Multi-Analyte Liquid Biopsies for Treatment Guidance in Advanced Refractory Cancers: Findings of the LIQUID IMPACT Trial

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Abstract

Background

LIQUID IMPACT (CTRI/2019/02/017548) is a single arm, open label, phase II/III study to evaluate the feasibility of providing therapeutic guidance in cancers based on non-invasive interrogation of circulating tumor analytes in peripheral blood (Encyclopedic Liquid Biopsy: eLB). The study enrolled patients with solid organ cancers where the disease had progressed following systemic therapy failure, where no (further) viable standard of care (SoC) therapy options were available, and where invasive biopsies to obtain tumor tissue (for molecular profiling) were contraindicated.

Methods

Encyclopedic Liquid Biopsy (eLB) interrogated gene alterations in cell-free tumor DNA (ctDNA), and differentially expressed genes in exosomal mRNA. eLB also evaluated Circulating Tumor Associated Cells for expression of therapeutically relevant cell-surface signaling receptors as well as the chemoresistance profile towards systemic anticancer agents. Patients who received personalized combination therapy regimens based on eLB findings were evaluated radiologically to determine response to treatment, Objective Response Rate (ORR) and Quality of Life (QoL).

Results

At the time of submission of this manuscript, Partial Response (PR) was observed in 14 of 43 patients evaluable *per protocol* (ORR = 32.6%). Majority of patients reported stable to improved QoL.

Conclusion

The present study demonstrated that refractory cancers have latent vulnerabilities which can be identified via non-invasive eLB to design personalized label- and organ-agnostic treatment regimens to yield meaningful treatment benefit.

Trial registration

Clinical Trial Registry – India, CTRI/2019/02/017548 [Registered on: 02/08/2019] - http://ctri.nic.in/Clinicaltrials/pdf_generate.php?trialid=31265&EncHid=&modid=&compid=%27,%2731265det%27; Trial Registered Prospectively.

Background

The outcomes of the RESILIENT trial[1] conclusively established that an integrational multi-analyte evaluation tumor tissue (Encyclopedic Tumor Analysis: ETA) can facilitate safe and efficacious treatment options in refractory cancers to yield significant response rates. ETA required access to fresh tumor tissue, which was obtained by an invasive biopsy. During the course of the trial, the investigator encountered patients who were unable to undergo an invasive biopsy for various reasons such as proximity of the lesion to vital organs, co-morbidities or patients' reluctance. Outside of the setting of a clinical trial, patients may also refuse an invasive biopsy due to procedural expenses. Where invasive biopsies are indicated and feasible, they are invariably associated with various procedural risks[2,3].

Though the potential of non-invasive liquid biopsies (LBx) has been described previously[4], there are presently no meaningful clinical applications of LBx apart from the limited scope of companion diagnostics which evaluate a predefined set of mutations in cell free tumor DNA (ctDNA) for selection of single targeted therapy agents in a few cancers[5]. We hypothesized that combining the strengths of ETA with the convenience and safety of LBx can not only yield viable treatment guidance, but also significantly mitigate the risks and expenses linked to invasive biopsies. Based on this hypothesis, we developed an Encyclopedic Liquid Biopsy (eLBx) which interrogated multiple tumor-derived (circulating) analytes in peripheral blood.

Tumors secrete various components into the vasculature such as DNA, sub-cellular vesicles and intact viable malignant cells[6] which remain in circulation for finite period of time and may be harvested for downstream interrogations which can reveal vulnerabilities of the cancer. Circulating Tumor Associated Cells (C-TACs) can be harvested and profiled by immunocytochemistry (ICC) to reveal expression of targetable signalling receptors, e.g., VEGF(R), AR or HER2. Viable C-TACs can also be assayed *in vitro* to determine chemoresistance characteristics of the cancer against a panel of systemic anticancer agents. These investigations provide a deeper understanding of the tumor interactome to design patient-specific treatment regimens without the need for an invasive biopsy. The LIQUID-IMPACT trial was designed to evaluate this hypothesis. We present data from this trial whish shows that it is possible to achieve a significant Objective Response Rate (ORR) in patients with refractory malignancies based on an integrational non-invasive battery of investigations.

