exacta®
ENCYCLOPEDIC TUMOR ANALYSIS

93.9%
Progression Free Survival*
*At 90 Days

90.5%
Disease Control Rate

42.9%
Objective Response Rate

THE RESILIENT PROTOCOL

BREAKTHROUGH TECHNOLOGY
For Refractory, Metastatic
Solid Organ Cancers

- Publications
- Data
- Evidence
- Accreditations

EXACTA®- RESILIENT PROTOCOL

Exacta® is an intensive and in depth tumor gene expression analysis. It analyses 100s of millions of data points at the molecular level to reveal all possible targets for precision drugs. Exacta® is one of the most powerful tumor investigation and is an extreme analytical tool that reveals the driver mutations and pathways driving a person's cancer. Exacta is best molecular analysis for difficult cancers.

EXACTA®- RESILIENT PROTOCOL IS USEFUL FOR CANCER PATIENTS WHERE:

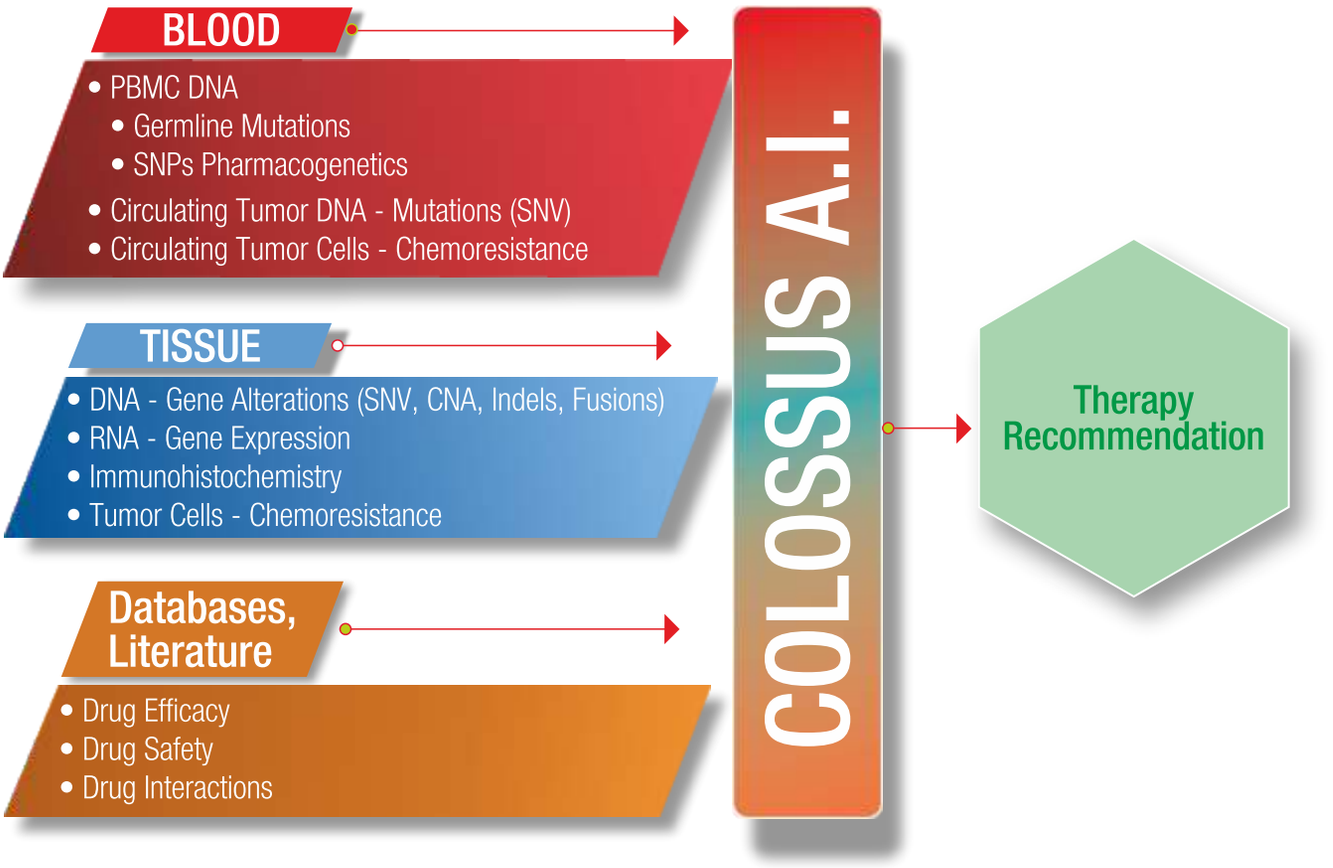
- ✓ First-line therapy has failed
- ✓ Risk of therapy failure is high
- ✓ Cancer has relapsed
- ✓ For newly diagnosed patients to explore best therapy options
- ✓ Cancer is high-grade / metastatic

THE RESILIENT PROTOCOL

DATAR CANCER GENETICS

datarpgx.com

Exacta®: Encyclopedic Tumor Analysis



DNA:	Tumor DNA (453 genes), ctDNA (52 genes)
RNA :	Gene fusions, Gene Expressions, Pathways : Tumor/ mRNA + Exosomal mRNA (20,800 transcripts) Exosomal miRNA (756 transcripts)
IHC :	MSI / MMR + TMB + PD-L1 and other IHC markers
PGX :	Pharmacogenetics: Drug efficacy and safety.
CHEMO-SENSITIVITY:	In Vitro Chemosensitivity on viable tumor cells (NCCN + Off Label)
A.I. :	Integrative, Multi-Level, Iterative algorithm to determine optimum therapy.

Oncotarget

Encyclopedic tumor analysis for guiding treatment of advanced, broadly refractory cancers: results from the RESILIENT trial.

ANNALS OF ONCOLOGY

- Encyclopedic Tumor Analysis for organ agnostic treatment with Axitinib in combination regimens for advanced cancers.
- Encyclopedic Tumor Analysis Guided Treatments with ConventionalDrugs Outperform Available Alternatives in Refractory Cancers.
- Encyclopedic Tumor Analysis (ETA) Guided Combination Regimens of Hormone Receptor Antagonists with Other Systemic Agents for Treatment of Refractory Cancers
- mTOR Inhibitors in Combination Regimens Guided by Encyclopedic Tumor Analysis Show Superior Outcomes Compared to Monotherapy in Refractory Cancers
- Clinical Utility of Encyclopedic Tumor Analysis to Treat Patients Advanced Refractory Head and Neck Cancers.

OXFORD ACADEMIC
Neuro-Oncology

Clinical Utility of Encyclopedic Tumor Analysis to Treat Patients with Brain Metastasis in Refractory Cancers.

Journal of Global Oncology®
An American Society of Clinical Oncology Journal

Clinical efficacy of combination therapies with androgen receptor antagonists for treatment of multiple refractory cancers.

Journal of *Personalized Medicine* MDPI

Adaptive, Iterative, Long-Term Personalized Therapy Management in a Case of Stage IV Refractory NSCLC.

NCCN National Comprehensive Cancer Network®

Clinical Utility of Encyclopedic Tumor Analysis to Treat Breast Cancer Patients who have Failed Standard of Care Treatments.

www.oncotarget.com Oncotarget, 2019, Vol. 10, (No. 54), pp: 5605-5621

Research Paper

Encyclopedic tumor analysis for guiding treatment of advanced, broadly refractory cancers: results from the RESILIENT trial

Rajnish Nagarkar[1], Darshana Patil[2], Timothy Crook[3], Vineet Datta[2], Sagar Bhalerao[1], Sonal Dhande[1], Vijay Palwe[1], Shirsendu Roy[1], Prakash Pandit[1], Ashwini Ghaisas[2], Raymond Page[4], Harjeetsingh Kathuria[1], Ajay Srinivasan[2] and Dadasaheb Akolkar[2]

[1]HCG Manavata Cancer Centre, Nasik, India

[2]Datar Cancer Genetics Limited, Nasik, India

[3]St. Luke's Cancer Center, Royal Surrey County Hospital, Guildford, UK

[4]Worcester Polytechnic Institute, Worcester, USA

***Correspondence to**: Ajay Srinivasan, **email:** ajays@datarpgx.org*

***Keywords:** precision oncology; encyclopedic tumor analysis; personalized cancer treatment; objective response rate; progression free survival*

***Received:** April 09, 2019 **Accepted:** August 16, 2019 **Published:** September 24, 2019*

ABSTRACT

RESILIENT (CTRI/2018/02/011808) was a single arm, open label, phase II/III study to test if label agnostic therapy regimens guided by Encyclopedic Tumor Analysis (ETA) can offer meaningful clinical benefit for patients with relapsed refractory metastatic (r/r-m) malignancies. Patients with advanced refractory solid organ malignancies where disease had progressed following ≥2 lines of systemic treatments were enrolled in the trial. Patients received personalized treatment recommendations based on integrational comprehensive analysis of freshly biopsied tumor tissue and blood. The primary end points were Objective Response Rate (ORR), Progression Free Survival (PFS) and Quality of Life (QoL). Objective Response (Complete Response + Partial Response) was observed in 54 of 126 patients evaluable per protocol (ORR = 42.9%; 95% CI: 34.3%–51.4%, $p < 0.0001$). At study completion, Disease Control (Complete Response + Partial Response + Stable Disease) was observed in 114 out of 126 patients evaluable per protocol (CBR = 90.5%; 95% CI: 83.9% - 95.0%, $p < 0.00001$) and Disease Progression in 12 patients. Median duration of follow-up was 138 days (range 31 to 379). Median PFS at study termination was 134 days (range 31 to 379). PFS rate at 90 days and 180 days were 93.9% and 82.5% respectively. The study demonstrated that tumors have latent vulnerabilities that can be identified via integrational multi-analyte investigations such as ETA. This approach identified viable treatment options that could yield meaningful clinical benefit in this cohort of patients with advanced refractory cancers.

INTRODUCTION

It has been popularly believed [1] that analyzing the molecular structure of cancer would yield definitive strategies and therapeutic direction for improved outcomes. However, translation of this seemingly axiomatic deduction into meaningful improvements in systemic therapy has proved to be persistently elusive. While platforms and solutions for molecular analysis of tumors have become ubiquitous, widespread adoption of treatment strategies based on evidence of molecular hallmarks appears to be stymied for want of definitive data and lack of demonstrable, quantifiable clinical benefits.

Targeted treatments have been confined to their labelled indications and efforts at replication of therapeutic benefit in an organ-agnostic setting [2, 3] appear to be limited, the most notable example yet being of the checkpoint inhibitor Pembrolizumab, which was

recently approved for solid organ malignancies with mismatch repair deficiency (dMMR). It is also pertinent to mention the pan-cancer drug Larotrectinib [4] which has been approved for use across solid organ malignancies with neurotrophic receptor tyrosine kinase (NTRK) gene fusion, and Tisotumab vedotin [5], which has shown promise in clinical trials to treat all tumors that express tissue factor.

There have been other [6–13] efforts to improve outcomes in hard to treat cancers using a putative correlation between molecular analysis and treatment selection in off-label or organ agnostic settings. However, these studies were either based on univariate analysis of biomarkers and/or constrained in design by restricting inclusion to patients who were positive for a predefined molecular feature of the tumor. Most of these studies [6, 7, 9] also treated patients with single agents selected on the basis on available molecular indications, even in instances where multiple drug indications may have been available. Though there is evidence [14–18] from prior trials that multi-drug combinations of cytotoxic and targeted agents may yield improved therapeutic benefit, fewer prior studies [8, 11, 12] appear to have evaluated multi-drug combinations based on tumor molecular profiling. As a consequence of these restrictions, the outcomes reported in prior precision medicine trials (such as the ones enlisted above) have either fallen short of expectations [6] or have merely suggested equivocal to incremental improvements in efficacy [7–12], and have indicated the need for further evaluation of molecular guided therapy selection approach. Only the IPREDICT Study has reported a significantly higher ORR [13].

Presently, no evidence exists on treatment strategies for r/r m-cancers based on comprehensive, multi-analyte molecular analysis with synchronous *in vitro* chemo-sensitivity profiling, in a label-agnostic manner. Accordingly, we designed the RESILIENT Study, where label-and organ-agnostic treatment strategies for patients with r/r m-cancers were based on an integrative, multi-analyte Encyclopedic Tumor Analysis (ETA) which captures in depth information about the multi-layered tumor interactome. In the RESILIENT Study, participants received personalized multi-drug therapy recommendations based on inputs from multiple molecular biomarkers as well as *in vitro* chemosensitivity testing on viable tumor cells. We present the study outcomes which demonstrate the efficacy of ETA-guided treatment options which target latent vulnerabilities of the tumor to afford meaningful clinical benefit to patients.

RESULTS

Patients

Between December 2017 and October 2018, 231 patients were screened for recruitment, of whom, 190 patients were recruited and 143 patients eventually started treatment as per ETA; 47 patients were excluded prior to start of treatment for various reasons including withdrawal of consent (n = 23), death (n = 16), deterioration of Eastern Cooperative Oncology Group (ECOG) performance status (n = 6) or unavailability of lesions measurable on a CT/PET-CT scan (n = 2). Treatment for the first patient commenced on January 05, 2018 and for the most recent patient on November 16, 2018. Out of the 143 patients who started treatment, 17 patients were excluded prior to any follow-up evaluation for various reasons including patient being lost to follow-up (n = 7), death (n = 5), withdrawal of consent (n = 4) and deterioration of health (n = 1). A total of 126 patients were evaluable as per study criteria and 65 patients were continuing treatment in accordance to the TR as on the lock-in date of January 25, 2019. The CONSORT diagram (Figure 1) depicts the study structure and flow. Patient demographics, cancer types and prior treatments are indicated in Table 1 with expanded and additional details in Supplementary Table 1 and Supplementary Table 2 Patient-wise extent of disease and sites of metastases are indicated in Supplementary Table 3. The distribution of cancer types among the study population was an accurate representation of the locoregional prevalence rates [19].

Landscape of genomic alterations

Figure 2 depicts the landscape of genomic alterations in the Intent to Treat (ITT) population. Point mutations in TP53 were most frequently encountered (56%) gene variations in the ITT population, which also included two instances of copy loss. Similarly, point mutations in PTCH1 (16%) and PIK3CA (15%) were the second and third most frequently encountered variants. Gain of gene copy was observed most often in MYC, ERBB2, NBN, PDE4DIP, EXT1, NCOA2, RUNX1T1, UBR5, CCNE1, PLAG1, PRKDC and RECQL4. Loss of gene copy was most frequently encountered in genes such as ARID1A, KRAS, ATM, NF1, LAMP1, APC, RB1, FLT3, FGFR3, ERCC5, PTEN, SMARCA4 and JAK3. Patient-wise actionable gene alterations that formed the basis for therapy selection are indicated in Supplementary Table 4. Additionally, gene expression data in terms of mRNA as well as immunohistochemistry (IHC) were also considered for therapy selection and are also indicated (where actionable) in Supplementary Table 4.

Treatments

Among the 143 patients who received ETA-guided treatment under RESILIENT, 45 patients received combinations of cytotoxic agents, 5 patients received combinations of targeted agents and 93 patients received combinations of cytotoxic and targeted agents. Endocrine therapy agents were administered to 21 patients in

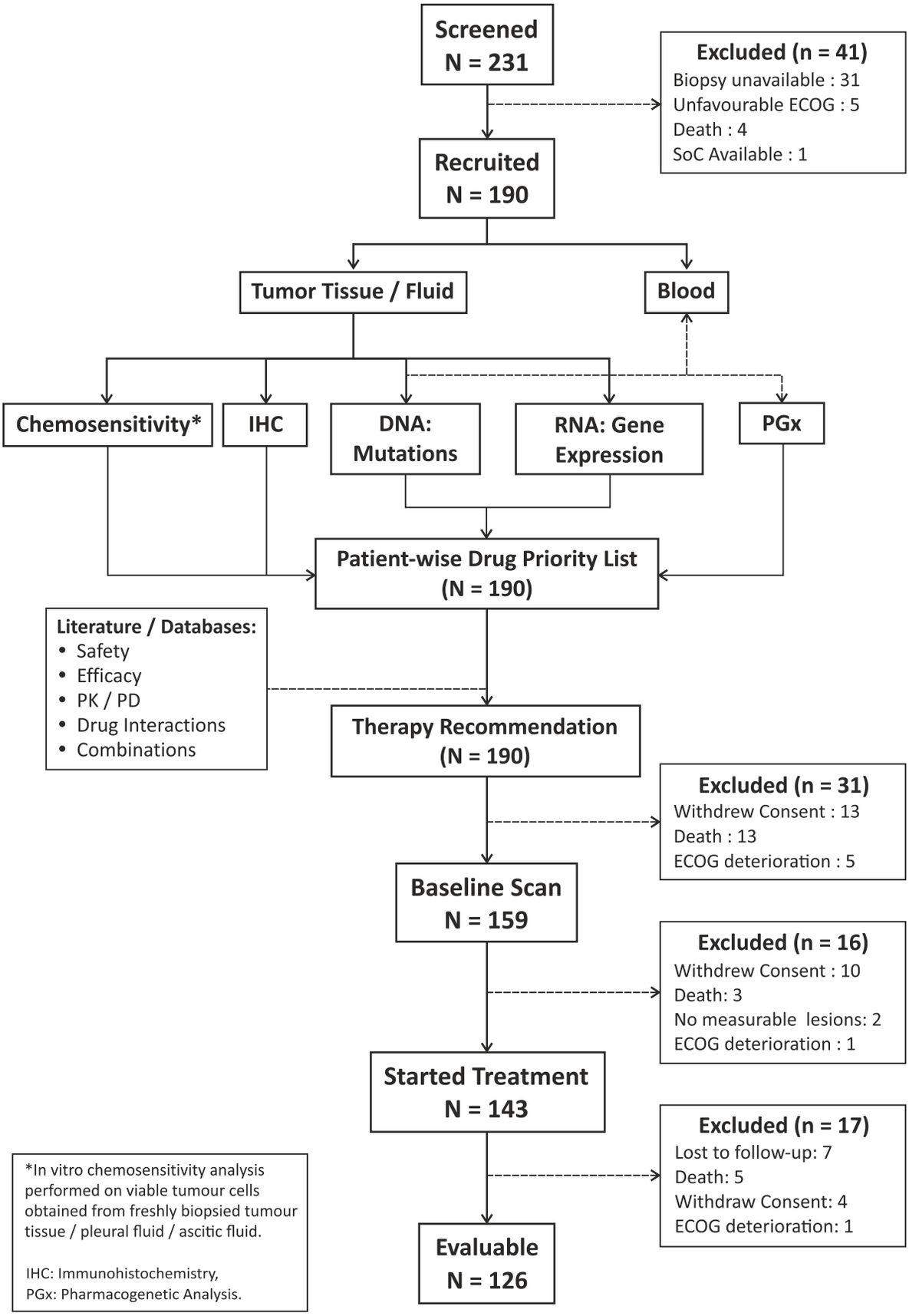


Figure 1: CONSORT diagram.

Table 1: Baseline characteristics of Intent to Treat (ITT) and evaluable patients

Parameter	ITT	Evaluable
	Number (%)	Number (%)
Ethnicity		
South Asian (Indian)	143 (100%)	126 (100%)
Gender		
Male	73 (51.0%)	65 (51.6%)
Female	70 (49.0%)	61 (48.4%)
Age		
Min	25	24
Max	75	72
Median	50	50
Cancer Types and Organs		
Bone	4 (2.1%)	3 (2.4%)
Breast	26 (18.2%)	21 (16.7%)
Cervical	5 (3.5%)	5 (3.9%)
Colorectal	14 (9.8%)	14 (11.1%)
Oesophagus	2 (1.4%)	2 (1.6%)
Gastric	7 (4.9%)	6 (4.8%)
Head and Neck	36 (25.2%)	31 (24.6%)
Hepatobiliary	7 (4.9%)	6 (4.8%)
Kidney	4 (2.8%)	4 (3.2%)
Lung	7 (4.9%)	5 (4.0%)
Neuroendocrine tumors	3 (2.1%)	3 (2.4%)
Ovarian	9 (6.3%)	8 (6.3%)
Pancreatic	8 (5.6%)	8 (6.3%)
Prostate	1 (0.7%)	1 (0.8%)
Sarcoma	5 (3.5%)	4 (3.2%)
Skin	3 (1.8%)	3 (2.4%)
Testes	2 (1.2%)	2 (1.6%)
Grade of Tumor		
1 (Well-differentiated)	13 (9.1%)	11 (8.7%)
2 (Moderately differentiated)	54 (37.8%)	50 (39.7%)
3 (Poorly differentiated/undifferentiated)	52 (36.4%)	43 (34.1%)
(Grade unevaluable)	24 (16.8%)	22 (17.5%)
Total Prior Lines of Therapy		
1–2	38 (26.6%)	36 (28.6%)
3–4	61 (42.7%)	52 (41.3%)
≥ 5	44 (30.8%)	38 (30.2%)

addition to cytotoxic and/or targeted agents. Patients were administered treatments as per institutional protocols and treatments were continued until study completion or dose-limiting toxicity or progression or any other end-point, such as patient opting out/defaulting. Patient-wise details of prior treatments received, ETA-guided treatment combinations, and rationale for ETA guided agents are indicated in Supplementary Table 4.

Response to treatment

Among the 126 patients who underwent follow-up scans and were thus evaluable per protocol, Objective Response was observed in 54 patients (ORR = 42.9%), including 3 Complete Responses (CR) and 51 Partial Responses (PR). At study completion, 3 patients (2.4%) had continued CR, 45 patients (35.7%) had PR, 66 patients (52.4%) had Stable Disease (SD), and 12 patients (9.5%) showed Disease Progression. The Clinical Benefit Rate (CBR) was determined to be 90.5%. Response Evaluation in the Intent to Treat (ITT) population, which included 17 patients who were excluded prior to any follow-up scans, indicated an ORR of 37.8%, which was not significantly lower than the patients evaluable per protocol. Similarly, CBR was determined to be 68.5% when evaluated in the ITT population, in those patients where SD was determined to be ≥60 days. Characteristics of response are indicated in Table 2. Waterfall Charts depict the best radiological response (Figure 3A) and radiological response at study termination (Figure 3B) of all 126 patients. A Swimmer Plot (Figure 4) depicts temporal trends in response and duration of response.

Progression free survival (PFS)

Patients were followed up for a median duration of 138 days (range 31 to 379 days). The Kaplan Meier plot of PFS is depicted in Figure 5. PFS rates at 90 days and at 180 days were 93.9% and 82.5% respectively. A comparison of PFS on RESILIENT (PFS2) with that on last prior systemic line of treatment (PFS1) for the relevant patient was ascertainable as per trial criteria (PFS1 ≤90 days) in 62 patients where median PFS1 was 72 days (range 22 to 111) and PFS2 was 120 days (range 34 to 374). Of these 62 patients, 22 patients (35.5%) achieved a PFS2: PFS1 ratio of ≥2.5 and 47 patients (75.8%) achieved and PFS2: PFS1 ratio of >1.3.

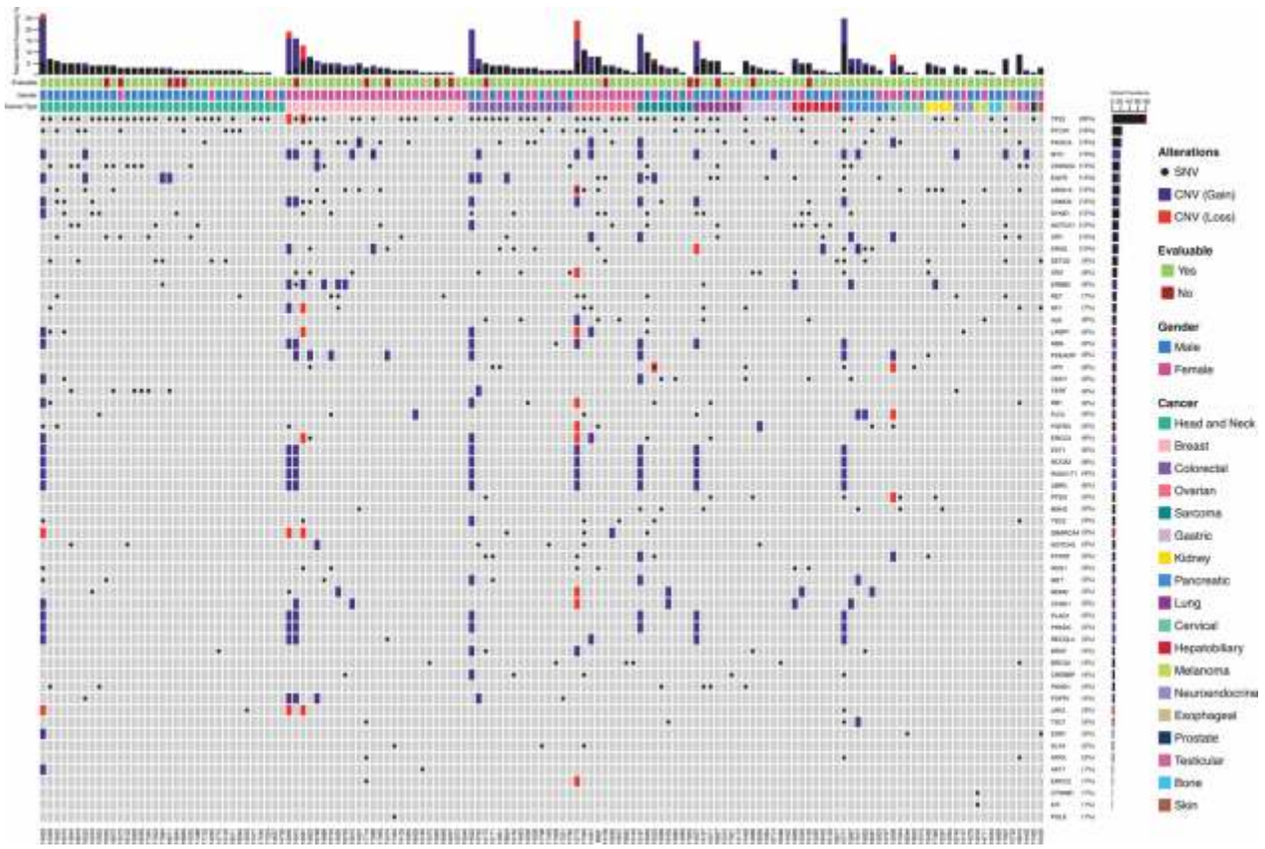


Figure 2: Landscape of genomic alterations in the Intent to Treat (ITT) population. Each vertical column indicates a single patient (5-digit numeric identifier in the bottom X-axis). Vertically stacked grey boxes in each column indicate individual genes (gene names on right Y-axis). Black dots within each box indicates a point mutation (single nucleotide variation), whereas blue and red shaded boxes indicate gain or loss of gene copy respectively. Patients are grouped according to cancer types – colour coded boxes immediately above the grey stacked boxes. Gender is indicated above the cancer type. Patients who were evaluable per protocol are indicated in the topmost row of colour-coded boxes. Bar graph on the top indicates combined variant frequency (%) per patient. Bar graph to the right indicates total frequency of occurrence of alterations in that particular gene in the ITT population.

Table 2: Clinical activity of ETA-guided therapies in patients with r/r-m solid organ malignancies

Parameter	Value
Objective Response Rate	
Number of patients	54
% of cohort (95% CI)	42.9 (34.3–51.5)
P value	<0.00001
Status at Study Completion	
Complete Response (%)	3 (2.4%)
Partial Response (%)	45 (35.7%)
Stable Disease (%)	66 (52.4%)
Disease Progression (%)	12 (9.5%)
Time to Objective Response (days)	
Median	64
Range	28–309
Duration of Follow-Up (days)	
Median	138
Range	31–379
Progression Free Survival (days)	
Median	134
Range	31–379

Metastases

Among the evaluable patients, (n = 126), the most commonly observed sites of metastases at baseline were lymph nodes (n = 84, 66.7%), lungs (n = 35, 27.8%), bones (n = 30, 23.8%) and liver (n = 31, 24.6%). Central nervous system involvement as brain metastases was observed in 9 patients (7.9%) and bone marrow involvement was observed in 3 patients (2.4%). Presence or absence of metastases in vital organs such as brain, lung or liver did not appear to impact outcomes in response to ETA-guided therapy; ORR or CBR in patients with brain, lung or liver metastases were not found to be significantly different from patients who did not have metastases to these organs (Supplementary Table 5). Significantly, all brain metastases were observed to be stable (n = 7) or had regressed (n = 2) at the most recent evaluation for these patients; none of the patients reported new or increase in size of brain metastases. At RESILIENT Study completion, 12 patients (9.5%) had progressed, among whom 9 patients (7.1%) progressed with no new distant metastases which were observed only in the other 3 patients (2.4%).

Therapy related adverse events

Adverse Events (AEs) were recorded as per the National Cancer Institute - Common Terminology Criteria for Adverse Events (NCI-CTCAE) v5.0 [20] (Table 3). All the 143 patients in the Intent to Treat (ITT) population were evaluated for therapy-related AEs. Onset of therapy related AEs was observed approximately up to 1 week, post therapy and time to resolution ranged between 1 to 2 weeks. The most common AEs (any grade) reported in ≥10% patients were Fatigue, Anorexia, Mucositis Oral, Edema, Diarrhoea, Pyrexia, Neutropenia, Myalgia, Vomiting, Anemia, Constipation, Thrombocytopenia and Pruritis/Rash. The only grade 3 AE in ≥10% patients was neutropenia (11.3%). Haematological toxicities of any grade were observed in 47 patients (32.9%) while grade 3 haematological toxicities were observed in 28 patients (19.6%). Patients with metastases to bone marrow (2.4%) did not appear to be at greater risk of haematological therapy-related toxicities as compared to the entire cohort. Overall, grade 3 and above therapy-related AEs were reported in 57 patients (39.9%) among whom dose readjustment or interruptions were necessitated in 47 (32.9%) patients due to Anemia, Edema, Hypotension, increased blood bilirubin, Mucositis Oral, Neutropenia, Thrombocytopenia and Vomiting. No grade 4 treatment-related AEs were reported in any of the patients. There were no mortalities that could be ascribed to treatments received. Owing to the patients receiving unique combinations of treatment agents, as well as individualized management of dosage and schedule, there were no discernible patterns in adverse events (or categories of adverse events) that could be ascribed to specific mechanistic classes (e. g., TKI/platins) or categories (e. g., cytotoxic/targeted/endocrine) of drugs. All AEs were managed by administration of standard of care agents or procedures as required.

Quality of Life (QoL)

Quality of Life was measured based on a brief questionnaire that evaluated the patients' functional and symptomatic status which are innately linked to the ECOG status. Patients' feedback was obtained on functional, symptomatic and overall health status at baseline and at most recent follow-up or at study termination. 83.9% patients indicated stable to improved functional status, 74.2% patients indicated stable to decreased symptomatic status and 90.3% patients indicated stable to improved overall health status.

