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14. ABSTRACT

Acquired drug resistance to specific KRAS G12C inhibitors sotorasib and adagrasib has been already been detected in patients and thus alternative drug treatment approaches are needed. In this project we have seen a strong correlation between drug resistance and NNMT overexpression. Innate sensitive cell lines and PDOs have higher NNMT levels and genetic knock-out of this protein modulates drug sensitivity. Similarly, the acquisition of drug resistance to either sotorasib or adagrasib in sensitive models has shown a relevant increase in NNMT expression. These models of acquired drug resistance mimic what happens in the clinic when tumors are no longer sensitive to the treatment and the tumor relapses. Genetic repression of NNMT in acquired resistant models also affects drug sensitivity, recapitulating what happens in innate resistant models.

NNMT is a metabolic enzyme that transfers methyl groups from universal donor SAM to nicotinamide, forming 1-MNA as a by-product. We have detected by UPLC-MS/MS that 1-MNA levels are higher in those models with higher levels of NNMT and significantly lower when NNMT protein is not detected. Finally, we have detected that those models with higher NNMT levels are more sensitive to NAMPT inhibition with daporinad.

15. SUBJECT TERMS
Acquired drug resistance, metabolic vulnerability, targeted therapy

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Introduction

The advances in targeted therapies have provided a shift of paradigm in the treatment of solid tumors. The recent FDA-approval of specific KRAS G12C inhibitors sotorasib and adagrasib have shown that KRAS is no longer undruggable and patients with this specific mutation can benefit from these treatments. However, acquired drug resistance is expected and understanding the mechanisms underlying this process and how to overcome is in dire need to provide alternative therapeutic approaches.

Body

Key words
Acquired drug resistance, metabolic vulnerability, targeted therapy

Accomplishments

- What were the major goals of the project?

The major goals for this project were the following:
Major task 1: Establishment of cellular models. Drug resistant models from NSCLC sensitive cells were developed.

Major task 2: Description of metabolic adaptations driven by NNMT in G12C resistant models. Evaluation of major adaptations in the metabolome of sensitive and drug resistant cells was performed. These results were used to define drug treatments that could target vulnerable pathways.

Major task 3: Evaluate the anti-tumor effect of daporinad. Differences in the cytotoxic effect of daporinad has been performed in vitro. In vivo evaluation od daporinad stills needs to be assessed.

- What was accomplished under the goals?

During the course of the project the following results have been obtained:
Major task 1: Establishment of cellular models.
Different approaches have been followed to accomplish this Task. First, acquired drug resistant cells were generated by increasing inhibitor concentration of either sotorasib or adagrasib until cell lines were growing normally at 10 μM. Second, genetic modulation of NNMT was performed to ectopically overexpress NNMT or knock-out the gene with CRISPR-Cas9 technologies. Cell viability of the obtained cell models was evaluated with different approaches (Fig. 1).

