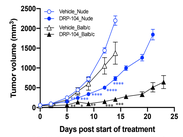


Figure 3. CT26 in immunocompetent (BALB/c) or immunodeficient (BALB/c nude) mice were treated with DRP-104 (0.5 mg/kg) or vehicle *s.c., q.d.* x5 days ON+2days OFFx4cycles. n=8, *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001.
Tumor growth inhibition (TGI) of DRP-104 was significantly reduced in immunodeficient nude mice (68.4%) as compared to the TGI in the immunocompetent mice (88.1% at day14) suggesting that immune cells contribute to DRP0-104 anti-tumor activity.

Combination of DRP-104 with Anti-CTLA-4 Ab or Anti-TIGIT Ab Demonstrates Enhanced Anti-Tumor Efficacy

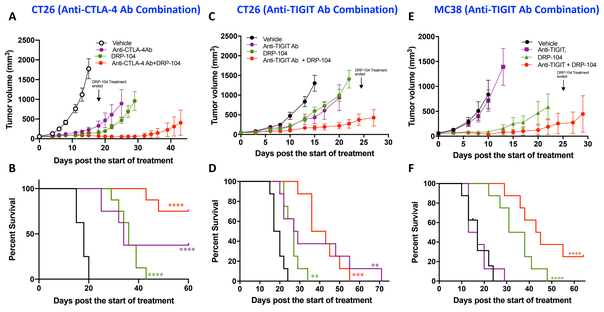


Figure 5. CT26 bearing BALB/c mice or MC38 bearing C57BL/6 mice were treated with DRP-104 (**A, B**; 1.4 mg/kg, **C-F**, 0.5 mg/kg) or vehicle *s.c., q.d.* x5 days ON+2days OFFx 3cycles (**A, B**) or 4cycles (**C-F**) and/or anti-CTLA-4 Ab (2.5 mg/kg), *i.p., q4dx*3 (**A, B**), or anti-TIGIT Ab 10 mg/kg, *i.p., biwx*4 (**C-F**). **A**, **C**, and **E** ; Tumor Growth, **B**, **D**, and **F**; Survival. n=8, **: p<0.01; ***: p<0.001; ****: p<0.0001.
DRP-104 and anti-CTLA4 Ab monotherapies showed similar TGI. Combining DRP-104 with anti-CTLA-4 demonstrated improved tumor growth inhibition and tumor growth delay (p<0.001 vs vehicle), leading to significant increase in survival including 6 out of 8 mice (75%) being tumor free at day 60 versus 3 out of 8 mice (37.5%) for the anti-CTLA-4 Ab monotherapy group.
In both anti-TIGIT Ab resistant MC38 and sensitive CT26 mouse models, DRP-104 showed improved TGI (p<0.01) in combination with anti-TIGIT Ab leading to enhanced median survival as well as increased long term durable cures in the MC38 tumor model.

Combination of DRP-104 with Anti-PD-1 Ab Demonstrates Enhanced Anti-Tumor Efficacy and is Associated with Cytokine Modulation

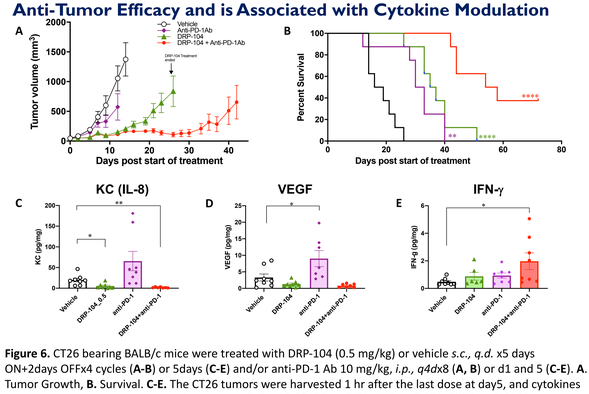
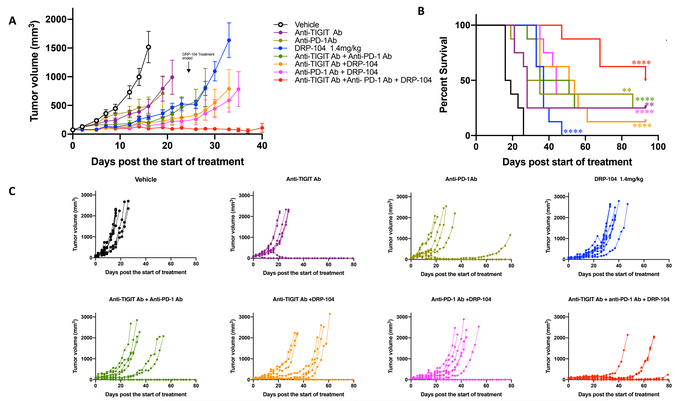


