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

Abstract B012

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BACKGROUND

The recent approval of KRAS G12C inhibitors has provided novel treatment options for lung, colorectal and other cancer patients harboring this mutation and has changed the landscape of research for these cancers. Current priorities in the field include characterizing the mechanism of action of KRAS G12C inhibitors and the mechanisms underlying resistance to KRAS inhibition.

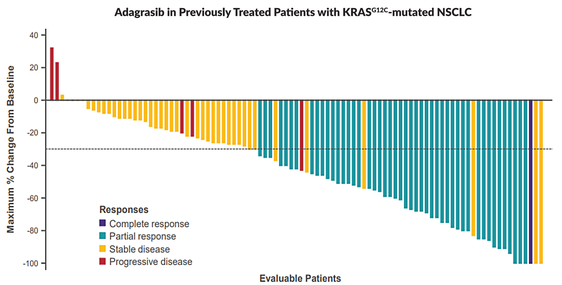
Utilizing access to pre and post-treatment patient samples from KRYSTAL-1, preliminary gene expression analyses from baseline and post-adagrasib (Cycle 1, Day 8) treated patient samples demonstrated multiple oncogenic pathway gene signatures, including MYC, mTOR, cell cycle, EMT and inflammation-related signatures, were significantly regulated by adagrasib.

KRAS/MAPK pathway and cell cycle-related genes including ETV4, CCND1, DUSP6, TOP1, and CENPA, as well as the Singh KRAS dependency signature were significantly downregulated in Cycle1, Day 8 compared to baseline tumor biopsies. Genes implicated in inflammation and the immune response were significantly upregulated in Cycle 1, Day 8 compared to baseline tumor biopsies.

In agreement with the gene expression data as well as findings from syngeneic mouse models, preliminary immunohistochemical analysis on matched baseline, post-adagrasib treated (Cycle 1, Day 8), 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. Finally, flow cytometry and TCRb sequencing data from adagrasib and pembrolizumab-treated patient blood samples demonstrated an increase in several activated CD8+ T cell populations and the emergence of new T cell clones in a subset of patients after combination treatment. Together, these data suggest robust evaluation of patient biopsies pre and post adagrasib treatment may better predict outcomes that can be achieved in patients harboring *KRAS*^{G12C} mutant cancers and can guide early clinical development strategies, including support for combining adagrasib with immune checkpoint inhibitors.

Figure 1. Adagrasib Demonstrates Objective Responses in *KRAS* G12C-Mutant Patients



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. Janne, P, NEJM, June 2022.

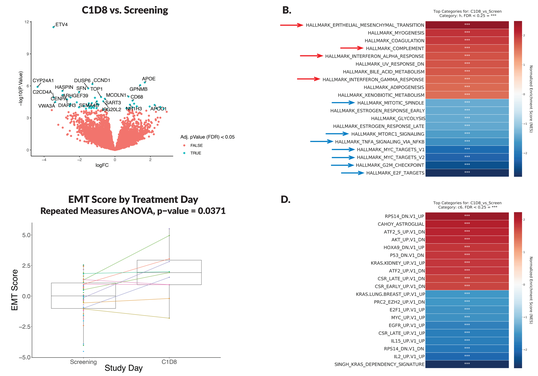
- Similar depth of anti-tumor activity with adagrasib treatment observed in preclinical models (Hallin et al, Cancer Discovery, 2020) and NSCLC patients
- Nonsignificant trend observed wherein *STK11* and/or *KEAP1* mutant models are partially adagrasib-resistant (4th RAS Initiative Symposium, Oct 17-19, 2022, poster #37)

Key Translational Questions:

- 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

Figure 2. Adagrasib-Regulated Gene Expression Signatures in Patients Includes EMT, Oncogenic, Immune and Proliferation-Related Gene Set Modulation Post Treatment Relative to Baseline



74 baseline tumor samples and 11 C1D8 samples which included 10 pretreatment and C1D8 tumor biopsy pairs from adagrasib monotherapy treated patients were run on the HTG EdgeSeq Transcriptome panel (HTG Molecular).

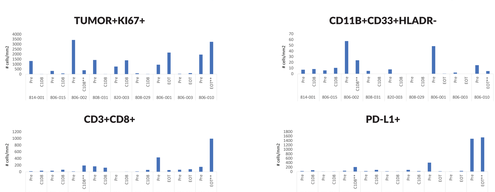
A. 51 genes are differentially expressed in C1D8 vs screening samples, with ETV4, CCND1, DUSP6 and CENPA among top hits of significantly down regulated transcripts. Key regulators of lipoprotein metabolism and macrophage function including ApoE, NR1H3, CD68, and APOC1 are significantly upregulated.

B. EMT, interferon alpha and gamma, and innate immune system among the highest significantly enriched pathways in C1D8 as compared to screening in GSEA Hallmark pathways following adagrasib treatment. MYC, G2M checkpoint, mitotic spindle, E2F, mTORC1, and TNF alpha signaling among the highest significantly enriched pathways in screening compared to C1D8.

C. Adagrasib treatment results in significantly increased EMT score as measured by treatment day in screening vs C1D8 adagrasib-treated patients.