Methods

Study Design

LIQUID IMPACT (CTRI/2019/02/017548) was a single arm, single centre, non-randomized phase II/III prospective trial for evaluation of treatment response to therapy based on eLBx recommendation in patients with refractory malignancies where an invasive biopsy to obtain tumor tissue (for molecular and functional profiling) was contraindicated. The trial was approved by the Institutional Review Boards (IRB) and Ethics Committees of the Study Sponsor (Datar Cancer Genetics, DCG) as well as the trial site (HCG-

Manavata Cancer Centre, HCG-MCC). The trial was conducted in accordance with all applicable ethical guidelines and the Declaration of Helsinki. Details of the trial are available at WHO-ICTRP.

Patients

The trial recruited patients with solid organ cancers who had either failed at least two prior lines of Standard of Care (SoC) treatments **or** where (further) SoC treatment options were unviable, **and** where an invasive biopsy to obtain tumor tissue (for molecular / functional profiling) was contraindicated. Eligible patients had radiologically detectable and measurable lesions with an Eastern Co-operative Oncology Group (ECOG) performance status of ≤ 2 and fitness as ascertained by the treating clinician. Patients who fulfilled the above criteria were counselled regarding the potential benefits and risks of the trial. Thereafter, patients who provided signed, informed consents were enrolled.

Blood Collection

20 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.

Isolation circulating tumor DNA (ctDNA)

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).

Exosomal mRNA Isolation

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 extraction of total exosomal RNA using an ExoRNeasy serum/plasma kit (QIAGEN, Germantown, USA) according to the manufacturer's protocol. Purified exosomal RNA was quantified via the miRNA Qubit assay (Life Technologies, Carlsad, USA).

Molecular Profiling of the Cancers

Profiling of mutations in ctDNA was performed as described previously[1]. Gene expression profiling of exosomal mRNA was performed as described previously[1].

Enrichment of Circulating Tumor Associated Cells

C-TACs were enriched and harvested from Peripheral Blood Mononuclear Cells (PBMCs) as described previously[9]. Briefly, PBMCs were obtained from whole blood and treated with an epigenetically activating media for up to 100 hours at 37°C under 5% CO_2, 4% O_2. This process induces cell death in normal (non-malignant) cells with functional apoptotic machinery while simultaneously conferring survival privilege

on apoptosis-resistant cells of tumorigenic origin, i.e. Circulating Tumor Associated Cells (C-TACs) and their heterotypic clusters (C-ETACs: Circulating Ensembles of Tumor Associated Cells). Enriched and harvested C-TACs were identified by immunocytochemistry (ICC) profiling.

Immunocytochemistry (ICC) Profiling of C-TACs

Harvested C-TACs were used for preparation of cytospin slides by using standard procedures. One slide was used for identification of C-TACs by immunofluorescent staining using anti-EpCAM, anti-panCK and anti-CD45 antibodies, as well as DAPI to confirm intact (nucleated) cells. Additional slides were used for ICC profiling for expression of theranostic cell surface antigens including AR, ER, HER2, VEGF, EGFR, VEGFR and PDGFR. Cells were fixed with 4% paraformaldehyde solution (pH 6.9) for 20 min at room temperature with intermittent vertexing. Cell permeabilization was achieved with 0.3% Triton-X 100 for 15 min and was followed by blocking using 3% BSA for 30 min. Cells were immunostained with primary antibodies at room temperature for 60 min. Cells were washed with PBS (pH 7.4) and then incubated with appropriate secondary antibodies. Cells were washed with PBS (pH 7.4) and then incubated with 4',6-Diamidino-2-phenylindole dihydrochloride (DAPI) in a dark at room temperature for 15 min. Positive and negative cell line controls were also processed with each batch of samples. Plates were scanned by Cell Insight CX7 High-Content Screening (HCS) Platform (Thermo Fisher Scientific, USA). All antibodies were used at manufacturer recommended dilutions with dilutions being prepared in manufacturer provided or recommended dilution buffers.

