Selection of Targeted Therapeutics in Colorectal Cancer Spheroids for Clinical Prediction

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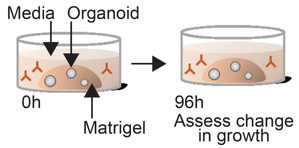
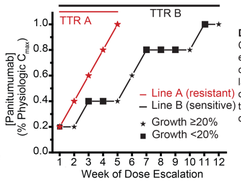
Background

-Precision oncology requires translational tools predictive of clinical benefit.
-Epidermal growth factor receptor inhibition (EGFRi) yields a survival advantage in RAS/RAF wildtype (wt) colorectal cancer (CRC), however clinical tools do not predict individual patient response or resistance mechanisms.
-Patient-derived cancer organoids (PDCOs) allow for *ex vivo* tracking of response. Here, we present methods to develop EGFRi resistance for PDCO as a novel biomarker to differentiate treatment sensitivities.

Methods

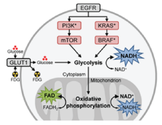
A) Dose Escalation Design

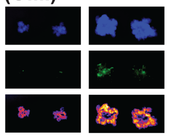




Dose Escalation (A): Change in diameter assessed every 96h. If threshold for escalation met (●), therapy escalated to next tier as a function of physiologic C_{max}, if growth threshold not met timepoint denoted (■).

B) Optical Metabolic Imaging (OMI)



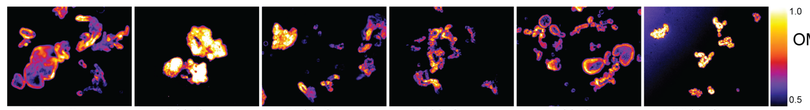


Optical Metabolic Imaging: Changes in EGFR signaling result in transition in ratio of NADH/FAD. During glycolysis increase in NADH signal intensity versus oxidative phosphorylation which results in increase in FAD. Representative NADH signal intensity used for calculation of mean NADH and FAD with resultant redox ratio.

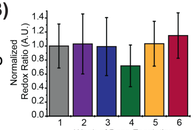
C) Validation with Clinical Outcomes

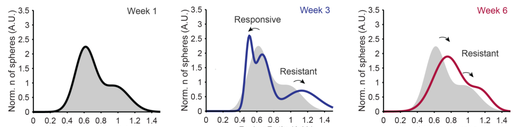
-Validation of RAS status in PDCOs resistance
-Stratification of clinical response for RAS^{WT} against prospective clinical outcomes with variable reponse rate to single agent EGFRi estimated at 10-30%

Metabolic Response to Dose Escalation



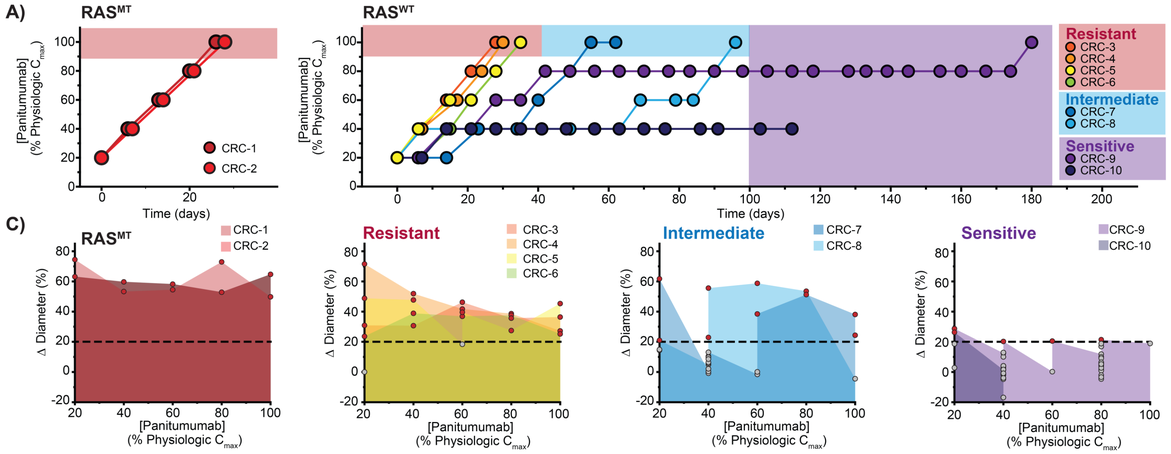
Sphere level widefield OMI Imaging: Intrinsic autoflourescence overlay of signal of NADH over FAD over timecourse of dose escalation (**A**).