DISCUSSION

Data from RESILIENT shows that r/r m-cancers have unexplored vulnerabilities amenable to treatment. Consequently, it is possible to obtain durable objective response and disease control in a significant proportion of the total patient population, by guiding treatments based on ETA. Contrary to the discouraging or equivocal data from previous studies, the ORR and CBR observed in RESILIENT demonstrates the clinical impact of ETA

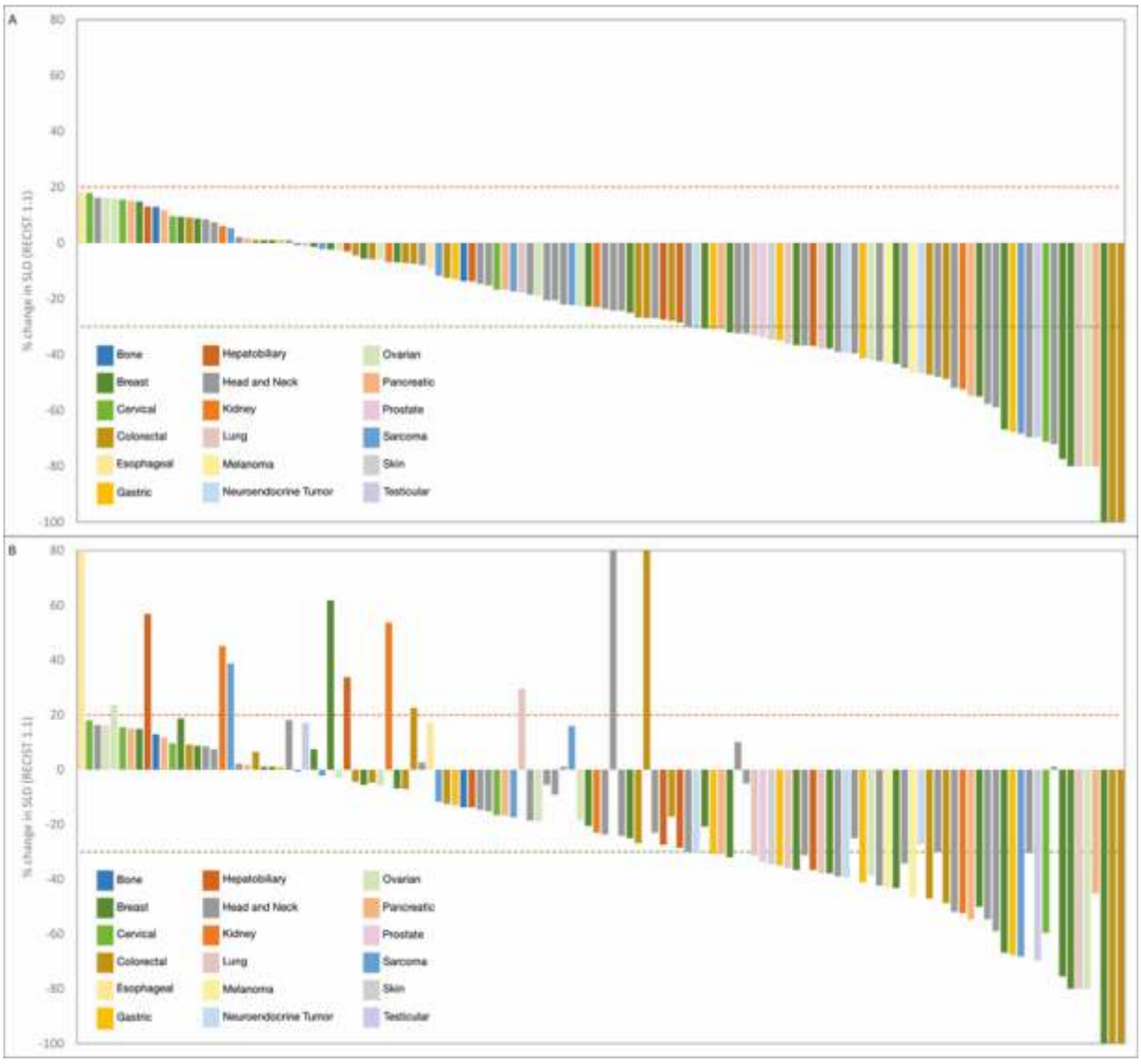


Figure 3: Summary of outcomes in RESILIENT. (**A**) Waterfall chart of best response. Treatment response was evaluated as per RECIST 1.1. Percent change in dimensions of target lesions (Sum of Largest Diameters, SLD) between baseline and at evaluation are graphically represented. Patients are arranged in descending order of change (%) in SLD. (**B**) Waterfall chart of response at study completion. Treatment response was evaluated as per RECIST 1.1. Percent change in dimensions of target lesions (Sum of Largest Diameters, SLD) between baseline and at evaluation are graphically represented. Sequence of patients is same as in Figure 2A to indicate change in status (if any) at study completion.

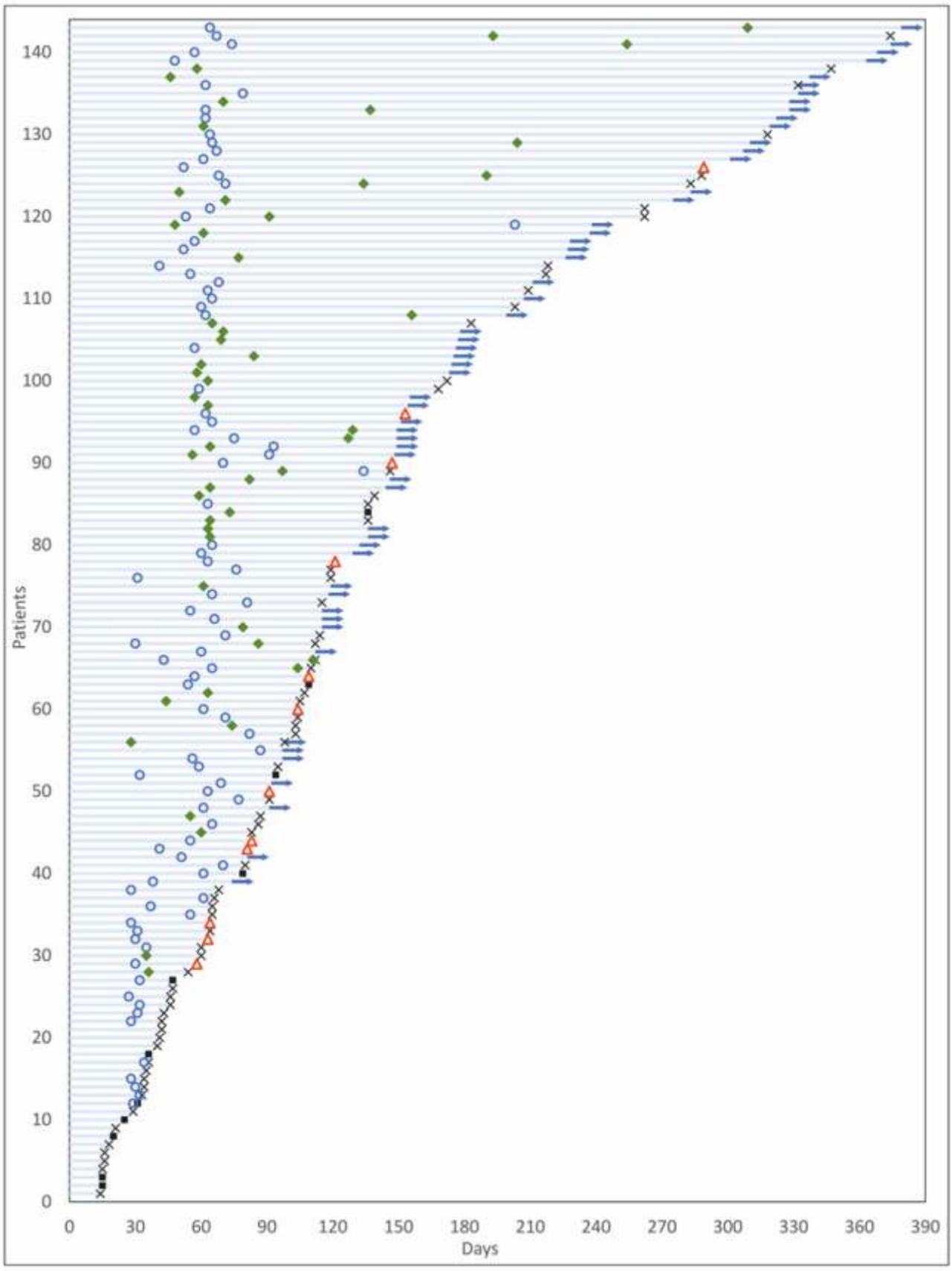


Figure 4: Swimmer plot of patient response. The Y-axis indicates patients while the X-axis indicates time (days). ♦: Partial Response/Complete Response; O: Stable Disease; Δ: Disease Progression; ×: Lost to follow-up/Withdrew Consent; ■: Death; ➡: Progression Free Survival. For radiological response status, only the first scan and subsequent scan where response status changed are indicated.

and potential benefits or label- and organ-agnostic therapy selection in clinical practice.

The discouraging outcomes of prior trials have been often used as a benchmark for vocal skepticism [21, 22] and to dissuade label-agnostic individualized treatment selection. A comparison of RESILIENT with 6 such widely reviewed studies/trials is hence relevant (Supplementary Table 6). The SHIVA trial [6] targeting 3 molecular pathways with Molecular Targeting Agents (MTAs) reported weak outcomes and went on to discourage the use of MTAs outside their current indications. The MyPathway trial [7] reported ORR of 23% across 14 different tumor types. A pilot study by Von Hoff et al [8] showed PFS ratio of ≥1.3 in 27% of patients treated with cytotoxic and targeted/endocrine agents based on limited molecular profiling. The MOSCATO trial [9] reported ORR of 11% in patients following molecular profiling and treatment with cytotoxic, targeted and endocrine therapies. The M. D. Anderson Cancer Center reported [10] an ORR of 27% in a retrospective analysis of several Phase-I clinical trials in personalized medicine where a limited set of molecular aberrations were targeted using approved cytotoxic, targeted and investigational agents. Interim data from 4 arms of the NCI – MATCH [11] study showed an aggregate ORR of 7.5% among patients who received targeted therapy based on molecular changes. More recently, the IPREDICT study [12] was significant in that it indicated an ORR of 45% and 75% of patients indicated a potential gain in PFS by ~30% based on molecularly matched treatments. The WINTHER study [13] reported an ORR of 13% and 9% respectively in the two arms where patients were treated on the basis of molecular features in DNA and RNA respectively.

While these studies relied on univariate molecular marker analysis for therapy selection, they also generally had restrictive inclusion criteria which recruited only those patients who had a predefined molecular target for treatment with preselected choice of agents. This inclusion qualification rate was factored in for an indexed comparison between results of some of these trials [6–11] and RESILIENT (Supplementary Table 7) to evaluate the parameters of the various studies which necessitated bias correction in their reported ORRs. The advantage of an ETA-guided approach to therapy selection, as in RESILIENT, is evident in absence of prequalifying molecular features, due to which potentially all patients with solid organ malignancies stand to benefit rather than just the limited proportion of the real-world patient population where tumors harbor the predefined feature. Analysis of differentially (over) expressed genes based on mRNA or IHC provided additional therapy options for several patients, including those where actionable gene alterations were unavailable. As opposed to the WINTHER trial [13] where actionable information from DNA and RNA competed with each other for efficacy analysis, they were complementary to one another in the RESILIENT protocol. Similarly, *in vitro* chemosensitivity analysis using viable tumor cells provided direct functional evidence of drug efficacy which aided therapy selection. There have been concerns about the suitability of *in vitro* chemosensitivity analysis for therapy selection [23] in cancers based on outcomes of prior studies. However, prior efforts appear to be based on single agents and regimens included in Standard of Care (SoC) for the cancer types. On the other hand, we evaluated a comprehensive panel of FDA-approved agents in an organ agnostic setting

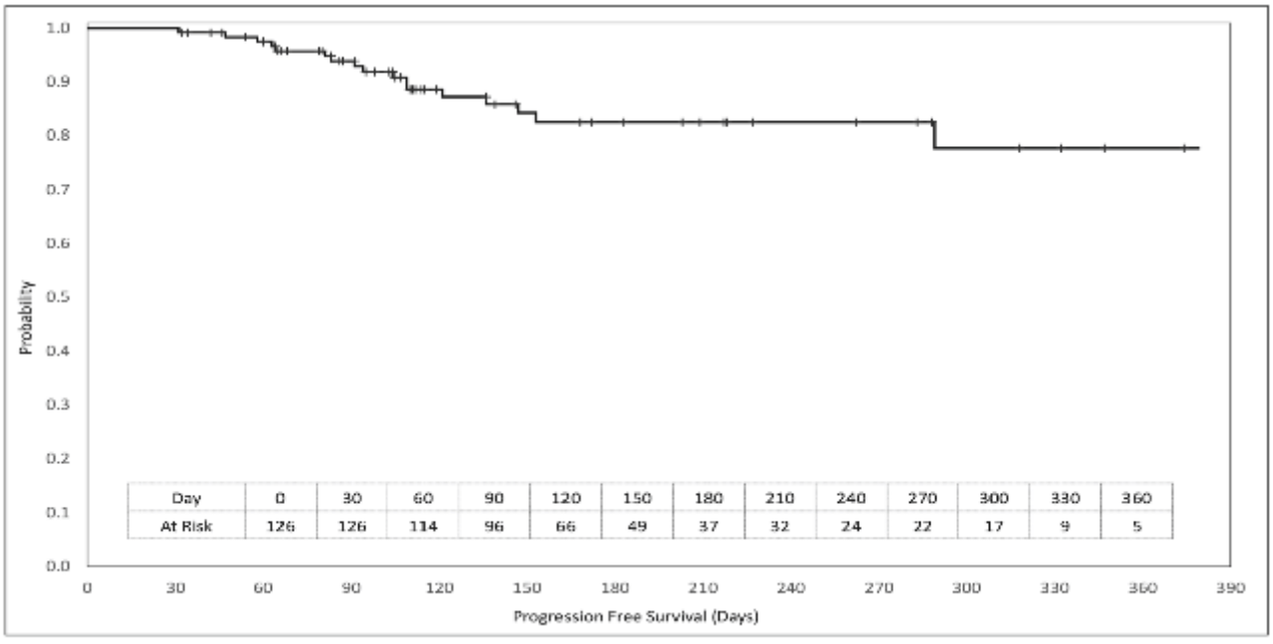


Figure 5: Kaplan Meier plot of progression free survival. Patients at risk at each milestone are indicated in the inset table. Vertical cross-bars indicate censoring events.

Table 3: Therapy-related adverse events in intent to treat population

Adverse events	Any grade		Grade ≥3	
	No of patients	%	No of patients	%
Fatigue	121	84.6%	9	6.3%
Anorexia	92	64.3%	6	4.2%
Mucositis Oral	57	39.9%	13	9.1%
Edema	39	27.3%	4	2.8%
Pyrexia	35	24.5%	8	5.6%
Diarrhoea	35	24.5%	1	0.7%
Neutropenia	32	22.4%	16	11.2%
Myalgia	30	21.0%	3	2.1%
Vomiting	26	18.2%	6	4.2%
Anemia	22	15.4%	12	8.4%
Constipation	20	14.0%	1	0.7%
Thrombocytopenia	18	12.6%	12	8.4%
Pruritis/Rash	16	11.2%	1	0.7%
Nausea	14	9.8%	2	1.4%
Peripheral neuropathy	11	7.7%	2	1.4%
Pain at site of biopsy	9	6.3%	3	2.1%
Hyper-/Hypotension	8	5.6%	6	4.2%
Alopecia	6	4.2%	0	0.0%
Increased blood bilirubin	4	2.8%	3	2.1%
Eletrolyte Imbalance	3	2.1%	2	1.4%
Hoarseness	2	1.4%	1	0.7%
Pneumonitis	2	1.4%	1	0.7%
Dysuria	1	0.7%	0	0.0%
Any Event	**143**	**100%**	**57**	**39.9%**

based on which optimum agent (s) were selected. Thus, the outcomes in RESILIENT were superior to the next line SoC treatment options and indicated the possibility of viable alternatives to checkpoint inhibitors [24–28] (Supplementary Table 8) for representative cancer types.

The durability of response in RESILIENT, in terms of the 90-day PFS rate, appears to be significant as deduced from Kaplan Meier plots (Supplementary Table 9). It is generally believed that PFS decreases with every subsequent line of therapy. Thus, contemporary precision oncology trials have benchmarked PFS (while on trial) to the last failed systemic line of treatment to determine therapeutic advantage. While a 30% increase has been indicated as significant, outcomes of RESILIENT show that it is possible to achieve significant (2.5×) increase in PFS as compared to the last line. A significant number of patients were progression free at study completion, and hence the median reported PFS in RESILIENT reflects the status at study completion and not the final outcome. Duration of Response (DoR) and Overall Survival (OS) are presently not mature for reporting.

Most of the study population had experienced progression of disease with new distant metastases of the last failed treatment, whereas on the RESILIENT protocol, appearance of distant metastases on progression was well controlled; out of the 12 patients where progression was seen, local progression was observed in 9 patients while only 3 patients presented new distant metastases. The suppression of metastatic tendency of the disease is significant and its impact cannot be overemphasized in view of the shifting appreciation of late stage disease management as recognized in Prostate cancer where Metastasis Free Survival (MFS) has been described as a relevant clinical trial endpoint [29].

Though objective response is a desirable aim at every treatment threshold, an equally relevant consideration for treatment of advanced refractory cancers is achievement of stable disease (SD) with associated improvements not only in time-dependent end points but in quality of life measures. In this respect, the disease control achieved in RESILIENT is clearly encouraging.

Though there have been several reports [14–18] of de novo combinations of targeted and cytotoxic agents yielding improved therapeutic benefits, patients in most prior studies received single agents. Only a few precision medicine studies included combination treatments which were administered to all (or limited set of) patients based on molecular profiling. The MD Anderson Study [10] retrospectively evaluated patients from several drug trials where patients received single agents and combinations of approved as well as experimental agents. In case of the WINTHER trial [13], though genomic and transcriptomic molecular marker data were evaluated, majority of patients were treated with single agents based on either DNA or RNA in the respective study arms. In the RESILIENT Study, all patients received combinations of cytotoxic, targeted or endocrine agents based on cellular and molecular evidence evaluated by the ETA. The authors seek to draw attention of the reader to the fact that none of the study patients received experimental or unapproved drugs. All patient-specific therapy combinations recommended through the ETA included only those drugs that have already been approved for treatment of (same/different) cancers with well characterized toxicity profiles. Thus, AEs were well controlled even in this heavily pre-treated population with tumor evolution and systemic deterioration following multiple prior lines of treatments. Notably, there were no grade 4 treatment related AEs in RESILIENT; for comparison a prior meta-analysis of Phase I trials between 2001 to 2012 of cytotoxic drugs reported Grade 4 AEs in 19.9% in patients [30].

Having discussed the benefits of ETA, an insight into the limitations is also pertinent. ETA requires fresh tissue from a *de novo* biopsy tissue where the quality and quantity of biopsied tissue could be of concern. Patients who have progressed on multiple lines of treatment are often psychologically fatigued for further invasive procedures and possible hospitalization. The impact of previous treatments on the overall health of the patients, especially on the bone marrow reserve can impede compliance with ETA guided treatments. However, adoption of ETA-guided approach at an earlier treatment stage could obviate limitations associated with less beneficial SoC treatments. In the ITT population (n = 143), among the 17 patients who were excluded prior to any follow-up, 6 patients were lost to follow-up due to inability to travel from other cities for treatment. Similarly, among the 17 patients who were excluded after the first evaluation, 12 patients were lost to follow-up for the same reason. Due to these early exclusions, the median follow-up duration appears underrepresented in the study population. As a corrective action, for all subsequent enrolments into RESILIENT, priority and preference were given to patients living within the same city, or within a reasonable distance with access to direct transportation. Another significant impediment towards achieving improved outcomes was the non-availability of USFDA or EMA approved treatment agents for incorporation in the TR, as several such drugs are not approved in India and possibly in several other countries. RESILIENT is also confined to the south Asian – Indian population, although it seems unlikely that the outcomes would vary across ethnicities.

METHODS

All laboratory processes were conducted at a CAP and ILAC accredited institution.

Study design

RESILIENT was a single arm, single centre, non-randomized phase II/III prospective trial for evaluation of treatment response to therapy based on ETA recommendation in r/r m-cancers patients. The Ethics Committees of the participating institutes had approved the trial. The design of the trial acknowledged the rationale [8] that owing to the diversity in cancer types and unique treatment history of each patient, there can be no accurate external control for each patient. Therefore, rather than a randomised two arm trial design, a single arm design would more accurately evaluate and represent treatment benefits from the ETA guided approach, when benchmarked against the patients last (failed) line of treatment. Thus, prior treatment response of patients served as the virtual control arm [8]. The Progression Free Survival (PFS) on ETA-guided treatment (PFS2) was benchmarked against that (PFS1) on the last (failed) line of systemic treatment in those patients where PFS1 was ≤ 90 days.

Patients

RESILIENT recruited patients with solid organ malignancies who had either failed at least two prior lines of Standard of Care (SoC) treatments or where SoC treatment options were unavailable or further unviable. Eligible patients had radiologically evident and measurable disease, an Eastern Co-operative Oncology Group (ECOG) performance status of ≤2 and who consented to provide tissue and blood/fluid samples. Patients who fulfilled the above criteria were counselled regarding the potential benefits and risks of the trial. Thereafter, willing patients provided duly signed, informed consents. The complete eligibility criteria are available at http://apps.who.int/trialsearch/Trial2.aspx?TrialID=CTRI/2018/02/011808.

Encyclopedic tumor analysis (ETA)

Tumors employ myriad mechanisms, feedback loops and redundancies at each of the functional layers of coding, transcription, regulation and protein synthesis, such as reactivation of signalling pathways, cross-talk between various pathways, post-translational modification, heterogeneity of tumors, clonal evolution

of resistant variants and more [31, 32]. Consequently, each functional layer influences the processes towards sustaining survival and proliferation singularly and cumulatively. Thus, any drug - feature conjugation that looks towards a single layer of the process will inevitably miss the interactions and context in the other layers of this interactome. The ETA captures and contextualizes data from multi-layered tumor interactome, including *in vitro* response/resistance of viable cells. Individual procedures as part of the ETA are described in the sub-sections below.

Tissue and blood collection

Approximately 5 × 5 × 5 mm freshly biopsied tumor tissue was transferred into 5 mL transport medium and stored at 4°C during transit. Fresh tissue was either processed immediately or cryopreserved at −80° C.

10 mL peripheral blood was collected by venous puncture in Cell-Free DNA BCT® and EDTA vacutainer tubes. Blood was stored and transported at 4° C. Plasma was separated by centrifugation at 3000× *g* for 20 min at 4° C, followed by 16000× *g* for 10 min at 20–25° C. Plasma samples without hemolysis were processed immediately.

Histopathology and immunohistochemistry

Formalin-Fixed Paraffin-Embedded (FFPE) blocks were prepared as per standard procedures. Histopathological (HPE) and immunohistochemical (IHC) analyses were carried out as per standard procedures. Tumor content of freshly biopsied tissue was determined by HPE evaluations. Tissue samples with ≥80% tumor content were considered as acceptable for molecular evaluations.

DNA isolation

Genomic DNA was isolated from fresh tissue samples using the PureLink® Genomic DNA Mini Kit and MagMAX FFPE DNA isolation kit (Thermo Fisher Scientific, USA) as per the manufacturer's instructions. DNA was quantified at 260 nm and quality was determined by measuring the ratio of absorbance at 260/280 nm using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, USA).

Total ctDNA was purified from 2 mL plasma using a Circulating Nucleic Acid kit (QIAGEN, Germantown, USA) as per the manufacturer's protocol. ctDNA was quantified using an HS DNA Qubit assay (Life Technologies, Carlsad, USA).

RNA isolation

Total tumor RNA was isolated from fresh tumor tissue by using mirVana miRNA isolation kit (Ambion, Austin, USA) as per the manufacturer's instruction. Total RNA was quantified using a Qubit 2 Fluorometer (Thermo Fisher Scientific, Waltham, USA) with the manufacturer's RNA assay kit.

RNA from exosomes were isolated from peripheral blood plasma. Plasma samples (2 ml) from EDTA tubes were centrifuged at 16000× *g* for 10 min at 4° C and filtered via a 0.45 µm membrane to remove larger vesicles. The filtrate was used for the extraction of total exosomal RNA using an ExoRNeasy serum/plasma kit (QIAGEN, Germantown, USA) according to the manufacturer's protocol [17]. Purified exosomal RNA was quantified using an miRNA Qubit assay (Life Technologies, Carlsad, USA).

Tumor DNA profiling

Tumor DNA was sequenced for 453 genes using Oncomine Comprehensive Assay v3 and Ion AmpliSeq Comprehensive Cancer Panel (Thermo Fisher, USA) as per user recommended protocols. Briefly, 40 ng DNA was used for NGS library preparation via PCR-based Ampliseq target enrichment protocol. Libraries of 100 pmol were sequenced using Ion Proton (Thermo Fisher Scientific, Waltham, USA). Torrent Suite™ v5.2 (Thermo Fisher Scientific, Waltham, USA) software was used to perform primary analysis, including signal processing and base calling. Primary QC parameters were: minimum read length of 25 bases, read quality trimming of 17 QV, window size for quality trimming 30 bp. The processed sequenced data were aligned to the reference genome GRCh37/hg19 to generate Binary Alignment/Map (BAM) files. Sequencing data were considered for downstream analysis with coverage at ≥10,000× depth and >80% amplicons with at least 600 reads. The aligned data were analyzed using Torrent Variant Caller software with optimized parameters such as minimum allele frequency (0.003), minimum mapping quality (4), minimum coverage (600), down sample to coverage (10,000) and position bias (1). Reported somatic variants of >0.5% allele frequency (AF) were compared to the reference genome hg19. The Integrative Genomics Viewer (IGV) was used to visualize the read alignment and the presence of variants against the reference genome and to confirm the veracity of the variant calls by checking for possible strand biases and sequencing errors. All the germline variants found in the 1000 Genomes Project or The Exome Aggregation Consortium (ExAC) with a frequency of >0.1% were excluded. All somatic mutations were annotated, sorted and interpreted using COSMIC and/or TCGA data. Variants with <0.5% AF were confirmed orthogonally with digital droplet polymerase chain reaction (ddPCR, BioRad) using the rare mutation assay as per the manufacturer's protocol.

Targeted whole transcriptome analysis

The Ion AmpliSeq™ Transcriptome Human Gene Expression Research Panel was used to determine the expression of 20,802 genes including 18,574 coding genes and 2228 non-coding genes based on University of California Santa Cruz (UCSC) hg19 annotation. Historical RNA from normal adjacent tissue was used as

a control for transcriptome analysis. A barcoded cDNA library was generated with a SuperScript® VILO™ cDNA Synthesis kit from 40 ng of total RNA. The cDNA was amplified using Ion AmpliSeq™ technology as per the manufacturer's instructions (Thermo Fisher Scientific). Amplified cDNA libraries were evaluated for quality on a Bioanalyzer 2100E using a high sensitivity DNA 1000 chip (Agilent Technologies) and quantified using an Ion Library TaqMan™ Quantitation Kit (Thermo Fischer Scientific. Pooled libraries of 100 pM were amplified using emulsion PCR on an Ion Torrent OneTouch2 and enriched as per the manufacturer's instructions. Templated libraries were sequenced on an Ion Torrent Proton™ sequencing system, using an Ion PI sequencing kit and an Ion PI chip (Thermo Fisher Scientific). Analysis of AmpliSeq RNA sequencing data was performed using the AmpliSeq-RNA plugin available for Ion Torrent sequencing platforms. This plugin uses the Torrent Mapping Alignment Program (TMAP—https://github.com/iontorrent/TMAP), which is optimized for aligning raw sequencing reads (from Ion Torrent) against the hg19 transcriptome reference sequence against regions defined in the Browser Extensible Display (BED) file (hg19_AmpliSeq_Transcriptome_21K_v1.bed). The quality of the raw data was evaluated based on three parameters: number of reads, mean read length and target detected (% of all amplicons that had ≥10 assigned reads). Differential gene expression analysis was performed using R/Bioconductor package edgeR with raw read counts from AmpliSeq. Read count normalization was performed using the Counts Per Million (CPM). Significant differential expressed genes were called using the following threshold: absolute log fold-change ≥2 and Benjamini–Hochberg adjusted $p < 0.05$. The commercial software iPathway Guide (Advaita) was used for pathway analysis to explore significantly affected pathways.

In vitro chemosensitivity profiling of viable tumor cells

Viable tumor cells were isolated from freshly biopsied tumor tissue by standard procedures and maintained *in vitro*. Viable cells were seeded into multi-well plates and allowed to adhere. Adherent viable cells *in vitro* were treated with a panel of FDA-approved anti-cancer drugs for 24 hours after which apoptotic cell death events were determined. All assays included positive and negative controls as well as untreated cell controls to determine baseline apoptotic events. Response to each drug was determined after subtracting baseline apoptosis in untreated controls. Data from all investigations were integrated to identify agents and their combinations with maximum projected efficacy and safety.

Therapy recommendation

An interdisciplinary tumor board comprising of oncologists, pathologists, other clinicians, molecular biologists, and bioinformaticians evaluated tumor-data including somatic and germline mutations in DNA for actionable gene alterations, pharmacogenetics analysis of alterations in drug metabolism enzymes (DME), differentially expressed genes and pathways for targeting, immunohistochemistry and *in vitro* chemosensitivity profiling of viable tumor derived cells. Drug indications derived from all evaluations were integrated and harmonized to create a drug preference list based on maximum projected efficacy and identification of potential risks due to alterations in DME. All drugs in the preference list were evaluated for further safety and efficacy based on information in published literature and public databases, and included sources such as (i) safety and efficacy findings from Phase I/II/III trials and meta analyses, (ii) analyses of multi-drug combinations [14–16] including targeted-cytotoxic drug combinations, (iii) pharmacokinetic and pharmacodynamics studies, and (iii) reported drug-interactions. This approach yielded patient-specific priority list of drugs and their combinations with projected efficacy and safety profiles, i. e., the Therapy Recommendation (TR). Availability of treatment agents in India was also considered in design of patient-specific regimens. The patient-specific TRs did not exclude drugs or combinations that may have been indicated in Standard of Care (SoC) for that cancer; ETA did not aim to deny regimens merely because of indication in SoC, rather ETA sought to identify optimum regimens with putative benefit for the patient, irrespective of the empirical nature of existing guidelines.

Treatments

Patient specific TRs were submitted to the treating clinician within 7 to 10 days of receipt of patient samples. The treating clinician evaluated the suitability of the suggested treatments and oversaw therapy administration. Being a single site study, TRs and treatments for all patients were evaluated by the Principal Investigator and other treating clinicians, due to which there were no subjective differences in interpretations for therapy management. Clinicians and patients were not blinded to the treatment. The treating clinicians exercised their discretion with regard to the optimum starting dose on a case by case basis considering patient safety, risk of therapy related adverse events (AEs), prior treatments and history of AEs. Treatments were administered/continued as per the standard practice and protocols of the treating institution until guideline-imposed limitations for duration of treatment were encountered, or until dose-limiting toxicity or disease progression or in the event of patient exclusion.

Evaluations

The baseline status of the disease was determined by a ^{18}F-Fluorodeoxyglucose Positron Emission Tomography – Computed Tomography (FDG PET-CT) scan before

initiation of treatment. A baseline MRI scan was also performed to identify any brain metastases. Response was evaluated on the lines of RECIST 1.1 criteria [33] through a further scan after the patient completed at least two treatment cycles or 60 days of treatment, except in cases where the treating clinician advised evaluation in the interim. Thereafter, follow-up scans were performed every 6 to 10 weeks. Status of brain metastases was determined by follow-up MRI scans. All scans were independently reviewed by a panel of external expert radiologists.

Patient monitoring

All study participants underwent periodic investigations such as complete blood counts, hepatic and renal function tests, urinalysis and left ventricular ejection fraction (LVEF) to determine fitness to receive or continue treatment as per study protocol. Other investigations such as ultrasonography, x-ray or endoscopy was carried out on recommendation of the treating oncologist. Adverse events were recorded during patient admissions as well as via telephonic follow-up during those weeks where patients did not visit the hospital. All adverse events were reported as per NCI-CTCAE v5 criteria. All grade 3 adverse events were flagged and followed up on a daily basis until resolution. Patients received printed instructions of subsequent treatment and imaging appointments as well as telephonic reminders. Patients had 24-hour telephonic access to study-coordinators as well as access to emergency/ambulance services.