Figure 6. CT26 bearing BALB/c mice were treated with DRP-104 (0.5 mg/kg) or vehicle *s.c., q.d.* x5 days ON+2days OFFx4 cycles (**A-B**) or 5days (**C-E**) and/or anti-PD-1 Ab 10 mg/kg, *i.p., q4dx*8 (**A, B**) or d1 and 5 (**C-E**). **A.** Tumor Growth, **B.** Survival. **C-E.** The CT26 tumors were harvested 1 hr after the last dose at day5, and cytokines in tumor lysates was measured by Luminex. n=8, *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001.
While anti-PD-1Ab showed only partial TGI, DRP-104 inhibited tumor growth significantly (p<0.01). The efficacy was further pronounced when DRP-104 was combined with anti-PD-1Ab (p<0.01) with significantly improved tumor growth delay. Median survival was also significantly enhanced in the combination setting including 3/8 (37.5%) long term durable cures at day 77 (49 days after the last treatment) versus 0% in the monotherapy arms. DRP-104 treatment was associated with anti-tumorigenic cytokine modulations in the TME, which were distinct from anti-PD-1Ab treatment effects.

Triplet Combination of DRP-104 with Anti-PD-1Ab and anti-TIGIT Ab Further Enhances Anti-Tumor Efficacy



	Vehicle	Anti-TIGIT Ab	Anti-PD-1 Ab	DRP-104	Anti-TIGIT Ab +Anti-PD-1 Ab	Anti-TIGIT Ab +DRP-104	Anti-PD-1 Ab +DRP-104	Anti-TIGIT Ab +Anti-PD-1 Ab +DRP-104
Median Survival (day)	17.5	28.0	31.5	37.0	43.0	52.5	44.0	93.0

Figure 7. CT26 bearing BALB/c mice were treated with DRP-104 1.4 mg/kg or vehicle *s.c., q.d.* x5 days ON+2days OFFx4cycles and/or anti-TIGIT Ab 10 mg/kg, *i.p.* and/or anti-PD-1 Ab 3 mg/kg, *i.p., biwx*4. **A.** Tumor Growth, **B.** Survival, **C.** Spider Plots. n=8, *: p<0.05; **: p<0.01; ****: p<0.0001.
The triple combination of DRP-104 with anti-PD-1 Ab and anti-TIGIT Ab further enhanced TGI and showed improved survival.

Conclusions

- ❖Immuno-phenotypical analyses showed that DRP-104 induced substantial and broad changes in various immune cell infiltrates to tumors, such as increased TIL, proliferative T, NK and NKT cells. TAMs were polarized to M1 phenotype.
- ❖Efficacy of DRP-104 was significantly reduced in immunodeficient mice as compared to the TGI in the immunocompetent mice.
- ❖$CD8^+$ cell and NK cell depletion reduced TGI of DRP-104, while $CD4^+$ cell depletion enhanced anti-tumor efficacy of DRP-104.
- ❖DRP-104 enhanced efficacy of checkpoint inhibitors including anti-CTLA-4 Ab, anti-PD-1/PD-L1 Ab, and anti-TIGIT Ab, and showed significantly increased survival.
- ❖DRP-104 induced anti-tumorigenic cytokine modulations in TME distinct from anti-PD-1 Ab.
- ❖Triplet combination of DRP-104 with anti-PD-1 Ab and anti-TIGIT Ab, showed further enhanced TGI leading to an increase in long-term durable cures.

These data support clinical development of DRP-104 (sirpiglenastat) as a single agent and in combination with immune checkpoint inhibitors - including in settings of nonresponse to IO therapies. A first-in-human clinical trial is currently ongoing.

References

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