In Vitro Chemosensitivity Profiling of Viable C-TACs

Approximately 100 C-TACs/well were seeded into 96 well culture plates and incubated for 24 hours (37°C, 5% CO_2, 4% O_2). Viable cells were stained with Calcein AM and treated with optimized concentrations of a panel of cytotoxic anticancer agents. Each plate included control wells where cells were not exposed to drugs to determine baseline cell mortality levels. The plates were placed in the on stage incubator of fluorescent microscope EVOS M7000 (Thermo Fisher Scientific) at 37°C with 5% CO_2, 4% O_2 and the wells imaged every 10 min for 12 h. Wells were imaged every 10 minutes for 12 hours and the extent of cell death was determined based on cell morphology changes and time required for fade out of live cell tracking dye in selected frames.

Therapy Recommendation and Treatments

Patient-specific therapy recommendations (TR) were generated as described previously[1] and provided to the treating clinicians (TC) within 7 to 10 days of receipt of patients' blood samples. Individual TRs were evaluated by the TC, who also reviewed fitness of each patient based on the clinical history of prior treatments and known therapy related adverse events (AEs). Drug safety information was referred to generate patient-wise list of expected AEs which guided appropriate starting dose as well as dose escalation in each patient. In all patients, the treatment agents were initially administered at lower (≤50%) doses, and sequentially escalated based on an individualised dose escalation schedule. Other factors which guided patient-specific dosage and schedule included institutional guidelines and protocols

as well as clinical assessment of patients' health. Patient-specific regimens were administered as per the standard practice and protocols of the study site until either disease progression, death or dose limiting toxicity was encountered. Patients who showed durable response were maintained with suitable dose reduction as decided by the treating clinician.

Evaluations

Patients underwent an ^{18}F-Fluorodeoxyglucose Positron Emission Tomography – Computed Tomography (FDG PET-CT) scan before initiation of treatment to determine baseline status of disease. A Magnetic Resonance Imaging (MRI) scan was performed to identify any brain metastases. Response was evaluated on the lines of RECIST 1.1 criteria[7] from follow-up scans following at least two treatment cycles or 60 days of treatment, except in cases where the treating clinician advised evaluation in the interim.

Patient Monitoring

All patients underwent periodic investigations including complete blood counts, hepatic and renal function tests, urinalysis and left ventricular ejection fraction (LVEF) to assess fitness for treatment as per study protocol. Adverse events (AEs) were recorded every week either during patient admissions or by telephonic follow-up. All AEs were reported as per NCI-CTCAE v5 criteria[8]. Grade 3 and above AEs, if any, were followed up till resolution. Patients had a 24-hour telephonic access to study-coordinators, emergency services and ambulance.

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. Progression Free Survival (PFS) was also evaluated in patients and was defined as time from commencement of treatment under ETA to disease progression or death during the active study phase. Quality of Life (QoL) was evaluated based on patient's feedback on symptomatic and functional status at baseline and at study termination or most recently available follow-up.

Patient Follow-Up

Patients were followed up until study termination or patient exclusion (death / loss to follow-up / withdrawal of consent), whichever was earlier to determine Progression Free Survival (PFS).

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 20 patients were required to accrue; if there were 2 or fewer responses, the study was required to be stopped. Else, at least 20 additional patients were required to accrue for a total minimum of 40 patients. The null hypothesis would

be rejected if 6 or more responses were observed in 40 patients. With 40 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.

Results

Patients

Between Feb 2019 and Oct 2019, 74 patients were screened for recruitment, of whom, 65 patients were recruited (received therapy recommendations) and 51 patients eventually started treatment as per eLBx; 23 patients were excluded prior to start of treatment for various reasons including withdrawal of consent (n = 4), death (n = 1), deterioration of ECOG performance status (n = 9), unfit for therapy (n = 5) or unavailability of lesions measurable on a CT / PET-CT scan (n = 3). Out of the 51 patients who started treatment, 8 patients were excluded within the first week (prior to any follow-up evaluation) for various reasons including patient being lost to follow-up (n = 1), death (n = 2), withdrawal of consent (n = 1) and deterioration of ECOG performance status (n = 4). A total of 43 patients were evaluable as per study criteria at the time of submission. The CONSORT diagram (Fig. 1) depicts the study structure and patient flow. Patient demographics are provided in Table 1.