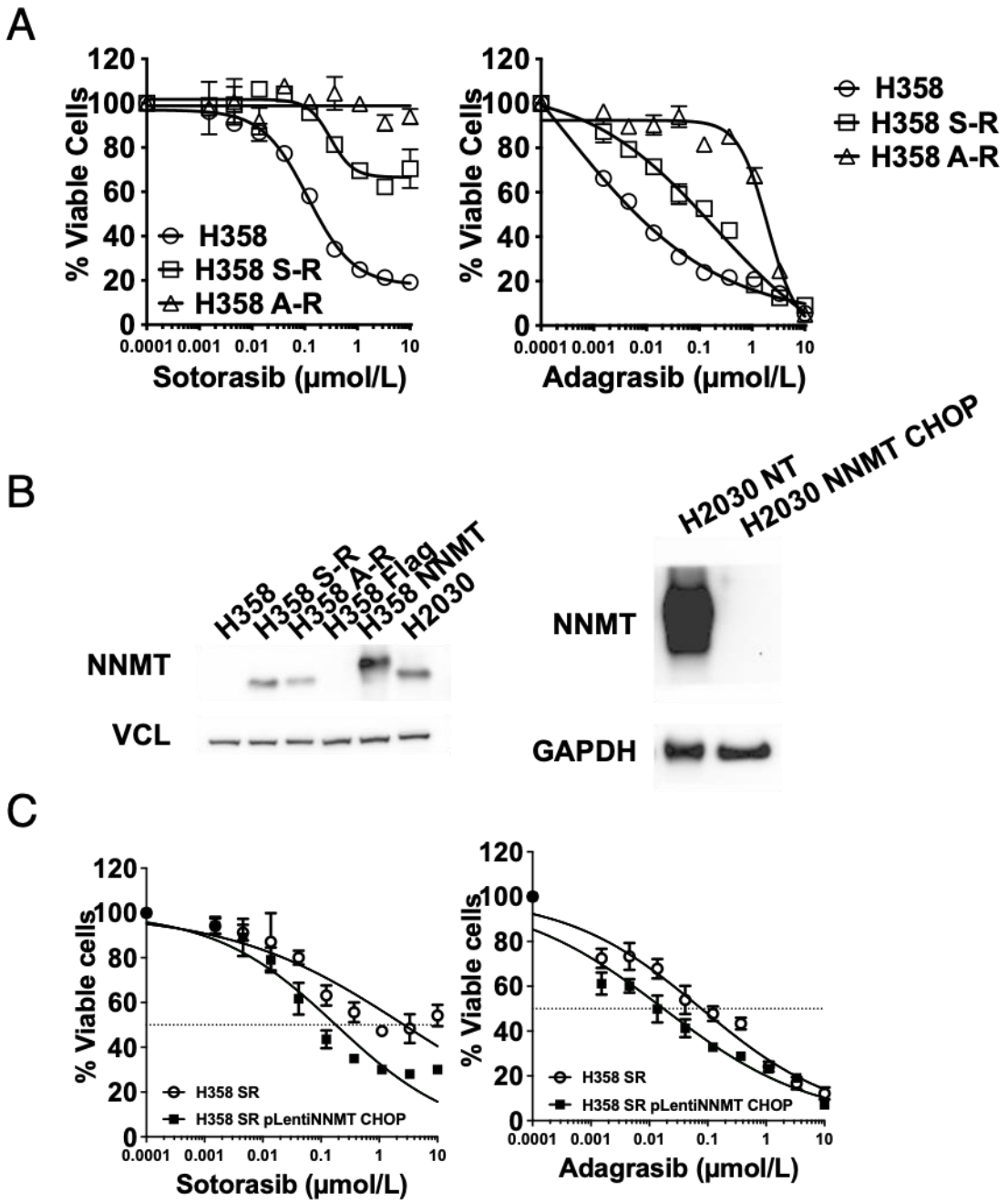

Fig. 1. Major task 1 goals. A. Acquired resistant cell lines were generated. B. Western blot evaluation of cell lines generated with genetic modulation. C. Cell viability assessment of the NNMT generated models.

Major task 2: Description of metabolic adaptations driven by NNMT in G12C resistant models.

NNMT is a metabolic enzyme that transfers methyl groups from SAM to nicotinamide, with 1-MNA as a by-product. Modulation of NNMT affects several metabolites of the methionine

pathway and we have seen that 1-MNA is the most significantly different between the analyzed models (Fig. 2).

