Effects of Adagrasib on Oncogenic Signaling, Immune Cell Regulation and Biomarkers of Response in Preclinical and Preliminary Clinical Analyses

Abstract #37

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BACKGROUND

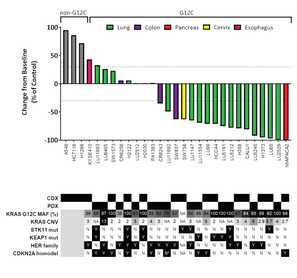
The recent advancement of KRAS^{G12C} inhibitors to the clinic has changed the landscape of research and led to novel treatment options for lung, colorectal and other cancer patients harboring this mutation. While treatment with KRAS^{G12C} inhibitors results in tumor regression or stable disease in the vast majority of patients, there is a range of responses both within and between cancer types and in lung and CRC in particular, which highlights the need for a better understanding of drug mechanism of action and the identification of rational combination strategies.

Extensive in vitro and in vivo preclinical evaluation was performed with adagrasib in KRAS^{G12C} mutated cell line and patient-derived xenograft models to evaluate drug-like properties, breadth of efficacy, and drug mechanism across tumor types to inform optimal clinical development. RNA sequence-based gene expression profiling coupled with functional CRISPR studies in preclinical models identified molecular biomarkers that correlated with anti-tumor activity and that also correlated with sensitivity to adagrasib in the clinic. Furthermore, preliminary gene expression analyses from baseline and post-adagrasib (C1D8) treated patient samples demonstrated multiple oncogenic pathway gene signatures, including MYC, mTOR, cell cycle, EMT and inflammation-related signatures, were significantly regulated by adagrasib and was similar to what was observed in preclinical studies.

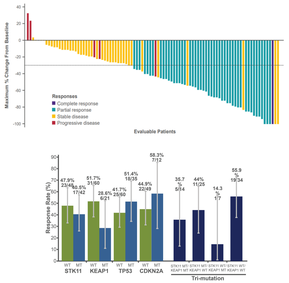
In agreement with the gene expression data and findings from syngeneic mouse models, preliminary immunohistochemical analysis on matched baseline, post-adagrasib treated (C1D8), and end of treatment FFPE patient samples revealed marked changes in tumor cell mechanistic biomarkers and immune cell types following adagrasib treatment. These observations included decreased tumor Ki67 and increased tumor PD-L1, as well as marked alterations in tumor immune cell composition, including increased CD8+ T cells and decreased MDSCs following adagrasib treatment in several patients. Together, these data suggest deep and robust preclinical evaluation of adagrasib may predict outcomes that can be achieved in patients harboring KRAS^{G12C} mutant cancers and can guide early clinical development strategies.

Fig. 1: Adagrasib Achieves Objective Responses in Models and Patients

A. Adagrasib in KRAS^{G12C}-mutated Tumor Xenograft Models



B. Adagrasib in Previously Treated Patients with KRAS^{G12C}-mutated NSCLC



A. 17 of 26 KRAS^{G12C} xenografts show >30% regression with MRTX849 at 100 mg/kg QD x ~21 days. Status of mutations and alterations in key genes are shown below each model. Hallin et al, Cancer Discovery, 2020.

B. All patient results based on BICR. Data as of Oct. 15, 2021. Objective responses observed in 43%, DCR was 80%. 75% of responders achieved >50% tumor reduction. Jänne, P, NEJM, June 2022. Bottom panel is ORR in patients harboring KRAS^{G12C} co-mutations.

- Similar activity of adagrasib observed in preclinical models and NSCLC patients
- Nonsignificant trend observed wherein *STK11* and/or *KEAP1* mutant models are partially adagrasib-resistant
- *KEAP1* mutant NSCLC patients exhibit reduced response rate to adagrasib

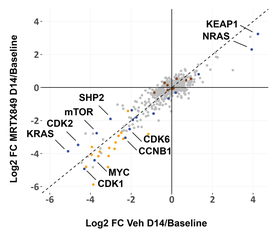
Key Translational Questions:

- Does *KEAP1* mutation confer resistance to adagrasib?
- What is the adagrasib mechanism of action and the effect on the anti-tumor immune response?
- What drives submaximal responses & can combinations improve responses and/or duration of response?

