Inflammatory and stem-like colorectal cancer (CRC) subtypes identified in patient-derived xenograft (PDX) models show tumor growth inhibition (TGI) by the combination of trametinib (T) and neratinib (N) irrespective of KRAS mutation (MT) status

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Abstract LB-087

Abstract

Background: KRAS mutant (MT) CRC tumors demonstrate constitutively activated RAF-MEK-ERK signaling pathways and are resistant to anti-EGFR therapies. In preclinical studies using KRAS MT CRC cell lines, resistance to MEK inhibitors (MEKi) lead to induction of ERBB3. Using kinome-centered synthetic lethality screen, suppression of ERBB3 receptor tyrosine kinase is strongly synergistic with MEKi in CRC cell lines. We previously showed that KRAS MT cell lines of intrinsic inflammatory subtype were sensitive to MEKi and neratinib (N), but stem-like subtype cell lines were resistant regardless of KRAS MT status. In this study, we evaluated treatment with trametinib + neratinib in 6 PDX models (Champions Oncology). PDX models were characterized by their genomic signature for intrinsic subtypes, and molecularly profiled for KRAS, BRAF, PI3K, NRAS, and DNA mismatch repair status (microsatellite instability-high, MSI-H; microsatellite stable, MSS).

Methods: An initial study was performed for dose tolerability. Efficacy studies were subsequently undertaken with 10 mice in each of 4 cohorts: vehicle, neratinib 40 mg/kg po qd , trametinib 1.0 mg/kg po qd and nertinib 40 mg/kg + trametinib 1.0 mg/kg. Doses were given until the model reached tumor endpoint of 1500 mm^3. Mean tumor volumes (MTV) of treatment groups were compared to control group to determine % tumor growth inhibition (TGI). Tissue specimens were obtained from vehicle and all treatment arms for WES, RNA seq, and phosphoprotein analysis.

Model No.	Subtype	MT profile	% TGI (T v N v T+N)*	p-value v control
CTG-0117	Inflammatory	KRAS WT	43% v 49% v 76%	0.215 v 0.17 v **0.009**
CTG-0382	Inflammatory	KRAS MT	62% v 40% v 80%	**0.009** v 0.11 v **0.001**
CTG-0406	Inflammatory	KRAS MT	43% v 46% v 67%	**0.001 v 0.0006 v 0.0001**
CTG-1170	Inflammatory	MKRAS MT/MSS	44% v 56% v 81%	0.043 v 0.0071 v **0.0001**
CTG-0069	Stem-like	KRAS MT	39% v 32% v 61%	0.12 v 0.31 v **0.015**
CTG-0079	Stem-like	KRAS MT	10% v 22% v 42%	0.91 v 0.51 v 0.068

*TGI was determined by calculating % TGI (100% x [1-(final MTV–initial MTV of a treated group)/ (final MTV–initial MTV of the control group)]).

Conclusions: Trametinib + neratinib was well tolerated as evaluated by body weight and demonstrated significant TGI in all three inflammatory models and in one of two stem-like CRC PDX models. Importantly, 4 out of 5 these tumors were KRAS MT and MSS suggesting that trametinib + neratinib may be a promising targeted therapy for this population of CRC patients. WES, RNA seq, and phosphoprotein analyses from vehicle and treatment arms will be performed. This analysis may define potential biomarkers related to sensitivity or resistance to trametinib + neratinib and inform additional targeted therapies.

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Methods

Patient-derived xenograft (PDX) models and in vivo treatments
Harlan NCr nude mice with a minimum weight of 20g were used for the study. Fragments from low passage CRC PDX models were utilized. Fragments (5x5x5) were surgically implanted subcutaneously. All mice were synchronized onto study with average tumor volumes of 250 mm^3. Tumor endpoint is reached at a volume of 1500 mm^3, unless specified differently. Mice were randomly assigned to different treatment groups (10 mice / group): vehicle control (0.5% carboxymethylcellulose); neratinib alone (40 mg/kg); trametinib alone (1 mg/kg); and trametinib in combination with neratinib. Each drug was suspended in 0.5% carboxymethylcellulose and orally administered. Mice were treated daily for 4 wks. Trametinib was dosed in 3 week cycle. Tumor volume and body weight were measured twice a week. Tumors were harvested and divided into two: (1) fixation in PAX gene kits, and (2) snap frozen in a dry ice/ethanol bath for immunoblot.

