KRAS Mutant Colorectal Cancer Organoid Models Generated from Patient-derived Xenografts Show Response to Combination of Trametinib, Neratinib, and Trastuzumab

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Rekha Pal[1], Ashok Srinivasan[1], Peter C. Lucas[1], Carmen J. Allegra[1], Angela Davies[2], Alshad S. Lalani[3], Samuel A. Jacobs[1], and Katherine L. Pogue-Geile[1]

[1]NSABP, Pittsburgh, PA; [2]Champions Oncology, Rockville, MD; [3]Puma Biotechnology, Inc., Los Angeles, CA

CTG-0079

ABSTRACT

BACKGROUND: KRAS-mutant (mt) colorectal cancer (CRC) has constitutively activated RAF-MEK-ERK pathways and resistance to anti-EGFR therapies. In pre-clinical models, we found that, regardless of KRAS mutations, cell lines with inflammatory subtype were sensitive to MEK162 plus neratinib (N), whereas CRC cell lines of the stem-like subtype were resistant to this combination. In the C-07 and C-08 clinical studies, patients (pts) with tumors of the stem-like subtype were resistant to chemotherapy and had a very poor prognosis. We hypothesized that dual-HER2 targeting may provide a more robust ERBB inhibition than N alone. We therefore tested trastuzumab (Tz) combined with trametinib (Tm) + neratinib (N) using PDX organoids (PDXOs) derived from KRAS-mt CRC tumors. PDXOs have emerged as powerful preclinical models to predict drug response. The goal of this study was to identify an efficacious drug combination for inflammatory and stem-like KRAS-mt tumors using PDXOs.

METHODS: PDXOs were generated using published methods from Hans Clevers' laboratory. Three KRAS-mt PDX tissues from Champions Oncology were used to generate four PDXOs for drug testing. For the CTG-0406 PDX model, tissues from two independent mice were used to develop two separate PDXOs.

RESULTS: All four PDXOs were KRAS-mt. All PDXOs tested were resistant to Tm, N, and Tz, as single agents, irrespective of whether they were of the inflammatory or the stem-like subtype. N+Tz inhibited cell viability in all four models; however, the triple combination of Tm+N+Tz resulted in greater inhibition (67-76%) of cell viability.

CONCLUSIONS: We demonstrate that KRAS-mt PDXOs were inhibited to a greater extent with Tm+N+Tz as compared to any of these drugs alone or with doublets. PDXOs provide a rapid and cost-effective preclinical platform for screening of unique drug combinations.

SUPPORT: NSABP Foundation, Inc.

Table 1. Percent Viability of Organoid Cultures after Treatment

Model	CTG-0406(1)	CTG-0406 (2)	CTG-1170	CTG-0079
Subtype	Inflammatory	Inflammatory	Inflammatory	Stem-like
Drug(s)	% Cell Viability			
Tm (10 nM)	96	116	95	94
N (125 nM)	109	97	96	92
Tz (20 µg/ml)	92	119	97	93
Tm (10 nM) + N (125 nM)	64	59	31	56
Tm (10 nM) + Tz (20 µg/ml)	74	75	41	70
N (125 nM) + Tz (20 µg / ml)	34	70	23	43
Tm (10 nM) + N (125 nM) + Tz (20 µg/ml)	26	33	24	31

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METHODS

PDX models were characterized by their gene-expression signature for intrinsic subtypes, using all three published classifiers (CRCA, CCS, and CMS) 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.

Organoid Culture

Tumor tissues from CTG-0406 (inflammatory model), CTG-1170 (inflammatory model), and CTG-0079 (stem-like subtype), were used to generate patient-derived xenograft organoids (PDXOs) (van de Wetering et al., Cell 2015 ; 161, 933-45).

Molecular Characteristics of CRC PDX Models

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

RESULTS

Fig 1. Cell viability of PDXOs tested after treatment with trametinib (MEKi), neratinib (pan-ERBBi), and trastuzumab (ERBB2i) as single agents neratinib and trastuzumab as single agents.