Table 1
Baseline Characteristics of Intent to Treat (ITT) and Evaluable Patients.

Parameter	ITT	Evaluable
Total Patients	51	43
Gender	28	26
Male	23	17
Female		
Age	49 (19–72)	49 (27–72)
Median (Range)		
Cancer Types and Organs	5	4
Breast	6	5
Colorectal	1	1
Endometrium	10	9
Head and Neck	4	3
Liver	5	5
Lung	1	1
Occult Primary	4	4
Oesophagus	5	3
Ovary	1	0
Pancreas	1	1
Prostate	2	2
Sarcoma	2	1
Skin	2	2
Stomach	2	2
Testes		
Prior Therapies	4 (2–9)	4 (2–9)
All Therapies: Median (Range)	3 (1–7)	3 (1–7)
Systemic Therapies: Median (Range)	32	25
Surgery (number of patients)	26	23
Radiation (number of patients)		

Treatments

Among the 43 patients (evaluable) who received eLBx-guided treatment, 9 patients received combinations of cytotoxic agents, 1 patient received combinations of targeted agents and 33 patients received combinations of cytotoxic and targeted agents. Endocrine therapy agents were administered to 2 patients in addition to cytotoxic and targeted agents. Patient-wise details of prior treatments, eLBx- indications and ETA-treatments are provided in Supplementary Table S1.

Response to Treatment

Among the 43 patients who were evaluable *Per Protocol*, Partial Response was observed in 14 patients (ORR = 32.6%) (Table 2). Waterfall Charts depict the best response (Fig. 2) of all 43 patients. Patients were followed up for a median duration of 3.8 months. Median Progression Free Survival (mPFS) was 3.8 months (95%CI: 2.95–4.55 months, data censored at the date of submission). The Kaplan Meier plot of PFS is depicted in Fig. 3.

Table 2
Clinical Activity of eLbx-guided therapies.

Parameter	Value
Status at Study Completion	0 (0%)
Complete Response (%)	14 (32.6%)
Partial Response (%)	18 (41.9%)
Stable Disease (%)	11 (25.6%)
Disease Progression (%)	
Progression Free Survival (days)	108
Median	(28–287)
Range	

Adverse Events

All the 51 patients in the Intent to Treat (ITT) population were evaluated for therapy-related AEs (Table 3). There were no Grade IV treatment related AEs or any treatment related deaths. The most common AEs (any grade) reported in ≥ 10% patients were Fatigue, Pain, Anorexia, Pyrexia, Edema, Mucositis Oral, Neutropenia, Anemia, Vomiting, Dyspnoea, Diarrhoea, Peripheral neuropathy, Nausea, Rash, Thrombocytopenia, Constipation and Hemorrhage. Grade 3 AEs in ≥ 10% patients were Fatigue, Pain, Anorexia, Mucositis Oral, Anemia, Edema, Neutropenia and Dyspnoea. Haematological toxicities of any grade were observed in 29 patients while grade 3 haematological toxicities were observed in 14 patients. Grade 3 therapy-related AEs which necessitated dose adjustments were reported in 28 patients. Onset of therapy related AEs was observed approximately up to 7 days post-therapy and time to resolution was up

to 2 weeks. All AEs were managed by administration of standard of care agents or procedures as required. There were no treatment-related mortalities.

Table 3
Therapy-related Adverse Events in Intent to Treat Population.

