Targeted Inhibition of the HGF/c-Met Pathway by Merestinib Augments the Effects of Albumin-Bound Paclitaxel in Gastric Cancer

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Abstract

Background and Hypothesis: Combination chemotherapy regimens are commonly used to treat gastric adenocarcinoma (GAC), but the median survival time remains less than one year. *Nab*-paclitaxel has demonstrated high antitumor activity in previous GAC studies. Many growth factors and their receptors are overexpressed in GAC and have been implicated in its pathophysiology. We hypothesize that merestinib, a small-molecule inhibitor targeting c-Met, Axl, and DDR1/2 pathways, will have significant antitumor effects and will enhance the response to *nab*-paclitaxel in GAC preclinical models.

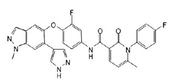
Project Methods: *In vitro* proliferation and protein expression were assessed using WST-1 and immunoblot assays. Subcutaneous xenografts of MKN-45 and SNU-1 cell lines were implanted in mice to study tumor growth inhibition. Immunohistochemistry was performed to examine intratumor proliferation and microvessel density.

Results: *In vitro* assays showed that *nab*-paclitaxel and merestinib decreased cell proliferation in all three cell lines, with an additive effect in combination. Reduction in cell proliferation at low doses of *nab*-paclitaxel (10 nM), merestinib (100 nM), and their combination was 87%, 82%, and 94% (MKN-45 cell line, high phospho-c-Met expression), 59%, 50%, and 82% (SNU-1 cell line, low phospho-c-Met expression), and 53%, 19%, and 66% in gastric fibroblasts. Immunoblot analysis of merestinib treated MKN-45 cells revealed increased expression of apoptotic proteins and decreased expression of phospho-c-Met, phospho-EGFR, phospho-IGF-1R, phospho-ERK, and phospho-AKT. In gastric fibroblasts, merestinib decreased phospho-ERK and increased apoptotic protein expression. Phospho-c-Met and phospho-EGFR were not detected in SNU-1 immunoblots; however, phospho-ERK, phospho-VEGFR, and apoptotic protein expression increased after treatment. In MKN-45 xenografts, net tumor growth in control, *nab*-paclitaxel, merestinib, and combination groups was 503 mm^3, 115 mm^3, 91 mm^3, and -9.7 mm^3. Immunohistochemistry analysis of tumor cell proliferation and microvessel density corroborated tumor growth study results.

Conclusion: The data suggest that merestinib in combination with nab-paclitaxel carry a promising potential for improving clinical GAC therapy.

Background

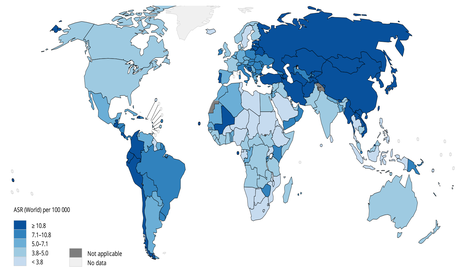
- **Gastric Cancer**
  - Fifth most common cancer and third most common cause of cancer-related deaths worldwide
  - Previously most common cause of cancer-related deaths worldwide up to the mid-1990s.
  - Decline in prevalence and mortality reflects changes in diet, smoking, and *H. pylori* infections, which are risk factors for gastric cancer development
  - In US, 33% five-year survival rate overall.
  - For those with distant metastasis, which is most common at diagnosis, five-year survival rate is 5.9%
- **Nab-Paclitaxel (NPT)**
  - Water soluble, solvent-free, albumin-bound paclitaxel
  - Cell mitosis inhibitor
  - Enhanced delivery to tumor leading to superior antitumor activity and less toxicity
- **Merestinib**
  - Small-molecule type II ATP competitive, slow-off inhibitor of MET kinase, as well as many other receptor tyrosine kinases implicated in cancer
  - Low dissociation constants for relevant receptor tyrosine kinases, including Met, Axl, and DDR1/2
  - Current clinical trial for non-small cell lung cancer and advanced biliary tract cancer
- **C-met signaling pathway**
  - Receptor tyrosine kinases are often overactivated or overexpressed in many types of cancer, including gastric adenocarcinoma
  - Involved in cell proliferation, survival, motility, cell cycle progression, and invasion
  - Activates Ras, PI3K, STAT, beta-catenin, and Notch signal transduction pathways
  - Activation of the c-Met pathway reported in a significant proportion of gastric adenocarcinoma patients (10-50%)



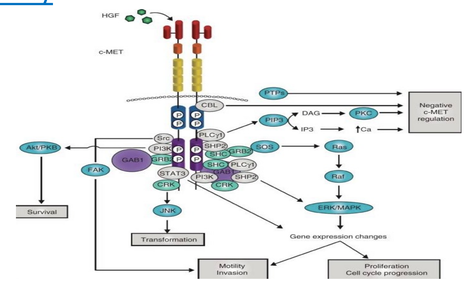
Methods

- Cell lines used: MKN-45, SNU-1, and human gastric fibroblasts
- Protein expression: Immunoblot analysis
- *In vitro* cell proliferation: WST-1 assay
- Tumor growth: Subcutaneous xenografts in NOD/SCID mice using MKN-45 and SNU-1 cells
- Cancer cell proliferation and microvessel density: Immunohistochemistry