Endpoints

The primary efficacy end point of the study was Objective Response Rate (ORR) defined as the percentage of patients who achieved Complete Response (CR) or Partial Response (PR) during the active study phase. Other end points were Clinical Benefit Rate (CBR) and Progression Free Survival (PFS). CBR was defined as the percentage of patients who achieved CR, PR or Stable Disease (SD). PFS was defined as time from commencement of treatment under ETA to disease progression or death during the active study phase. PFS on ETA-guided treatment (PFS2) was compared against that (PFS1) on the last (failed) systemic line. The qualitative end point was Quality of Life (QoL), based on patient's feedback on symptomatic and functional status at baseline and at study termination or most recently available follow-up.

Statistical methods and analysis

The sample size of the study was determined on the basis of the ORR, assuming that the ORR in such refractory advanced stage cancer patients is <10%. Simon's 2-stage design was used to validate adequacy of cohort size for assessment of ETA based therapy. The null hypothesis that the true response rate is 10% was tested against a one-sided alternative. Initially, at least 21 patients were required to accrue; if there were 2 or fewer responses, the study was required to be stopped. Else, at least 45 additional patients were required to accrue for a total minimum of 66 patients. The null hypothesis would be rejected if 11 or more responses were observed in 66 patients. With 66 evaluable patients, this design yields a type I error rate of 5% and power of 90% when the true response rate is 25%. The 95% CI of ORR was constructed using binomial distribution (Clopper-Pearson estimation method). Patient demographics were analysed with descriptive statistics. Contingency tables described the categorical data with counts and percentages. Continuous data was summarized using median and range. CONSORT diagram, waterfall plot and bar graphs were used to summarize the data. Kaplan-Meier estimator was used to estimate survival function.

Abbreviations

ETA: Encyclopedic Tumor Analysis; r/r-m: relapsed refractory metastatic; ORR: Objective Response Rate; PFS: Progression Free Survival; QoL: Quality of Life; dMMR: mismatch repair deficiency; NTRK: neurotrophic receptor tyrosine kinase; ECOG: Eastern Cooperative Oncology Group; RECIST: Response Evaluation Criteria in Solid Tumors; CONSORT: Consolidated Standards of Reporting Trials; CR: Complete Responses; PR: Partial Responses; SD: Stable Disease; CBR: Clinical Benefit Rate; AE: Adverse Events; NCI: National Cancer Institute; CTCAE: Common Terminology Criteria for Adverse Events; ITT: Intent to Treat; DoR: Duration of Response; MFS: Metastasis Free Survival; SoC: Standard of Care (SoC); DCGL: Datar Cancer Genetics Limited; HCG-MCC: HCG Manavata Cancer Centre; CAP: College of American Pathologists; cfDNA: cell free DNA; PCR: Polymerase Chain Reaction; NGS: Next Generation Sequencing; mRNA: messenger RNA; FFPE: Formalin Fixed Paraffin Embedded; IHC: immunohistochemistry; SNP: Single Nucleotide Polymorphisms; TR: Therapy Recommendations; FDG: ^{18}F-Fluorodeoxyglucose; PET-CT: Positron Emission Tomography – Computed Tomography; MRI: Magnetic Resonance Imaging.

Author contributions

RN: Principal Investigator, Overall Study Oversight, Counselling of Patients, Review of Treatment Recommendations and Clinical Management; DP: Design of Study Protocol, Review of Treatment Recommendations, Review of AEs, Data Analysis and Drafting; TC: Review of Clinical Data and Drafting; VD: Review of Data and Drafting; SB: Counselling of Patients, Review of Treatment Recommendations and Clinical Management; SD: Counselling of Patients, Review of Treatment Recommendations and Clinical Management; VP: Counselling of Patients,

Review of Treatment Recommendations and Clinical Management; SR: Counselling of Patients, Review of Treatment Recommendations and Clinical Management; PP: Counselling of Patients, Review of Treatment Recommendations and Clinical Management; AG: Review of Study by Ethics Committee and Approvals; RP: Review of *in vitro* Chemosensitivity Protocols, Review of Data, Drafting; AS: Data Compilation, Data Analysis and Drafting; DA: Design of Study Protocol, Design of Encyclopedia Tumor Analysis Protocols, Review of Treatment Recommendations, Data Analysis.

ACKNOWLEDGMENTS

The authors express gratitude towards the patients as well as families of patients who consented to participate in the RESILIENT study; the staff at HCG-MCC for patient care and management; Dr. Navin Srivastava, Dr. Pradip Devhare, Dr. Revati Patil, Dr. Smita Wagh, Dr. Shalom Syed, Mr. Pradip Fulmali, Mr. Sachin Apurwa, Mr. Sanket Patil, Mr. Sushant Pawar, Ms. Swati Deshpande, Ms. Prachi Inamdar, Ms. Ashwini Pawar, Ms. Rimple Shah, Ms. Mohini Garte, Mr. Pankaj Porje, Mr. Milind Agnihotri and Mr. Rajan Datar for managing various critical operational aspects of the trial.

CONFLICTS OF INTEREST

TC and HK have no conflict of interest to declare. RP receives consultation fees from time to time from the sponsor. DP, VD, AG, AS and DA are in the employment of the sponsor. The entire team from HCG-Manavata Cancer Centre, Nasik, *viz.* RN, SB, SD, VP, SR and PP report grants from DCGL during the conduct of the study; multiple research grants from Novartis, Dr. Reddy's Laboratories, Celltrion Healthcare, Intas Pharmaceutical Industries, Sun Pharmaceuticals, Amgen, Zydus Cadilla, US Vitamins and Lupin Laboratories, outside the submitted work, and educational support from Intas Pharmaceuticals, Fresenius Kabi and Dr. Reddy's Laboratories.

FUNDING

No external or public funding was received for this study. The study was funded wholly by Datar Cancer Genetics Limited (DCGL).

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144:646–74. https://doi.org/10.1016/j.cell.2011.02.013. [PubMed]
2. Lacombe D, Burock S, Bogaerts J, Schoeffski P, Golfinopoulos V, Stupp R. The dream and reality of histology agnostic cancer clinical trials. Mol Oncol. 2014; 8:1057–63. https://doi.org/10.1016/j.molonc.2014.06.002. [PubMed]
3. Yan L, Zhang W. Precision medicine becomes reality-tumor type-agnostic therapy. Cancer Commun (Lond). 2018; 38:6. https://doi.org/10.1186/s40880-018-0274-3. [PubMed]
4. Drilon A, Laetsch TW, Kummar S, DuBois SG, Lassen UN, Demetri GD, Nathenson M, Doebele RC, Farago AF, Pappo AS, Turpin B, Dowlati A, Brose MS, et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. N Engl J Med. 2018; 378:731–39. https://doi.org/10.1056/NEJMoa1714448. [PubMed]
5. de Bono JS, Concin N, Hong DS, Thistlethwaite FC, Machiels JP, Arkenau HT, Plummer R, Jones RH, Nielsen D, Windfeld K, Ghatta S, Slomovitz BM, Spicer JF, et al. Tisotumab vedotin in patients with advanced or metastatic solid tumours (InnovaTV 201): a first-in-human, multicentre, phase 1-2 trial. Lancet Oncol. 2019; 20:383–393. https://doi.org/10.1016/S1470-2045(18)30859-3. [PubMed]
6. Le Tourneau C, Delord JP, Gonçalves A, Gavoille C, Dubot C, Isambert N, Campone M, Trédan O, Massiani MA, Mauborgne C, Armanet S, Servant N, Bièche I, et al, and SHIVA investigators. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): a multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. Lancet Oncol. 2015; 16:1324–34. https://doi.org/10.1016/S1470-2045(15)00188-6. [PubMed]
7. Hainsworth JD, Meric-Bernstam F, Swanton C, Hurwitz H, Spigel DR, Sweeney C, Burris H, Bose R, Yoo B, Stein A, Beattie M, Kurzrock R. Targeted Therapy for Advanced Solid Tumors on the Basis of Molecular Profiles: Results From MyPathway, an Open-Label, Phase IIa Multiple Basket Study. J Clin Oncol. 2018; 36:536–42. https://doi.org/10.1200/JCO.2017.75.3780. [PubMed]
8. Von Hoff DD, Stephenson JJ Jr, Rosen P, Loesch DM, Borad MJ, Anthony S, Jameson G, Brown S, Cantafio N, Richards DA, Fitch TR, Wasserman E, Fernandez C, et al. Pilot study using molecular profiling of patients' tumors to find potential targets and select treatments for their refractory cancers. J Clin Oncol. 2010; 28:4877–83. https://doi.org/10.1200/JCO.2009.26.5983. [PubMed]
9. Massard C, Michiels S, Ferté C, Le Deley MC, Lacroix L, Hollebecque A, Verlingue L, Ileana E, Rosellini S, Ammari S, Ngo-Camus M, Bahleda R, Gazzah A, et al. High-Throughput Genomics and Clinical Outcome in Hard-to-Treat Advanced Cancers: results of the MOSCATO 01 Trial. Cancer Discov. 2017; 7:586–95. https://doi.org/10.1158/2159-8290.CD-16-1396. [PubMed]
10. Tsimberidou AM, Iskander NG, Hong DS, Wheler JJ, Falchook GS, Fu S, Piha-Paul S, Naing A, Janku F, Luthra R, Ye Y, Wen S, Berry D, Kurzrock R. Personalized medicine in a phase I clinical trials program: the MD Anderson Cancer Center initiative. Clin Cancer Res. 2012;

18:6373–83. https://doi.org/10.1158/1078-0432.CCR-12-1627. [PubMed]

11. Harris L, Chen A, O'Dwyer P, Flaherty K, Hamilton S, McShane L, Gray R, Li S, Mitchell E, Dragaud D, Williams M, Sklar J, Iafrate AJ, et al. Abstract B080: Update on the NCI-Molecular Analysis for Therapy Choice (NCI-MATCH/EAY131) precision medicine trial. Mol Cancer Ther. 2018 (Suppl 1); 17:B080. https://doi.org/10.1158/1535-7163.TARG-17-B080.

12. Sicklick JK, Kato S, Okamura R, Schwaederle M, Hahn ME, Williams CB, De P, Krie A, Piccioni DE, Miller VA, Ross JS, Benson A, Webster J, et al. Molecular profiling of cancer patients enables personalized combination therapy: the I-PREDICT study. Nat Med. 2019; 25:744–50. https://doi.org/10.1038/s41591-019-0407-5. [PubMed]

13. Rodon J, Soria JC, Berger R, Miller WH, Rubin E, Kugel A, Tsimberidou A, Saintigny P, Ackerstein A, Braña I, Loriot Y, Afshar M, Miller V, et al. Genomic and transcriptomic profiling expands precision cancer medicine: the WINTHER trial. Nat Med. 2019; 25:751–58. https://doi.org/10.1038/s41591-019-0424-4. [PubMed]

14. Liu S, Nikanjam M, Kurzrock R. Dosing de novo combinations of two targeted drugs: towards a customized precision medicine approach to advanced cancers. Oncotarget. 2016; 7:11310–20. https://doi.org/10.18632/oncotarget.7023. [PubMed]

15. Nikanjam M, Liu S, Kurzrock R. Dosing targeted and cytotoxic two-drug combinations: lessons learned from analysis of 24,326 patients reported 2010 through 2013. Int J Cancer. 2016; 139:2135–41. https://doi.org/10.1002/ijc.30262. [PubMed]

16. Nikanjam M, Liu S, Yang J, Kurzrock R. Dosing Three-Drug Combinations That Include Targeted Anti-Cancer Agents: analysis of 37,763 Patients. Oncologist. 2017; 22:576–84. https://doi.org/10.1634/theoncologist.2016-0357. [PubMed]

17. Rugo HS, Stopeck A, Joy AA, Chan S, Verma S, Lluch A, Liau KF, Kim S, Bycott P, Soulieres D. A randomized, double-blind phase II study of the oral tyrosine kinase inhibitor (TKI) axitinib (AG-013736) in combination with docetaxel (DOC) compared to DOC plus placebo (PL) in metastatic breast cancer (MBC). J Clin Oncol. 2007; 25:1003–1003.

18. Ganesan P, Moulder S, Lee JJ, Janku F, Valero V, Zinner RG, Naing A, Fu S, Tsimberidou AM, Hong D, Stephen B, Stephens P, Yelensky R, et al. Triple-negative breast cancer patients treated at MD Anderson Cancer Center in phase I trials: improved outcomes with combination chemotherapy and targeted agents. Mol Cancer Ther. 2014; 13:3175–84. https://doi.org/10.1158/1535-7163.MCT-14-0358. [PubMed]

19. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I, Bray F. (2018). Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today, accessed [30 Dec 2018].

20. NCI, NIH, DHHS. Common Terminology Criteria for Adverse Events v5.0. 2017. Available from: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_50.

21. Tannock IF, Hickman JA. Limits to Personalized Cancer Medicine. N Engl J Med. 2016; 375:1289–94. https://doi.org/10.1056/NEJMsb1607705. [PubMed]

22. Prasad V. Perspective: the precision-oncology illusion. Nature. 2016; 537:S63. https://doi.org/10.1038/537S63a. [PubMed]

23. Burstein HJ, Mangu PB, Somerfield MR, Schrag D, Samson D, Holt L, Zelman D, Ajani JA, and American Society of Clinical Oncology. American Society of Clinical Oncology clinical practice guideline update on the use of chemotherapy sensitivity and resistance assays. J Clin Oncol. 2011; 29:3328–30. https://doi.org/10.1200/JCO.2011.36.0354. [PubMed]

24. Chow LQ, Haddad R, Gupta S, Mahipal A, Mehra R, Tahara M, Berger R, Eder JP, Burtness B, Lee SH, Keam B, Kang H, Muro K, et al. Antitumor Activity of Pembrolizumab in Biomarker-Unselected Patients With Recurrent and/or Metastatic Head and Neck Squamous Cell Carcinoma: Results From the Phase Ib KEYNOTE-012 Expansion Cohort. J Clin Oncol. 2016; 34:3838–45. https://doi.org/10.1200/JCO.2016.68.1478. [PubMed]

25. Ferris RL, Blumenschein G Jr, Fayette J, Guigay J, Colevas AD, Licitra L, Harrington K, Kasper S, Vokes EE, Even C, Worden F, Saba NF, Iglesias Docampo LC, et al. Nivolumab for Recurrent Squamous-Cell Carcinoma of the Head and Neck. N Engl J Med. 2016; 375:1856–67. https://doi.org/10.1056/NEJMoa1602252. [PubMed]

26. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. N Engl J Med. 2015; 372:2509–20. https://doi.org/10.1056/NEJMoa1500596. [PubMed]

27. Overman MJ, Lonardi S, Leone F, McDermott RS, Morse MA, Wong KY, Neyns B, Leach JL, Garcia Alfonso P, Lee JJ, Hill A, Lenz HJ, Desai J, et al. Nivolumab in patients with DNA mismatch repair deficient / microsatellite instability high metastatic colorectal cancer: update from CheckMate 142. J Clin Oncol. 2017; 35:519–519. https://doi.org/10.1200/JCO.2017.35.4_suppl.519.

28. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, Desai J, Hill A, Axelson M, Moss RA, Goldberg MV, Cao ZA, Ledeine JM, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. Lancet Oncol. 2017; 18:1182–91. https://doi.org/10.1016/S1470-2045(17)30422-9. [PubMed]

29. Beaver JA, Kluetz PG, Pazdur R. Metastasis-free Survival - A New End Point in Prostate Cancer Trials. N Engl

J Med. 2018; 378:2458–60. https://doi.org/10.1056/NEJMp1805966. [PubMed]

30. Fukuda Y, Huang E, Finnigan S, Ivy SP, Rubinstein L, Takebe N. Risks and benefits of phase 1 oncology trials, 2001 through 2012. J Clin Oncol. 2014; 32:2552–2552. https://doi.org/10.1200/jco.2014.32.15_suppl.2552.

31. Groenendijk FH, Bernards R. Drug resistance to targeted therapies: déjà vu all over again. Mol Oncol. 2014; 8:1067–83. https://doi.org/10.1016/j.molonc.2014.05.004. [PubMed]

32. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. Cell. 2010; 141:1117–34. https://doi.org/10.1016/j.cell.2010.06.011. [PubMed]

33. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009; 45:228–47. https://doi.org/10.1016/j.ejca.2008.10.026. [PubMed]

https://doi.org/10.1093/annonc/mdz268.061
Published: 01 October 2019

ANNALS OF ONCOLOGY

abstracts

1934P Encyclopedic tumor analysis for organ agnostic treatment with axitinib in combination regimens for advanced cancers

T. Crook[1], D. Akolkar[2], D. Patil[2], A. Bhatt[3], A. Ranade[4], V. Datta[2], S. Schuster[2], A. Srinivasan[2], R. Datar[2]

[1]St. Luke's Cancer Center, Royal Surrey County Hospital, Guildford, UK, [2]Research and Innovations, Datar Cancer Genetics Limited, Nasik, India, [3]Medical Oncology, Avinash Cancer Clinic, Pune, India, [4]Medical Oncology, Jupiter Hospital, Pune, India

Background: Anti-angiogenic agents are approved for treatment of various cancers like Colon, Ovary, Breast, Glioma, Lung, Kidney and Liver. Axitinib, a selective inhibitor of Vascular Endothelial Growth Factor Receptors (VEGFR 1 / 2 / 3) was initially approved as a single agent for treatment of advanced Renal Cell Carcinomas (RCC), following failure of one prior line of systemic therapy, and recently as frontline treatment for RCC in combination with Pembrolizumab. Currently, Axitinib is not approved by US FDA or recommended by NCCN for use in combination with cytotoxic/targeted or endocrine therapies. We show that Axitinib can be combined for treatment of advanced solid organ tumors based upon Encyclopedic Tumor Analysis (ETA) with clinical benefit.

Methods: Between January 1, 2017 and November 30, 2018, 191 patients obtained ETA for considering precision treatment options to treat advanced broadly refractory solid organ tumors. Fresh tumor biopsies were submitted for evaluation. In a cohort of 30 patients who received combination treatment with Axitinib and cytotoxic and/or targeted and/or endocrine agents based on ETA, treatment response was evaluated as per RECIST 1.1 criteria. Objective Response Rate (ORR), Clinical Benefit Rate (CBR) and Progression Free Survival (PFS) were retrospectively determined. Therapy related adverse events were reviewed from clinical records.

Results: Out of 30 patients treated with combinations of Axitinib and either targeted, cytotoxic or endocrine drugs Partial Response (PR) was observed in 13 (43.3%) patients and Stable Disease (SD) was observed in 17 (56.7%) patients. ORR was 45.7% and CBR was 97.1%. Median PFS was 125 days (Range 35-368 days). There were no Grade IV treatment related Adverse Events (AEs) or any treatment related deaths. The most common Grade III treatment related AEs were anorexia, fatigue, and neutropenia.

Conclusions: Axitinib in combination with appropriate targeted and/or cytotoxic and/or endocrine drugs determined by ETA is well tolerated and is a potent precision therapeutic option for advanced broadly refractory solid organ cancers irrespective of the organ of origin.

Legal entity responsible for the study: The authors.

Funding: Datar Cancer Genetics Limited.

Disclosure: T. Crook: Advisory / Consultancy: Datar Cancer Genetics Limited. D. Akolkar: Full / Part-time employment: Datar Cancer Genetics Limited. D. Patil: Full / Part-time employment: Datar Cancer Genetics Limited. A. Bhatt: Advisory / Consultancy: Datar Cancer Genetics Limited. A. Ranade: Advisory / Consultancy: Datar Cancer Genetics Limited. V. Datta: Full / Part-time employment: Datar Cancer Genetics Limited. S. Schuster: Full / Part-time employment: Datar Cancer Genetics Limited. A. Srinivasan: Full / Part-time employment: Datar Cancer Genetics Limited. R. Datar: Officer / Board of Directors: Datar Cancer Genetics Limited.

ANNALS OF ONCOLOGY

https://doi.org/10.1093/annonc/mdz413.098
Published: 07 November 2019

abstracts

94P Encyclopedic tumour analysis guided treatments with conventional drugs outperform available alternatives in refractory cancers

T. Crook[1], A. Vaid[2], S. Limaye[3], R. Page[4], D. Patil[5], D. Akolkar[5], V. Datta[5], A. Ghaisas[5], R. Patil[5], H. Singh[5], A. Srinivasan[5], S. Apurwa[5], R. Datar[5]

[1]Royal Surrey County Hospital, Guildford, UK, [2]Medical and Haemato Oncology, Medanta - The Medicity, Gurugram, India, [3]Medical Oncology, Kokilaben Dhirubhai Ambani Hospital, Mumbai, India, [4]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA, USA, [5]Research and Innovations Department, Datar Cancer Genetics, Nashik, India

Background: Refractory cancers pose formidable management challenges. We hypothesized that such malignancies have unexplored vulnerabilities that can be identified using Encyclopedic Tumor Analysis (ETA) and effectively targeted using conventional agents in a label- and organ-agnostic manner to yield treatment benefit. The pan-cancer RESILIENT trial addressed patients with advanced refractory malignancies who were treated with ETA guided treatments regimens without any restrictive eligibility criteria.

Methods: Molecular Profiling (MP) of patients' fresh tumor tissue interrogated gene alterations and differentially regulated metabolic pathways to identify molecular targets of approved anticancer agents in a label-agnostic manner. Immunohistochemistry (IHC) identified hormone receptors (HR) that could be targeted with endocrine agents. Chemoresistance and response (CRR) profiling of viable tumor derived cells (TDCs) identified functional vulnerabilities of the tumor against a panel of systemic anticancer agents. Synergistic integration of MP, IHC and CRR datasets (i.e., ETA) generated patient-specific drug priority lists with projected efficacy and safety. Patients who received such ETA-guided treatments were evaluated by PET-CT scans to determine treatment response as well as Objective Response Rate (ORR), Disease Control Rate (DCR) and Progression Free Survival (PFS).

Results: Among the 200 patients who were screened, 110 patients received ETA-guided treatments and were evaluable for response per protocol. PR was observed in 47 patients (ORR = 42.7%) and 99 patients continued to exhibit PR or SD at study termination (DCR = 90%). Median PFS was 125 days. Median PFS rate at 90 days was 94.0%. No significant therapy related adverse events (AEs) were noted – there were no grade IV AEs or treatment related deaths. Most patients reported stable to improved Quality of Life (QoL) in terms of disease-related symptoms and functional status.

Conclusions: ETA-guided treatments offer meaningful survival benefits and outperformed available alternatives including checkpoint inhibitors in this heavily pretreated pan-cancer population.

Legal entity responsible for the study: The authors.

Funding: Datar Cancer Genetics Limited.

Disclosure: D. Patil: Full / Part-time employment: Datar Cancer Genetics Limited. D. Akolkar: Full / Part-time employment: Datar Cancer Genetics Limited. V. Datta: Full / Part-time employment: Datar Cancer Genetics Limited. A. Ghaisas: Full / Part-time employment: Datar Cancer Genetics Limited. R. Patil: Full / Part-time employment: Datar Cancer Genetics Limited. H. Singh: Full / Part-time employment: Datar Cancer Genetics Limited. A. Srinivasan: Full / Part-time employment: Datar Cancer Genetics Limited. S. Apurwa: Full / Part-time employment: Datar Cancer Genetics Limited. R. Datar: Shareholder / Stockholder / Stock options, Licensing / Royalties, Officer / Board of Directors: Datar Cancer Genetics Limited. All other authors have declared no conflicts of interest.

https://doi.org/10.1093/annonc/mdz413.106
Published: 07 November 2019

ANNALS OF ONCOLOGY

abstracts

102P Encyclopedic tumour analysis (ETA) guided combination regimens of hormone receptor antagonists with other systemic agents for treatment of refractory cancers

A. Vaid[1], T. Crook[2], A. Ranade[3], S. Limaye[4], D. Patil[5], D. Akolkar[5], V. Datta[5], R. Page[6], S. Schuster[7], C. Sims[5], R. Patil[5], A. Srinivasan[5], S. Apurwa[5], R. Datar[5]

[1]Medical and Haemato Oncology, Medanta - The Medicity, Gurugram, India, [2]St Luke's Cancer Centre, Royal Surrey County Hospital, Guildford, UK, [3]Avinash Cancer Clinic, Pune, India, [4]Medical Oncology, Kokilaben Dhirubhai Ambani Hospital, Mumbai, India, [5]Research and Innovations Department, Datar Cancer Genetics, Nashik, India, [6]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA, USA, [7]Datar Cancer Genetics Europe GmbH, Bayreuth, Germany

Background: Hormone and Growth Factor Receptors (HR) such as ER, PR, HER2 and AR are involved in the pathogenesis of various cancers and are commonly targeted in treatment regimens. HR antagonists Standard of Care (SoC) are often administered as monotherapy or as combinations with selected cytotoxic or targeted agents. In the SHIVA trial, monotherapy with HR antagonists based on molecular profiling was reported with dismal outcomes. We hypothesized that Encyclopedic Tumor Analysis (ETA)-informed novel combinations of HR and synergistic cytotoxic or targeted agents could be efficacious in multiple cancers.

Methods: Molecular Profiling (MP) of patients' fresh tumor tissue interrogated gene alterations and differentially regulated metabolic pathways to identify molecular targets of approved anticancer agents in a label-agnostic manner. Immunohistochemistry (IHC) identified hormone receptors (HR) that could be targeted with endocrine agents. Chemoresistance and response (CRR) profiling of viable tumor derived cells (TDCs) identified functional vulnerabilities of the tumor against a panel of systemic anticancer agents. Molecular indications linked to ER, PR, HER2 and AR as well as IHC findings were linked to selection of HR antagonists. Synergistic integration of MP, IHC and CRR data (i.e., ETA) generated patient-specific drug priority lists with projected efficacy and safety. Patients who received such ETA-guided treatments were evaluated by PET-CT scans to determine treatment response.

Results: 37 patients underwent ETA from the study sponsor and received ETA-guided combination treatments of HR antagonists with other agents. PR was seen in 17 patients (ORR = 45.9%). 32 patients showed PR or SD at most recent follow-up (DCR = 86.5%) and progression was seen in 5 patients. Median PFS was 147 days (range 31 to 410). No significant therapy related adverse events were observed in any of these patients. Most patients reported stable to improved Quality of Life (QoL).

Conclusions: ETA-guided combination treatment regimens with HR antagonists offer a viable and efficient strategy in advanced refractory malignancies and outperform monotherapy options.

Legal entity responsible for the study: The Authors.

Funding: Datar Cancer Genetics Limited.

Disclosure: D. Patil: Full / Part-time employment: Datar Cancer Genetics Limited. D. Akolkar: Full / Part-time employment: Datar Cancer Genetics Limited. V. Datta: Full / Part-time employment: Datar Cancer Genetics Limited. S. Schuster: Full / Part-time employment: Datar Cancer Genetics Limited. C. Sims: Full / Part-time employment: Datar Cancer Genetics Limited. R. Patil: Full / Part-time employment: Datar Cancer Genetics Limited. A. Srinivasan: Full / Part-time employment: Datar Cancer Genetics Limited. S. Apurwa: Full / Part-time employment: Datar Cancer Genetics Limited. R. Datar: Shareholder / Stockholder / Stock options, Licensing / Royalties, Officer / Board of Directors: Datar Cancer Genetics Limited. All other authors have declared no conflicts of interest.

ANNALS OF ONCOLOGY

https://doi.org/10.1093/annonc/mdz413.115
Published: 07 November 2019

abstracts

111P mTOR inhibitors in combination regimens guided by encyclopedic tumour analysis show superior outcomes compared to monotherapy in refractory cancers

T. Crook[1], A. Vaid[2], S. Limaye[3], R. Page[4], D. Patil[5], D. Akolkar[5], V. Datta[5], A. Ghaisas[5], R. Patil[5], H. Singh[5], A. Srinivasan[5], S. Apurwa[5], R. Datar[5]

[1]Royal Surrey County Hospital, Guildford, UK, [2]Medical and Haemato Oncology, Medanta - The Medicity, Gurugram, India, [3]Medical Oncology, Kokilaben Dhirubhai Ambani Hospital, Mumbai, India, [4]Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA, USA, [5]Research and Innovations Department, Datar Cancer Genetics, Nashik, India

Background: Though mTOR inhibition is considered an attractive strategy for cancer management, anti-mTOR monotherapies have not shown meaningful benefits. We hypothesized that an Encyclopedic Tumor Analysis (ETA) can identify vulnerabilities in the tumor in addition to mTOR activation. We further hypothesized that tandem synergistic targeting of these vulnerabilities using combination of mTOR inhibitors and other systemic anticancer agents in a label- and organ-agnostic manner can improve outcomes in refractory solid organ cancers as compared to mTOR inhibition monotherapy alone.

Methods: Molecular Profiling (MP) of patients' fresh tumor tissue interrogated gene alterations and differentially regulated metabolic pathways to identify druggable molecular targets in a label-agnostic manner. Immunohistochemistry (IHC) identified targetable hormone receptors (HR). Chemoresistance and response (CRR) profiling of viable tumor derived cells (TDCs) identified vulnerabilities of the tumor against a panel of systemic anticancer agents. Molecular indications linked to PIK3CA, mTOR, PTEN or TP53 genes were used for selection of mTOR inhibitors. Synergistic integration of MP, IHC and CRR datasets (i.e., ETA) generated patient-specific drug priority lists with projected efficacy and safety. Patients who received such ETA-guided treatments were evaluated by PET-CT scan to determine treatment response.

Results: Among 41 patients who received combination treatments, 23 patients showed PR (ORR = 56.1%), 16 showed SD (DCR = 95.1%) and progression was observed in 2 patients. One patient who received monotherapy progressed at 27 days. Median PFS was 110 days (range 27 to 592). In the SHIVA trial where patients with mTOR activation (n = 46) received monotherapy with mTOR inhibitor, median PFS of 72 days (range 57 to 100) was reported. No significant therapy–related adverse events were reported in any patient. Most patients reported stable to improved Quality of Life (QoL).

Conclusions: ETA-guided combination regimens with mTOR inhibitors offer a viable and efficient strategy in advanced refractory malignancies and outperform mTOR inhibitor monotherapy.

Legal entity responsible for the study: The authors.

Funding: Datar Cancer Genetics Limited.

Disclosure: D. Patil: Full / Part-time employment: Datar Cancer Genetics Limited. D. Akolkar: Full / Part-time employment: Datar Cancer Genetics Limited. V. Datta: Full / Part-time employment: Datar Cancer Genetics Limited. A. Ghaisas: Full / Part-time employment: Datar Cancer Genetics Limited. R. Patil: Full / Part-time employment: Datar Cancer Genetics Limited. H. Singh: Full / Part-time employment: Datar Cancer Genetics Limited. A. Srinivasan: Full / Part-time employment: Datar Cancer Genetics Limited. S. Apurwa: Full / Part-time employment: Datar Cancer Genetics Limited. R. Datar: Leadership role, Shareholder / Stockholder / Stock options, Officer / Board of Directors: Datar Cancer Genetics Limited. All other authors have declared no conflicts of interest.

Annals of Oncology, Volume 30, Issue Supplement_9, November 2019, mdz431.008
https://doi.org/10.1093/annonc/mdz431.008
Published: 24 November 2019

ANNALS OF ONCOLOGY

Title

Clinical Utility of Encyclopedic Tumor Analysis to Treat Patients Advanced Refractory Head and Neck Cancers.

Authors

R. Nagarkar[1], D. Patil[2], V. Palwe[1], V. Datta[2], A. Ghaisas[2], N. Srivastava[2], A. Srinivasan[2], D.Akolkar[2], R. Datar[2]

Affiliations

1 HCG Manavata Cancer Centre, Nasik, India
2 Research and Innovations Department, Datar Cancer Genetics, Nashik, India

Abstract

Background

Head and Neck Squamous Cell Carcinomas (HNSCC) account for 4.5% of global cancer incidences and mortality respectively. In India however, HNSCC accounts for 17% of cancer related incidences and 15% of cancer related mortality. Standard of Care (SoC) systemic treatment approaches for HNSCC are based on randomized clinical trials which do not sufficiently consider patient specific features of the tumor. We evaluated the efficacy of personalized treatment in a cohort (n = 31) of advanced refractory HNSCC, where patient-specific treatment regimens were based on Encyclopedic Tumor Analysis (ETA).