Adverse Event	Any Grade	Grade III
Anorexia	30	12
Pyrexia	21	2
Mucositis	12	6
Edema	12	5
Neutropenia	12	5
Anemia	11	6
Vomiting	11	1
Diarrhoea	9	3
Peripheral Neuropathy	9	2
Nausea	7	0
Thrombocytopenia	6	3
Constipation	5	0
Paraesthesia	4	0
Hypotension	3	0
ANY	**46**	**28**

Quality of Life (QoL)

Quality of Life was measured based on a brief questionnaire that evaluated the patients' functional and symptomatic status as well as Eastern Co-operative Oncology Group (ECOG) Performance Status. Patients' feedback was obtained at baseline and every month. Within the evaluable cohort, 86% of patients indicated stable to decreased symptomatic status while 54% of patients indicated stable to improved functional status.

Discussion

Findings of the LIQUID IMPACT trial shows that non-invasive multi-analyte liquid biopsies can reveal latent actionable vulnerabilities of the cancer, which can be targeted with patient-specific combination

regimens to yield clinical benefits even in heavily pretreated populations where SoC options are unavailable.

We have previously reported the findings of the RESILIENT trial[1] which exposed the fallacy of the 'single drug based on a single molecular alteration' paradigm of several prior precision oncology trials[9–14], by demonstrating that multi-analyte tumor profiling can reveal latent weaknesses of the tumor and guide efficacious and safe treatment selection in advanced refractory solid organ cancers, especially in those cases where no actionable molecular alterations were available. We also acknowledged a limitation of the study in requiring fresh tissue from a *de novo* biopsy tissue, since the quality and quantity of biopsied tissue could be of concern. Obtaining representative tissue is a requirement of not just ETA, but also for routine workup in oncology including histopathological evaluation (HPE) and immunohistochemistry (IHC). Factors which impact tumor tissue procurement include inaccessibility of the tumor, proximity of the tumor to vital organs or vasculature, patients' comorbidities or even patients' reluctance due to fear of pain or procedural complications. Where a biopsy is feasible and advised, there may be significant procedural risks, e.g., for biopsies of lung lesions are associated with risks of pneumothorax and pulmonary hemorrhage[15–17]. Other complications of biopsies include sepsis, air embolism, bile leak, viscus perforation and risk of tumor seeding[18,19]. We observed that patients who have progressed on multiple lines of treatment are often psychologically fatigued for further invasive procedures and possible hospitalization. Based on this background, we designed an Encyclopedic Liquid Biopsy (eLBx) which aimed to offer all the be benefits of a multi-analyte investigation as in case of the ETA, coupled with the safety and convenience of a simple blood draw thus avoiding all the risks of an invasive biopsy.

The primary endpoint of the trial was Objective Response Rate. While Complete Response (CR) was not observed in any patient until the date of submission, Partial Response (PR) as per RECIST 1.1 was observed in 14 out of 43 patients evaluable *per protocol*, yielding an Objective Response Rate (ORR) of 32.6%. Though not an endpoint of the study, Stable Disease (SD) for more than 60 days was observed in 18 other patients. yielding a Disease Control Rate (DCR) of 74.4% in the evaluable population. Median Progression Free Survival (mPFS) was 3.8 months. Among the evaluable cohort, PFS rates were 74%, 48% and 44% at 3 months, 6 months and 12 months respectively.

The outcome data from the RESILIENT trial as well as the present study demonstrate the clinical utility and efficacy of integrational multi-analyte-based approach (ETA / eLBx) in making available efficacious personalized combination regimens even in a heavily pretreated cohort of patients where further SoC options are unavailable. In routine clinical setting, where the SoC options have been exhausted, patients are often offered clinician's choice of treatment regimens. Such treatment options may be label-agnostic and based on prior observations but lack evidence in support of efficacy and safety. On the other hand, the advantage of ETA / eLBx lies in providing the clinician with contemporaneous evidence on the tumors strengths and vulnerabilities. Thus, non-SoC treatment decisions based on such evidence have a higher probability of treatment success and clinical benefits than arbitrary 'roll of the dice' selection of treatment agents.