Quantitive assessment of redox ratio: Median value of redox ratio across population with error bars denoting standard deviation (**B**). Population modeling (**C**) with gaussian distributions of individual sphere populations plotted as a function of week of dose escalation.

Dose Escalation of EGFRi



Tracking EGFRi Dose Escalation: Plot showing time course of EGFRi dose escalation plotted by a function of dosing of EGFRi (panitumumab). Lines were escalated stepwise by 20% physiologic C_{max} provided growth threshold met over treatment course. Plots stratified by RAS^{MT} (**A**) and RAS^{WT} (**B**) to define thresholds of treatment sensitivity including resistant (red), intermediate (blue) and sensitive (violet). Corresponding plots of growth assessments plotted as a function of EGFRi dosing (**C**) with threshold of growth (20%) at 96h (dashed line).

Conclusions

-*Ex vivo* resistance to EGFRi can be acheived in CRC PDOCs in clincially relevant timeframes.

-RAS^{MT} PDCOs show serial resistance over dose escalation

-RAS^{WT} PDCOs have range of treatment sensitivities over the course of dose escalation including serial resistance, intermediate sensitivities and growth arrest.

-Sphere level metabolism suggests population differences in therapeutic sensitivity over course of dose escalation.

-Preliminary clinical correlates suggest potential as integrative biomarker for targeted therapies.

Future Directions

-Comprehensive molecular profiling in pairwise comparison between baseline and EGFRi resistant cultures to assess mechanism of therapeutic resistance

-Prospective investigation of single agent EGFRi in RAS-^{WT}/RAF^{WT} CRC with PDCOs developed and assessed by dose escalation for evaluation against clinical outcomes.

Patient Characteristics

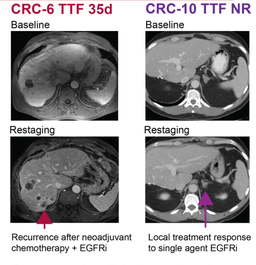
Name	Site of Tissue	Tissue Sampling	Grade	Stage
CRC-1	Liver	Surgical Resection	Moderate	IVA
CRC-2	Sigmoid Colon	Surgical Resection	Moderate	IIC
CRC-3	Rectum	Biopsy	Moderate	IIIB
CRC-4	Colon	Biopsy	Moderate	IVB
CRC-5	Rectum	Biopsy	Moderate	IIIC
CRC-6	Liver	Biopsy	Well	IVB
CRC-7	Rectum	Biopsy	Well-Moderate	IIIB
CRC-8	Liver	Surgical Resection	Moderate	IVB
CRC-9	Liver	Surgical Resection	Moderate	IVA
CRC-10	Lung	Biopsy	Moderate	IVB

Clinical chacteristics for PDCOs selected for EGFRi dose escalation.

Molecular Profile and Clinical Response

Name	*APC*	*KRAS*	*PIK3CA*	*TP53*	*SMAD4*	*Other*	TTR
CRC-1	R876*; E1464*	G12V	H1047L				26
CRC-2	E1309fs	G12D		R175H		*HNF1A* T156M	28
CRC-3	E418*; E1295*			G279fs; P152L			28
CRC-4	V97Afs*26						30
CRC-5	c.835-8A>G			R248Q		*PTEN* I32fs	35
CRC-6	K1370*			c.96+1G>A			35
CRC-7	Q1338*			E51*	R361C	*HER2* Amp CN 14	62
CRC-8	K311N						96
CRC-9	L548fs; F1491fs			R306*			>180
CRC-10	R213*; L1342fs			L257R; P34fs			NR

Molecular profiles of PDCOs stratified for treatment sensitivities by EGFRi dose escalation.



Representative clinical correlates in pts with PDCOs treated with EGFRi

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