Methods

Freshly biopsied tumor tissue and peripheral blood of patients were used for integrational multi-analyte investigations as part of ETA, which included gene alterations and gene expression, as well as in vitro chemosensitivity and response profiling (CRR) of viable tumor cells. Patients received individualized therapy recommendations based on ETA. All patients underwent whole body PET-CT and brain MRI scans prior to start of treatment, and follow-up scans every 6-8 weeks. Treatment response was evaluated as per RECIST 1.1 criteria.

Results

Among the 31 patients who received personalized treatment guided by ETA, partial response (PR) was observed in 14 patients and Stable Disease (SD) in 16 patients yielding an Objective Response Rate (ORR) of 45.2% and Clinical Benefit Rate of (CBR) 96.8%, respectively. Patients were followed up for a median of 146 days (Range 42 – 368). At most recent follow-up 1 patient showed disease progression, whereas Progression Free Survival was observed in 30 patients. Median Progression-Free Survival was 146 days. No grade IV adverse events were observed. There were no treatment related deaths. Most common Grade III adverse events included Fatigue, Anorexia, Thrombocytopenia, Neutropenia and Oral Mucositis. Most patients reported qualitative improvements in symptomatic and functional status.

Conclusions

ETA guided treatments can offer viable treatment options in advanced refractory HNSCC yielding meaningful ORR and disease control in majority of patients.

OXFORD ACADEMIC
Neuro-Oncology

Neuro-Oncology, Volume 21, Issue Supplement_6, November 2019, Pages vi59–vi60,
https://doi.org/10.1093/neuonc/noz175.238
Published: 11 November 2019

Title

Clinical Utility of Encyclopedic Tumor Analysis to Treat Patients with Brain Metastasis in Refractory Cancers.

Authors

Timothy Crook (PhD), Darshana Patil (MD), Dadasaheb Akolkar (PhD), Anantbhushan Ranade (MD), Amit Bhatt (MD), Vineet Datta (MD), Jatinder Bhatia (MD), Stefan Schuster (PhD), Revati Patil (MD), Ajay Srinivasan (PhD), Harjeet Kathuria (DNB), Rajan Datar (BS)

Abstract

Brain metastasis in solid organ cancers is associated with adverse prognosis, which is further aggravated by limited systemic treatment options. Such patients are also often excluded from clinical trials since their poor prognosis is perceived to unfavorably impact trial outcomes and misrepresent efficacy data. We retrospectively evaluated the efficacy of treatment guided by Encyclopedic Tumor Analysis (ETA) in patients with advanced refractory malignancies and brain metastases to determine the impact on outcomes. Freshly biopsied tumor tissue (primary / lymph node / liver) and peripheral blood of patients were used for integrational multi-analyte investigations as part of ETA, which included gene mutations, gene expression, and in vitro chemosensitivity profiling of viable tumor cells. Based on ETA, patients received individualized therapy recommendations. All patients underwent a PET-CT scan as well as MRI scan prior to treatment start to determine extent of disease. All patients underwent follow-up PET-CT scans and brain MRI scans every 6-8 weeks. Of the ten patients with brain metastases, which were evaluated after receiving ETA-guided treatment, the median follow-up duration was 97 days (range 79 – 180 days) during which all ten patients remained progression-free. Median time to progression for these patients on the last (failed) line of treatment was 91 days (range 30 - 176 days). Five patients showed partial response and five patients showed stable disease while on ETA-guided treatment. During the follow-up period, all brain metastases were either stable (n=7) or had regressed (n=3), and none of the patients reported new brain lesions. Personalized ETA guided treatments imparted clinical benefit by halting disease progression in this cohort of high-risk patients who would have otherwise been considered for palliative regimens due to perceived unfavorable prognosis.

https://ascopubs.org/doi/abs/10.1200/JGO.2019.5.suppl.115?af=R&

Journal of Global Oncology®
An American Society of Clinical Oncology Journal

Title

Clinical efficacy of combination therapies with androgen receptor antagonists for treatment of multiple refractory cancers.

Authors

Dadasaheb Akolkar, Darshana Patil, Vineet Datta, Ajay Srinivasan, Rajan Datar

Affiliations

Datar Cancer Genetics Limited, Nasik, India

Abstract

Androgen Receptor (AR) antagonists have been the mainstay of prostate cancer treatments. However, there is increasing interest in the use of anti-AR agents in treatment of other cancers such as Triple Negative Breast Cancer and Lung Cancer. AR antagonists are usually administered as single agents and rarely in combination with other cytotoxic or targeted agents. We hypothesized that administration of AR antagonists indicated by Encyclopedic Tumor Analysis (ETA) in synergistic combination with cytotoxic, targeted or other endocrine agents may afford clinical benefit for refractory cancers.

We evaluated treatment response in a basket of 18 patients with various advanced refractory solid organ malignancies, who received personalized treatments based on ETA investigations. As part of ETA, freshly biopsied tumor tissue and blood samples were evaluated for various markers such as gene mutations (DNA), gene expression (RNA) and receptor proteins (immunohistochemistry). Finally, viable tumor cells from the freshly biopsied tissue were used in in vitro chemosensitivity analysis with a panel of cytotoxic and targeted therapy agents. Radiological disease status was evaluated retrospectively and treatment response as well as Progression Free Survival (PFS) was determined.

Among the 18 patients, there were 8 males (44%) and 10 females (56%) with median age of 58 years (range 28 – 79). Patients had received a median of 3 prior lines of treatment (range 1 – 14). All 18 patients received ETA guided combination treatments which included an AR blockade. 9 patients showed Partial Response (PR) with an Objective Response Rate (ORR) of 50%. 5 patients (28%) showed stable disease for ≥3 months (Clinical Benefit Rate = 77.8%), while 4 patients (22%) showed disease progression. In 2 patients (11%) disease progressed at ~60 days and in the remaining 2 patients (11%) progression was seen at > 120 days. Treatments were well tolerated without severe adverse events.

Androgen addicted, refractory solid organ tumors respond to combinations of cytotoxic, targeted and endocrine agents along with AR antagonists guided by ETA.

Journal of *Personalized Medicine*

MDPI

Case Report

Adaptive, Iterative, Long-Term Personalized Therapy Management in a Case of Stage IV Refractory NSCLC

Anantbhushan Ranade [1], Darshana Patil [2], Amit Bhatt [1], Rucha Dhasare [1], Vineet Datta [2], Rajan Datar [2] and Dadasaheb Akolkar [2,*]

[1] Avinash Cancer Clinic, Tilak Road, Pantancha Gate, Sadashiv Peth, Pune 411030, Maharashtra, India
[2] Datar Cancer Genetics Ltd., F-8 D-Road, Ambad, Nasik 422010, Maharashtra, India
* Correspondence: dadasaheb.akolkar@datarpgx.com

Received: 19 June 2019; Accepted: 3 July 2019; Published: 5 July 2019

Abstract: In this paper we report long-term therapy management based on iterative de novo molecular and cellular analysis in a case of metastatic non-small cell lung cancer (NSCLC), with prior history of treated colorectal cancer. In the described case temporal tumor evolution, emergent therapy resistance and disease recurrences were addressed via the administration of personalized label- and organ-agnostic treatments based on de novo tumor profiling. This adaptive and iterative treatment strategy countered disease progression at each instance and led to the durable regression of primary as well as metastatic lesions. Concurrently, serial evaluation of mutations in cell-free circulating tumor DNA (ctDNA) via liquid biopsy (LBx) was performed to monitor disease status, ascertain treatment response, identify emergent drug resistance and detect recurrence at sub-radiological levels. The treatment management strategy described herein effectively addressed multiple, sequential clinical conundrums for which viable options were unavailable under the current Standard of Care (SoC).

Keywords: non small cell lung cancer (NSCLC); precision oncology; personalized therapy management

1. Introduction

Standard of Care (SoC) approaches for the diagnosis and treatment of cancers are generally based on parameters such as anatomy, histology and the stage and grade of the disease. In some cancers, the evaluation of selected signaling proteins by immunohistochemistry (IHC) or limited gene variants are additional parameters that guide diagnosis and treatment. In cancers where molecular features are considered for diagnosis or treatment, these are restricted to univariate analyses, which may not sufficiently represent patient-specific cellular and molecular features of the tumor. Likewise, most targeted treatment options under SoC are tethered to the primary organ and are generally based on safety and efficacy data from randomized drug-centric clinical trials. Use of molecularly targeted treatment options in an organ-agnostic setting is encountered less frequently [1,2], with drugs such as Pembrolizumab and Larotrectinib [3] being notable exceptions.

SoC approaches also do not sufficiently (if at all) factor in the molecular dynamics associated with tumor evolution and drug resistance [4,5]. It then follows that the molecular characterization of the tumor from archival tissue (e.g., from a foundational biopsy) may not be representative of the present status of the malignancy and that treatment choices based on retrospective molecular information may be associated with risks of treatment failure [6]. De novo evaluation of the tumor's molecular and metabolic dynamics from freshly biopsied tissue or analysis of circulating tumor biomarkers in blood are thus expected to provide the most relevant evidence for treatment selection.

Similarly, SoC approaches towards the monitoring of disease status or response to treatment tends to be based on the manifestation of clinical symptoms of disease or an increase in the severity of symptoms. Though radiological monitoring of disease status is sensitive, it is not viable for high-frequency monitoring due to risks of radiation exposure [7]. Specific serological assays based on tumor antigens are unavailable for many malignancies, and where available may be associated with risks of low accuracy/specificity [8].

Precision Oncology aims to overcome several conundrums associated with SoC approaches in the diagnosis of cancers and treatment selection, by evaluating molecular features of the tumor [9]. The selection of appropriate treatment agents based on molecular evidence and in a label-/organ-agnostic manner is the mainstay of Precision Oncology. There have also been several clinical trials [10–12] attempting to match treatments to molecular features of the cancer in an organ-agnostic setting. While some of these trials reported equivocal benefits [10], some others have indicated potential for significant clinical benefits [12] from the administration of molecularly matched therapies. Despite the varying findings of various trials, label-agnostic cancer treatments are often encountered in existing treatment guidelines, e.g., those of the National Comprehensive Cancer Network (NCCN) [13], as well as in routine clinical practice [14] since this can offer treatment avenues for patients where (further) SoC options may be unavailable/unviable. In addition to therapy management, a concurrent aim of Precision Oncology is to improve means for monitoring disease status and treatment response via the evaluation of circulating tumor biomarkers in peripheral blood, viz., liquid biopsy (LBx). LBx interrogates the genomic and metabolic landscape of a tumor by qualitatively and quantitatively evaluating tumor-derived analytes such as cell-free circulating tumor DNA (ctDNA) and exosomal RNAs [15]. LBx can also provide real-time information on tumor dynamics [16] and emergent chemoresistance as well as newer vulnerabilities of the tumor.

Thus, a combinatorial strategy of de novo molecular profiling of cancer along with high-frequency non-invasive LBx monitoring of tumor molecular characteristics can bring an unprecedented level of precision in personalized treatment strategies, especially for advanced, refractory or difficult to treat cancers. In the present report, we describe a case of metastatic non-small cell lung cancer (NSCLC) where long-term therapy management based on de novo real-time evaluation of the multi-layered tumor interactome overcame sequential SoC-therapeutic roadblocks.

2. Patient and Methods

2.1. Patient

The case described in this manuscript is a retrospective observational report of a single patient who opted to receive personalized cancer treatment. The patient was not part of any prospective interventional clinical trial. The patient provided signed informed consent for the publication of deidentified data and results. Sample collections and therapeutic interventions were carried out at Sanjeevan Hospital, Pune, India and Joshi Hospital, Pune, 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. As part of standard clinical practices, the patient consented to receive personalized cancer treatment via the treating oncologists at all hospitals where therapy was administered. All interventional procedures including therapy administration were approved as per standard hospital practices and in concordance with existing ethical, medical and legal requirements.

2.2. Tissue Collection

Approximately 5 × 5 × 5 mm freshly biopsied tumor tissue was transferred into 5 mL transport medium (that preserved the viability of tumor cells) and stored at 4 °C during transit. Fresh tissue was either processed immediately or cryopreserved at −80 °C.

2.3. Histopathology and Immunohistochemistry

Formalin-Fixed Paraffin-Embedded (FFPE) blocks were prepared as per standard procedures. Histopathological (HPE) and immunohistochemical (IHC) analyses were carried out as per standard procedures. Tumor content of freshly biopsied tissue was determined by HPE evaluations. Tissue samples with ≥80% tumor content were considered as acceptable for molecular evaluations.

2.4. Blood Collection and Processing

First, 8–10 mL peripheral blood was collected by venous puncture in each of the Cell-Free DNA BCT® and EDTA vacutainer tubes. Blood was stored and transported at 4 °C. Plasma was separated by centrifugation at 3000× *g* for 20 min at 4 °C, followed by 16,000× *g* for 10 min at 20–25 °C. Plasma from Cell-Free DNA BCT® tubes without hemolysis were processed for cell-free nucleic acid isolation.

2.5. Tumor DNA Isolation

Genomic DNA was isolated from the FFPE tumor blocks using a GeneRead DNA FFPE kit (Qiagen, Germantown, MD, USA) as per the manufacturer's instructions. DNA was quantified at 260 nm and quality was determined by measuring the ratio of absorbance at 260/280 nm using a NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

2.6. Cell-Free DNA (ctDNA) Isolation

Total ctDNA was purified from 2 mL plasma using a Circulating Nucleic Acid kit (QIAGEN, Germantown, MD, USA) as per the manufacturer's protocol. ctDNA was quantified using an HS DNA Qubit assay (Life Technologies, Carlsad, CA, USA).

2.7. Tumor RNA Isolation

Total tumor RNA was isolated using an mirVana miRNA isolation kit (Ambion, Austin, TX, USA) as per the manufacturer's instruction. Total RNA was quantified using a Qubit 2 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) with the manufacturer's RNA assay kit.

2.8. Exosomal RNA Isolation

Plasma samples (2 mL) from EDTA tubes were centrifuged at 16,000× *g* for 10 min at 4 °C and filtered via a 0.45 µm membrane to remove larger vesicles. The filtrate was used for the extraction of total exosomal RNA using an ExoRNeasy serum/plasma kit (QIAGEN, Germantown, MD, USA) according to the manufacturer's protocol [17]. Purified exosomal RNA was quantified using an miRNA Qubit assay (Life Technologies, Carlsad, CA, USA).

2.9. Whole-Exome Sequencing (WES)

Tumor genomic DNA was subjected to target enrichment with high multiplex PCR amplification using an Ion Ampliseq™ Exome RDY Kit (Thermo Fisher Scientific, Waltham, MA, USA) and analyzed via an Ion Proton Next Generation Sequencing (NGS) system (Thermo Fisher Scientific, Waltham, MA, USA). DNA reads with a Q17 quality score were aligned to the reference human genome GRCh37/hg19. The average mean depth targeted for the submitted sample was 192× with minimum 100× read depth criteria accepted for variant detection. NGS data were processed using Ion Torrent Suite v5.2 and the Torrent Server was used to successively map the human genome sequence (build GRCh37/hg19) with a Torrent Mapping Alignment Program (TMAP v5.2) optimized for Ion Torrent data. Clinically relevant variants were annotated and classified using American College of Medical Genetics (ACMG) guidelines [18] and variants reported in the literature and databases including HGMD, ClinVar, OMIM, GWAS and COSMIC. Copy number variations were determined with medium sensitivity using Ion Reporter 5.2 software.

2.10. Cell-Free tumor DNA (ctDNA) Profiling

A 50-gene NGS panel (Supplementary Table S1) consisting of 207 amplicons and covering over 22,000 bases was designed to detect somatic hotspot mutations reported at high frequency in multiple cancer types as identified from TCGA, COSMIC, ICGC, MD Anderson Cancer Center and My Cancer Genome databases.

ctDNA (20 ng) was used for NGS library preparation via PCR-based Ampliseq target enrichment protocol. Libraries of 100 pmol were sequenced using Ion Proton (Thermo Fisher Scientific, Waltham, MA, USA). Torrent Suite™ v5.2 (Thermo Fisher Scientific, Waltham, USA) software was used to perform primary analysis, including signal processing and base calling. Primary QC parameters were: minimum read length of 25 bases, read quality trimming of 17 QV, window size for quality trimming 30 bp. The processed sequenced data were aligned to the reference genome GRCh37/hg19 to generate Binary Alignment/Map (BAM) files. Sequencing data were considered for downstream analysis with coverage at ≥10,000× depth and >80% amplicons with at least 600 reads. The aligned data were analyzed using Torrent Variant Caller software with optimized parameters such as minimum allele frequency (0.003), minimum mapping quality (4), minimum coverage (600), down sample to coverage (10,000) and position bias (1). Reported somatic variants of >0.5% allele frequency (AF) were compared to the reference genome hg19. The Integrative Genomics Viewer (IGV) was used to visualize the read alignment and the presence of variants against the reference genome and to confirm the veracity of the variant calls by checking for possible strand biases and sequencing errors. All the germline variants found in the 1000 Genomes Project or The Exome Aggregation Consortium (ExAC) with a frequency of >0.1% were excluded. All somatic mutations were annotated, sorted and interpreted using COSMIC and/or TCGA data. Variants with <0.5% AF were confirmed orthogonally with digital droplet polymerase chain reaction (ddPCR, BioRad) using the rare mutation assay as per the manufacturer's protocol.

2.11. mRNA Profiling (Transcriptome Analysis)

The Ion AmpliSeq™ Transcriptome Human Gene Expression Research Panel was used to determine the expression of 20,802 genes including 18,574 coding genes and 2228 non-coding genes based on University of California Santa Cruz (UCSC) hg19 annotation. Exosomal RNA from asymptomatic individuals (male) was used as a control for cancer exosomal mRNA analysis. RNA prepared from normal tissue was used as a control for tumor mRNA analysis. A barcoded cDNA library was generated with a SuperScript® VILO™ cDNA Synthesis kit from 20 ng of exosomal RNA. The cDNA was amplified using Ion AmpliSeq™ technology as per the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). Amplified cDNA libraries were evaluated for quality on a Bioanalyzer 2100E using a high sensitivity DNA 1000 chip (Agilent Technologies, Santa Clara, CA, USA) and quantified using an Ion Library TaqMan™ Quantitation Kit (Thermo Fischer Scientific, Waltham, MA, USA)/KAPA Library Quantification Kits (KAPA Biosystems/Roche, Basel, Switzerland). Pooled libraries of 100 pM were amplified using emulsion PCR on an Ion Torrent OneTouch2 and enriched as per the manufacturer's instructions. Templated libraries were sequenced on an Ion Torrent Proton™ sequencing system, using an Ion PI sequencing kit and an Ion PI chip (Thermo Fisher Scientific, Waltham, MA, USA). Analysis of AmpliSeq RNA sequencing data was performed using the AmpliSeq-RNA plugin available for Ion Torrent sequencing platforms. This plugin uses the Torrent Mapping Alignment Program (TMAP—https://github.com/iontorrent/TMAP), which is optimized for aligning raw sequencing reads (from Ion Torrent) against the hg19 transcriptome reference sequence against regions defined in the Browser Extensible Display (BED) file (hg19_AmpliSeq_Transcriptome_21K_v1.bed). The quality of the raw data was evaluated based on three parameters: number of reads, mean read length and target detected (% of all amplicons that had ≥10 assigned reads). Differential gene expression analysis was performed using R/Bioconductor package edgeR with raw read counts from AmpliSeq. Read count normalization was performed using the counts per million (CPM) method. Significant differential expressed genes were called using the following threshold: absolute log fold-change ≥2

and Benjamini–Hochberg adjusted $p < 0.05$. The commercial software iPathway Guide (Advaita) was used for pathway analysis to explore significantly affected pathways.

2.12. In Vitro Chemosensitivity Profiling of Viable Tumor Cells

Viable tumor cells were isolated from fresh tissue and treated in vitro with chemotherapy agents and their synergistic combinations. Apoptotic cell death events were determined to evaluate the response to such drug(s). Data from all investigations were integrated to identify agents and their combinations with maximum projected efficacy and safety.

3. Results

This section is divided into four sub-sections, each describing a diagnostic or therapeutic roadblock that was encountered during the management of this case, where SoC approaches may have been unviable or inappropriate. Each sub-section further describes the strategy adopted to overcome the conundrum.

3.1. Overcoming Clinical Conundrum #1: Molecular Investigations Facilitated Accurate Diagnosis and Appropriate Therapy Selection

A 72-year-old never-smoker male patient, known case of diabetes mellitus, was diagnosed in July 2012 with KRAS.pG12D-positive T1N0M0 adenocarcinoma of the ascending colon and cecum. The patient underwent right hemicolectomy with an end to end anastomosis and received oral chemotherapy of Capecitabine (500 mg, Once Daily (OD)) for 4 months. Follow-up (October 2014) 18F-fluorodeoxyglucose (FDG) positron emission tomography-computed tomography (PET-CT) detected hypermetabolic nodular lesion with spiculated margins in the anterior segment of the upper lobe of the left lung, suspected of metastasis from primary Ca colon; the patient received oral chemotherapy of Capecitabine (500 mg, OD) for 2 months. In October 2015, radiological follow-up (chest X-ray) indicated persistent and increased size of lesion in the anterior segment of the left upper lobe of the lung, indicating non-response to Capecitabine. Under SoC treatment strategy, the patient was considered for next systemic treatments for colorectal cancers, which included combinations of 5-fluorouracil, oxaliplatin or irinotecan as well as Bevacizumab.

The status of previously reported KRAS as well as other actionable mutations was evaluated via liquid biopsy (LBx) analysis of circulating cell-free tumor DNA (ctDNA) in the patient's peripheral blood using a commercial multi-gene NGS panel. Interestingly, LBx indicated the absence of the KRAS.pG12D mutation, but the presence of an exon 19 deletion mutation (pE746-A750del) in the *Epidermal Growth Factor Receptor* (*EGFR*) gene. This mutation was previously reported in non-small cell lung cancers (NSCLC) and indicated potential benefit from EGFR tyrosine kinase inhibitor (TKI) therapies [19]. In view of this molecular evidence, the lung mass was biopsied and evaluated by histopathological examination (HPE), which indicated that the tumor was adenosquamous (ADS) subtype, positive for CK7, P63, CK5/6 and CEA, but negative for TTF1. In view of molecular and HPE evidence, the diagnosis was confirmed as a second primary of NSCLC. Based on the sensitizing EGFR mutation, the patient was assigned a regimen of Gefitinib (tablet, 250 mg, OD) for 3 months. In January 2016, the patient underwent upper lobectomy and mediastinal lymph node dissection through a posterolateral thoracotomy, and later continued to receive Gefitinib therapy. High-frequency serial monitoring of EGFR exon-19 mutation burden in ctDNA indicated decreasing mutant allele frequency (MAF) (Figure 1A) concurrent with therapy response.

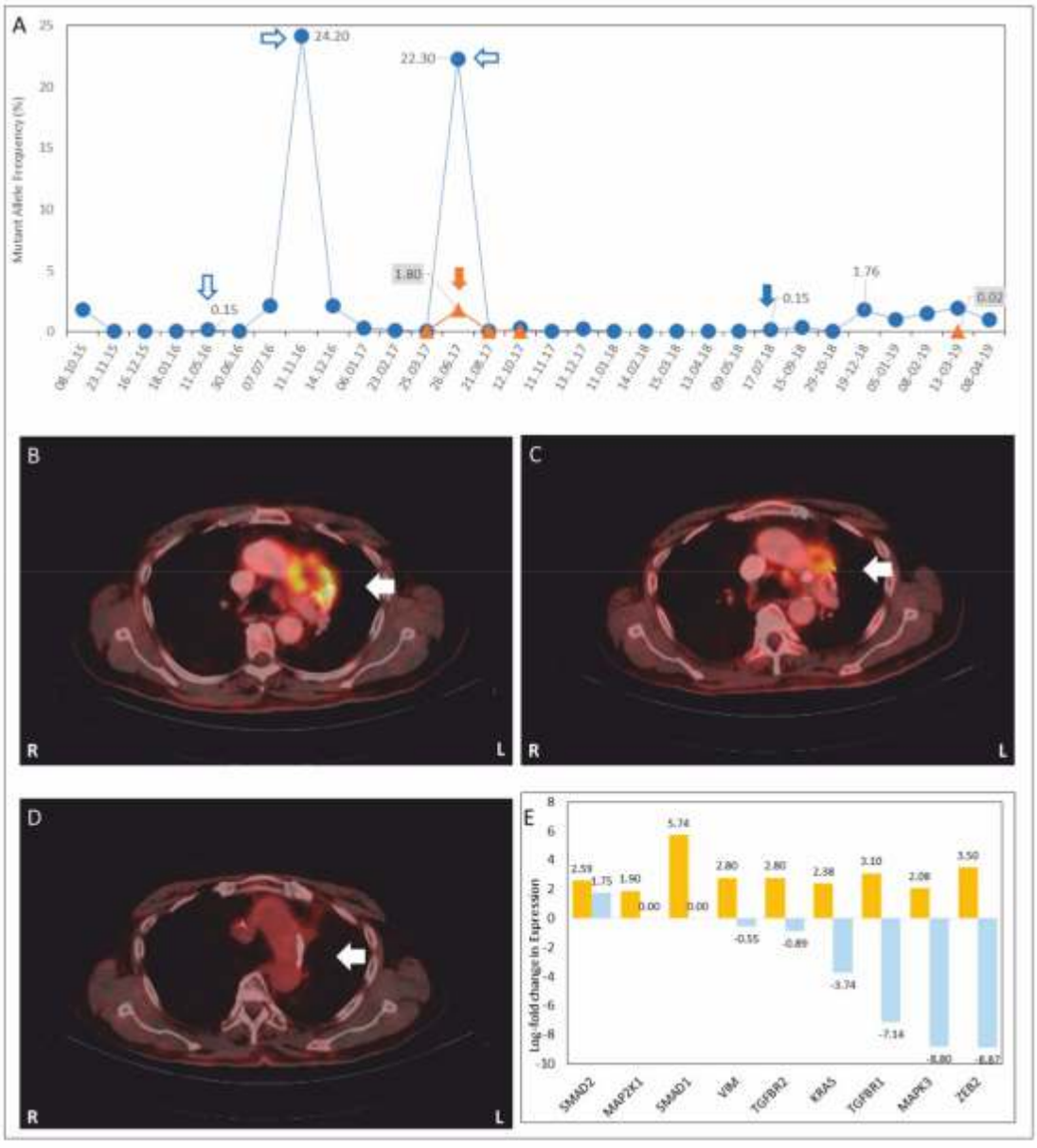


Figure 1. Trends in mutant allele frequency of Epidermal Growth Factor Receptor (EGFR) as determined by liquid biopsy analysis of ctDNA. Variations in allele frequencies of EGFR.pE746-A750del (●) and EGFR.T790M (▲) mutations in ctDNA. A spike in EGFR.pE746-A750del (⇩) was observed that was predictive of recurrence. A significant increase in EGFR.pE746-A750del at first recurrence (⇨), second recurrence (⇦) and third recurrence (⬇) was also noted. Detection of EGFR.T790M (⬇) at second recurrence is indicated. By July 2018, the ctDNA EGFR-mutation burden was undetectable (**A**). Treatment response: A regression of 18F-fluorodeoxyglucose (FDG)-avid left prevascular nodal lesion (white arrow) was observed between November 2016 (**B**), January 2017 (**C**) and April 2017 (**D**). L and R indicate left and right sides in the positron emission tomography-computed tomography (PET-CT) transverse sections. Trends in exosomal mRNA profile between November 2016 and January 2017 showed a downregulation of mRNA transcripts, which was suggestive of the reduction in invasiveness and metastatic potential (**E**).

3.2. Overcoming Clinical Conundrum #2: Monitoring for Sub-Radiological Disease and Recurrence

SoC approaches to monitoring of disease status and treatment response are based on the manifestation of clinical symptoms, in absence of which the disease status remains unknown. In the

present case, disease status was monitored at high frequency via LBx evaluation of mutation burden in ctDNA while the patient was on Gefitinib therapy. In May 2016, follow-up LBx detected a transient spike (Figure 1A) in EGFR exon-19 MAF, though the patient was clinically asymptomatic. At the next scheduled PET-CT scan (July 2016) there was no radiological evidence of recurrence or progression. The patient continued to receive Gefitinib therapy. Subsequently, EGFR exon-19 MAF was undetectable by LBx (June–October 2016) until November 2016, when a significant increase (Figure 1A) in EGFR exon-19 MAF was detected by LBx. Based on this observation, PET-CT scan was performed which showed equally significant increase in size and metabolic activity (maximum specific uptake value, SUV_{Max}) of the left lung upper lobe mass lesion, which contiguously infiltrated into the mediastinum. An additional FDG-avid (metastatic) lesion was detected in the left adrenal gland. In the present instance, the detection of the transient spike appeared to be indicative of disease recurrence at sub-radiological levels and prompted close monitoring of the patient, which led to timely detection and radiological confirmation of disease recurrence.

3.3. Overcoming Clinical Conundrum #3: Personalized Treatment Selection when Viable SoC Treatment Options Were Unavailable

At recurrence, surgical resection of the lung lesion as well as irradiation therapy were deemed unviable owing to the size and location of the lesions. HPE of freshly biopsied tumor tissue from the lung lesion indicated squamous-cell (SCC) morphology and appeared to be suggestive of a histopathological shift. Analysis of mutations in ctDNA indicated significant EGFR exon-19 MAF. However, whole-exome sequencing (WES) analysis of DNA obtained from SCC tissue was unable to detect EGFR exon-19 deletion mutation, suggesting that SCC tissue was a consequence of discrete clonal evolution. Thus, the patient appeared to harbor at least two subtypes of the malignancy based on EGFR mutation, one being the EGFR-positive ADS and the other being the EGFR-negative adenocarcinoma (ADC). However, since the adrenal lesion was unsuitable for biopsy and due to the inability to radiologically identify other metastatic sites, the simultaneous co-existence of histopathologically heterogeneous tumor subtypes could not be ascertained.

SCC subtypes of NSCLC are generally associated with poorer prognosis and an absence of viable treatment options [20]. Actionable molecular indications are also generally unknown in SCC. In order to address this therapeutic roadblock, comprehensive evaluation of circulating tumor biomarkers in peripheral blood was carried out, which identified multiple potentially targetable features such as the overexpression of *EGFR* and *ERBB2* genes as well as the upregulation of pathways such as *MAPK/ERK* pathway (genes including *KRAS, MAP2K1, MAPK3*) and Epithelial to Mesenchymal Transition (*EMT*) pathway (genes including *MMPs TGFBR1, TGFBR2, ZEB, SMAD* and *Vim*).

A combinatorial therapeutic strategy was designed to achieve: (a) the inhibition of *MMPs* via Doxycycline [21,22], (b) the suppression of EMT via Atorvastatin [23,24], (c) the perturbation of microtubule dynamics by Paclitaxel and (d) the targeting of *EGFR* as well as *ERBB2* with Afatinib.

The combination of Afatinib and Paclitaxel has been reported to be beneficial in Gefitinib/Erlotinib-resistant NSCLC with upregulated *ERBB*-family signaling receptors [25,26]. The anti-tumor activity of these drugs (single agents) as well as their combinations were determined by in vitro chemosensitivity analysis using viable tumor cells obtained by fresh tissue biopsy. Based on these findings, the patient was assigned (November 2016) a regimen of Afatinib (40 mg, OD), Paclitaxel (80 mg/m^2, weekly), Atorvastatin (20 mg, 1 Thrice Daily (TD)) and Doxycycline (100 mg, 1 Twice Daily (Bis Daily, BD)).