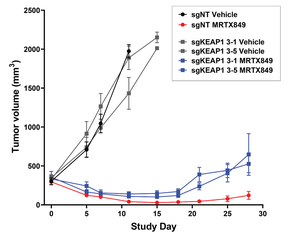
RESULTS

Fig. 2: *KEAP1* Knockout Confers Partial Resistance to Adagrasib in Preclinical Models *in vivo*

A. H2122 *in vivo* vs Baseline



B. LU99 CRISPR Clones

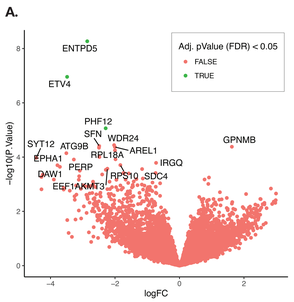


A. An sgRNA library targeting ~500 genes was transduced into H2122 cells and grown as tumor xenografts. *KEAP1*-targeting sgRNAs were enriched in vehicle or adagrasib-treated tumors suggesting *KEAP* LOF confers partial resistance to adagrasib treatment.

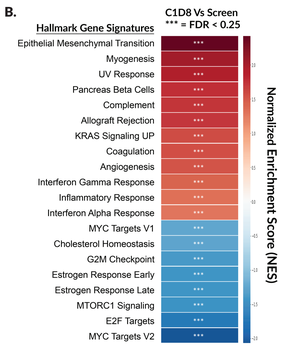
B. Lentivirus containing *KEAP1* or non targeting sgRNAs were transduced into LU99s and KEAP1 protein knock out was confirmed. *KEAP1* KO tumor xenografts demonstrated partial resistance to adagrasib in vivo.

Fig. 3: Adagrasib-Regulated Gene Expression Signatures in Patients Includes EMT, Oncogenic, Immune and Proliferation-related Gene Sets

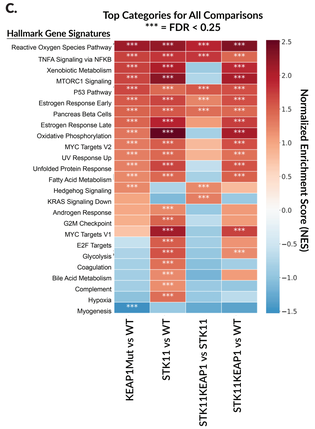
A.



B.



C.



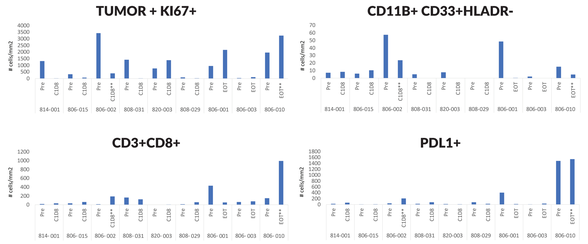
66 baseline tumor samples and 5 pretreatment and C1D8 tumor biopsy pairs from adagrasib monotherapy-treated NSCLC patients were run on the HTG EdgeSeq Transcriptome panel (HTG Molecular).

A. The MAP kinase regulated pathway gene ETV4 was among 3 significantly down regulated transcripts following adagrasib treatment.

B. GSEA analysis of Hallmark pathways significantly regulated following adagrasib treatment. Similar effects were observed by adagrasib treatment in nonclinical models (Hallin, J, Cancer Discovery, 2020) with additional immune-related effects observed in patients.

C. GSEA analysis of Hallmark pathways significantly differentially expressed in baseline samples from patients harboring *KEAP1* and/or *STK11* co-mutations. Data suggests co-mutations drive stronger oncogenic and proliferation gene expression programs.

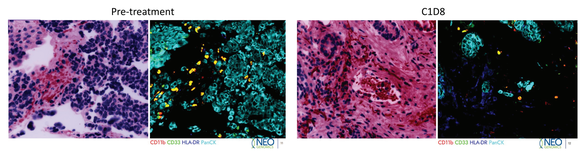
Fig. 4: Adagrasib Treatment Decreases Tumor Proliferation and Alters Key Intratumoral Immune Cell Populations in NSCLC Patient Biopsies



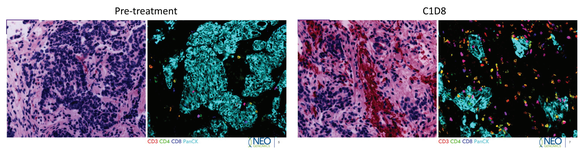
Pre and post adagrasib with 600 mg/kg monotherapy-treated (C1D8) and end of treatment (EOT) NSCLC biopsies were analyzed by immunofluorescence (Multiomyx, NeoGenomics) for 19 immune and cancer associated biomarkers within the tumor field.

