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Case Report

Re-establishing Responsiveness in a Case of Refractory Metastatic Rectal Cancer with a Personalized de novo Combination Regimen

Chirantan Bose, Pradip Devhare, Dadasaheb Akolkar*, Darshana Patil, Vineet Datta, Ashwini Ghaisas, Revati Patil, Sanket Patil, Sachin Apurwa, Navin Srivastava, Raja Dhasarathan, Shabista Khan, Shankar Somashekar, Kiran Bendale, Jinumary John, Rahul Gosavi, Ajay Srinivasan, Rajan Datar.

Datar Cancer Genetics, Nasik, India.

Abstract:

Introduction: Encyclopedic Tumor Analysis (ETA) is multi-analyte, molecular and functional interrogation to identify latent vulnerabilities in solid tumors which can then be targeted in organ- and label-agnostic combination treatment regimens.

Case Presentation: We describe here a case of metastatic rectal cancer in a 61-year-old male who was progressed on all prior Standard of Care (SoC) treatment modalities including surgery, chemotherapy and radiotherapy. We addressed disease recurrence via personalized therapy guided by ETA which revealed characteristic molecular heterogeneity in primary and metastatic lesions in terms of single nucleotide variations (SNVs) and gene copy number variations (CNVs). Notably, a novel TBL1XR1 (Exon1) – PIK3CA (Exon 2) gene fusion was identified in the tumor along with gene copy number gains in TERT, IGF-1R, MYC, FGFR1 and EGFR genes.

Conclusion: ETA based molecular analysis with synchronous in vitro chemo-sensitivity profiling strategy helped to define de novo combinatorial therapy regimen of targeted and cytotoxic drugs which countered disease progression at each instance and led to the durable regression of primary as well as metastatic lesions.

Keywords: Colorectal Cancer; TBL1XR1-PIK3CA; Abiraterone; Cetuximab; Everolimus

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***Correspondence to**: Dadasaheb Akolkar

Email: dadasaheb.akolkar@datarpgx.com

Introduction

Current Standard of Care (SoC) modalities for management of colorectal cancer (CRC) are surgery, chemotherapy, radiotherapy and targeted therapy mainly comprising anti-EGFR and anti-VEGF agents [1]. However, the treatment of metastatic CRC remains challenging with limited options after progression on SoC measures. Surgery is rarely feasible in metastatic disease and may be further restricted owing to patient's health and other co-morbidities. Chemotherapy regimens with fluoropyrimidines (e.g., FOLFOX, FOLFIRI, FOLFIRINOX) which form the backbone of initial line therapies have limited utility in advanced refractory cancers with onset of resistance [2]. There appears to be limited utility of tyrosine kinase inhibitors (TKI) in CRC; Regorafenib, though indicated in SoC has low response rates. The use of anti-EGFR monoclonal antibody (mAb) Cetuximab is restricted to RAS wild type CRC, that too to left sided CRC where outcomes are better than right sided CRC [1]. EGFR inhibitors in combination with chemotherapy agents have shown limited efficacy in treatment of advanced CRC and the clinical use of such combinations are not common [3]. Finally, though Checkpoint Inhibitor Therapies (CIT) are approved for advanced CRC, these are limited to use in a setting of deficient mismatch repair (dMMR) / high microsatellite instability (MSI-high) / high tumor mutation burden (TMB), the prevalence rates of which have been reported to be <5% in colorectal cancers [4].

Molecular heterogeneity accounts for the large variations in prognosis and response to chemotherapy in (CRC) patients [5]. Presently, molecular investigations in CRC are restricted to variations in RAS and RAF for the purposes of prognostication. A recent effort identified 4 consensus molecular subtypes of biological relevance that were associated with different patient outcomes (CRC ESMO guidelines) [6]. However, such investigations have limited therapeutically actionable relevance in CRC. Multi-gene variant profiling panels for Next Generation Sequencing (NGS) have no application in SoC, and are considered for personalized or label-agnostic therapy selection as physician's choice of treatment.

We have previously described the clinical benefits of a multi-analyte, molecular and functional interrogation (ETA: Encyclopedic Tumor Analysis) to identify latent vulnerabilities in solid tumors which can then be targeted in organ- and label-agnostic combination treatment regimens [7-8]. In the present case report, we describe the case of an advanced refractory metastatic rectal cancer where personalized de novo combination regimen based on the findings of ETA yielded durable response.

Case presentation

The case described in this manuscript is a retrospective observational report of a single patient who opted to receive ETA-guided personalized treatment [7]. The patient consented for publication of deidentified data and results.