Follow-up (January 2017) LBx, while the patient was receiving therapy, indicated (Figure 1A) a concomitant decrease in ctDNA *EGFR* mutation load as well as the downregulation of transcripts associated with *EMT* (Figure 1B) and *MAPK/ERK* pathways (Figure 1B), indicating reduced potential for invasion and metastasis. Supplementary Figure S1A–D depicts the changes in pathways and regulatory associations between pathway intermediates associated with *EMT* and *MAPK/ERK* over the period of November 2016 to January 2017. Simultaneously, follow-up PET-CT scan showed a regression

of prevascular mass (Figure 1C) and adrenal lesions. Radiological regression of the metastatic lesions rendered them amenable to CyberKnife® radiosurgery; a dose of 48 Gy was administered in eight fractions (January–February 2017) to the prevascular lesion and pretracheal node, followed by 12 Gy across two fractions to the prevascular lesion and 48 Gy across three fractions to the adrenal gland. Radiosurgery was followed by a continuation of combination therapy. Further reduction in ctDNA EGFR exon-19 MAF (March 2017) was accompanied by near complete radiological resolution of all previously noted lesions, the resolution of pericardial effusion and the absence of new lesions.

In absence of SoC treatment options, the clinicians availed of evidence-based label-agnostic treatment options for the patient which not only led to disease regression, but also to the re-establishment of the viability of an established loco-regional SoC treatment option.

3.4. Overcoming Clinical Conundrum #4: Combination of Agents Addresses EGFR Resistance and Target Latent Vulnerability of Tumor

Follow-up (June 2017) LBx evaluation of EGFR mutations in ctDNA detected EGFR.pT790M [27] mutation in ctDNA. The subsequent PET-CT (July 2017) showed radiological evidence of disease progression with new metastatic lesion in the liver and suspicious FDG-avid focus in pericardium. Biopsy of metastatic tissue from the liver and HPE analysis indicated that the tumor cells were adenocarcinoma—a second histopathological shift which indicated the presence of multiple histopathological subtypes. Immunohistochemistry (IHC) analysis was indicative of Androgen Receptor (AR) overexpression. Recent studies have indicated the therapeutic relevance of targeting AR where it is overexpressed in malignancies other than that of the prostate [28]. The patient was assigned a combination therapy with the third generation EGFR-TKI Osimertinib (80 mg, 1 OD) and the AR-antagonist Bicalutamide (50 mg, 1 OD), which led to steady regression of metastatic lesions (June 2017–August 2018) along with a concomitant decrease in ctDNA mutation burden.

3.5. Recent Status

In the end of July 2018, follow-up LBx indicated a marginal spike in EGFR exon-19 deletion MAF (Figure 1A). Follow-up PET-CT (Aug 2018) indicated a decrease in the size and extent of liver lesions and a stable size of the pericardial lesion, but the appearance of new FDG-avid focus in the right adrenal gland, which was not amenable to biopsy. Due to the deterioration of the Eastern Cooperative Oncology Group (ECOG) performance score, the patient was considered unfit for aggressive treatment regimens. Combination treatment with Bicalutamide and Osimertinib was paused between September 2018 and February 2019, during which ctDNA EGFR exon-19 deletion MAF was observed to increase. Follow-up PET-CT in December 2018 did not report liver lesions, but indicated interval increase in the size of FDG-avid foci in the right adrenal gland and pericardium. In February 2019, the patient resumed a regimen of Osimertinib + Bicalutamide, which led to a decrease in EGFR MAF between February and April 2019. However, due to significant systemic deterioration, the patient was taken off therapy in April 2019. The patient subsequently passed away in April 2019 following terminal cardiorespiratory arrest possibly due to long-term exposure to Osimeritinib [29]. The sequence of events is summarized in Figure 2.

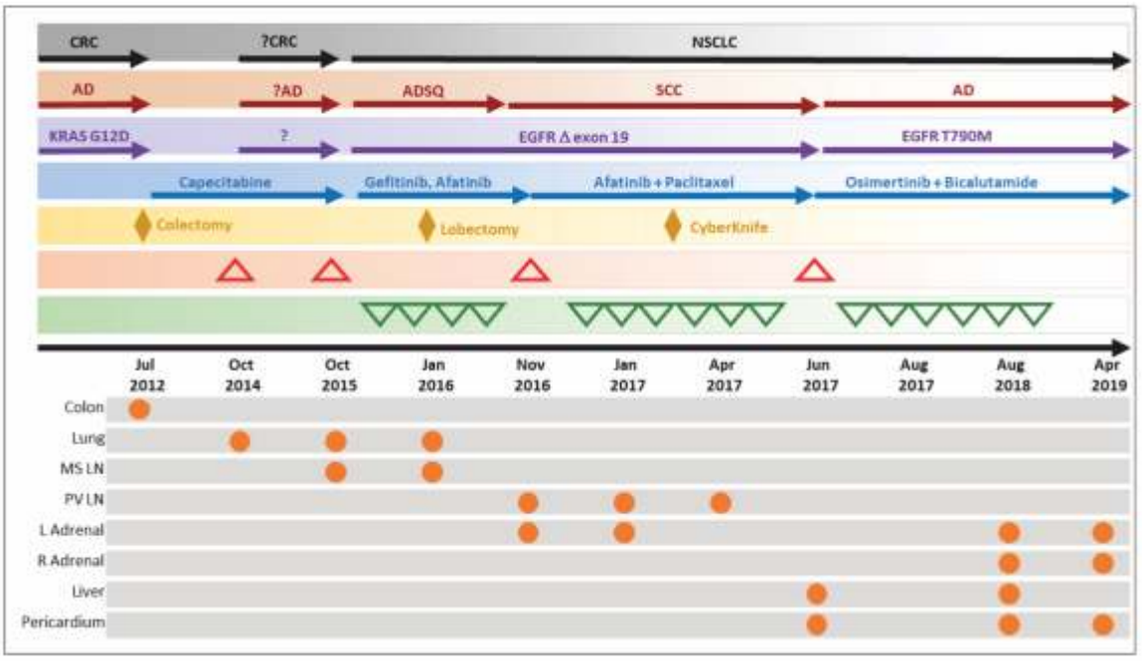


Figure 2. Timeline of events. CRC: colorectal cancer; NSCLC: non-small cell lung cancer; AD: adenocarcinoma; ADSQ: adenosquamous carcinoma; SCC: squamous cell carcinoma; : locoregional treatment; ∆: progression/recurrence; ∇: treatment response/regression; presence of malignant mass at various sites; MS LN: mediastinal lymph node; PV LN: prevascular lymph node; L Adrenal: left adrenal; R Adrenal: right adrenal.

4. Discussion

Discrete clonal evolution under the selection pressure exerted by the targeted molecules pose a significant challenge in controlling the disease at the recurrence stage. In this patient, at the first recurrence, existence of discreet EGFR-negative and -positive clonal sub-populations were identified by comprehensive tissue- and blood-based analyses. Such populations could have responded differently if the single drug approach would have followed. The combined use of Paclitaxel and Afatinib effectively controlled the disease at all the locations. The liquid biopsy analysis confirmed treatment response by demonstrating a decrease in *EGFR* mutation load and the downregulation of EMT markers. At the second recurrence, the reappearance of an *EGFR*-positive population in the liver lesions with selective evolution to T790M further enabled the successful use of Osimertinib. Approximately 30–70% of NSCLC cases have AR expression [28]. As AR signaling has been shown to be intact in such patients, AR blockade could be a potential endocrine treatment. In the present case, the combination of Osimertinib and Bicalutamide was well tolerated at a standard dose, with no instance of dose-limiting toxicity, and demonstrated clinical efficacy in controlling the multiple clonal population.

The adaptive, iterative evidence-based treatment approach that guided treatment selection helped overcome successive therapeutic challenges, avoided unfavorable outcomes and unambiguously contributed to life extension for the patient.

It is pertinent to state that during the timeline of events (2012–2019) described in this manuscript, Osimertinib was first approved by the United States Food and Drug Administration (USFDA) in 2015 for use in non-small cell lung cancers (NSCLC) harboring mutations in exon 20 (T790M). However, it was only in April 2018 [30] that the USFDA approved Osimertinib for use as front-line treatment for NSCLC harboring *EGFR* mutations targetable via the TKI class of drugs.

Dosages of *EGFR* TKIs Gefitinib, Afatinib and Osimertinib were based on labeled indication in lung cancer. The dosage of Paclitaxel (when given in combination with Afatinib) was based on safety and efficacy data reported in the original trial. The dosages of Bicalutamide, Doxycycline and

Atorvastatin were based on safety data from the drug labels. All drug dosages were finalized by the treating oncologists based on the patient's fitness and risk of adverse events.

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-4426/9/3/34/s1, Figure S1: Molecular pathways linked to invasion and metastasis. TGF-β signaling pathway intermediates in November 2016 (A) and January 2017 (B). ERBB2 and MAPK/ERK pathway intermediates in November 2016 (C) and January 2017 (D), Table S1: List of genes in multigene NGS panel.

Author Contributions: A.R. and A.B.: Treating Oncologists, Therapy Selection, Therapy Administration, Response Monitoring. R.D.: Clinical Management. D.P., D.A., V.D., R.D.: Evaluation of Molecular and Cellular Data, Therapy Recommendations.

Funding: The patient did not receive any financial compensation from Datar Cancer Genetics Limited (DCGL) for consenting to receive personalized cancer therapy. The patient did not receive any financial assistance for any therapy, interventions or procedures from DCGL. This manuscript does not report findings from a funded study. No external funding was received by any of the authors or their institutions in relation to the data presented in this manuscript.

Data Availability: Deidentified data may be made available by the authors upon reasonable request, and may require the execution of appropriate non-disclosure agreements.

Conflicts of Interest: A.R., A.B. and R.D. received financial compensation from DCGL for consultations. D.P., V.D. and D.A. 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. The patient and oncologists availed of personalized therapy guidance service from DCGL.

References

1. Hierro, C.; Matos, I.; Martin-Liberal, J.; Ochoa de Olza, M.; Garralda, E. Agnostic-Histology Approval of new drugs in Oncology: Are we already there? *Clin. Cancer Res.* **2019**, *25*, 3694–3707. [CrossRef] [PubMed]
2. Yan, L.; Zhang, W. Precision medicine becomes reality—Tumor type-agnostic therapy. *Cancer Commun.* **2018**, *38*, 6. [CrossRef] [PubMed]
3. Drilon, A.; Laetsch, T.W.; Kummar, S.; Dubois, S.G.; Lassen, U.N.; Demetri, G.D.; Nathenson, M.; Doebele, R.C.; Farago, A.F.; Pappo, A.S.; et al. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *N. Engl. J. Med.* **2018**, *378*, 731–739. [CrossRef] [PubMed]
4. Wu, C.I.; Wang, H.Y.; Ling, S.; Lu, X. The Ecology and Evolution of Cancer: The Ultra-Microevolutionary Process. *Annu. Rev. Genet.* **2016**, *50*, 347–369. [CrossRef] [PubMed]
5. El-Deiry, W.S.; Taylor, B.; Neal, J.W. Tumor Evolution, Heterogeneity, and Therapy for Our Patients with Advanced Cancer: How Far Have We Come? *Am. Soc. Clin. Oncol. Educ. Book* **2017**, *37*, e8–e15. [CrossRef] [PubMed]
6. Maeda, H.; Khatami, M. Analyses of repeated failures in cancer therapy for solid tumors: Poor tumor-selective drug delivery, low therapeutic efficacy and unsustainable costs. *Clin. Transl. Med.* **2018**, *7*, 11. [CrossRef]
7. Sodickson, A.; Baeyens, P.F.; Andriole, K.P.; Prevedello, L.M.; Nawfel, R.D.; Hanson, R.; Khorasani, R. Recurrent CT, Cumulative Radiation Exposure, and Associated Radiation-induced Cancer Risks from CT of Adults. *Radiology* **2009**, *251*, 175–184. [CrossRef] [PubMed]
8. Sharma, S. Tumor markers in clinical practice: General principles and guidelines. *Indian J. Med. Paediatr. Oncol.* **2009**, *30*, 1–8. [CrossRef]
9. Renfro, L.A.; An, M.-W.; Mandrekar, S.J. Precision oncology: A new era of cancer clinical trials. *Cancer Lett.* **2017**, *387*, 121–126. [CrossRef]
10. Le Tourneau, C.; Delord, J.P.; Gonçalves, A.; Gavoille, C.; Dubot, C.; Isambert, N.; Campone, M.; Trédan, O.; Massiani, M.A.; Mauborgne, C.; et al. Molecularly targeted therapy based on tumour molecular profiling versus conventional therapy for advanced cancer (SHIVA): A multicentre, open-label, proof-of-concept, randomised, controlled phase 2 trial. *Lancet Oncol.* **2015**, *16*, 1324–1334. [CrossRef]
11. Tsimberidou, A.-M.; Iskander, N.G.; Hong, D.S.; Wheler, J.J.; Falchook, G.S.; Fu, S.; Piha-Paul, S.; Naing, A.; Janku, F.; Luthra, R.; et al. Personalized Medicine in a Phase I Clinical Trials Program: The MD Anderson Cancer Center Initiative. *Clin. Cancer Res.* **2012**, *18*, 6373–6383. [CrossRef] [PubMed]
12. Sicklick, J.K.; Kato, S.; Okamura, R.; Schwaederle, M.; Hahn, M.E.; Williams, C.B.; De, P.; Krie, A.; Piccioni, D.E.; Miller, V.A.; et al. Molecular profiling of cancer patients enables personalized combination therapy: The I-PREDICT study. *Nat. Med.* **2019**, *25*, 744–750. [CrossRef] [PubMed]

13. Saiyed, M.M.; Ong, P.S.; Chew, L. Off-label drug use in oncology: A systematic review of literature. *J. Clin. Pharm. Ther.* **2017**, *42*, 251–258. [CrossRef] [PubMed]
14. Wagner, J.; Marquart, J.; Ruby, J.; Lammers, A.; Mailankody, S.; Kaestner, V.; Prasad, V. Frequency and level of evidence used in recommendations by the National Comprehensive Cancer Network guidelines beyond approvals of the US Food and Drug Administration: Retrospective observational study. *BMJ* **2018**, *360*, k668. [CrossRef] [PubMed]
15. Wang, J.; Chang, S.; Li, G.; Sun, Y. Application of liquid biopsy in precision medicine: Opportunities and challenges. *Front. Med.* **2017**, *11*, 522–527. [CrossRef]
16. Von Bubnoff, N. Liquid Biopsy: Approaches to Dynamic Genotyping in Cancer. *Oncol. Res. Treat.* **2017**, *40*, 409–416. [CrossRef] [PubMed]
17. Enderle, D.; Spiel, A.; Coticchia, C.M.; Berghoff, E.; Mueller, R.; Schlumpberger, M.; Sprenger-Haussels, M.; Shaffer, J.M.; Lader, E.; Skog, J.; et al. Characterization of RNA from Exosomes and Other Extracellular Vesicles Isolated by a Novel Spin Column-Based Method. *PLoS ONE* **2015**, *10*, e0136133. [CrossRef]
18. Sukhai, M.A.; Craddock, K.J.; Thomas, M.; Hansen, A.R.; Zhang, T.; Siu, L.; Bedard, P.; Stockley, T.L.; Kamel-Reid, S. A classification system for clinical relevance of somatic variants identified in molecular profiling of cancer. *Genet. Med.* **2016**, *18*, 128. [CrossRef]
19. Politi, K.; Lynch, T.J.; Lynch, T.; Lynch, T.J. Two sides of the same coin: EGFR exon 19 deletions and insertions in lung cancer. *Clin. Cancer Res.* **2012**, *18*, 1490–1492. [CrossRef]
20. Heist, R.S.; Sequist, L.V.; Engelman, J.A. Genetic Changes in Squamous Cell Lung Cancer: A Review. *J. Thorac. Oncol.* **2012**, *7*, 924–933. [CrossRef]
21. Saikali, Z.; Singh, G. Doxycycline and other tetracyclines in the treatment of bone metastasis. *Anti-Cancer Drugs* **2003**, *14*, 773–778. [CrossRef] [PubMed]
22. Cathcart, J.; Pulkoski-Gross, A.; Cao, J. Targeting matrix metalloproteinases in cancer: Bringing new life to old ideas. *Genes Dis.* **2015**, *2*, 26–34. [CrossRef] [PubMed]
23. Jiang, H.; Fan, Z.; Wang, Z.; Qu, J. Atorvastatin partially inhibits the epithelial-mesenchymal transition in A549 cells induced by TGF-β1 by attenuating the upregulation of SphK1. *Oncol. Rep.* **2016**, *36*, 1016–1022.
24. Chen, J.; Bi, H.; Hou, J.; Zhang, X.; Zhang, C.; Yue, L.; Wen, X.; Liu, D.; Shi, H.; Yuan, J.; et al. Atorvastatin overcomes gefitinib resistance in KRAS mutant human non-small cell lung carcinoma cells. *Cell Death Dis.* **2013**, *4*, e814. [CrossRef] [PubMed]
25. Suzawa, K.; Toyooka, S.; Sakaguchi, M.; Morita, M.; Yamamoto, H.; Tomida, S.; Ohtsuka, T.; Watanabe, M.; Hashida, S.; Maki, Y.; et al. Antitumor effect of afatinib, as a human epidermal growth factor receptor 2-targeted therapy, in lung cancers harboring HER2 oncogene alterations. *Cancer Sci.* **2016**, *107*, 45–52. [CrossRef] [PubMed]
26. Schuler, M.; Yang, J.C.; Park, K.; Kim, J.H.; Bennouna, J.; Chen, Y.M.; Chouaid, C.; De Marinis, F.; Feng, J.F.; Grossi, F.; et al. Afatinib beyond progression in patients with non-small-cell lung cancer following chemotherapy, erlotinib/gefitinib and afatinib: Phase III randomized LUX-Lung 5 trial. *Ann. Oncol.* **2016**, *27*, 417–423. [CrossRef] [PubMed]
27. Minari, R.; Bordi, P.; Tiseo, M. Third-generation epidermal growth factor receptor-tyrosine kinase inhibitors in T790M-positive non-small cell lung cancer: Review on emerged mechanisms of resistance. *Transl. Lung Cancer Res.* **2016**, *5*, 695–708. [CrossRef]
28. Munoz, J.; Wheler, J.J.; Kurzrock, R. Androgen receptors beyond prostate cancer: An old marker as a new target. *Oncotarget* **2015**, *6*, 592–603. [CrossRef]
29. Anand, K.; Ensor, J.; Trachtenberg, B.; Bernicker, E. Osimertinib induced cardio-toxicity: A retrospective review of FDA adverse events reporting system (FAERS). *J. Clin. Oncol.* **2019**, 9044.
30. United States Food and Drug Administration. Available online: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-osimertinib-first-line-treatment-metastatic-nsclc-most-common-egfr-mutations (accessed on 19 June 2019).

JNCCN.org Journal of the National Comprehensive Cancer Network

Title

Clinical Utility of Encyclopedic Tumor Analysis to Treat Breast Cancer Patients who have Failed Standard of Care Treatments.

Authors

Dadasaheb Akolkar[1], Darshana Patil[1], Anantbhushan Ranade[2], Revati Patil[1], Sachin Apurwa[1], Sanket Patil[1], Pradip Fulmali[1], Pradip Devhare[1], Navin Srivastava[1], Ajay Srinivasan[1], Rajan Datar[1]

Affiliations

Datar Cancer Genetics Limited, Nasik, India ; Avinash Cancer Clinic, Pune, India.

Abstract

Background

Post failure of 3 lines of systemic treatments in breast cancers, Standard of Care guidelines recommend palliative care or clinical trials for such patients. We retrospectively evaluated the efficacy of ultra-personalized treatment in a cohort of patients (n = 27) with advanced refractory breast cancers. These patients had availed of Encyclopedic Tumor Analysis (ETA), based on which each patient received individualised organ- and label-agnostic treatment regimens.

Methods

ETA was performed on freshly biopsied tumor tissue and peripheral blood. ETA interrogated gene alterations, gene expression, immunohistochemistry (IHC) in tumor tissue and in vitro chemoresistance profiling (CRP) of viable tumor cells. Findings of ETA were integrated to generate patient-specific therapy recommendations. All patients underwent whole body PET-CT and brain MRI scans prior to start of treatment. Treatment response was determined from follow-up PET-CT scans and used to calculate Objective Response Rate (ORR), Disease Control Rate (DCR) and Progression Free Survival (PFS).

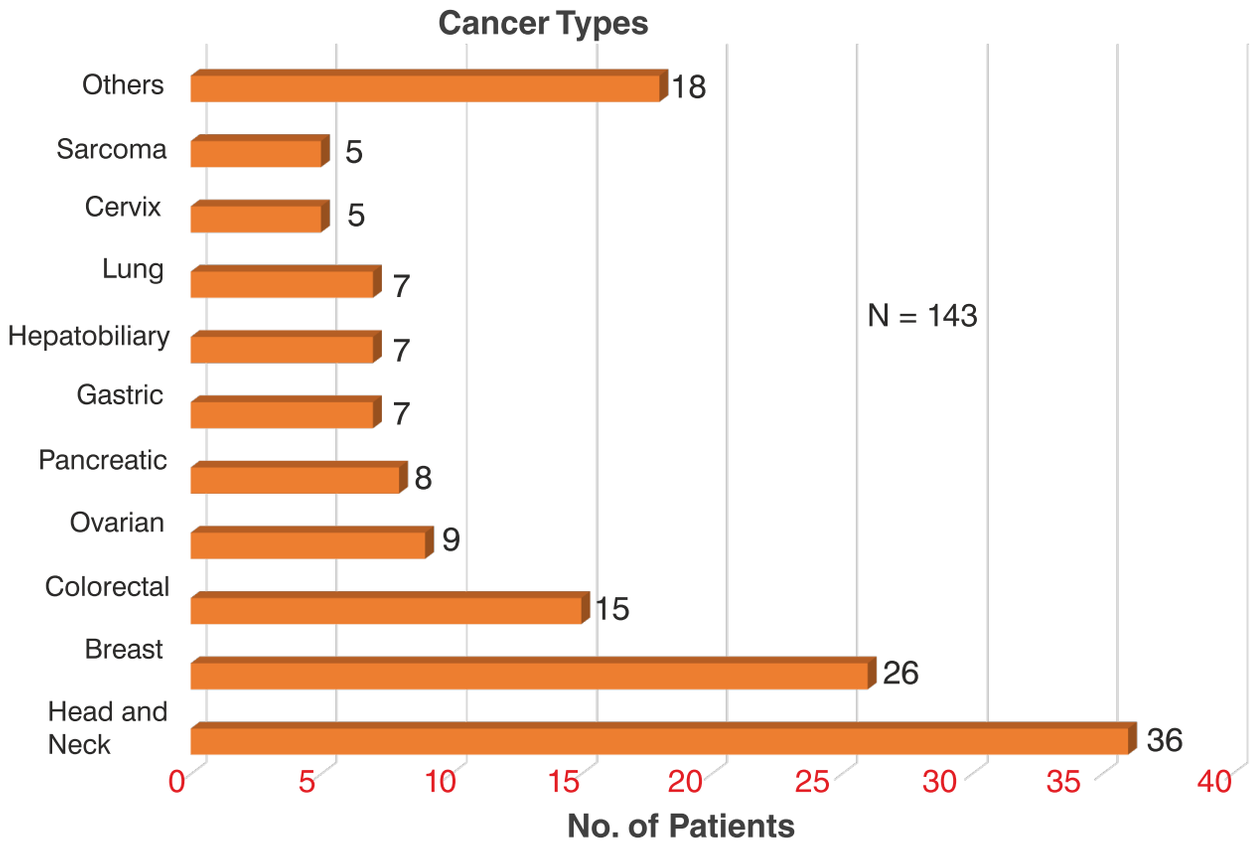
Results

Among the 27 patients who received personalized treatment guided by ETA, partial response (PR) was observed in 16 patients (ORR = 59.1%). At the most recent follow-up 26 patients continued to exhibit PR or Stable Disease (SD) (DCR = 96.3%). Median PFS was 115 days. There were no grade IV adverse events or any treatment related deaths. Most patients reported qualitative improvements in symptomatic and functional status.

Conclusion

ETA guided treatments can offer viable treatment options in advanced refractory breast cancers yielding meaningful ORR and disease control in majority of patients.

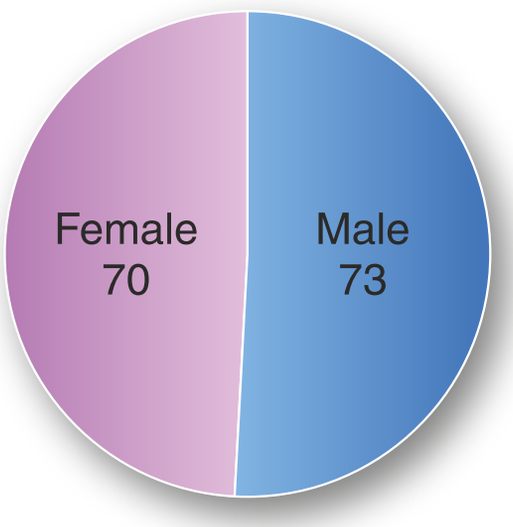
Demographics and Cancer Types



Other Cancer Types

Kidney	4
Bone	3
NET	3
Esophageal	2
Melanoma	2
Testes	2
Prostate	1
Pilomatrical	1

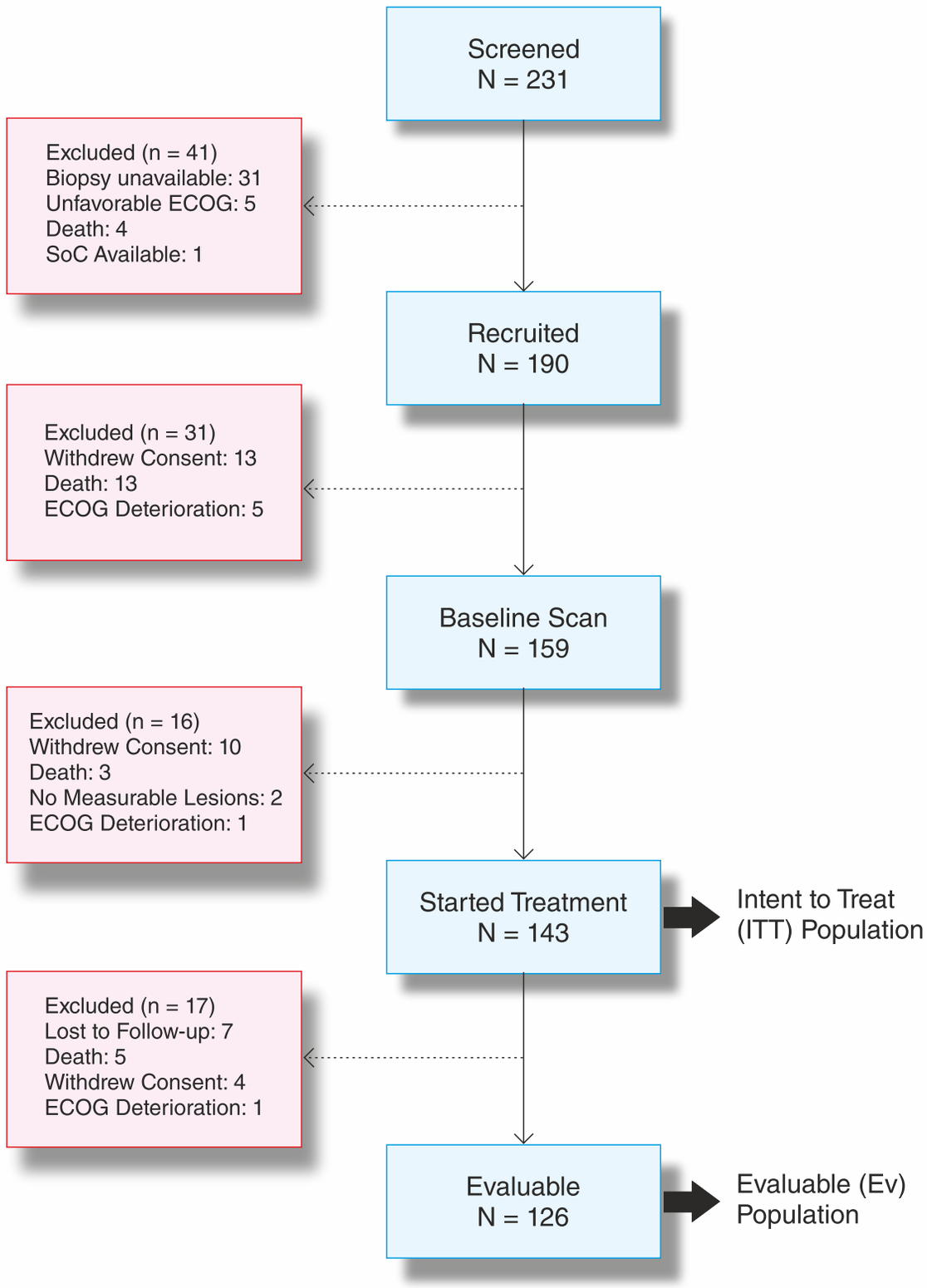
Gender



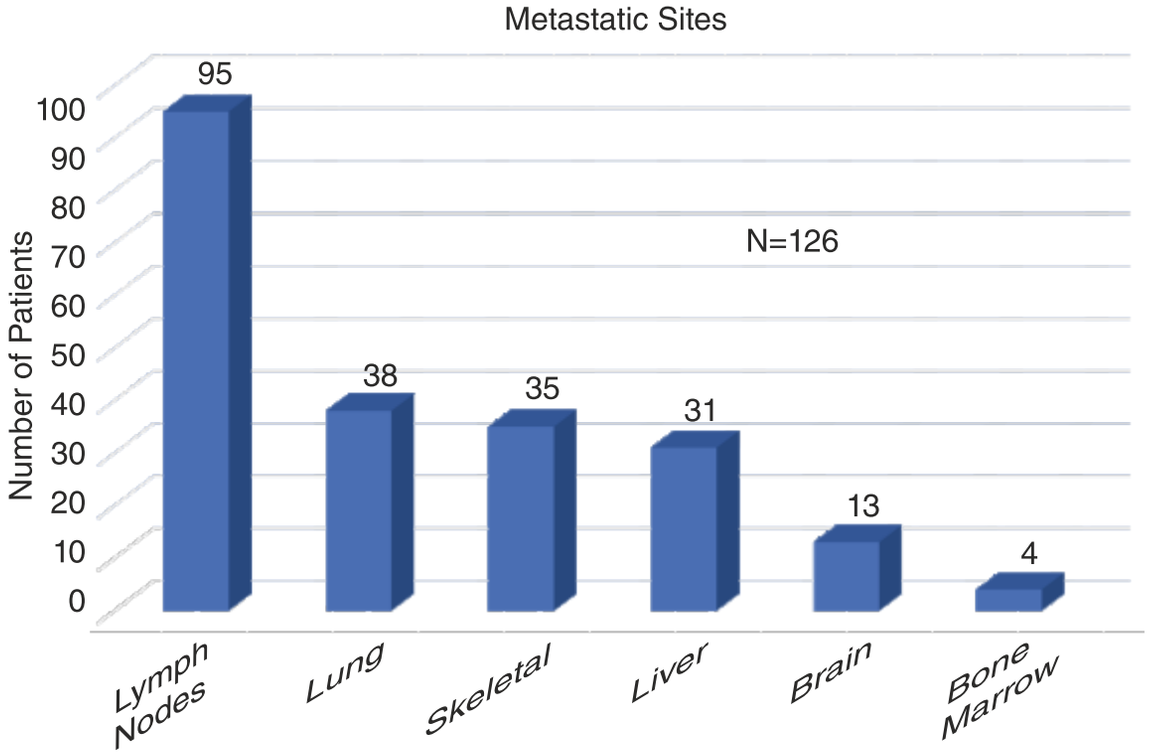
Age

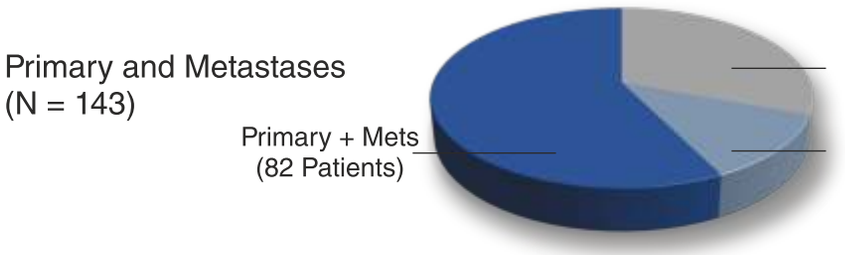
Minimum	24 years
Maximum	75 years
Median	50 years