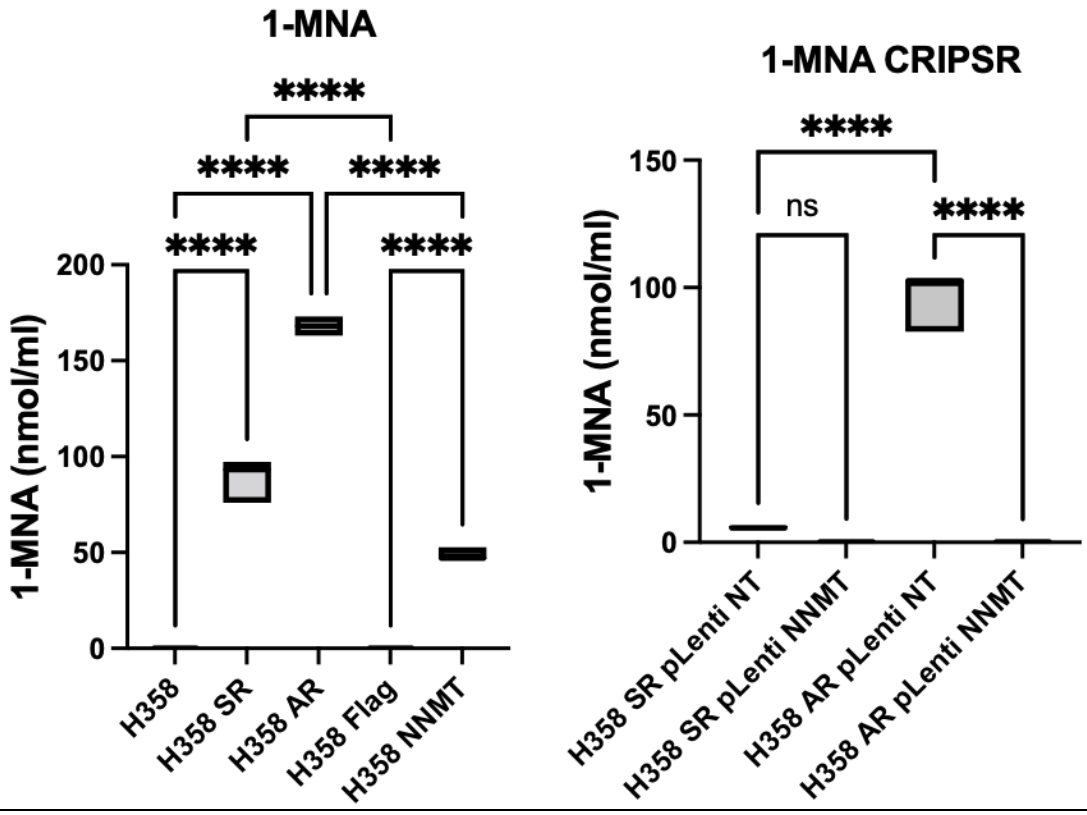

Fig. 2. Major Task 2 goals. UPLC-MS/MS analysis of 1-MNA, one the main metabolites that significantly changes in a NNMT-dependent manner.

Major task 3: Evaluate the anti-tumor effect of daporinad.

In vitro long-term evaluation of drug sensitivity was performed in the above-mentioned models with clonogenic assays. In these assays a low number of cells is seeded and treated with the required drug treatment for a long period of time. This approach allows for the growth of clones that are able to survive the treatment (Fig. 3).

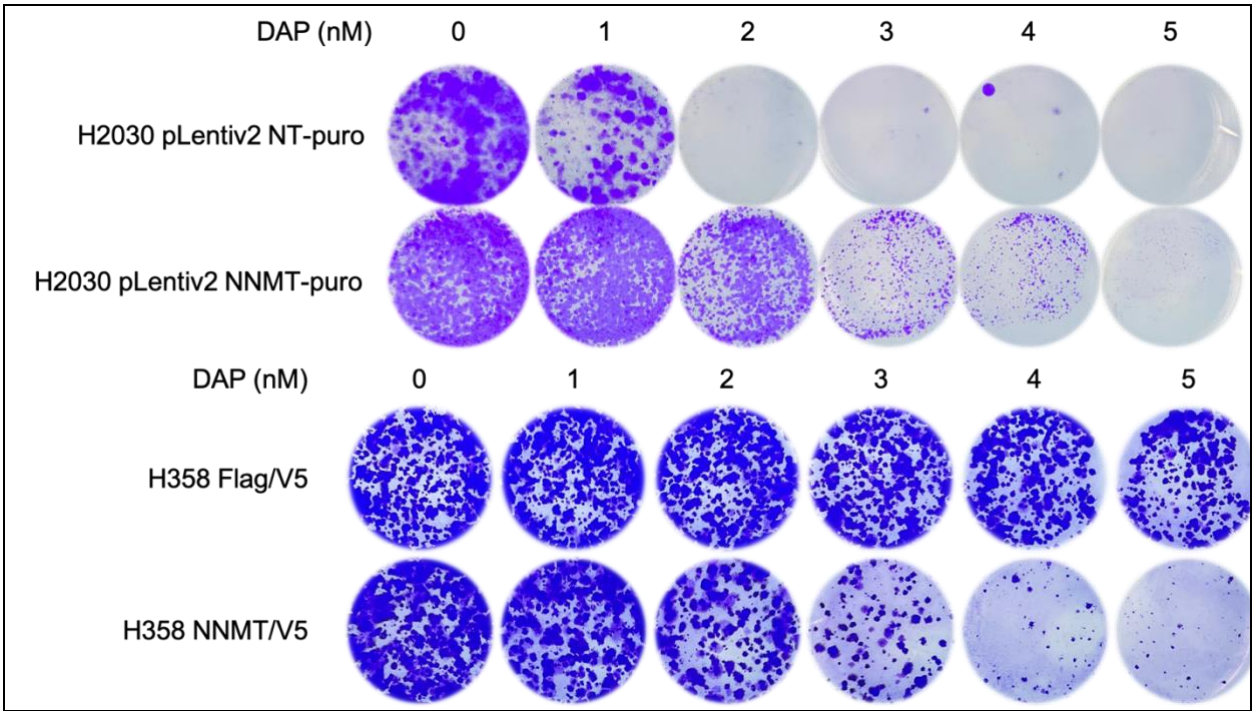

Fig. 3. Major Task 3 goals. Anti-tumor effect of daporinad. Cytotoxic effect of daporinad was evaluated in the generated cell lines.