Organoid culture
Tumor tissues from CTG-0406 (inflammatory model) were collected from left over pre-study animals that were not randomized for study and shipped overnight in hypothermosol FRS. Patient-derived xenograft organoid (PDXO) were derived and cultured as described. (van de Wetering et al., Cell 2015)

Results

PDX models were characterized by their genomic signature for intrinsic subtypes, using all three published classifiers (CRCA, CCS and CMS) by using RNAseq data and molecularly profiled for KRAS, BRAF, PI3K, NRAS, and MSI status. The mutation status and subtype designations of selected PDX colorectal models are shown below in **Table 1.**

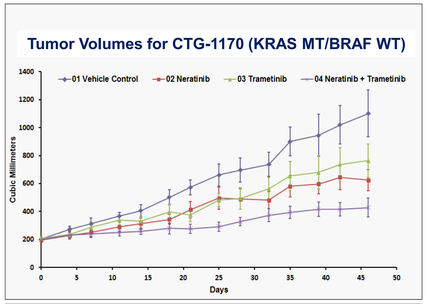
Table 1. Molecular characteristics of CRC PDX models

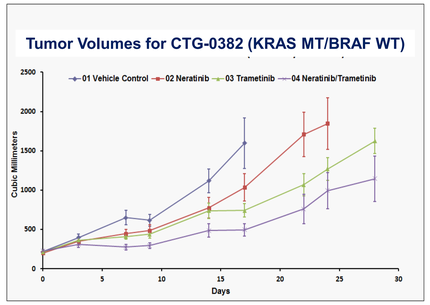
#	Model	Tumor type	Subtype	KRAS status	NRAS status	BRAF status	MSI status
1	CTG-0117	Colorectal	Inflammatory	Wild type	Wild type	Wild type	MSS
2	CTG-0406	Colorectal	Inflammatory	Mutant	Wild type	Wild type	MSS
3	CTG-1170	Colorectal	Inflammatory	Mutant	Wild type	Wild type	MSS
4	CTG-0382	Colorectal	Inflammatory	Mutant	Wild type	Wild type	MSS
5	CTG-0069	Colorectal	Stem-like	Mutant	Wild type	Wild type	MSS
6	CTG-0079	Colorectal	Stem-like	Mutant	Wild type	Wild type	MSS

Single agent trametinib ($p \leq 0.05$) inhibited tumor growth in three of four inflammatory PDX models, whereas single-agent neratinib ($p \leq 0.05$) inhibited tumor growth in two of four inflammatory models. However, the combination of trametinib plus neratinib resulted in the greatest tumor inhibition (vehicle control v combination, $p \leq 0.05$) in all four inflammatory models. In PDX models with a stem-like subtype (two models, CTG-0069 and CTG-0079), no significant tumor growth inhibition was seen with single-agent trametinib or neratinib compared to the vehicle control. Furthermore, combination of trametinib plus neratinib inhibited tumor growth in one stem-like subtype model, CTG-0069. There was no change in body weight of the treated PDX mice indicating that the combination was tolerated by the mice.