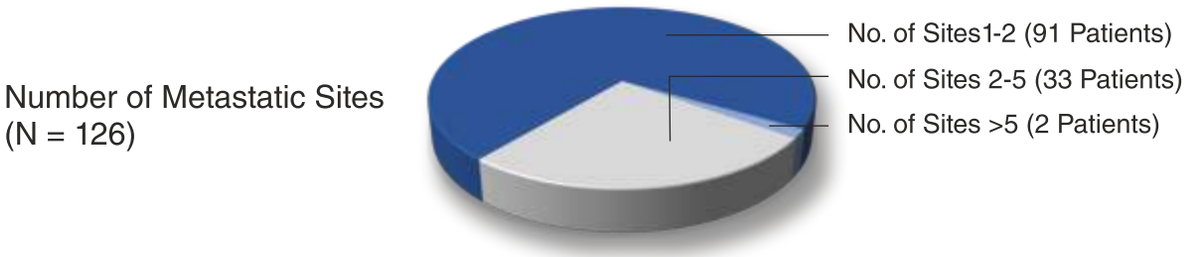
RESILIENT: CONSORT Diagram

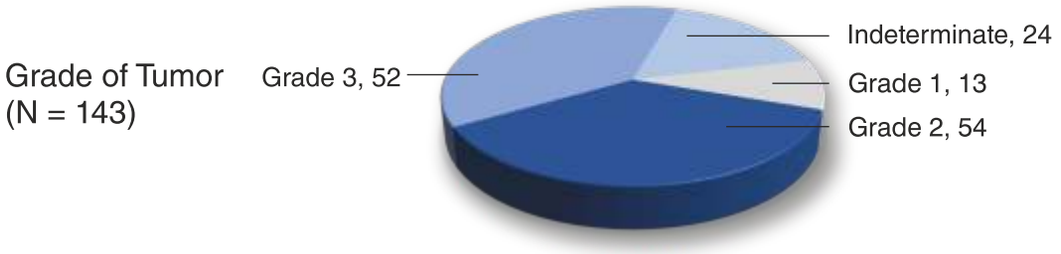


Baseline Disease Status



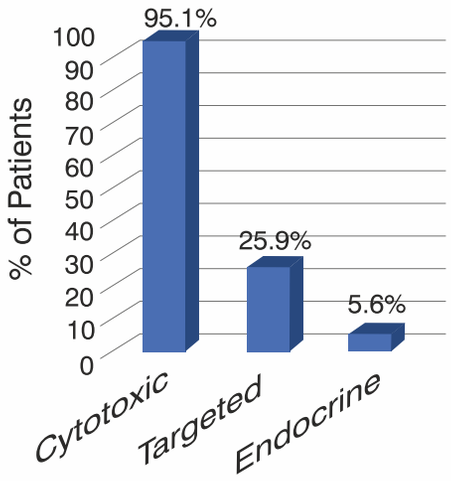




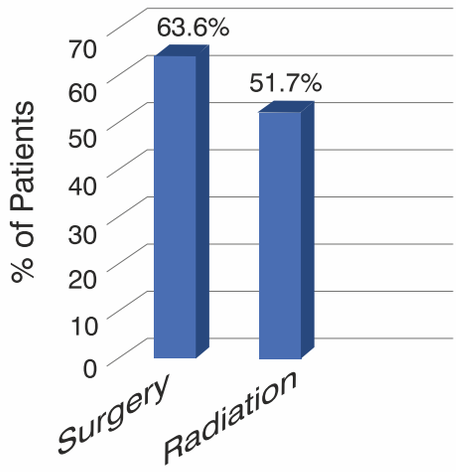


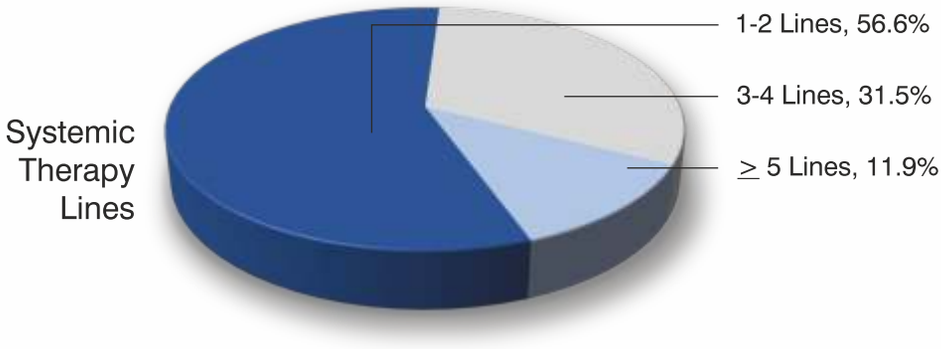
Prior Treatments

Systemic Agents

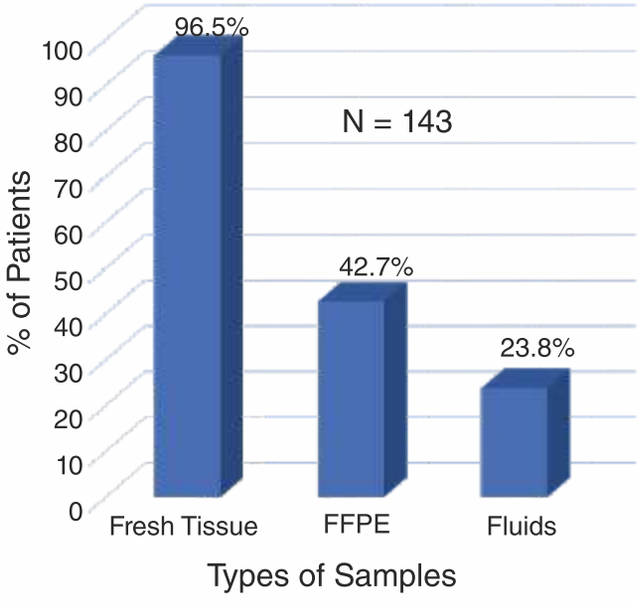


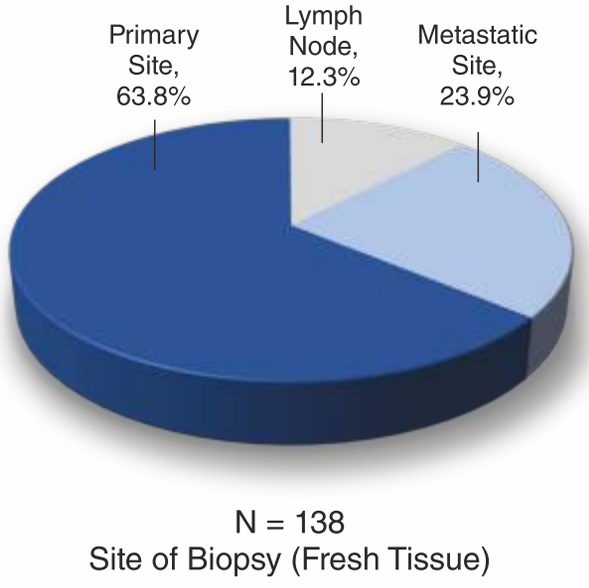
Surgery and Radiation



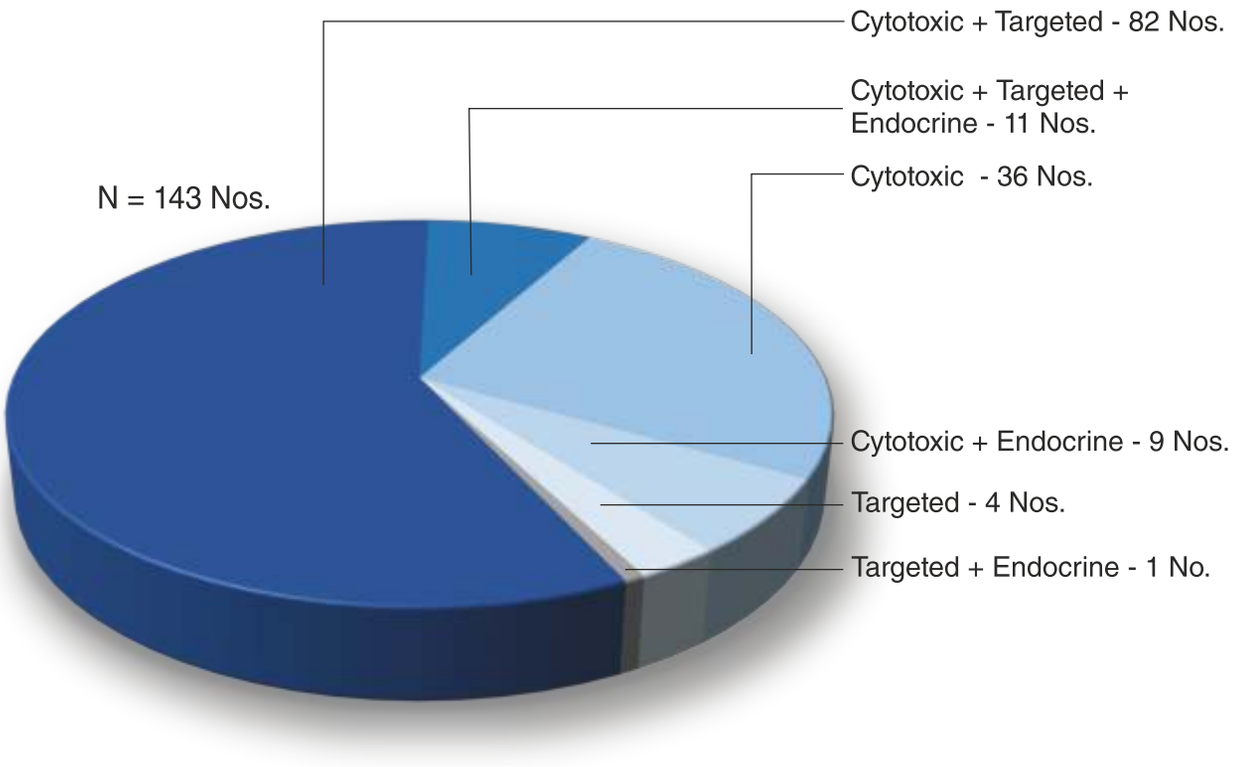


Patient Samples





ETA-Guided Treatment Profile



Therapy Type	Number	%
Cytotoxic + Targeted ± Endocrine	93	65.0 %
Cytotoxic ± Endocrine	45	31.5 %
Targeted ± Endocrine	5	3.5 %

- Patients in RESILIENT were administered combinations of Cytotoxic, Targeted and Endocrine Agents.
- Treatment regimens included only FDA-approved anticancer agents,
- None of the patients received experimental or unapproved agents.
- Checkpoint Inhibitors were not included in the RESILIENT Trial.

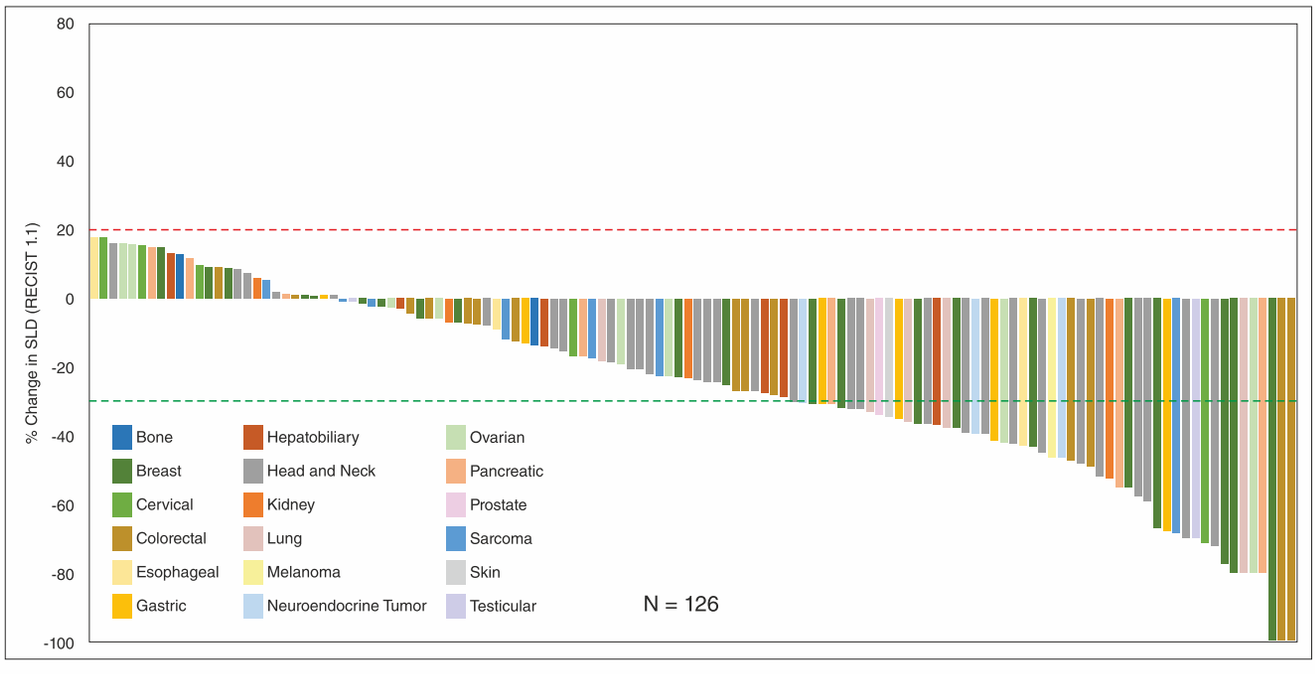
Treatment Response

- All patients underwent baseline PET-CT scan.
- Treatment response evaluated by follow-up PET-CT scan.
- Brain MRI: Baseline and follow-up (where indicated).
- Treatment Response Evaluated as per RECIST 1.1 criteria.

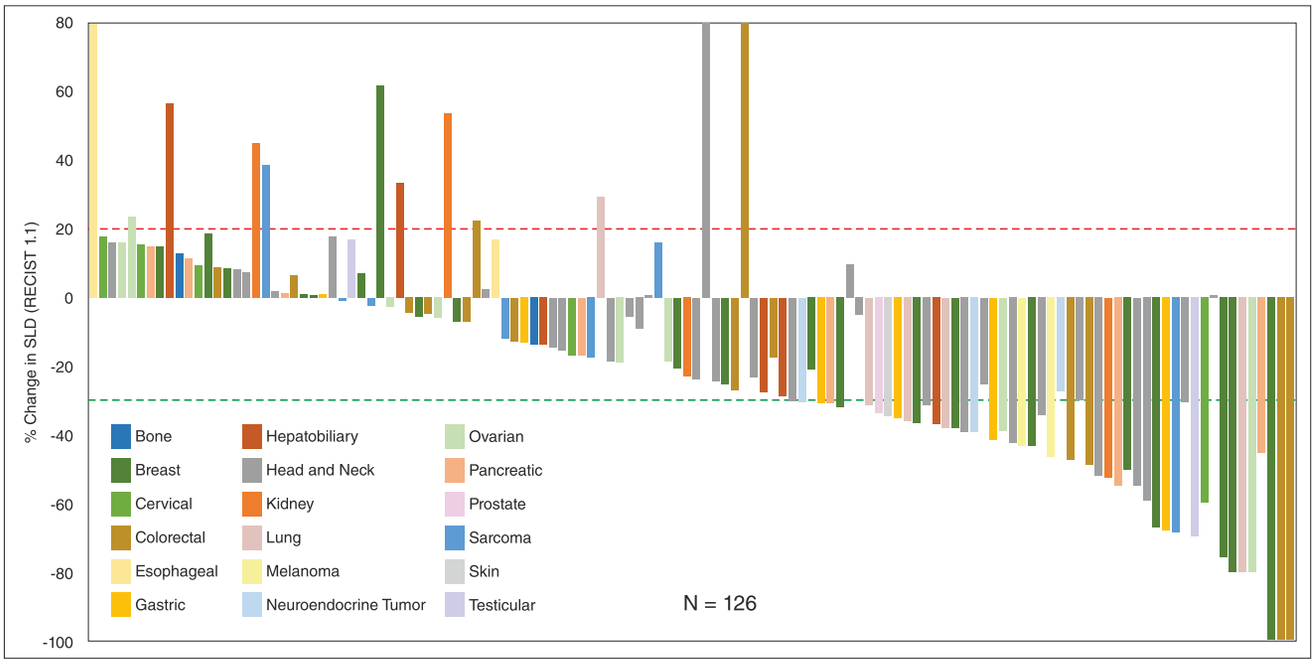
Complete Response	CR	Resolution of all target + non target lesions + no new lesions.
Partial Response	PR	>30% decrease in SLD of all target lesions + no new lesions.
Stable Disease	SD	<20% increase to <30% decrease in SLD of target lesions no new lesions.
Disease Progression	PD	>20% increase in SLD of target lesions &/or new lesions.

Objective Response Rate	ORR	CR + PR (%)
Clinical Benefit Rate	CBR	CR + PR + SD (%)
Disease Control Rate	DCR	

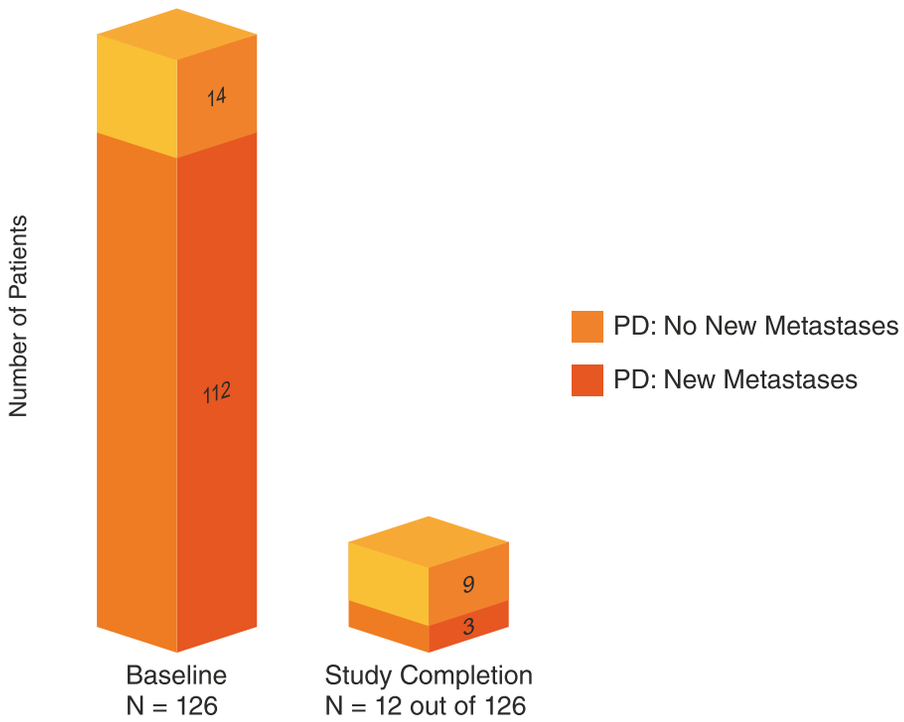
Best Response



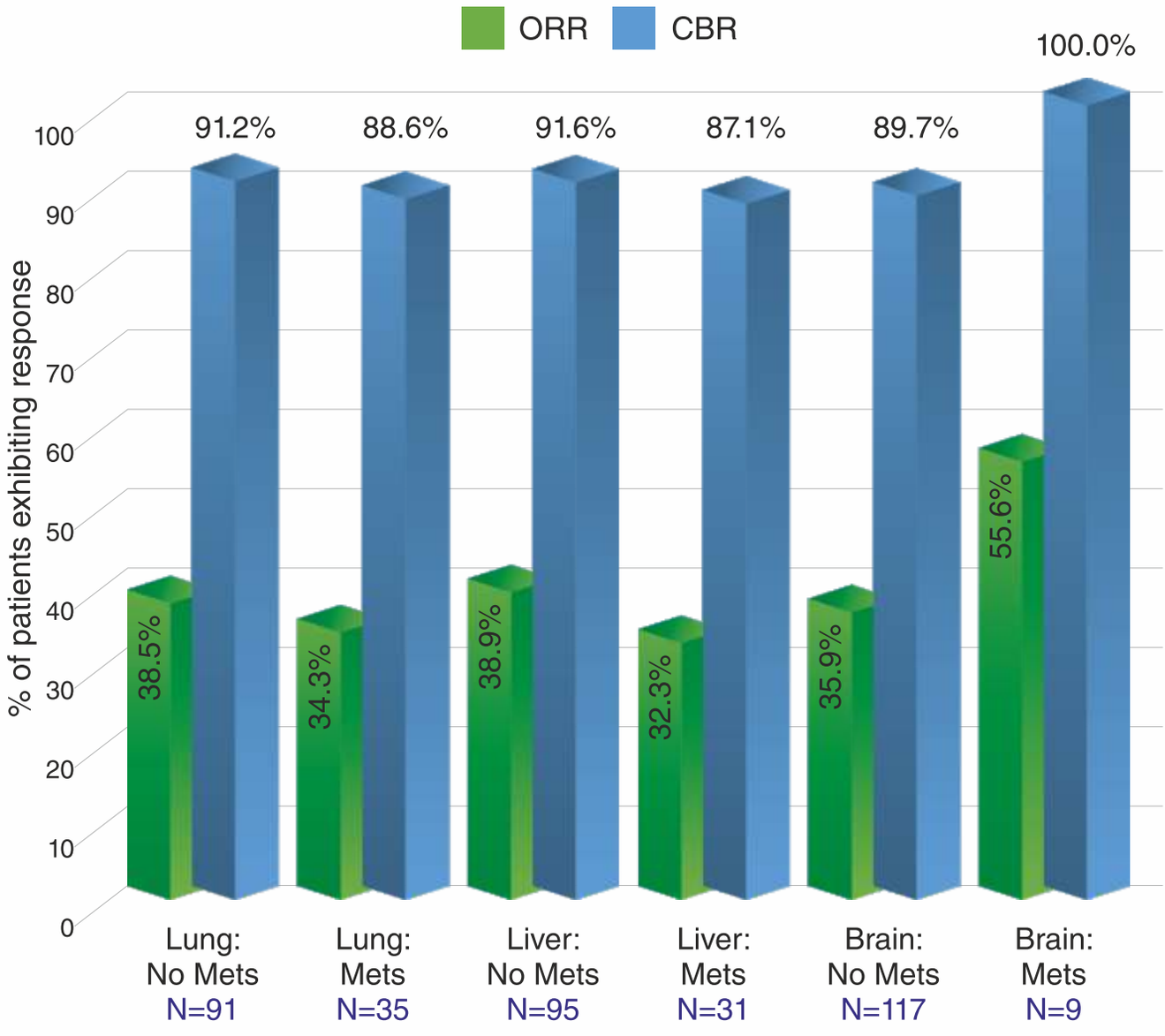
Final Outcome



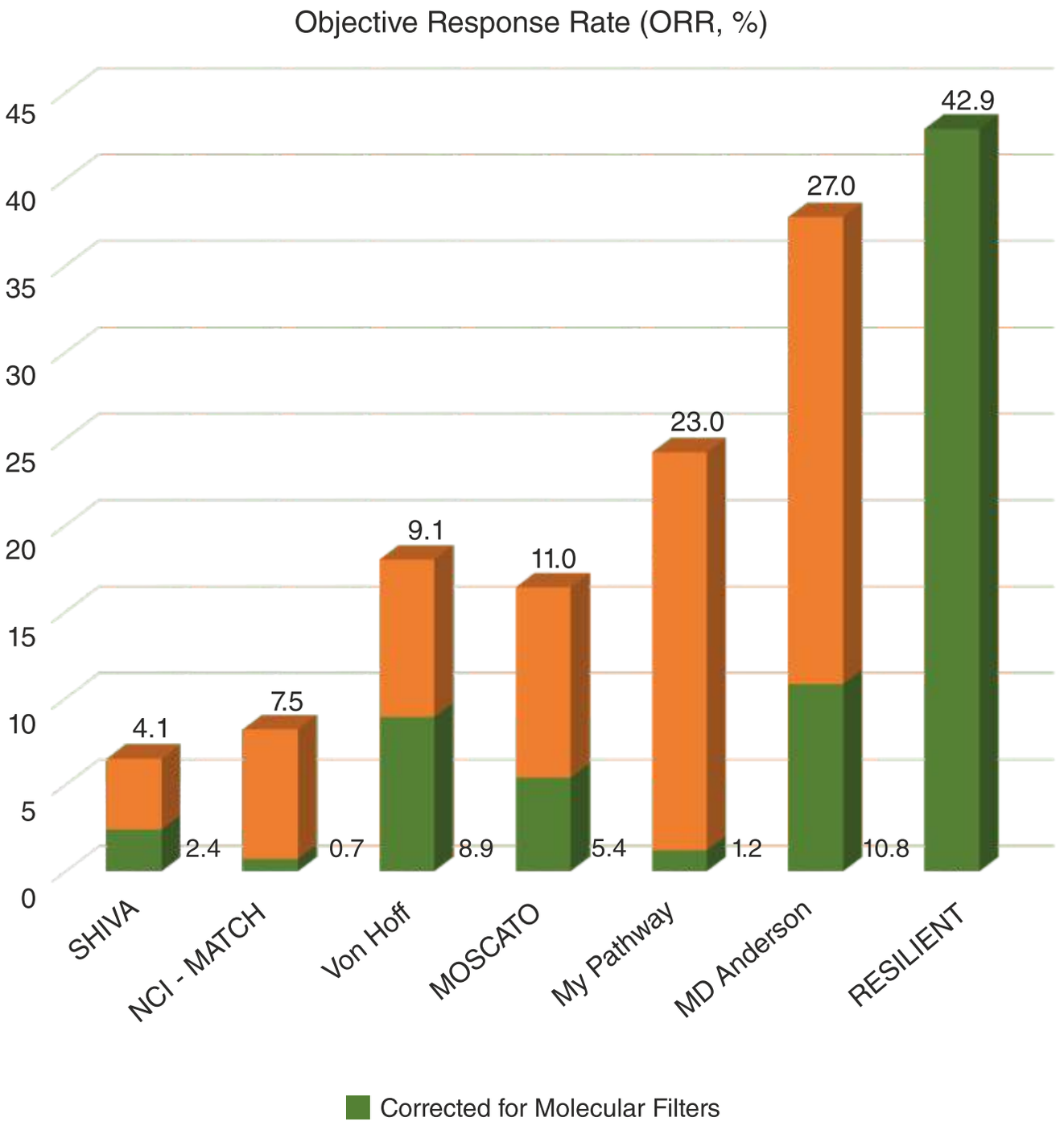
Control of Metastases



Baseline Metastases did not influence Outcomes

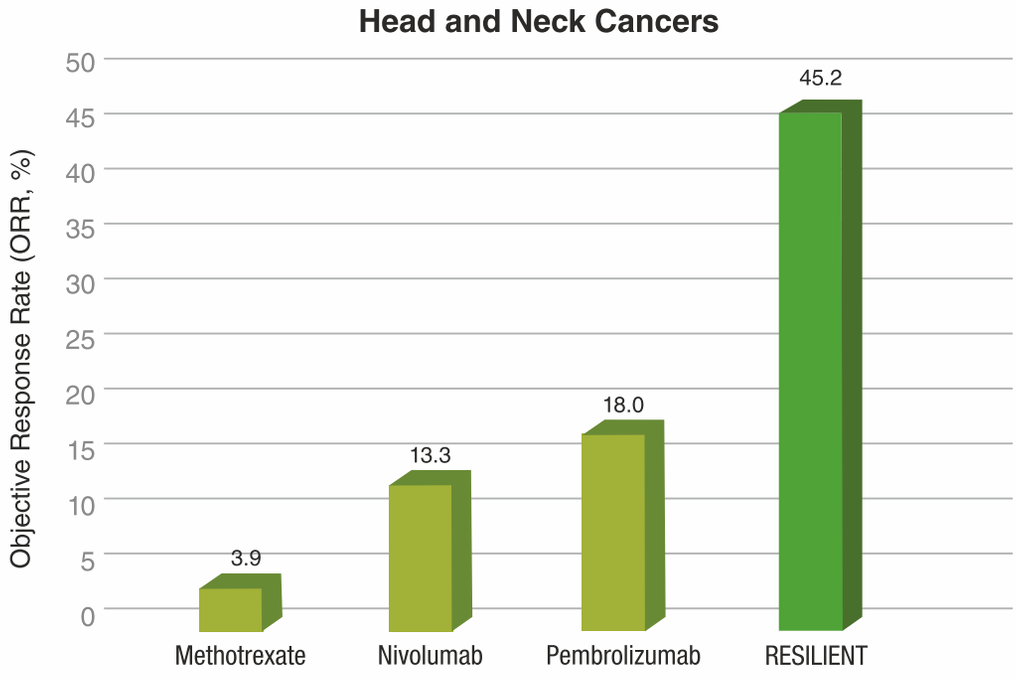


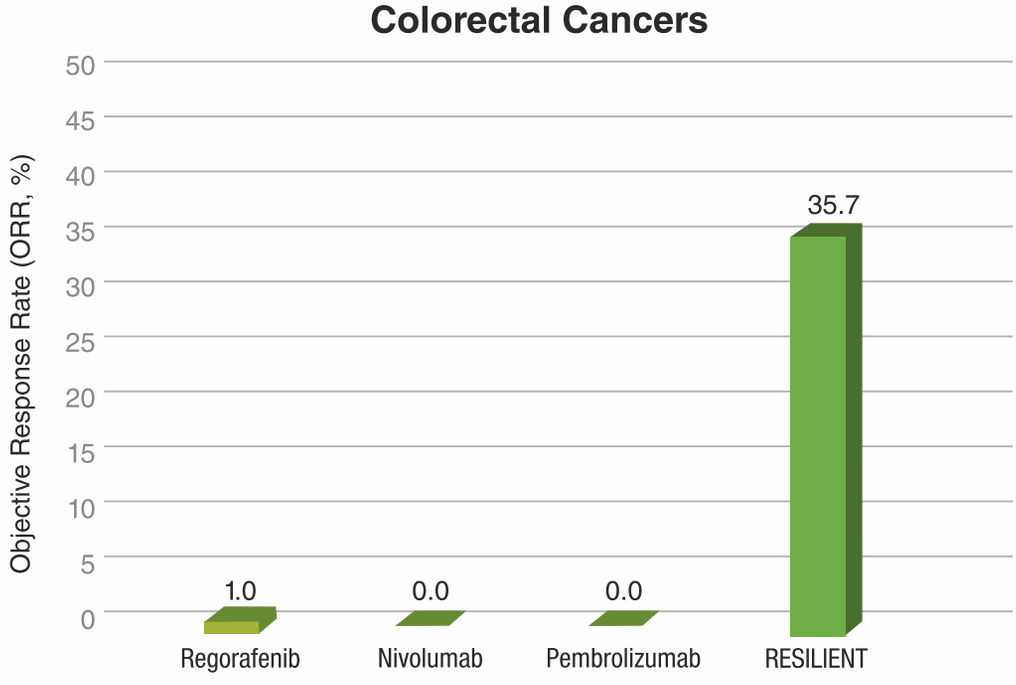
RESILIENT v/s Other Studies



- Other Studies excluded patients where the malignancy did not harbour predefined molecular features.
- Hence reported response rates were corrected for proportion of patients recruited.
- Resilient Study did not include any such filter, hence ORR remains unaffected.

