Correlation of *nab*-sirolimus tumor drug levels and improved tumor suppression in *KRAS G12C* non-small cell lung cancer xenografts treated with *nab*-sirolimus in combination with KRAS inhibitors

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Objective

- **To compare the tumor-targeting effects of *nab*-sirolimus and everolimus when administered alone or in combination with KRAS G12C inhibitors, and to correlate these effects with the impact on tumor growth, tumor drug accumulation, and inhibition of key downstream biomarkers of the mTOR pathway**

KEY FINDINGS

- **The combination of *nab*-sirolimus and KRAS G12C inhibitors significantly improved antitumor activity compared with single agents and the combination of everolimus and KRAS G12C inhibitors**
- **Corresponding with greater antitumor activity, *nab*-sirolimus was associated with higher intratumoral drug concentration and stronger mTOR target suppression than everolimus when combined with KRAS G12C inhibitors**
- **These findings suggest more efficient tumor targeting with *nab*-sirolimus plus a KRAS G12C inhibitor may lead to improved target inhibition and improved clinical outcomes**
- **Clinical dose-finding studies are needed to determine potential drug–drug interactions between *nab*-sirolimus and KRAS inhibitors**

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BACKGROUND

- The *KRAS G12C* mutation is the most common *KRAS* variant in non-small cell lung cancer (NSCLC), present in 10–13% of advanced non-squamous NSCLC[1]
- Two selective KRAS G12C inhibitors, sotorasib and adagrasib, are approved to treat *KRAS G12C*-mutated, locally advanced or metastatic NSCLC[2,3]; however, resistance to KRAS G12C inhibitors poses an ongoing challenge[4,5]
- Novel therapeutic approaches, including combination therapy across parallel pathways, are under investigation[5,6]
- The mTOR pathway is often activated in patients with *KRAS* mutations and may contribute to resistance to KRAS G12C inhibitors[5,6]
- *nab*-Sirolimus is a nanoparticle, albumin-bound, IV-administered mTOR inhibitor (mTORi) approved in the United States for the treatment of adults with advanced malignant PEComas[7,8]
- This study investigated the antitumor activity and the effects on signaling pathways of *nab*-sirolimus, everolimus, sotorasib, and adagrasib, alone or in combination, in *KRAS G12C*-mutated NSCLC xenografts

METHODS

- Athymic mice (n = 5 per treatment group) bearing subcutaneous NSCLC xenografts (NCI-H2122; *KRAS G12C*, *STK11* null, *TP53 C176F*) in both flanks were treated with the following, alone or in combination (**Table 1**):
  - Saline (control)
  - mTORi (*nab*-sirolimus or everolimus)
  - KRAS G12C inhibitor (sotorasib or adagrasib)
- Body weight and tumor volume were recorded three times/week
- Animals were sacrificed if a tumor exceeded 2000 mm^3 or at the end of the study (6 weeks)
- Tumor and blood samples were harvested for analysis of trough drug levels by liquid chromatography-tandem mass spectrometry
- Tumor lysates were analyzed for suppression of downstream markers of mTOR inhibition by Western blot

Table 1. Treatment regimens in a *KRAS G12C*- and *STK11*-mutated mouse NSCLC xenograft model

Treatment	Dose/ frequency[a]	Weekly dose (mg/kg)	% Clinical weekly dosing	Route of administration
Saline (control)	10 mL/kg, twice weekly	0	NA	IV
nab-Sirolimus	7.5 mg/kg, twice weekly	15	45	IV
Everolimus	3 mg/kg, 5 days/week	15	115	PO
Sotorasib	30 mg/kg, 5 days/week	150	11	PO
Adagrasib	30 mg/kg, 5 days/week	150	9	PO

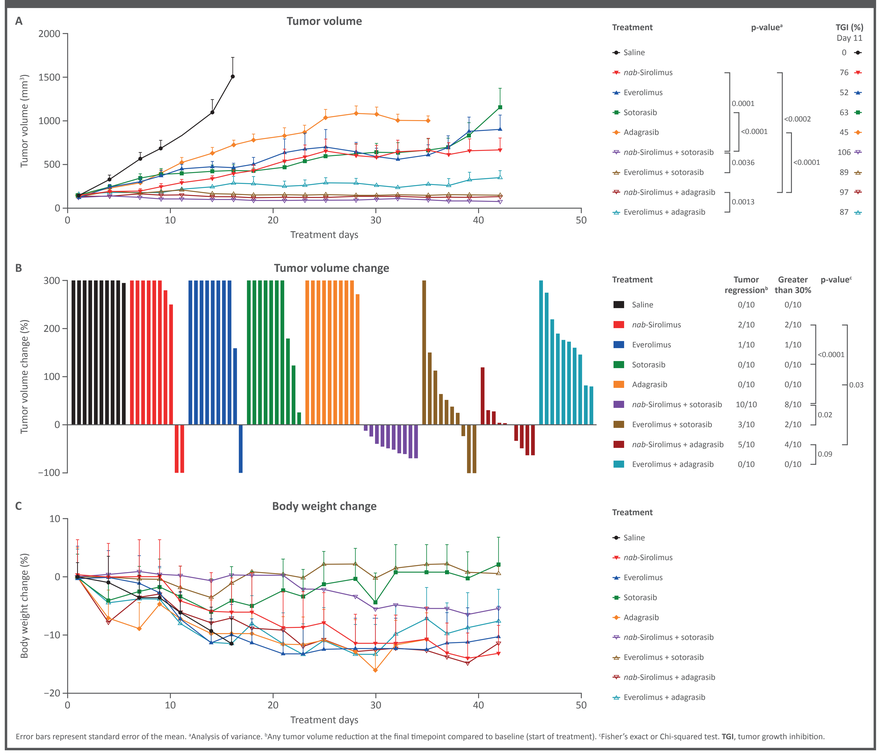
[a]Doses of mTORis are clinically relevant and equal weekly doses. **IV**, intravenous; **NA**, not applicable; **PO**, oral. Note: single-agent dosing regimens were employed in combination treatment groups.