- Strong decrease in the number of proliferating tumor cells post adagrasib treatment
- MDSC cell populations decreased with adagrasib treatment in a subset of patients
- CD8+ T cells increased with adagrasib treatment in most patients
- Evidence for an immune-related response to adagrasib treatment (increased PD-L1 expressing cells)
- Data correlates with nonclinical findings wherein adagrasib treatment led to decreased MDSCs, increased CD4 and CD8 T cells, and increased anti-tumor activity in combination with anti-PD-1 therapy (Briere, D. et al. MCT, 2021)

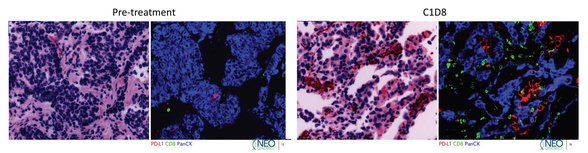
Fig. 5: Representative Immunohistochemistry Images from Patient Samples



A. CD11b/CD33/HLA-DR/PanCK staining in patient 001-806-002 pre-treatment vs Cycle 1 Day 8 (C1D8). CD11b+/CD33+/HLA-Dr^{low} signature of myeloid-derived suppressor cells (MDSC) markers are modulated in response to adagrasib treatment, indicating a reduction in the immunosuppressive state.

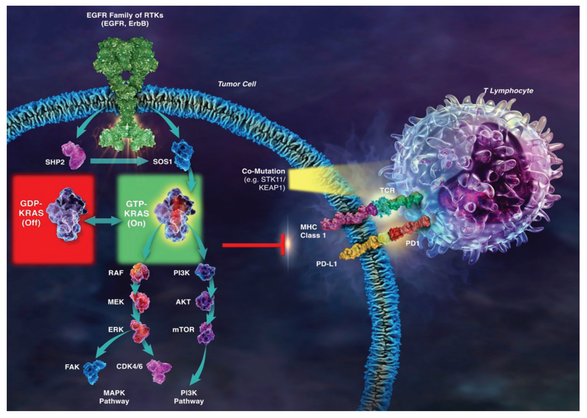


B. CD3/CD4/CD8/PanCK staining in patient 001-806-002 pre-treatment vs C1D8. CD3+/CD4+ and CD8+ T-cell populations are increased, following adagrasib treatment.



C. PD-L1/CD8/PanCK staining in patient 001-806-002 pre-treatment vs C1D8. PD-L1+ and CD8+ cell populations are increased with adagrasib treatment, indicating the potential of adagrasib to recondition the tumor immune microenvironment.

Fig. 6: Potential Combination Approaches with KRAS^{G12C} Inhibition



EGFR:
The EGFR signaling pathway is upstream of KRAS G12C and is often activated in tumor cells to bypass KRAS inhibition

SOS1:
SOS1 inhibition suppresses growth of xenograft KRAS-mutant tumors and prevents adaptive resistance to MEK inhibition

SHP2:
KRAS-mutant NSCLC has been found to be dependent on SHP2 activity in vivo

PD-1/PD-L1:
In preclinical models, the combination of KRAS G12C and PD-1 inhibition resulted in remission in more mice than either monotherapy approach alone

MAPK or PI3K Pathway Targets:
Several MAPK (eg, RAF, MEK, or CDK4/6) and PI3K (eg, PI3K, AKT, or mTOR) pathway targets lie downstream of KRAS G12C oncogenic signaling and may be activated in tumors resistant to KRAS G12C inhibition

CONCLUSIONS

- KEAP1 loss confers partial resistance to adagrasib in preclinical models
- Preliminary biomarker analysis of gene expression data from pre and post treated patient samples demonstrates adagrasib regulates EMT, KRAS/MAPK signaling, proliferation, and immune-related Hallmark signatures, similar to nonclinical models
- Key innate and adaptive immune cell populations are altered following adagrasib treatment in patient biopsies and nonclinical models
- Several rational combinations to complement the adagrasib mechanism of action and address identified mechanisms of resistance to monotherapy are currently ongoing

ACKNOWLEDGEMENTS

- We thank the patients and care givers for their participation, as well as clinical trial site staff and clinical study teams for their work and contributions
- NeoGenomics Laboratories for discussion and image production

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