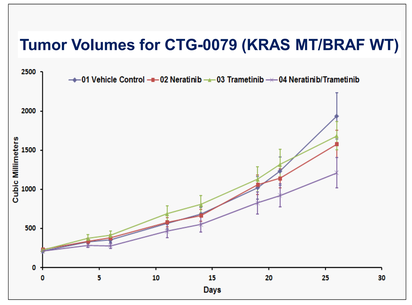
Fig 1. Tumor growth of inflammatory PDX models are more sensitive to T+N combination than stem-like subtypes

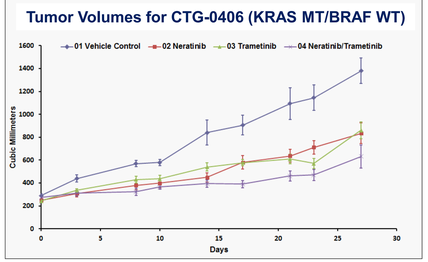
Inflammatory subtypes

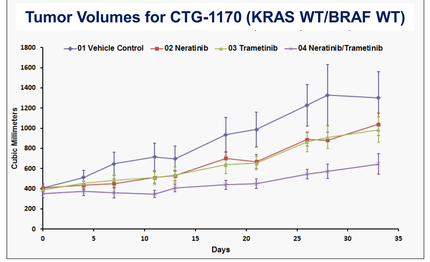




Stem-like subtypes







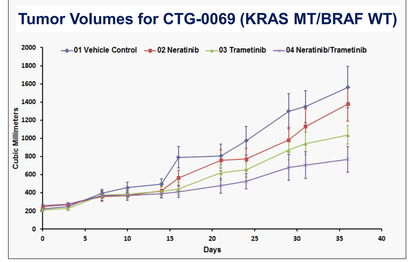
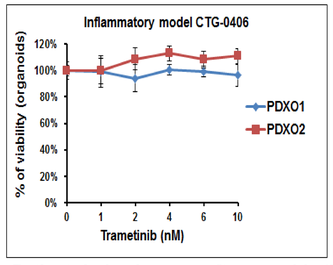
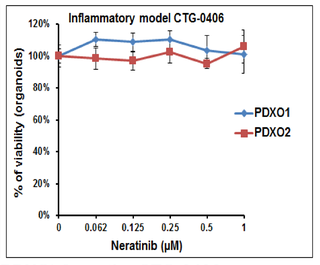
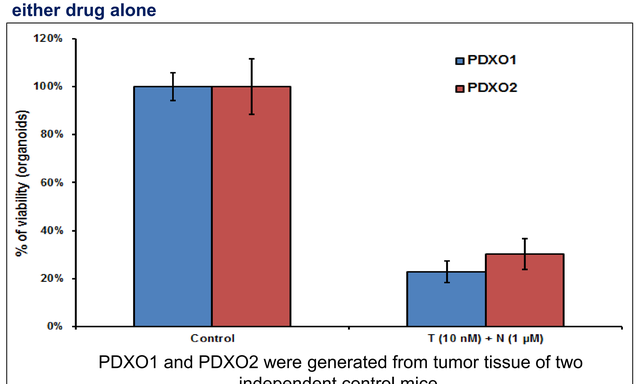


Fig 2. PDXO CTG-0406 resistant to single-agent T+N across all dose levels



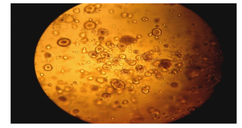


Patient-derived xenograft organoids (PDXO) from the CTG-0406 inflammatory PDX models are more sensitive to T+N combination than either drug alone

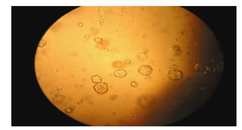


PDXO1 and PDXO2 were generated from tumor tissue of two independent control mice.

Fig 3. Image of PDXO CTG-0406, control v T+N



Control



T (10nM)+ N (1μM)

Conclusions

- We show that four PDX models with inflammatory subtype of CRC independent of KRAS status are sensitive to the combination of trametinib plus neratinib.
- One of two PDX models with stem-like subtype of CRC show tumor growth inhibition with the combination of trametinib plus neratinib.
- Both patient-derived xenografts and organoid models of KRAS MT CRC tumors demonstrated a combined treatment effect with trametinib plus neratinib.
- These preclinical models suggest that the combination of trametinib plus neratinib warrants investigation in metastatic CRC patients with MSS regardless of KRAS status.