RESULTS

nab-Sirolimus plus a KRAS G12C inhibitor reduced tumor growth

- *nab*-Sirolimus in combination with sotorasib or adagrasib demonstrated significantly greater tumor growth suppression compared with each as single-agent treatment and combinations of either KRAS G12C inhibitor with everolimus (**Figure 1A**)
  - There was no significant difference in tumor growth suppression between *nab*-sirolimus plus sotorasib and *nab*-sirolimus plus adagrasib
- The combination of everolimus plus a KRAS G12C inhibitor failed to improve tumor regression rates over single-agent treatment with either sotorasib or adagrasib (**Figure 1B**)
- Significantly higher rates of tumor regression >30% were observed for *nab*-sirolimus plus sotorasib or adagrasib compared with single-agent sotorasib ($p < 0.0001$) or adagrasib ($p = 0.03$) and for *nab*-sirolimus plus sotorasib versus everolimus plus sotorasib ($p = 0.02$) (**Figure 1B**)
  - A similar nonsignificant trend was observed for *nab*-sirolimus plus adagrasib versus everolimus plus adagrasib ($p = 0.09$) (**Figure 1B**)
- Treatments were tolerable with no overt signs of toxicity and produced a similar body weight change pattern when compared with the saline control (**Figure 1C**)

Figure 1. Tumor volume (A), tumor volume change (B), and body weight change (C) following treatment with *nab*-sirolimus, everolimus, sotorasib, or adagrasib, alone or in combination

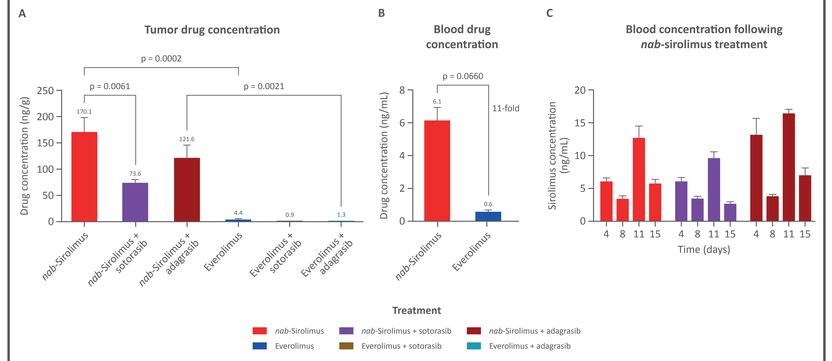


Error bars represent standard error of the mean. [a]Analysis of variance. [b]Any tumor volume reduction at the final timepoint compared to baseline (start of treatment). [c]Fisher's exact or Chi-squared test. **TGI**, tumor growth inhibition.

Tumor and blood pharmacokinetics of mTOR inhibitors alone or in combination with KRAS G12C inhibitors

- For single-agent mTORi, *nab*-sirolimus trough concentrations were higher than everolimus trough concentrations in both tumor (39-fold, $p = 0.0002$) (**Figure 2A**) and blood (11-fold, $p = 0.0660$) (**Figure 2B**)
  - The tumor/blood extraction ratio was significantly higher with *nab*-sirolimus than with everolimus (30.2 vs 7.7, $p = 0.030$), indicating preferred tumor drug uptake
- Compared with single-agent *nab*-sirolimus, treatment with *nab*-sirolimus plus sotorasib resulted in significantly reduced *nab*-sirolimus trough tumor concentrations (170.1 vs 73.6 ng/g, $p = 0.0061$), but did not impact trough blood concentrations (**Figures 2A** and **2C**)
- *nab*-Sirolimus trough tumor concentrations following single-agent treatment or treatment with *nab*-sirolimus plus adagrasib were similar (170.1 vs 121.6 ng/g, $p = 0.0061$), as were trough blood concentrations (**Figures 2A** and **2C**)
- When *nab*-sirolimus and everolimus were combined with a KRAS G12C inhibitor, significantly greater trough tumor concentrations were observed with *nab*-sirolimus plus sotorasib (85-fold, $p < 0.0001$) or adagrasib (91-fold, $p = 0.0021$) (**Figure 2A**), corresponding with the greater antitumor activity observed with the *nab*-sirolimus combinations

Figure 2. Comparison of trough drug concentrations in (A) tumor and (B, C) blood from mice treated with mTOR inhibitors alone or in combination with KRAS G12C inhibitors

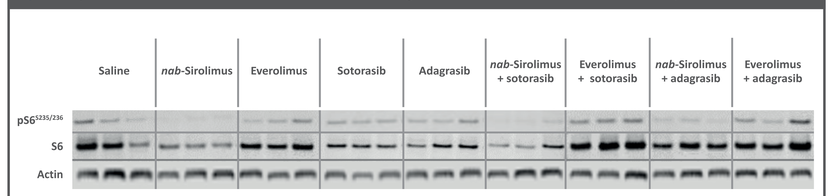


Error bars represent standard error of the mean.

Inhibition of downstream mTOR signaling with mTOR inhibitors and KRAS G12C inhibitors

- Stronger mTOR downstream target inhibition of phosphorylated ribosomal protein S6 (pS6) was observed for *nab*-sirolimus plus KRAS G12C inhibitor-treated groups compared with everolimus plus KRAS G12C inhibitor-treated groups (**Figure 3**)

Figure 3. Expression of downstream markers of mTOR inhibition following treatment with mTORi and KRAS G12C inhibitors, alone or in combination



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