Clinical history of this case is traceable to 2007 when the patient was diagnosed with Adenocarcinoma of the Rectum. The patient underwent abdominoperineal resection followed by standard dose chemotherapy with FOLFOX followed by external beam radiotherapy (EBRT) and 2 fractions of high dose-rate (HDR) brachytherapy. Limited molecular profiling indicated that the tumor was KRAS wild-type (wt). The patient was asymptomatic for 4 years until recurrence was detected in July 2011 as appearence of enlarged hypermatabolic periprostatic nodules on PET-CT. The patient

underwent radiofrequency ablation (RFA) in July 2011 as well as in Dec 2011, both of which showed complete metabolic response. Between 2012 and 2017, the patient reported recurrent urinary symptoms associated with periprostatic nodules for which he underwent RFA and other palliative procedures.

Between February 2016 and February 2017, follow-up PET-CT scans documented increase in size of the persistent mass in retro-prostatic region which was infiltrating the base of urinary bladder along with perilesional necrotic deposits. Between February and August 2017, the patient was administered 6 cycles of FOLFIRI. A subsequent PET-CT scan in September 2017 showed increase in size of the necrotic retro-prostatic lesion which was extending into the obturator muscles and also infiltrating into the base of urinary bladder.

A detailed exploratory workup in December 2017 via cystoscopy revealed necrosis in the prostatic fossa and evidence of cystitis. A whole-body PET-CT scan revealed further increase in tumor activity in the retroprostatic area along with evidence of necrosis along with persistent pelvic and inguinal lymphadenopathy. The patient was administered oral Tab Capecitabine (500 mg, 3 – 0 – 2 daily) until March 2018, when a follow-up PET-CT revealed further increase in the size of the tumor, stemming from the retroprostatic site, involving the anorectal and pre-sacral regions adjacent to the base of urinary bladder, and extension of the lesion into bilateral obturator muscles along with metastatic iliac lymph nodes.

Encyclopedic tumor analysis and personalized regimen

In March 2018, the patient underwent a biopsy at the left inguinal node to obtain tumor tissue sample. The freshly biopsied tissue sample along with 15 mL of peripheral blood was provided to the study sponsor to perform ETA. Evaluation of mononucleotide repeat markers (NR-21, BAT-26, BAT-25, NR-24, MONO-27) in tumor tissue DNA showed stable microsatellites (MS-S). There were no detectable actionable or significant gene variations in tumor tissue by NGS; APC p.L1489fs*18 variant (MAF 3.7%) was detected in NGS profiling of mutations in cell free tumor DNA (ctDNA) (Table 1). Differential gene expression profiling of the tumor transcriptome indicated significant overexpression of CYP17A1 indicating potential benefit from Abiraterone. In vitro chemo-resistance / sensitivity profiling of viable tumor cells indicated sensitivity towards several drugs including Methotrexate and Vinorelbine. Based on these findings the patient received a combination regimen of Methotrexate (70 mg; D1 and D8, 21-day cycle), Vinorelbine (40 mg; D1 and D8, 21-day cycle) and Abiraterone (250 mg, 1 OD), between March and September 2018 (7 cycles). Radiological follow-up (PET-CT) between March and September 2018 showed stable disease indicating that the treatment regimen had effectively halted further disease progression for up to 6 months along with stable serum CEA and CA19-9 levels. The regimen was well tolerated with minimal toxicity profile. Therapy related Adverse Events (AEs) were moderate (Grade II), transient and included Fatigue, Anorexia and Mucositis, all of which were clinically managed.

In September 2018, increase in serum CEA levels (278 ng/ml) was observed even though there were no radiological or clinical indications of progression. The patient underwent a second biopsy to obtain tumor tissue from the Pelvic mass for ETA re-evaluation of the tumor. NGS mutation profiling of ctDNA showed persistence of the previously detected APC.pL1489fs*18 mutation (MAF 1.9%) and emergence of new mutations in TP53. NGS mutation profiling of tumor tissue DNA revealed other single nucleotide variations (SNV) in TP53 p.T125T (MAF 66%), NOTCH3 p.R1190C (MAF 18%) and ATR p.R177* (MAF 3.9%) as well as copy number alterations (CNA, gain) in TERT (n = 17), IGF-1R (n = 15), MYC (n = 14), FGFR1 (n = 9) and EGFR (n = 8). Transcriptome analysis

showed overexpression of MMP7, MMP9, VEGFA, MAPK15, PTGS2 gene transcripts. Notably, a TBL1XR1-PIK3CA (T1:P2) gene fusion (209 transcript reads) was also detected in tumor tissue (Table 1). In vitro chemosensitivity profiling of viable tumor cells indicated low response towards Vinorelbine and Methotrexate, but increased response towards Pemetrexed.

Table 1 Molecular alterations identified after encyclopaedic tumor analysis (ETA) of blood and tumor tissue samples.