Gastric Cancer Incidence Rates by Country

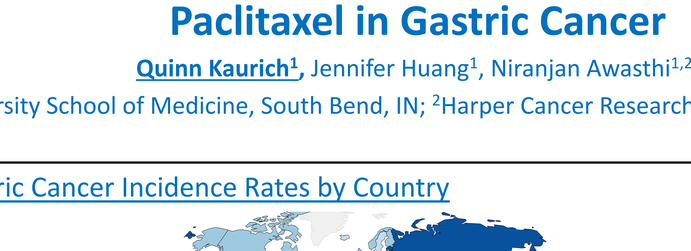


HGF/c-Met Pathway

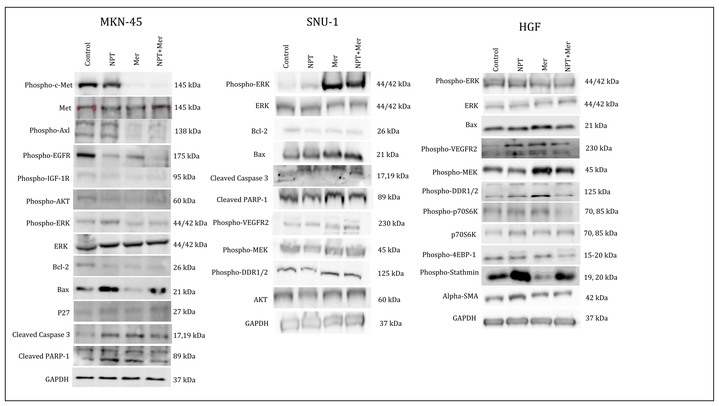


Results

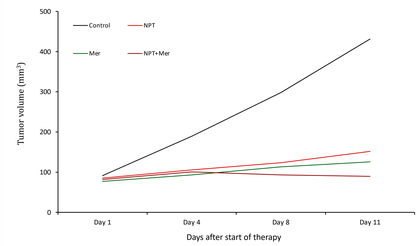
Proliferation Assays

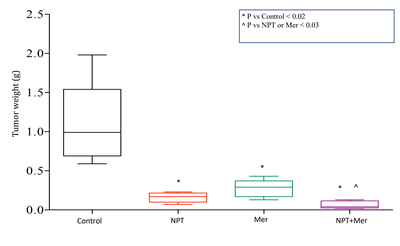


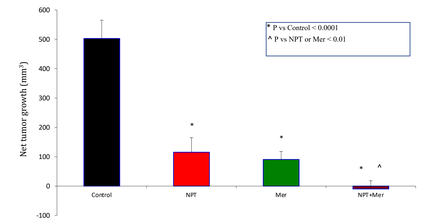
Immunoblot Analysis

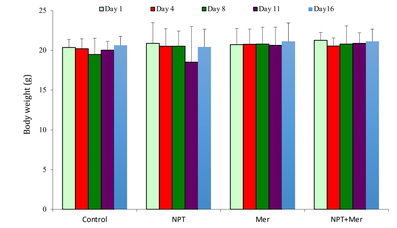


Tumor Growth in MKN-45 Xenografts (high phospho-c-Met expression)

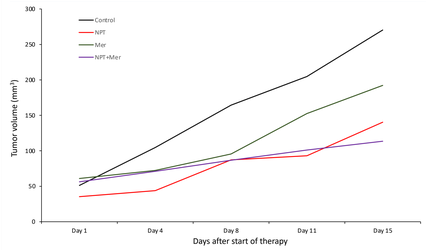


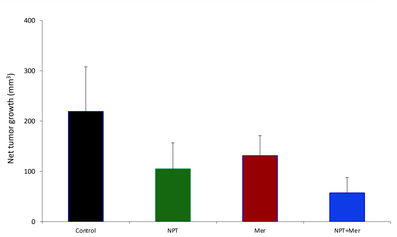




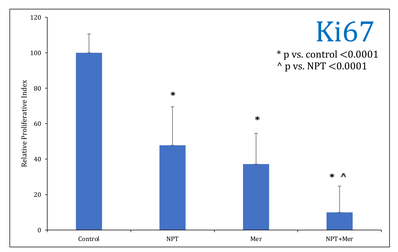


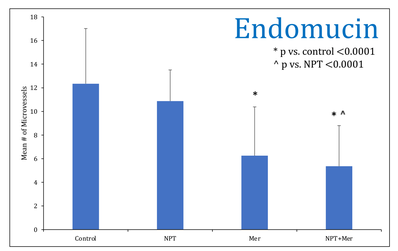
Tumor Growth in SNU-1 Xenografts (low phospho-c-Met expression)

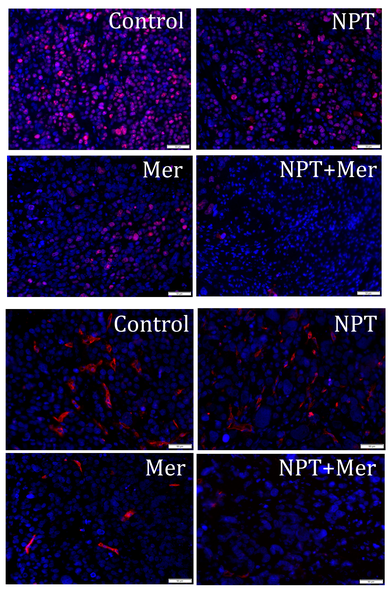




MKN-45 IHC Analysis







Conclusions

This study underlines the importance of the pathways inhibited by merestinib in gastric adenocarcinoma cell survival and proliferation. The favorable results from *in vitro* cell proliferation assays in multiple cell lines, *in vivo* tumor growth inhibition studies, and IHC analysis of MKN-45 tumor xenografts suggest that merestinib has strong antitumor activity and exhibits an additive effect when administered with *nab*-paclitaxel. Along with the immunoblots, which present a possible theoretical outline for the mechanism of these drugs, the results provide compelling evidence for further study in clinical trials.

Acknowledgements

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