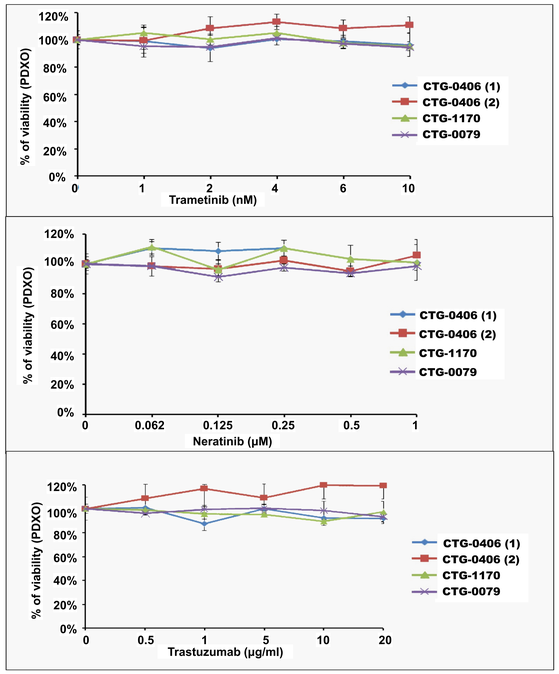


Fig 2. PDXOs from: (A) CTG-0406(1) & (2), (B) CTG-0079, and (C) CTG-1170, are sensitive to the triple combination of trametinib (Tm) + neratinib (N) + trastuzumab (Tz)

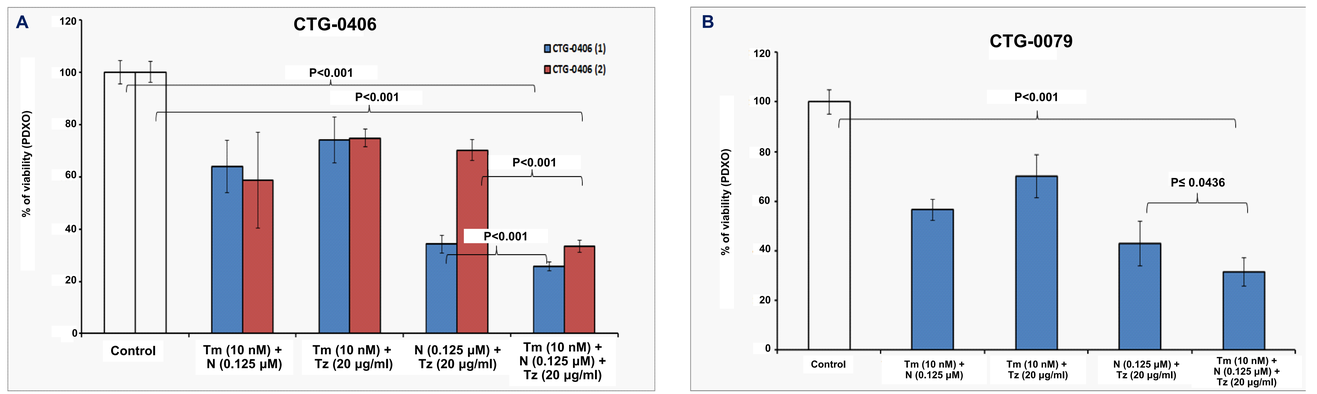
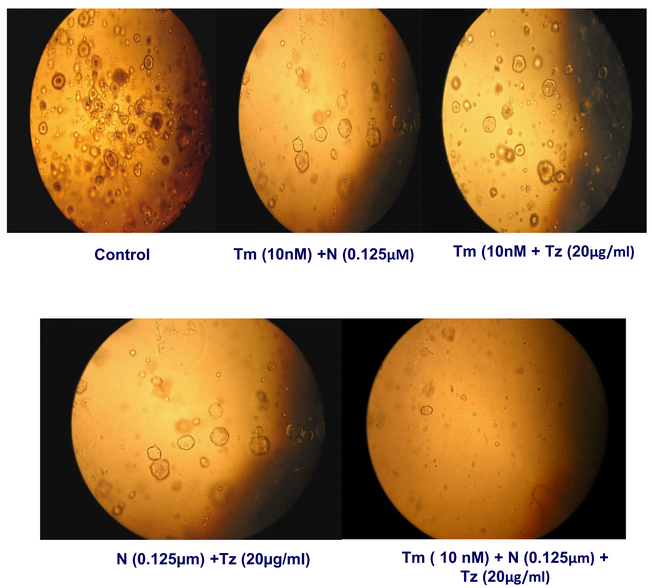


Fig 3. Image of PDXO CTG-0406, Control *vs* treatment groups



CONCLUSIONS

- KRAS mt PDXOs were inhibited to a greater extent with the triple combinations of trametinib+neratinib+trastuzumab compared to neratinib+trastuzumab in 3 out of 4 PDXO models.
- These preclinical models suggest that trastuzumab combinations warrant investigation in metastatic CRC pts with KRAS mutation.
- PDXOs provide a rapid and cost-effective preclinical platform to screen unique combinations for sensitivity and resistance in CRC.