RESILIENT v/s SoC v/s Checkpoint Inhibitors

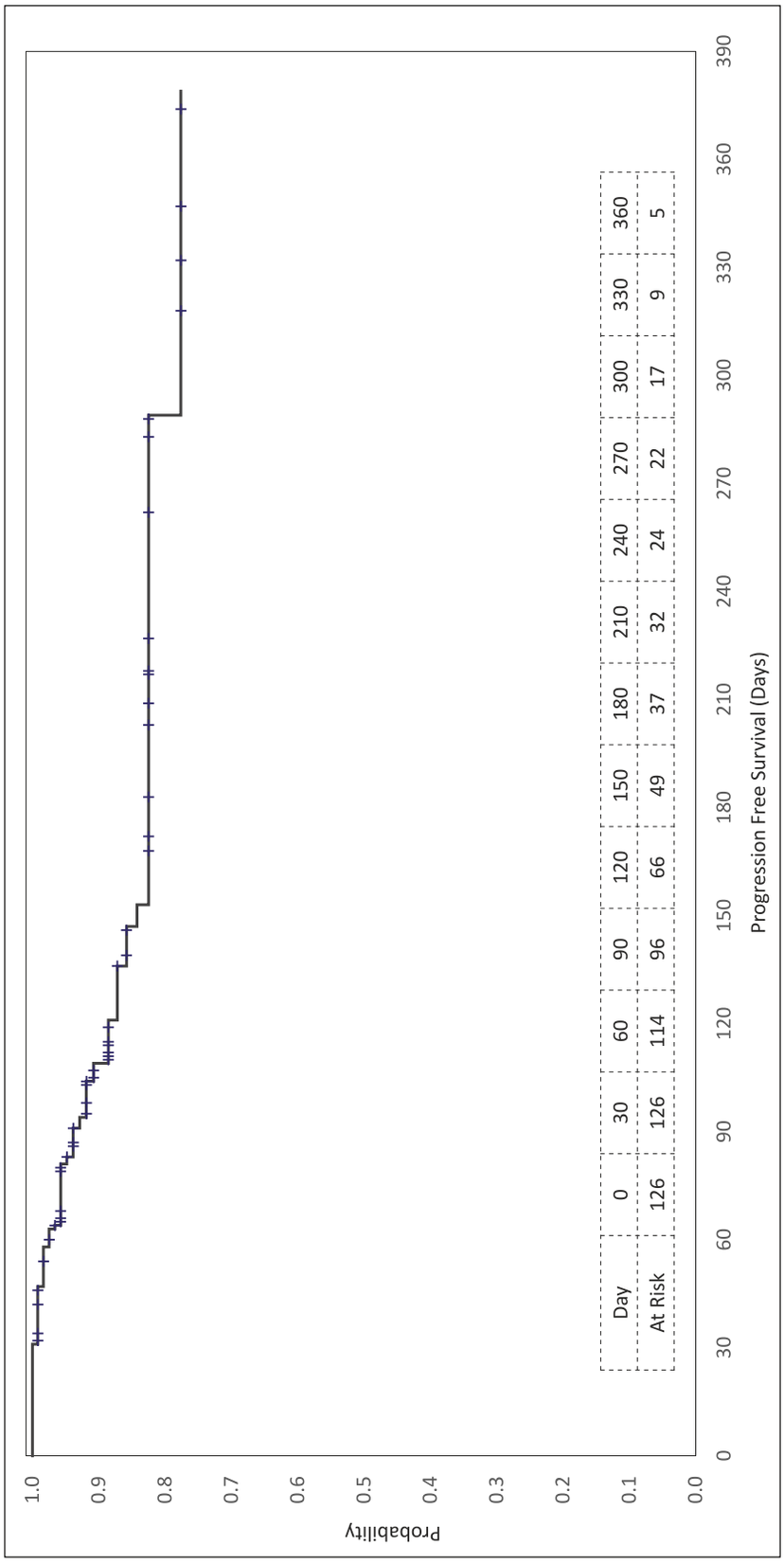




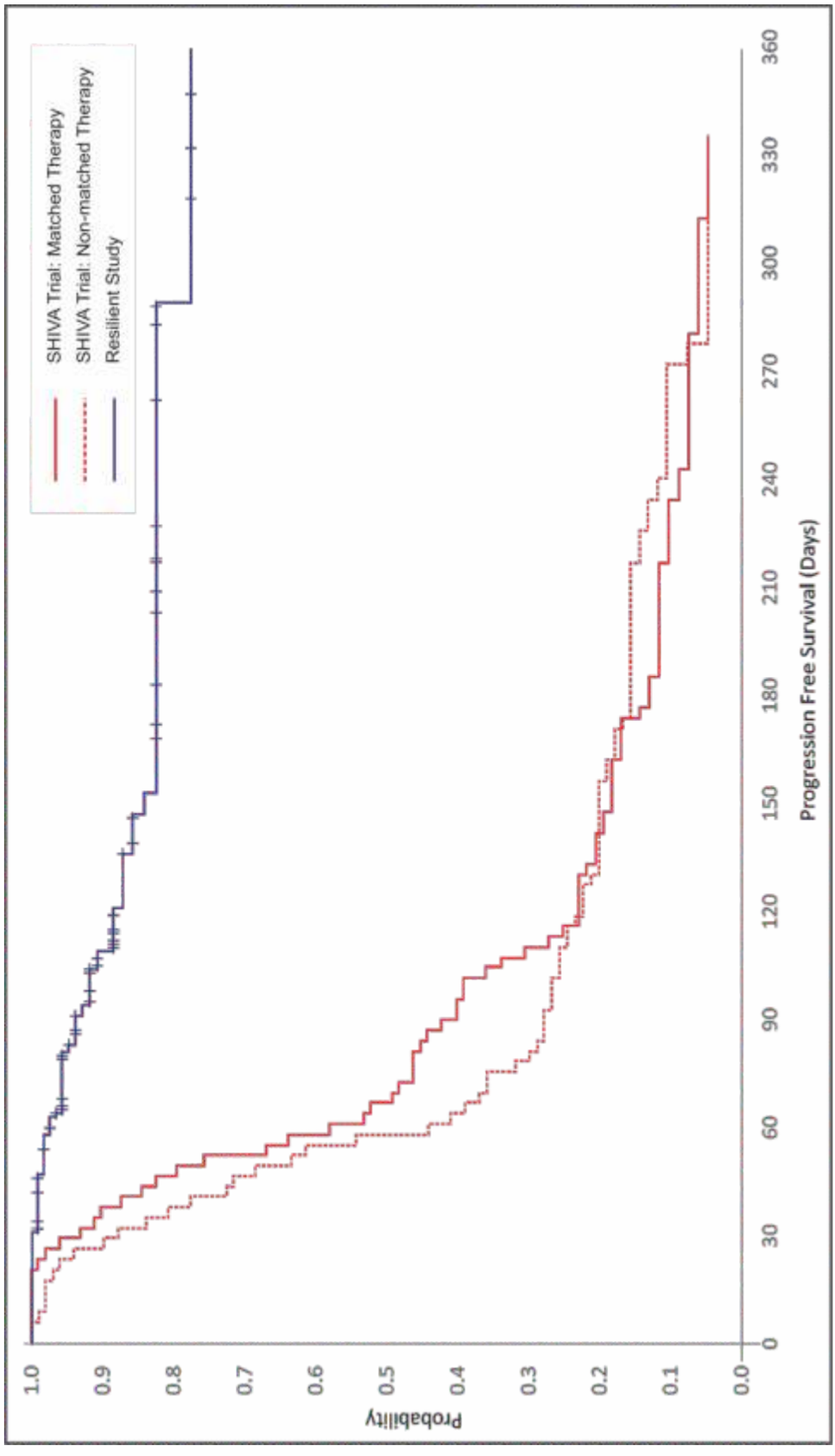
Objective Response Rate for Methotrexate and Regorafenib are in a setting of stage IV malignancy at recurrence / progression following failure of initial line(s) of therapy.

All colorectal cancers in Resilient were MMR-Proficient (MSS), where Immunotherapy agents have limited or no benefit.

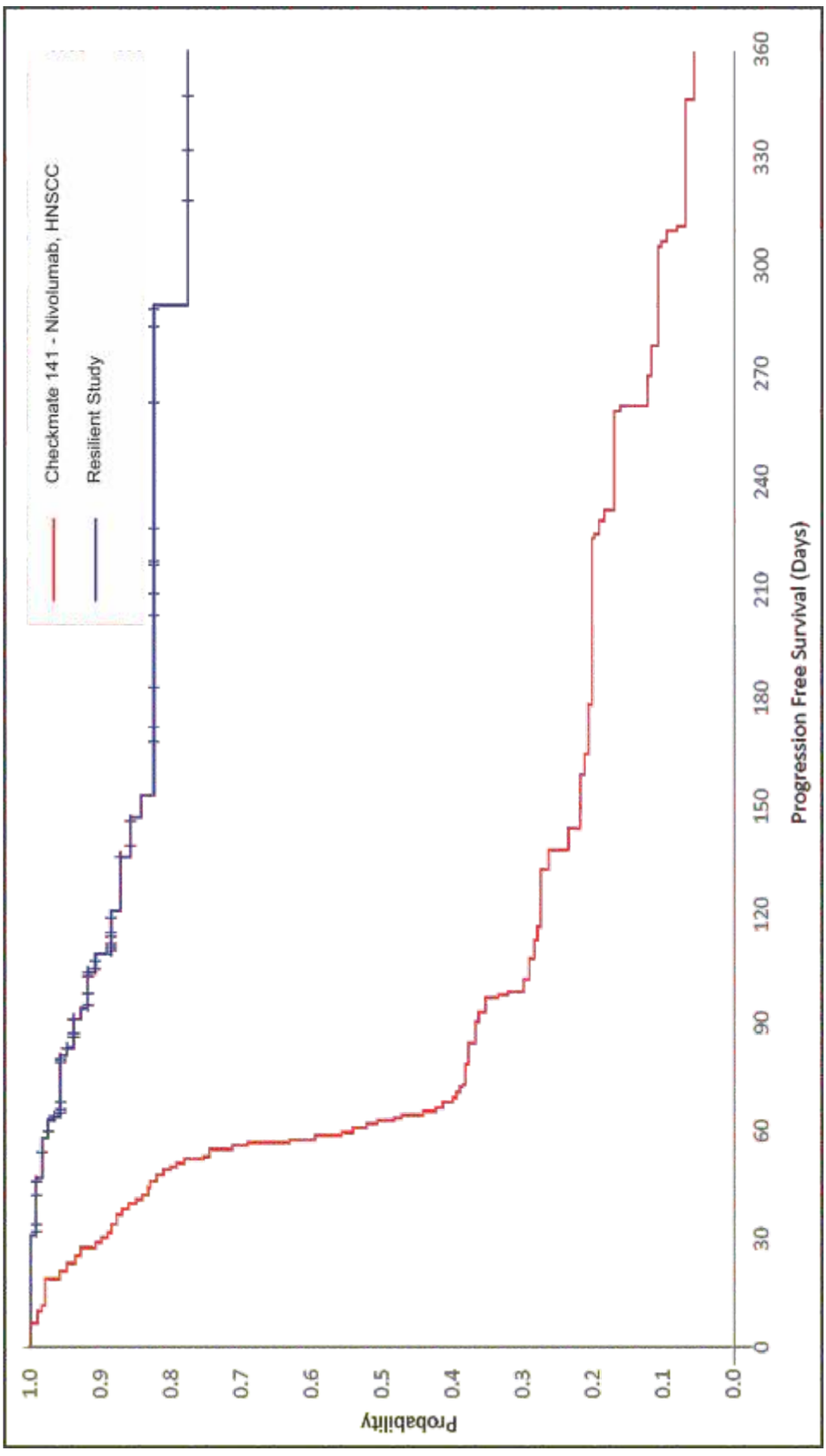
Progression-Free Survival (PFS)



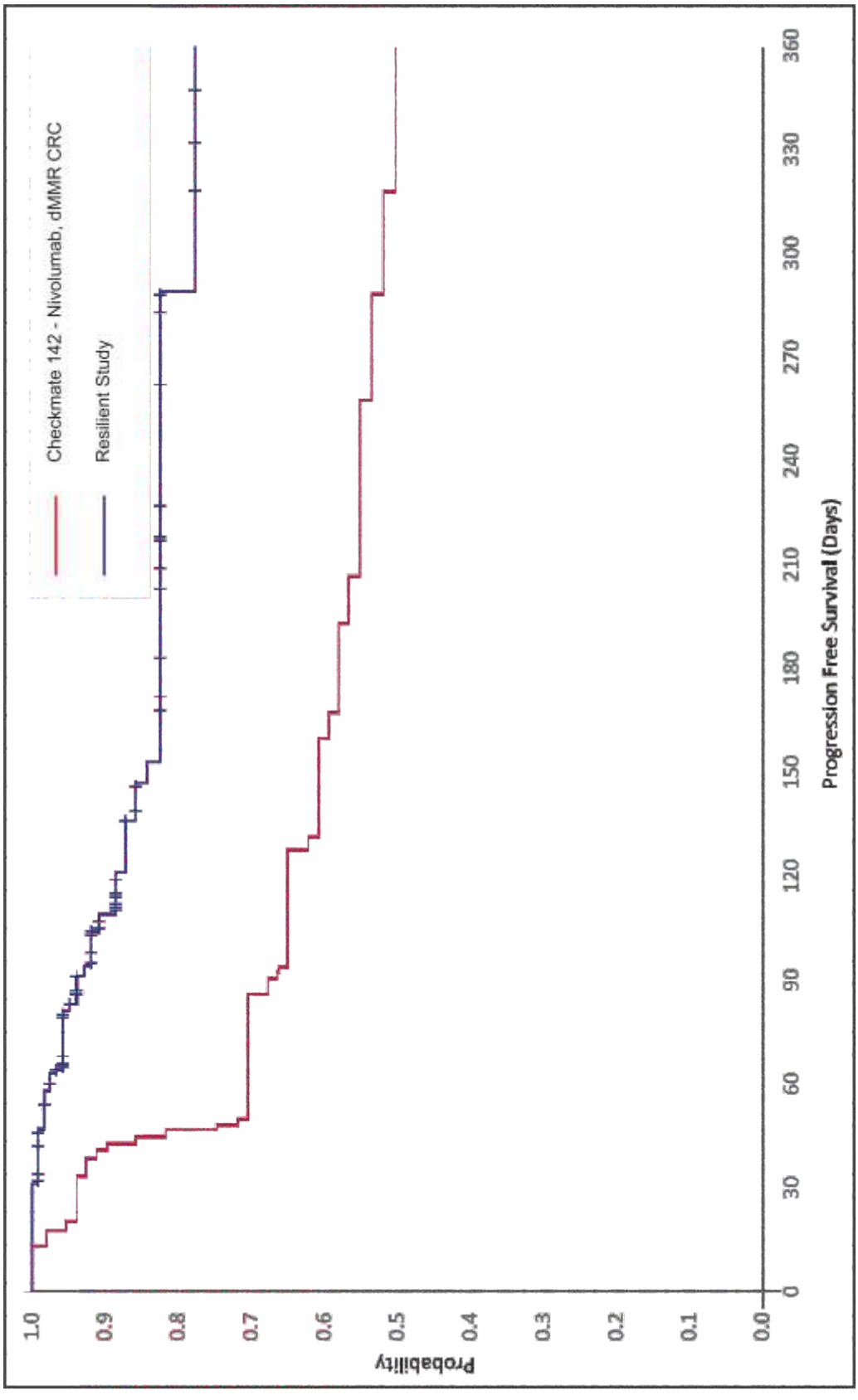
PFS: RESILIENT v/s SHIVA



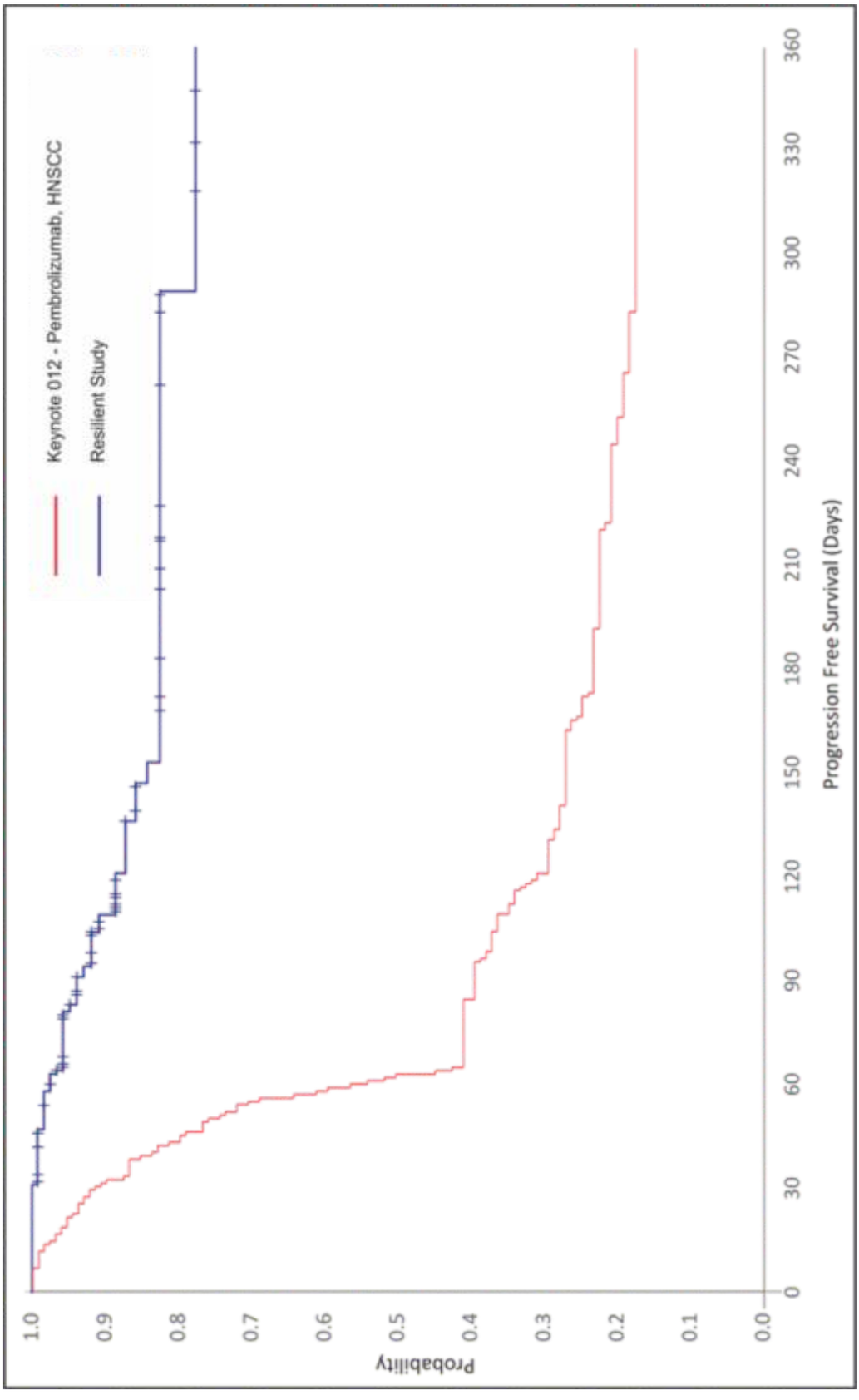
PFS: RESILIENT v/s CHECKMATE 141



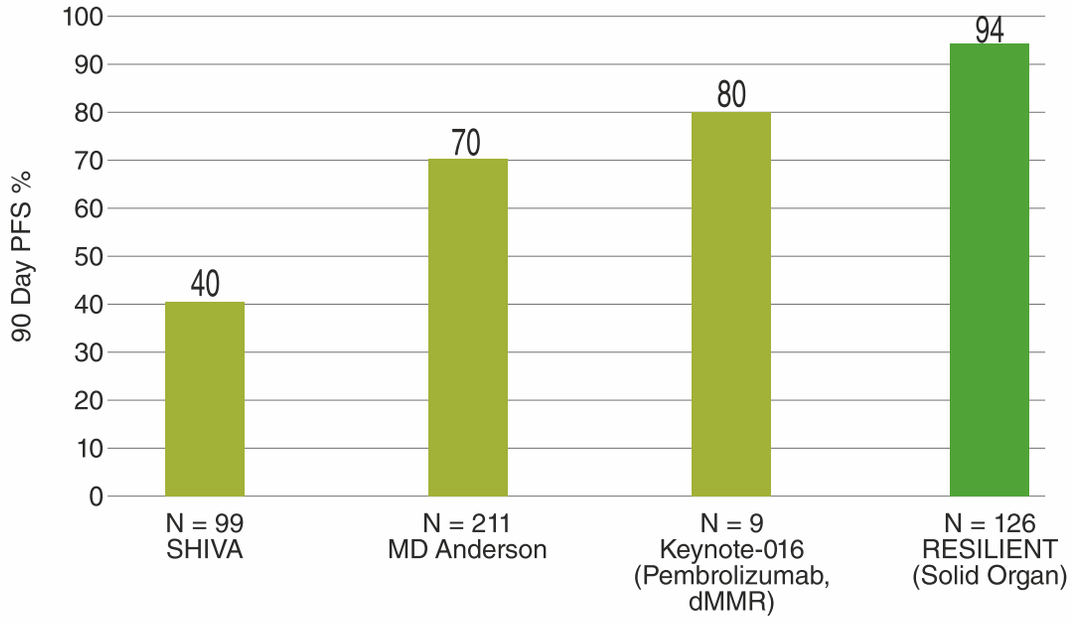
PFS: RESILIENT v/s CHECKMATE 142



PFS: RESILIENT v/s KEYNOTE 012



All Solid Organ Malignancies

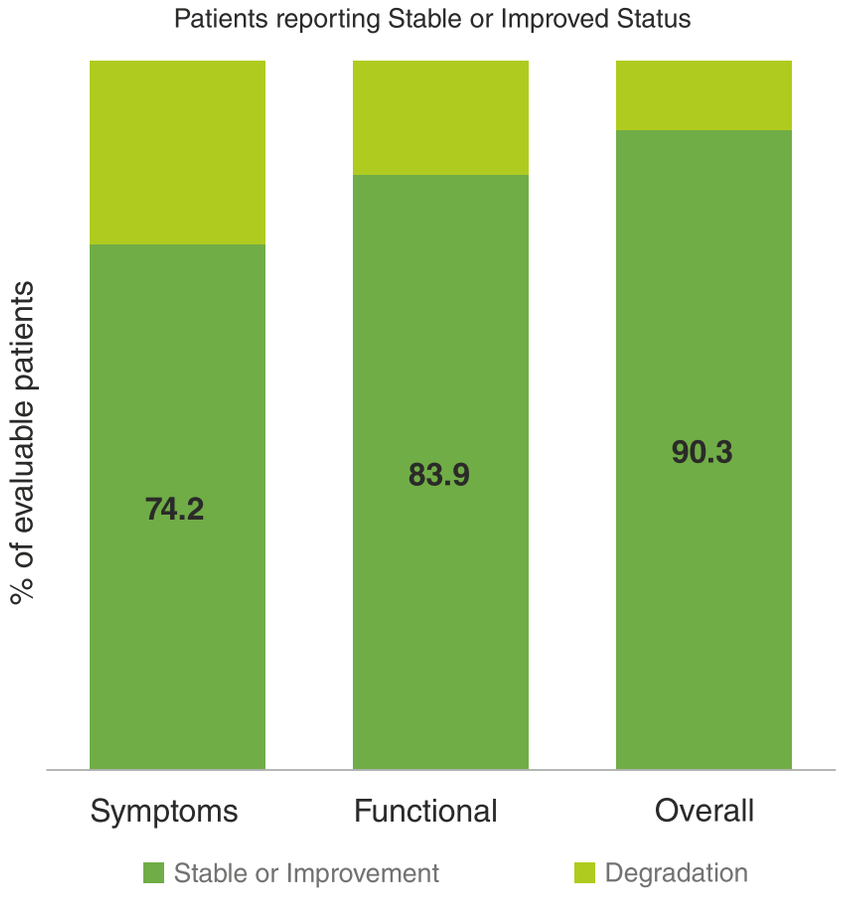


Adverse Events (AEs)

Adverse Events (Therapy Related)	Any Grade		Grade ≥ 3	
	No. of Patients	%	No. of Patients	%
Fatigue	121	84.6%	9	6.3%
Anorexia	92	64.3%	6	4.2%
Mucositis Oral	57	39.9%	13	9.1%
Edema	39	27.3%	4	2.8%
Pyrexia	35	24.5%	8	5.6%
Neutropenia	32	22.4%	16	11.2%
Vomiting	26	18.2%	6	4.2%
Anemia	22	15.4%	12	8.4%
Thrombocytopenia	18	12.6%	12	8.4%
Peripheral neuropathy	11	7.7%	2	1.4%
Hyper-/ Hypotension	8	5.6%	6	4.2%
Increased serum bilirubin	4	2.8%	3	2.1%
Any Event	**143**	**100%**	**57**	**39.9%**

- No Grade IV therapy related AEs.
- No therapy related deaths.
- All AEs managed by administration of Standard of Care Procedures and Agents

Quality of Life

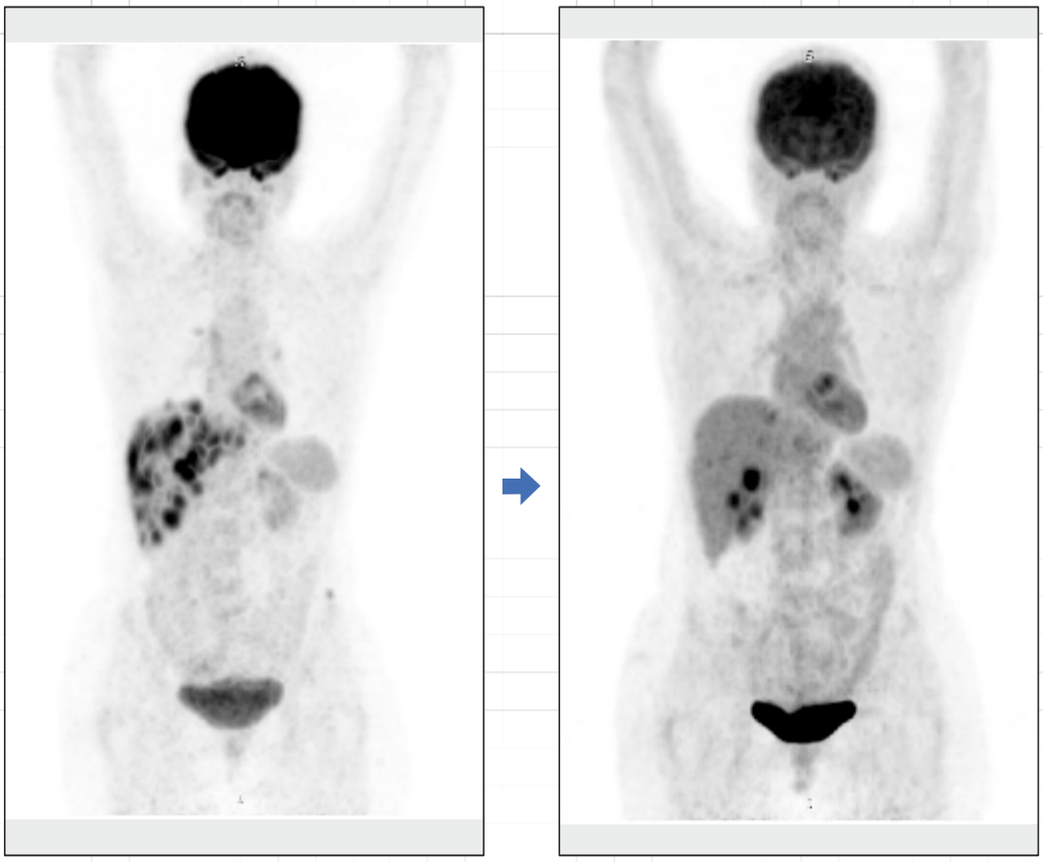


DATAR
CANCER GENETICS

datarpgx.com

Breast Cancer

39 year old female patient.
Cancer had progressed following 5 lines of therapy.

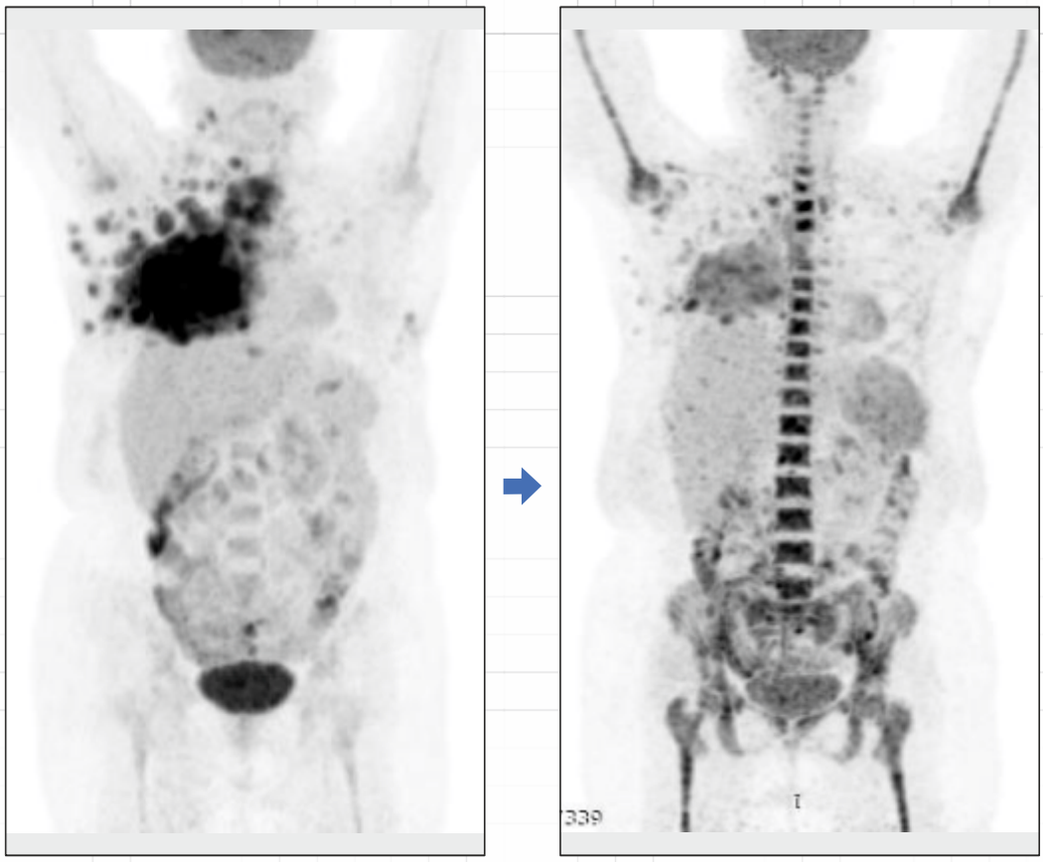


Baseline | Follow-up

Molecular Aberrations
PIK3CA SNV
TP53 SNV
VEGF Overexpression

Breast Cancer

53 year old female patient.
Cancer had progressed following 10 lines of therapy.