The following step in the project will be the in vivo assessment of daporinad efficacy in tumor xenografts.

- What opportunities for training and professional development has the project provided?

Nothing to report

- How were the results disseminated to communities of interest?

Nothing to report

- What do you plan to do during the next reporting period to accomplish the goals?

In the remaining period of the project, we aim to perform the animal studies that will evaluate the in vivo efficacy and feasibility of NNMT^{high} tumors with daporinad. Treated and untreated tumors will be measured and pharmacodynamic markers that evaluate daporinad effect will be performed.

After completion of the above-mentioned results, researchers anticipate that these results and the previously obtained will be ready to be presented in a conference.

Impact

- What does the impact on the development of the principal discipline of the project?

We have generated a relevant number of cell lines that have an enormous potential to explain acquired drug resistance to KRAS G12C inhibitors.

- What was the impact on other disciplines?

Nothing to report

- What was the impact on technology transfer?

Nothing to report

- What was the impact on society beyond science and technology?

Nothing to report

Changes/problems

- Changes in approach and reasons for change

Nothing to report

- Actual or anticipated problems or delays and actions or plans to resolve them

We required a no-cost extension in order to re-run crucial experiments whose unexpected outcomes have to be verified. This will be needed to complete a part of the original project's objectives. The extension is needed to accomplish some of the objectives that have been delayed because some technical issues.

- Changes that had a significant impact on expenditures

Nothing to report

- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

- Significant changes in use or care of human subjects

Nothing to report

- Significant changes in use or care of vertebrate animals

Nothing to report

- Significant changes in use of biohazards and/or select agents

Nothing to report

Products

During the course of the project several cell lines have been developed. Acquired resistant cells to sotorasib and adagrasib cells derived from H358 sensitive cells. Genetic overexpression of NNMT cell lines and genetically knock-out of NNMT cells have also been generated.

Participants & Other Collaborating organizations

Ines Pulido Endrino: no change
Takeshi Shimamura: no change

Key Research Accomplishments

- Generation of acquired resistant NSCLC models to KRAS G12C inhibitors. These newly developed cell lines will be extremely useful to understand how drug resistance to these inhibitors appears.
- Generation of genetically modified cell lines. Genetic knock-out of NNMT with CRISPR-Cas9 in naturally NNMT overexpressing cells was done. Overexpression of ectopic NNMT was done in cells without NNMT. These cell lines have been crucial to understand the role of NNMT in drug resistance and metabolic adaptations.
- Evaluation of metabolic adaptations in NSCLC during acquisition of drug resistance. Our resistance models have provided insight into the readaptations that cells undergo during the transition from sensitive to insensitive to G12C inhibitors.
- In vitro evaluation of DAP sensitivity. NNMT overexpressing cells are exquisitely sensitive to DAP whereas cells with lower levels of NNMT protein are not sensitive to this inhibitor. These results have been validated with the genetic modified cells.

Reportable Outcomes

- A relevant connection has been found between high levels of the NNMT protein and a higher resistance to KRAS G12C inhibitors
- Changes occurred during the acquisition of G12C inhibitors resistance cells undergo a series of molecular adaptations that affect several processes. In fact, drug resistant cells adapt their metabolism.
- The specific metabolic characteristics of drug resistant cells can be used as a collateral but also druggable vulnerability.
- The study of the metabolome of sensitive and resistant cells provide a very helpful landscape to generate alternative therapeutic approaches when drug resistance appears.
- This results can be translated to the clinic to understand how drug resistance appears and how to tackle it.

Conclusion

Although the approval of KRAS G12C inhibitors have improved the outcome of this specific subset of patients it is expected that drug resistance will happen. Thus, it is tremendously important to find alternative therapeutic approaches that can solve this issue. Our results during the acquisition of the drug resistant phenotype and the genetic modulation of this protein further support the relevant role of NNMT in KRAS drug resistance.

NNMT is metabolic enzyme that reducing nicotinamide levels and modulating the balance of methyl groups. These adaptations make NNMT an incredibly interesting target since inhibitors of NAMPT are available. NNMT overexpressing models are exquisitely sensitive to DAP (daporinad) in vitro. These metabolic vulnerabilities derives from the compromised levels of NAD+, a key energetic molecule. The use of DAP can target specifically those cells that are resistant to the drug treatment providing an alternative therapeutic treatment when cells become resistant to G12C inhibitors.