ETA (Month/Year)	**Gene Name**	**Molecular alteration**	**MAF / Fold / gain**	**Source**	**Method**
ETA-1 Feb 2018	APC	p.L1489fs*18	MAF 3.7%	ctDNA	NGS
	CYP17A1	Overexpression	10 fold	tissue	Transcriptome
	IL-6	Overexpression	5 fold	tissue	Transcriptome
	MAP2K2	Overexpression	2.2 fold	tissue	Transcriptome
	PTGS2	Overexpression	2.4 fold	tissue	Transcriptome
ETA-2 Sept 2018	APC	p.L1489fs*18	MAF 3.7%	ctDNA	NGS
	TP53	p.C141Y	MAF 0.9%	ctDNA	NGS
	TP53	p.V216M	MAF 0.2%	ctDNA	NGS
	TP53	p.T125T	MAF 66%	tissue	NGS
	NOTCH3	p.R1190C	MAF 18%	tissue	NGS
	ATR	p.R177*	MAF 3.9%	tissue	NGS
	TERT	CNV	17 gain	tissue	NGS
	IGF1R	CNV	15 gain	tissue	NGS
	MYC	CNV	14 gain	tissue	NGS
	FGFR1	CNV	9 gain	tissue	NGS
	EGFR	CNV	8 gain	tissue	NGS
	TBL1XR1 – PIK3CA	Fusion (T1:P2)	209 reads	tissue	NGS
	MMP7	Overexpression	9.9 fold	tissue	Transcriptome
	MMP9	Overexpression	3.7 fold	tissue	Transcriptome
	MAPK15	Overexpression	4.6 fold	tissue	Transcriptome
	VEGFA	Overexpression	4.2 fold	tissue	Transcriptome
	IL-6	Overexpression	2.4 fold	tissue	Transcriptome
	PTGS2	Overexpression	2.2 fold	tissue	Transcriptome

CHP – Cancer hotspot panel; NGS – Next Generation Sequencing; MAF – Mutant allele frequency; ctDNA – circulating tumor DNA; Fold – Upregulation of target gene compared to expression in normal RNA control.

Based on these findings, the patient was advised a combination regimen of Cetuximab (EGFR gain), Everolimus (chemo-sensitivity and TBL1XR1-PIK3CA fusion) and Pemetrexed (chemo-sensitivity). The patient was administered weekly Cetuximab (700 mg loading dose, 450 mg maintenance dose), Pemetrexed (500 mg, 21 day cycle) and Everolimus (5 mg, 1 OD) between October 2018 to March 2019. Administration of this regimen led to Partial Response (PR) during the same period (shown in Figure 1). There were no significant therapy related AEs with the exception of transient Anemia (Grade 3) as well as transient Anorexia and Fatigue, both Grade 2.

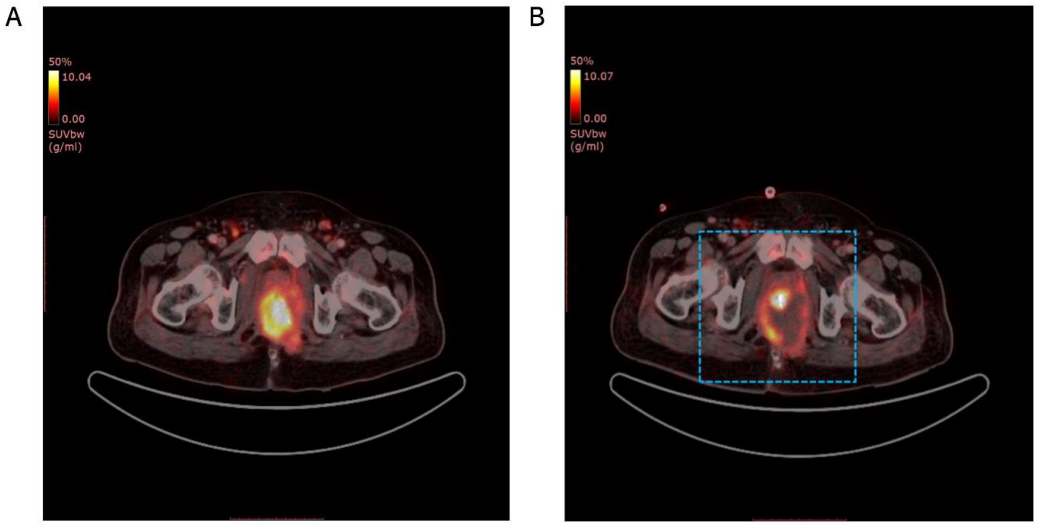

Figure 1 Significant regression of malignant rectal mass revealed in positron emission tomography-computed tomography (PET-CT) transverse sections. Compare follow-up scan after ETA guided therapy in right panel (B) Vs baseline scan left panel (A).