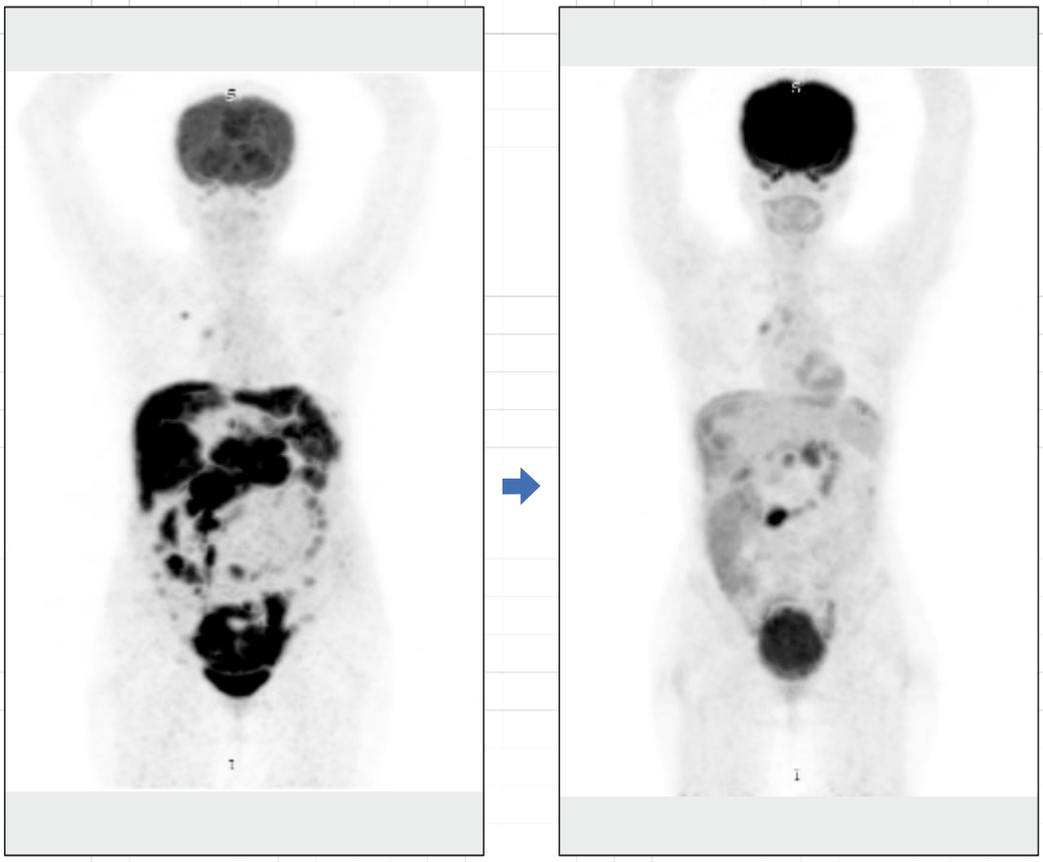


Baseline Follow-up

Molecular Aberrations	
FGFR1 CNV+	PARP1 SNV
SRC CNV+	TUBB3 Overexpression
CDK4 CNV+	TOP2A Overexpression
ESR1 SNV	

Ovarian Cancer

48 year old female patient.
Cancer had progressed following 3 lines of therapy.

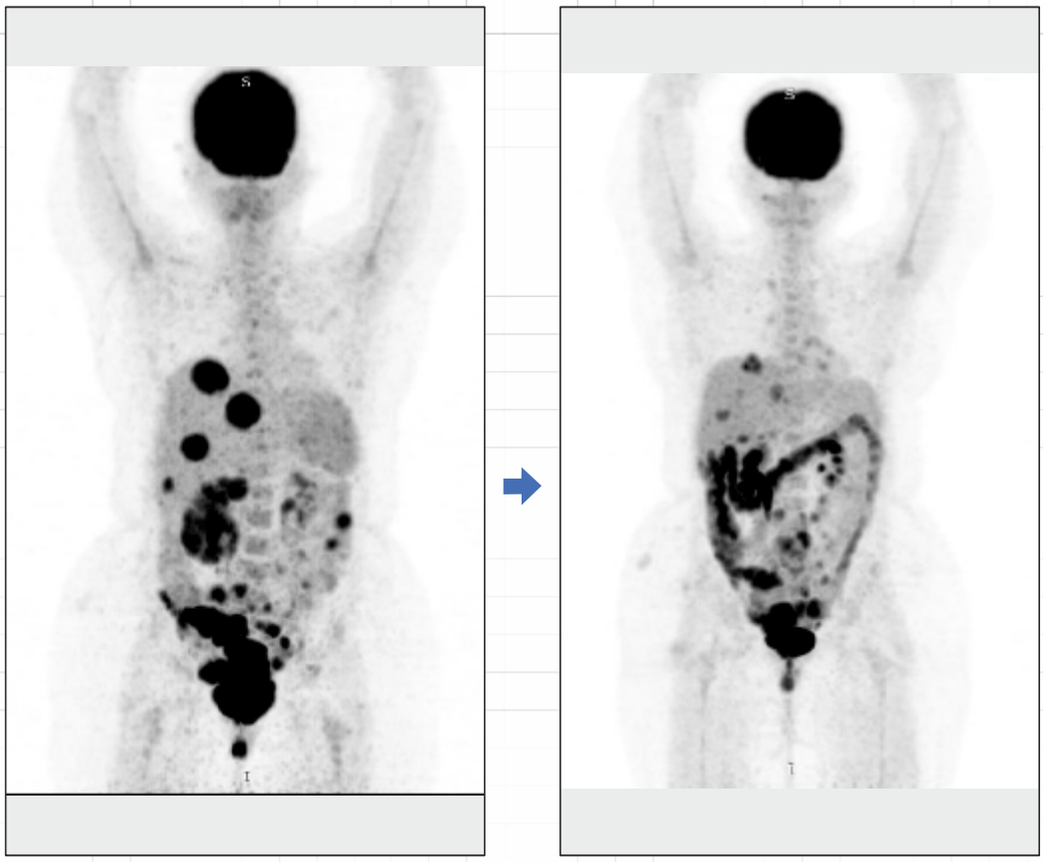


Baseline Follow-up

Molecular Aberrations
GNAS SNV
TP53 SNV
KRAS SNV
EGFR SNV
ALK SNV

Neuroendocrine Tumor of Cervix

41 year old female patient.
Cancer had progressed following 4 lines of therapy.



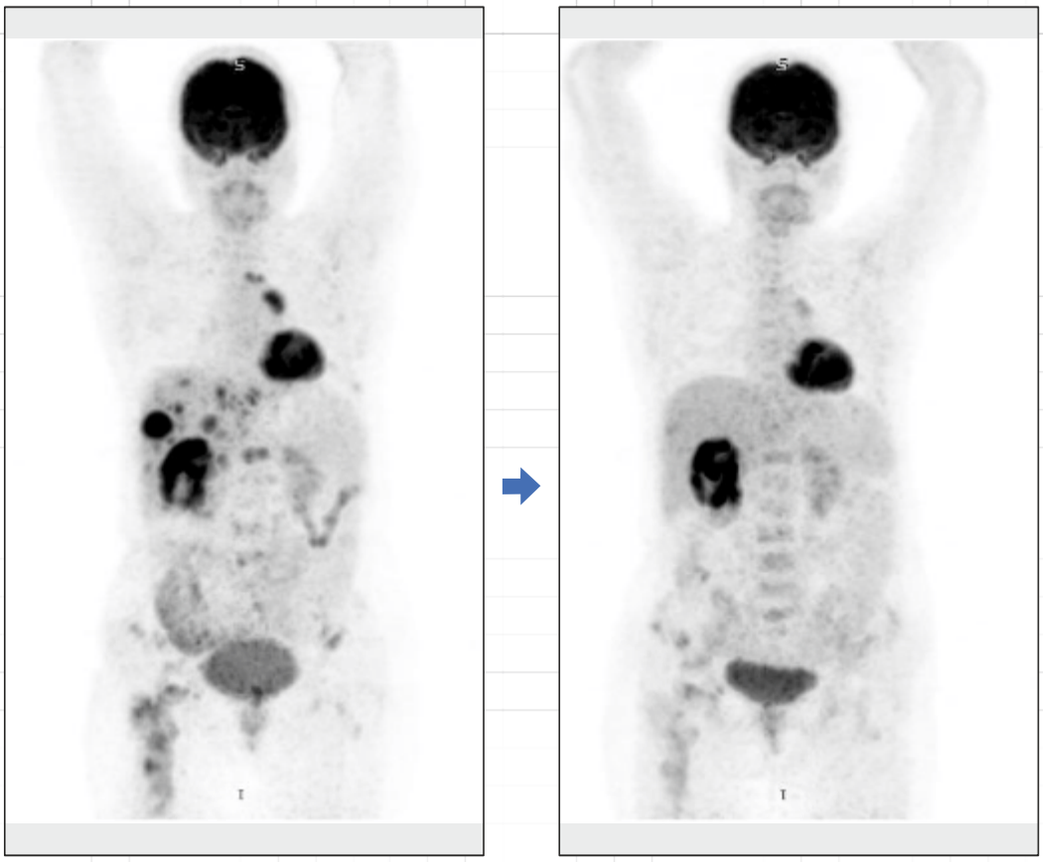
Baseline

Follow-up

Molecular Aberrations
EGFR Overexpression
ERBB2 Overexpression
KIT Overexpression
RET Overexpression

Gallbladder Cancer

32 year old female patient.
Cancer had progressed following 2 lines of therapy.

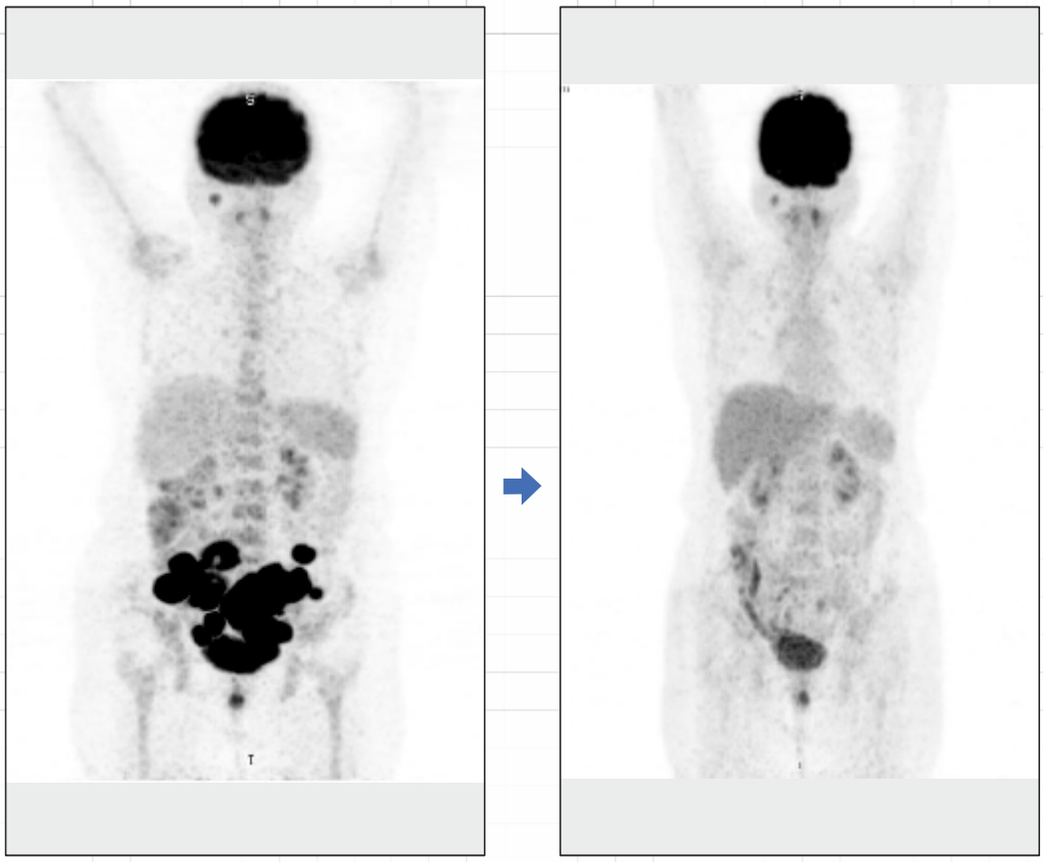


Baseline Follow-up

Molecular Aberrations
TP53 SNV
CDK4 CNV

Leiomyosarcoma.

53 year old female patient.
Cancer had progressed following 2 lines of therapy.

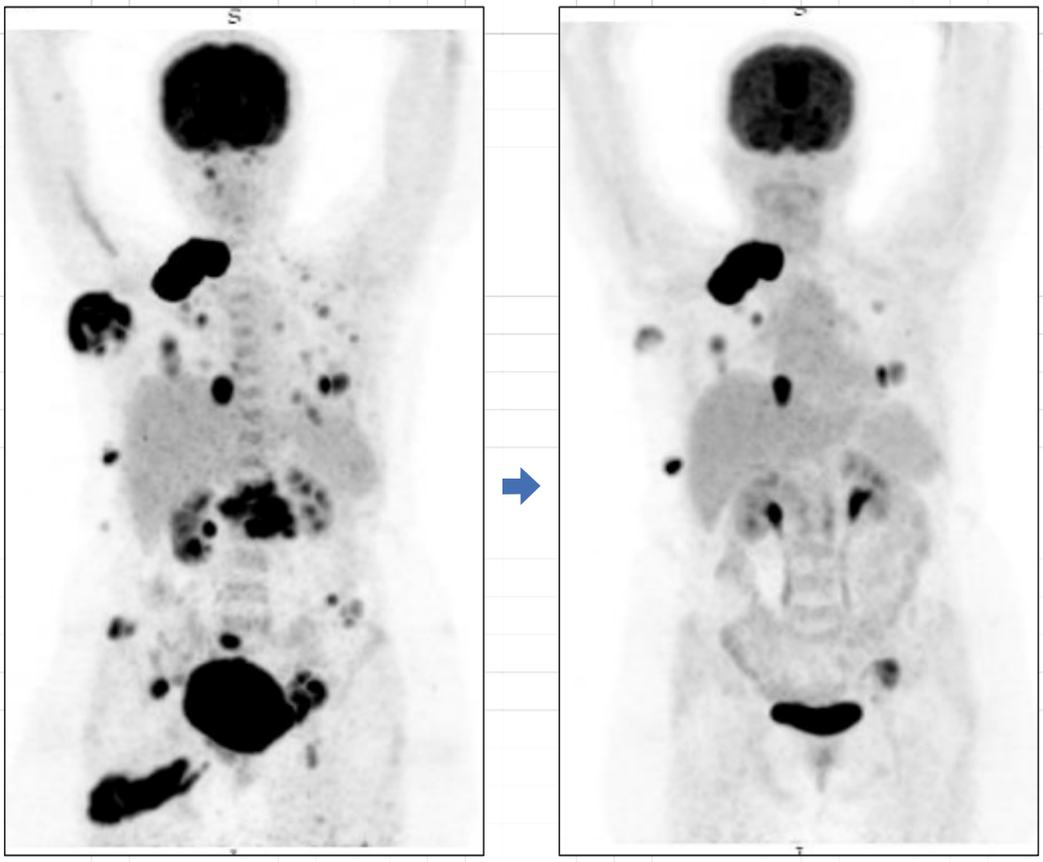


Baseline Follow-up

Molecular Aberrations
TP53 SNV
PTCH SNV

Melanoma

46 year old female patient.
Cancer had progressed following 5 lines of therapy.

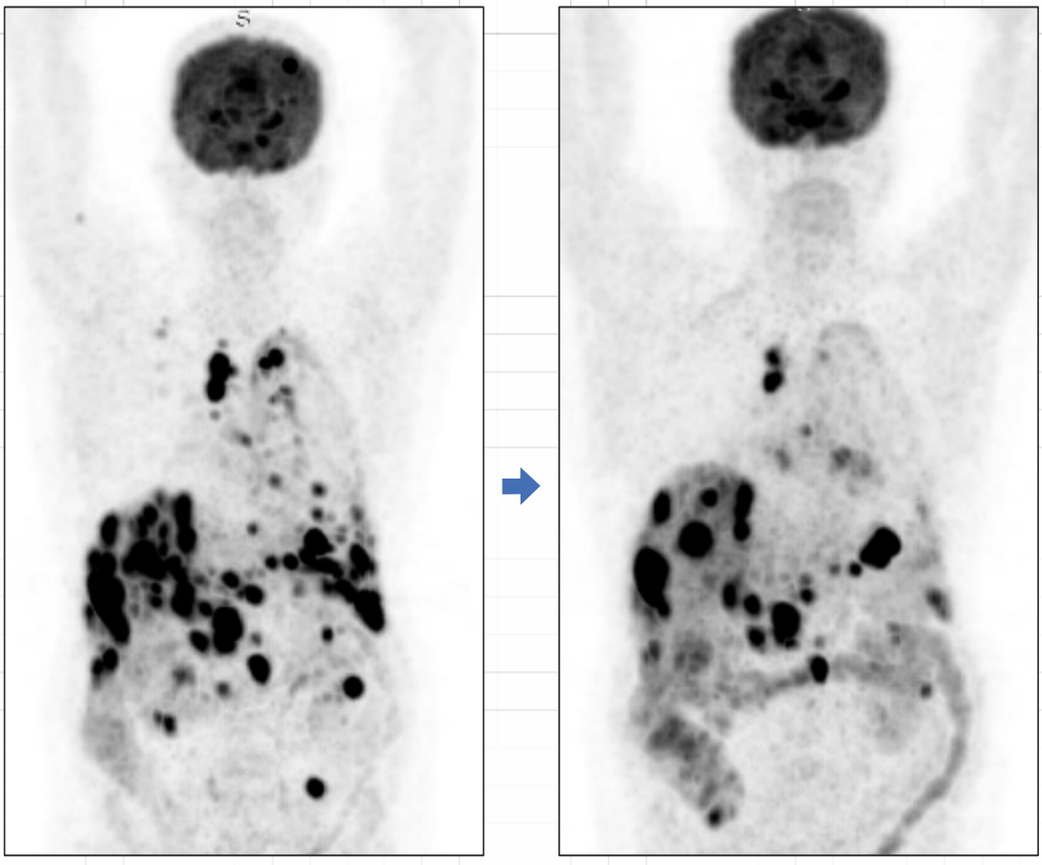


Baseline Follow-up

Molecular Aberrations
PIK3CA SNV
CDKN2A SNV
MYC CNV
PDGFR Overexpression
VEGF Overexpression

Lung Cancer

62 year old male patient.
Cancer had progressed following 4 lines of therapy.

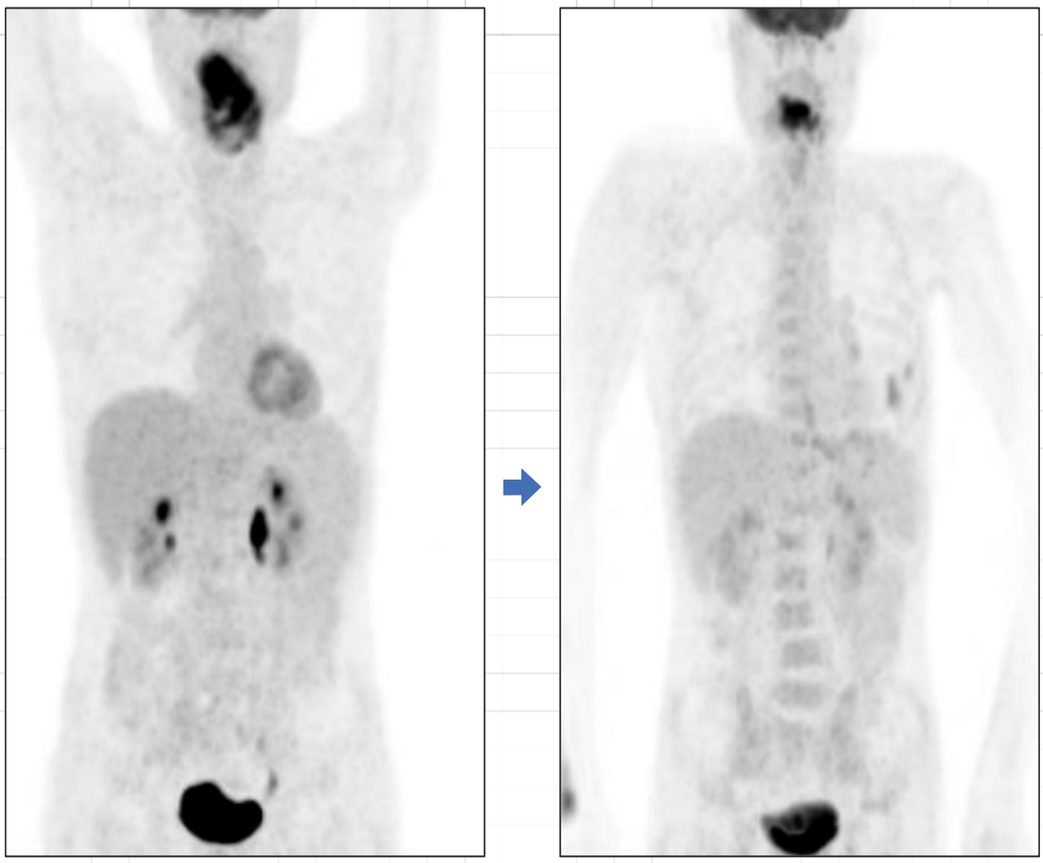


Baseline Follow-up

Molecular Aberrations
PIK3CA SNV
TP53 SNV (x6)
NRAS SNV
TOP2A Overexpression

Head & Neck Cancer

42 year old male patient.
Cancer had progressed following 5 lines of therapy.



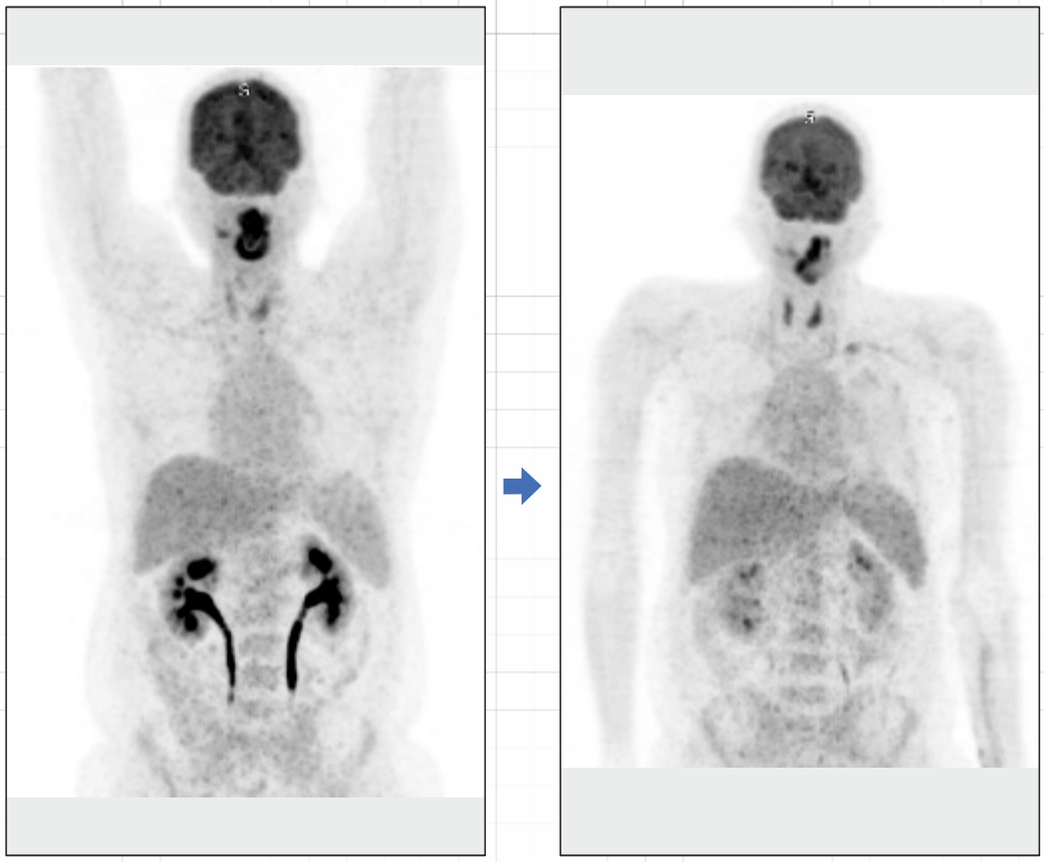
Baseline

Follow-up

Molecular Aberrations
PIK3CA SNV
EGFR SNV + CNV
PTEN SNV
MET SNV

Head & Neck Cancer

51 year old male patient.
Cancer had progressed following 3 lines of therapy.



Baseline

Follow-up

Molecular Aberrations
RB1 SNV (x2)
TP53 SNV (x3)
CDKN2A SNV
TUBB Overexpression
EGFR Overexpression

Head & Neck Cancer

65 year old female patient.
Cancer had progressed following 3 lines of therapy.

Day 0 | Day 30 | Day 79

Molecular Aberrations
PIK3CA SNV
MAF SNV

DATAR
CANCER GENETICS

datarpgx.com

ACCREDITATIONS

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS
Accreditation No.: 1790695

CLIA
Accredited Laboratory
No.: 99D2171983

TÜV SÜD
ISO 27001:2013

TÜV SÜD
ISO 9001:2015

ilac-MRA
Certificate No.: MC-2309

BREAKTHROUGH RESEARCH PRESENTATIONS

1

- Encyclopedic Tumor Analysis Guided Personalized Treatments in Advanced Refractory Malignancies: A Paradigm Shift in Cancer Management.

13th-15, Sept. -2019
Ahmedabad

2

- Circulating Ensembles of Tumor Associated Cells are a Reliable Biomarker of Pancreatic Cancer.

24th-26, Sept. -2019
Stanford, California, USA

3

- In vitro functional interrogation of viable Circulating Tumor Associated Cells (C-TACs) for evaluating Platin resistance.
- Encyclopedic Tumor Analysis for organ agnostic treatment with Axitinib in combination regimens for advanced cancers.

27th Sept. to 1st Oct. -2019
Barcelona, Spain

4

- Diagnostic Non-Invasive Biopsy Can Substitute Conventional Tissue Dependent Procedures in Suspected Cases of Lung Cancer.
- Artificial Intelligence Can Detect Ling Cancer from High Resolution Microscopic Images of Conditioned Peripheral Blood.

10th-12, Oct.-2019
Chicago, Illinois

5

- Clinical Efficacy of Combination Therapies with Androgen Receptor Antagonists for Treatment of Multiple Refractory Cancers.
- Tumor Infiltrating Lymphocytes shows in vitro Cytotoxic Activity Against Tumor Cells in Multiple Cancers.

11th-13, Oct.- 2019
Bangkok, Thailand

6

- Circulating Ensembles of Tumor Associated Cells are Ubiquitous in Breast, Ovarian and Cervical Cancers and Atypical in Asymptomatic Individuals.

3rd - 5, Nov. - 2019
Glasgow, Scotland, UK

BREAKTHROUGH RESEARCH PRESENTATIONS

7

- Encyclopedic Tumor Analysis Guided Treatment with Conventional Drugs Outperform available alternatives in Refractory Cancers.
- Circulating tumor associated cells in esophageal cancer are resistance educated per previous chemo treatments.
- Encyclopedic Tumor Analysis (ETA) guided combination regimens of hormone receptor antagonists with other systemic agents for treatment of refractory cancers.
- mTOR Inhibitors in combination regimens guided by encyclopedic tumor analysis show superior outcomes compared to monotherapy in refractory cancers.

MOLECULAR ANALYSIS FOR PERSONALISED THERAPY CONGRESS MAP

07th-09, Nov.- 2019
London, UK

8

- Diagnostic Non-Invasive Biopsy Can Substitute Conventional Tissue Dependent Procedures in Suspected Cases of Renal Cell Carcinoma.

EMUC19

14th -17, Nov.- 2019
Austria

9

- Diagnosis of Gliomas Using Circulating Glial Cells.
- Clinical Utility of Encyclopedic Tumor Analysis to Treat Patients with Brain Metastasis in Refractory Cancers.
- Prospective, Blinded Plasma Based Analysis for Diagnosis of Newly Diagnosed Glioma.
- In vitro Chemo Resistance Profiles of Circulating Glial Cells Replicate Chemo Characteristics of Tumor Tissue.

SNO Society for NeuroOncology

20th -24, Nov. - 2019
Phoenix, Arizona

10

- Clinical Utility of Encyclopedic Tumor Analysis to Treat Patients Advanced Refractory Head and Neck Cancers.

SINGAPORE 2019 ESMO ASIA

22nd-24, Nov. - 2019
Singapore

11

- In vitro chemo interrogation of viable circulating tumor associated cells from breast cancer patients.
- Circulating Ensembles of Tumor Associated Cells for Detection of Breast Cancer
- Viable Circulating Ensembles of Tumor Associated Cells Persist in Patients with No Radiologically Detectable Disease After Treatment in Breast cancer.

SAN ANTONIO BREAST CANCER SYMPOSIUM
UT Health San Antonio AACR

10th-14, Dec. - 2019
San Antonia, Texas, USA

12

- PD-L1 Profiling of Circulating Tumor Cells is a Viable Companion Diagnostic for Checkpoint Inhibitor Therapy in Lung Cancer.

ESMO IMMUNO-ONCOLOGY
Annual Congress

11th-14, Dec. - 2019
Geneva, Switzerland

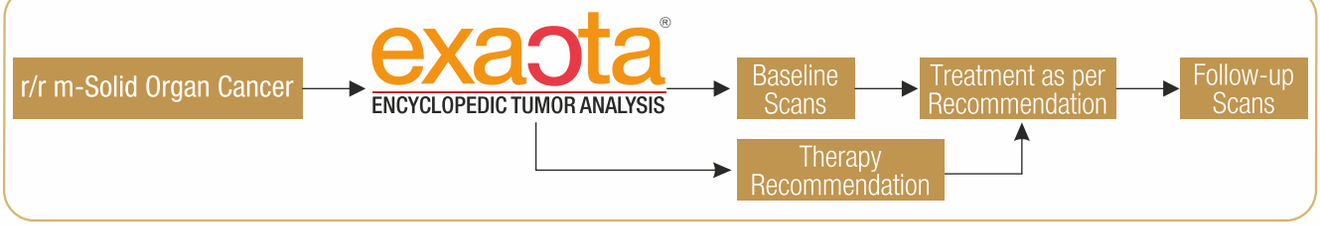
BREAKTHROUGH TECHNOLOGY

For Refractory, Metastatic Solid Organ Cancers

DIFFICULT CANCERS REQUIRE SMARTER STRATEGIES

- Tumors have latent vulnerabilities that can be identified through ETA.
- Agnostic to stage, grade, anatomy or morphology.
- Integrational multi-analyte interrogation of tumor interactome and tumor function.
- Tandem targeting of multiple vulnerabilities of the tumor using combination therapies to yield additive or synergistic benefits.

THE RESILIENT PROTOCOL



SUMMARY OF THE RESILIENT TRIAL

- World's First Precision Oncology Trial based on Integrational Multi-analyte Molecular and Cellular Analysis of Tumor.
- RESILIENT - single arm, open label, phase II / III study.
- Hypothesis: organ- and label- agnostic personalized therapy regimens guided by Encyclopedic Tumor Analysis (ETA) (Exacta) can offer meaningful clinical benefit for patients with relapsed refractory metastatic (r/r-m) malignancies.
- Study Population: Patients with r/r-m solid organ malignancies, disease progression following failure of (≥ 2 lines) systemic treatments.
- Intervention: Patients received personalized treatment recommendations based on ETA of de novo biopsy.
- ETA identified viable treatment options that could yield meaningful clinical benefit in patients with advanced refractory cancers.

DATAR
CANCER GENETICS
datarpgx.com

DATAR
CANCER GENETICS

datarpgx.com

Sitz in Eckersdorf
Schwester-Marie-Weg
11, 95488 Eckersdorf,
Germany.

Devonshire House,
60 Goswell Road,
London, EC1M 7AD,
United Kingdom (UK).

F-8, D Road, Ambad,
Nasik. 422 010,
India.