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Uncovering metabolic bottlenecks in KEAP1 mutant lung cancer

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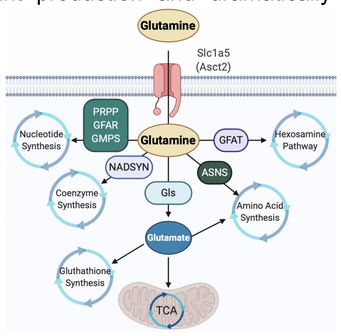
Abstract

During tumorigenesis, cancer cells continuously encounter metabolic bottlenecks as a result of accelerated growth, overall increased metabolic demand and increased oxidative stress due to the formation of reactive oxygen species (ROS). Lung cancer, the leading cause of cancer-related deaths worldwide, is the most common cancer type to acquire mutually exclusive gain-of-function mutations in the anti-oxidant transcription factor *NRF2* or loss-of-function mutations in its negative regulator *KEAP1.* Loss of *Keap1* activates Nrf2, increases antioxidant production and dramatically accelerates KRAS-driven lung cancer.

We observe that the ability of *KEAP1* mutant tumors to divert their metabolism towards antioxidant production comes with a cost, generating multiple metabolic vulnerabilities[1-3], including a dependency on glutamine metabolism that can be therapeutically exploited using DRP-104, a novel broad acting glutamine antagonist. DON (6-Diazo-5-oxo-L-norluecine) is the agonist moiety of DRP-104 that irreversibly inhibits all known enzymes in glutamine metabolism. Glutamine pathway inhibition by DON affects multiple pathways critical for tumor growth and survival (schematic). Broad inhibition of glutamine metabolism may provide a more effective therapeutic for glutamine addicted cancers and reduce the development of resistance.



Here we show DRP-104 is potent in reducing *KEAP1* mutant tumor growth in both murine and patient derived LUAD and squamous tumor models. Our data suggests that DRP-104 is a promising therapy to treat *KEAP1* mutant lung cancers and may offer further therapeutic potential when combined with standard of care or other novel metabolic strategies.

Results

DRP-104 reduces growth of Keap1 mutant murine LUAD

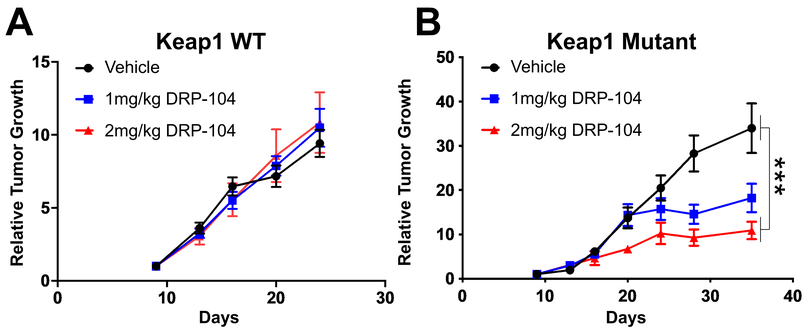


Figure 1: Relative tumor growth murine LUAD cell lines in immunocompromised animals. $Kras^{G12D/+}$;$p53^{-/-}$; (**A**) and $Kras^{G12D/+}$;$p53^{-/-}$;$Keap1^{-/-}$ (**B**) cell lines were injected subcutaneously into the flanks of nude mice. Animals were dosed subcutaneously in the opposite flank with either vehicle or DRP-104 daily for five days and then received a two day drug holiday.

Activation of Nrf2 induces sensitivity to DRP-104

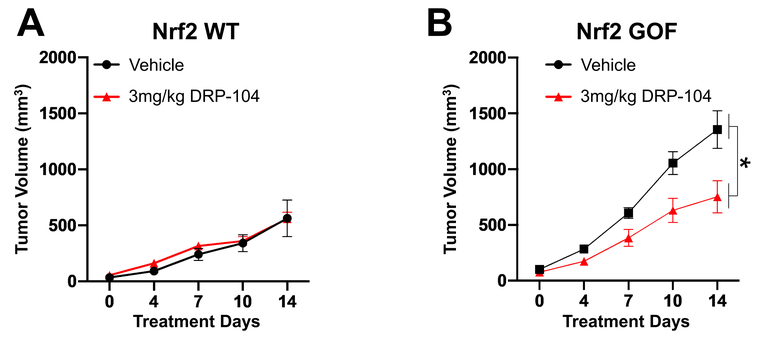


Figure 3: Tumor growth of murine LUAD cell line ($Kras^{G12D/+}$;$p53^{-/-}$) expressing an empty vector (**A**) or an Nrf2 gain of function construct (**B**) in immunocompetent animals. Cells were injected subcutaneously into the flanks of Black6 mice. Animals were dosed subcutaneously in the opposite flank with either vehicle or DRP-104 daily for five days and then received a two day drug holiday.