Follow-up radiological data for the patient was available until April 2019, following which only telephonic follow-up data was available. During a recent (May 2020) telephonic follow-up, the patient was surviving and asymptomatic. However, therapy and disease status are presently unknown.

Discussion

The present study describes the case of an advanced refractory colorectal cancer where ETA revealed latent molecular and functional vulnerabilities that could be targeted with personalized de novo combination regimens. Prior lines of therapy received by this patient were based on SoC and included locoregional (surgery, RFA, EBRT) as well as systemic (FOLFOX, FOLFIRI, Capecitabine) treatments. The inability of prior treatments to yield objective and durable response highlights the limitations of SoC approaches to treat cancers, especially those which are advanced and refractory. Such futile treatment are also associated with accumulated adverse effects which thus indirectly increase the costs of cancer care with no benefits to patient. This is a common dilemma faced by oncologists during routine clinical practice where treatments are assigned on the basis of limited profiling or sidedness and lead to varying outcomes.

Administration of the first iteration of ETA guided treatment regimen to the patient was beneficial in immediately halting further progression of the tumor. The use of Abiraterone [9] in this regimen was based on expression of CYP17A1 which is a target in prostate cancer therapy. While

Objective Response (OR) was not achieved, this regimen yielded a PFS of 6 months. Gene copy number gain of TERT , MYC and FGFR1 genes in subsequently biopsied tumor tissue indicates variations associated with poor prognosis in CRC [10-12]. The gain of EGFR gene copy number indicated potential benefit from Cetuximab, while gain of IGF-1R gene copy number indicated potential resistance to Cetuximab [13]. The tumor was found to harbour a novel gene fusion: TBL1XR1-PIK3CA since it has been reported to drive activation of PIK3CA [14]. Based on these findings, combination regimen included Cetuximab and Everolimus to achieve tandem targeting of multiple (EGFR, mTOR) pathways to improve treatment efficacy and Pemetrexed based on in vitro functional vulnerability. This multi-targeting de novo combination regimen led to durable partial response with significant regression in malignant rectal mass (shown in Fig. 1). The response to combination regimen is remarkable since recent clinical trials in metastatic KRAS-wt CRC have shown no benefit from the combination of anti-EGFR and anti-IGF1-R therapies over anti-EGFR monotherapy [15-16].

Traditional integration of univariate molecular data of the tumor into clinical practice have limited benefits in CRC. Similarly prior studies considering molecular indications have focussed on use of single agents such as Cetuximab in the NCI CCTG CO 17 trial [17], anti-EGFR antibody [17-23], or anti-VEGF Bevacizumab [20] despite possible availability of multiple indications. The utility of patient-specific combination therapies in advanced cancers has remained largely unexplored except the RESILIENT trial [7]. The present case study provides evidence of benefit to patients in routine clinical practice from combination treatment strategies guided by multi-analyte tumor profiling.

Conclusion

This trajectory of response in this case of recurrent metastatic rectal cancer proves utility of ETA guided personalized therapy and superiority of such treatments over SoC regimens. Personalized combination regimen in this patient was based on de novo patient-derived evidence and was associated with improved treatment efficacy, response rates and survival. It is pertinent to mention that though Cetuximab was approved by the FDA approval for CRC in 2004, the same could not be administered to the patient owing to non-availability in India. Though it was considered for administration at a later date, the decision to avoid it was based on the presence of potential resistance mechanism (IGF1R copy gain).

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Statement of ethics

Written informed consent was obtained from the patient for the publication (including images) of deidentified data and results. A copy of the written consent is available for review by the Editor-in-chief of this journal on request.

Sample collections and therapeutic interventions were carried out at HCG Manavata Cancer Centre, Nasik, India. Cellular and molecular investigations on the patient's samples were carried out at the College of American Pathologists (CAP)-accredited and International Organization for Standardization (ISO)-compliant facilities of Datar Cancer Genetics Limited (DCGL), Nasik, India.

All interventional procedures including therapy administration were approved as per standard hospital practices and in concordance with existing ethical, medical and legal requirements. In addition, the research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Conflict of interest

All enlisted authors are employees of DCGL which offers commercial services for onco-diagnosis and therapy management. R.D. is the Founder and Chairman and Managing Director of DCGL.

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Author contributions

C.B., P.D. and A.S.: Writing – Original Draft Preparation, Review and Editing; D.A., D.P., V.D., A.G., S.S., K.B.: Conceptualization, Supervision, Project administration; R.P., J.J., S.P., N.S., Raja. D., S.K., R.G.: Methodology, Data Curation; S.A.: Bioinformatics Analysis; R.D.: Resources, Project Administration and Review.

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