D. Top 20 most differentially expressed pathways in GSEA Oncogenic gene sets defined from microarrays of cancer perturbation significantly regulated following adagrasib treatment, including Singh KRAS Dependency as the most deeply enriched in screening samples.

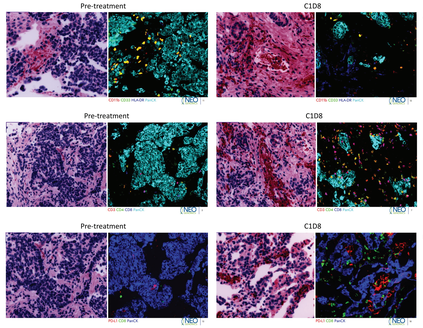
Figure 3. Adagrasib Treatment Decreases Tumor Proliferation and Alters Key Intratumoral Immune Cell Populations in NSCLC Patient Biopsies



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.

Data indicates adagrasib treatment results in decreased number of proliferating tumor cells, decreased MDSC cell populations, increased CD8+ T cells and increased PD-L1 expressing cells.

Figure 4. Representative Immunohistochemical Tumor Immune Alterations Following Adagrasib Treatment on Patient Tumor Biopsies



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.

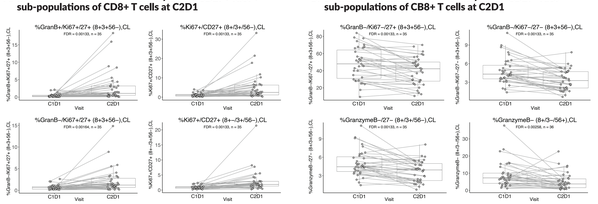
C. PD-L1/CD8/PanCK staining in patient 001-806-002 pre-treatment vs C1D8. PD-L1+ and CD8+ cell populations are increased with adagrasib treatment, indicating the potential of adagrasib to recondition the tumor immune microenvironment.

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)

Figure 5. Preliminary Data from Adagrasib + Pembrolizumab Treated Patients Shows Altered Proliferation and T-Cell Markers and Indicates Effects on T-Cell Repertoire Clonality

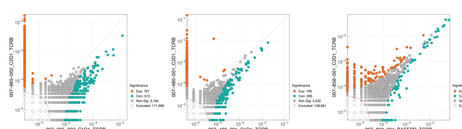
A. Increased abundance of % Granzyme B+ CD27+ Ki67+ sub-populations of CD8+ T cells at C2D1

B. Decreased abundance of % Granzyme B- CD27- Ki67- sub-populations of CB8+ T cells at C2D1



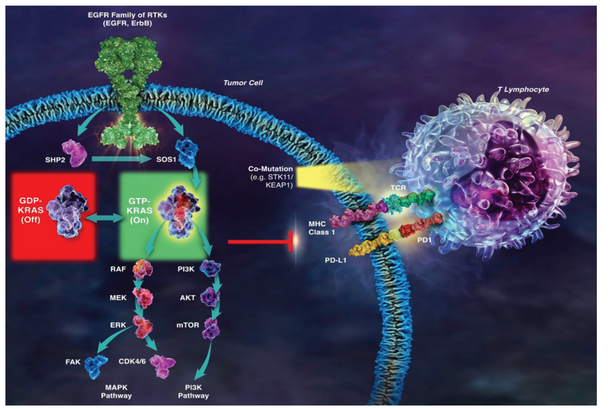
A and B. MRTX849-007: Blood from 64 patients treated with adagrasib + pembrolizumab were analyzed for immune cell populations by flow cytometry, with 35 or 36 patients with paired samples shown. GranzymeB+, Ki67+, CD27+ and CD8+ T-cell populations are increased at C2D1 indicating adagrasib + PD-1/L1 treatment leads to an increase in T cell infiltration in proliferating cells, an increase in costimulatory molecules, and a pro-inflammatory tumor microenvironment while the complementary negative populations are decreased at the C2D1 timepoint as compared to baseline.

C. Widespread expansion of novel TCR beta clones in 3 patients indicative of mounting an immune response



C. MRTX849-007: Whole blood from 34 patients treated with adagrasib + pembrolizumab were collected and had gDNA extracted at Adaptive Biotechnologies. 3 subjects exhibited notable expanded TCRb clones, including 2 patients with >98% of the expanded clones undetected at C1D1. Orange points represent clones significantly more abundant in C2D1. Teal clones are significantly more abundant in C1D1.

Figure 6: Rational 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

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

- Preliminary analysis of gene expression data from pre and post treated patient samples demonstrates adagrasib monotherapy regulates EMT, KRAS/MAPK signaling, proliferation, and immune-related signatures
- Key innate and adaptive immune cell populations are altered following adagrasib treatment in patient biopsies
- Adagrasib + pembrolizumab treatment results in altered proliferation and T-cell markers, including evidence of expansion of novel TCRb clones and elevated diversity in a subset of patients. This preliminary data indicates potential for effects on T-cell repertoire leading to heightened immune response and increased anti-tumor response in combination
- Several rational combinations to complement the adagrasib mechanism of action and address identified mechanisms of resistance to monotherapy are currently ongoing, including adagrasib + pembrolizumab

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