DRP-104 reduces growth of KEAP1 mutant patient derived xenografts

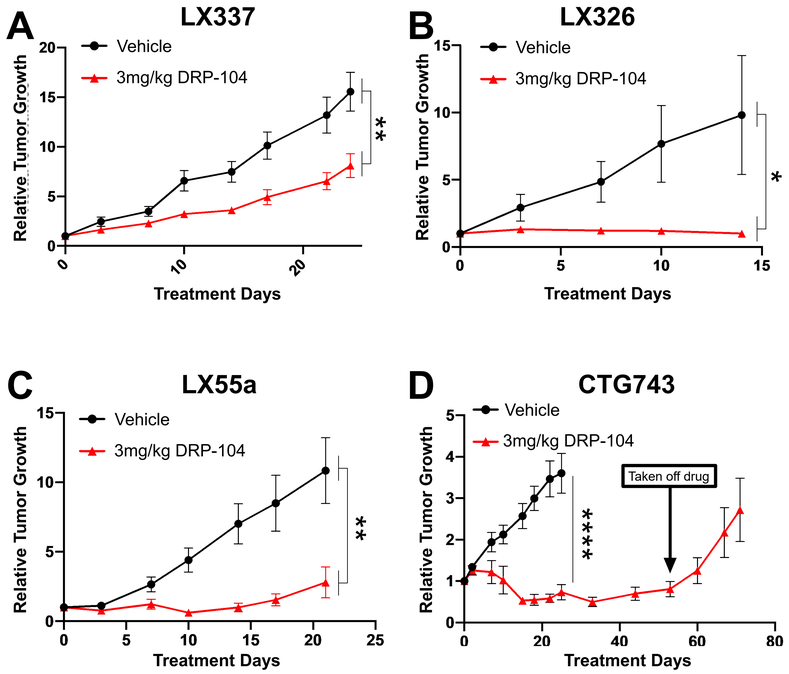


Figure 4: Relative tumor growth of patient derived KEAP1 mutant LUAD in immunocompromised animals. Tumors were implanted subcutaneously into the flanks of NSG mice. Animals were dosed subcutaneously with either vehicle or DRP-104 daily for five days and then received a two day drug holiday. CTG743 was treated until day 53 and then drug was removed. **A**)LX337: $Kras^{G12C}$; $Keap1^{G332}$ **B**) LX326: $Nras^{Q61L}$;$TP53^{R248L}$;$Keap1^{511C}$; **C**) LX55a: $Kras^{G12C}$; $TP53^{R248L}$; $Keap1^{D422N}$ D) CTG743: $Kras^{G12S}$;$TP53^{S215R}$; $Keap1^{H311R}$.

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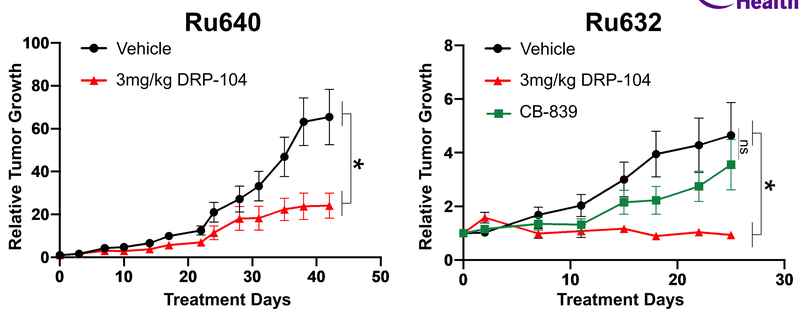

Figure 6: Relative tumor growth of patient derived KEAP1 mutant squamous cell carcinoma in immunocompromised animals. Tumors were implanted subcutaneously into the flanks of NSG mice. Animals were dosed subcutaneously in the opposite flank with either vehicle or DRP-104 daily for five days and then received a two day drug holiday. **A**) Ru640: $Keap1^{G511C}$ **B**) Where indicated, animals were dosed with 200mg/kg CB-839 twice daily by oral gavage. Ru632: $TP53^{-/-}$; $Keap1^{R320W}$.

DRP-104 reduces carbon flux to the TCA cycle in Keap1 mutant cell lines

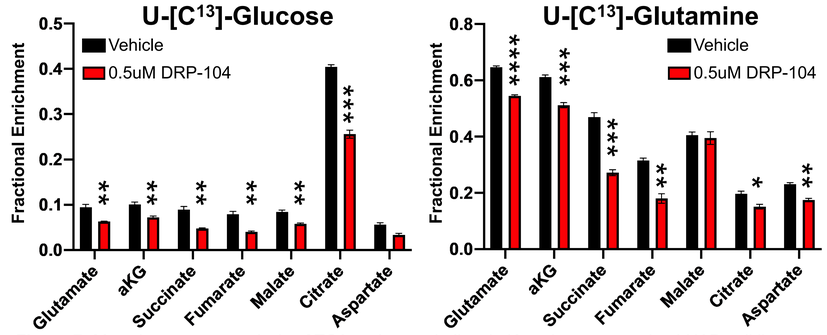

Figure 7: Mass isotopomer analysis of TCA cycle metabolites in Keap1 mutant murine LUAD cell lines cultured with U-[C^{13}]-Glucose (left) or U-[C^{13}]-Glutamine (right) and treated with vehicle or 0.5uM DRP-104.

Conclusions

- DRP-104 effectively inhibits tumor growth in Keap1 mutant murine LUAD tumor model
- Activation of Nrf2, which enhances glutamine dependence of tumor cells, sensitizes tumors to DRP-104
- DRP-104 effectively inhibits tumor growth in multiple Keap1 mutant LUAD and squamous PDXs, often resulting in tumor stasis or regression.
- DRP-104 is more effective in slowing tumor growth than glutaminase inhibition alone in mouse and PDX models[1].
- DRP104 reduces carbon flux into the TCA cycle.

References

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2. V. I. Sayin *et al.*, Activation of the NRF2 antioxidant program generates an imbalance in central carbon metabolism in cancer. *Elife* **6**, (2017).
3. S. E. LeBoeuf *et al.*, Activation of Oxidative Stress Response in Cancer Generates a Druggable Dependency on Exogenous Non-essential Amino Acids. *Cell Metab* **31**, 339-350.e334 (2020).