Apart from efficacy, safety of eLBx-guided treatments was an equally important consideration. Various prior meta-analyses[20–22] have reviewed the safety profile of multi-drug anticancer regimens and note that it has been largely possible to administer *de novo* drug combinations safely in most patients and that these patients had a manageable profile of adverse events (AEs). While the profile of Adverse Events (AEs) may not be predicted in any given patient prior to therapy administration, it is possible to anticipate AEs based on available literature as well as the history of AEs in each patient. As was observed during the RESILIENT Trial as well as in the present trial, patients where the cancer had progressed following failure of multiple systemic lines of therapy tend to be physiologically fatigued due to accumulated toxicity of prior treatments. This toxicity reflected as the patient drop-out between screening and start of therapy, as well as prior to the first follow-up scan. Thus, this heavily pretreated cohort was at an inherently higher risk of AEs due to cumulative toxicities from prior treatments. However, the administration of eLBx-guided therapy regimen did not appear to increase the risk of toxicity since there were no grade IV therapy related AEs or any treatment related mortalities. Grade III AEs, where observed, were limited and manageable by administration of standard procedures.

Patients were also assessed for Quality of Life at baseline and every month based on symptomatic and functional status; alleviation of disease related symptoms and improvements in functional status implied positive findings. Based on these parameters, all patients reported stable status or improvements in either of these parameters.

Challenges for eLBx may arise where patients are weak and unable to provide blood (inability to locate vein), in which case they would also be unfit for any treatment. In the present study, several patients were excluded prior to start of therapy as well as during the first week on therapy due to accumulated toxicitues from prior treatments. However, adoption of ETA- or eLBx-guided treatments at an earlier treatment stage could obviate such limitations associated with less beneficial prior SoC regimens. One challenge in the present trial (as was also reported in the RESILIENT Trial) was the non-availability of USFDA approved treatment agents for incorporation in the TR, as several such drugs are not approved in India and possibly in several other countries.

In conclusion, the LIQUID IMPACT Trial aimed to improve on the limitations faced during the RESILIENT trial and has been largely successful in being able to provide safe, efficacious treatment options based on non-invasive evaluations of the cancer in heavily pretreated patients.

Abbreviations

eLB
Encyclopedic Liquid Biopsy
PFS
Progression Free Survival
OS
Overall Survival

ORR
Objective Response Rate
QoL
Quality of Life
ctDNA
cell-free tumor DNA
CR
Complete Response
PR
Partial Response
ETA
Encyclopedic Tumor Analysis
LBx
Liquid biopsies
C-TACs
Circulating Tumor Associated Cells
C-ETACs
Circulating Ensembles of Tumor Associated Cells
ICC
immunocytochemistry
SoC
Standard of Care
ECOG
Eastern Co-operative Oncology Group
PBMCs
Peripheral Blood Mononuclear Cells
TR
Therapy recommendations
AEs
adverse events
PET-CT
Positron Emission Tomography – Computed Tomography
MRI
Magnetic Resonance Imaging

Declarations

Ethical statement

The trial was approved by the Institutional Review Boards (IRB) and Ethics Committees of the Study Sponsor (Datar Cancer Genetics, DCG) as well as the trial site (HCG-Manavata Cancer Centre, HCG-MCC).

The trial was conducted in accordance with all applicable ethical guidelines and the Declaration of Helsinki. Details of the trial are available at WHO-ICTRP. All enrolled patients provided signed informed consent for study participation and publication of deidentified data.

Data availability Statement

All datasets generated for this study are included in the main article and supplementary information. Deidentified data may be made available by the corresponding authors upon reasonable request, and may require the execution of appropriate non-disclosure agreements.

Author Contributions

RN: Principal Investigator, Overall Study Oversight, Counselling of Patients, Review of Treatment Recommendations and Clinical Management; D.P., and D.A.: Design of Study Protocol, Review of Treatment Recommendations, Review of AEs, Data Analysis and Drafting; T.C. and V.D.: Review of Data and Drafting; S.R., S.K. and V.P.: Counselling of Patients, Review of Treatment Recommendations and Clinical Management. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

TC have no conflict of interest to declare. DP and VD are in the employment of the sponsor. The entire team from HCG-Manavata Cancer Centre, Nasik, viz. RN, SR, SK and VP report grants from DCG 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.

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Figures

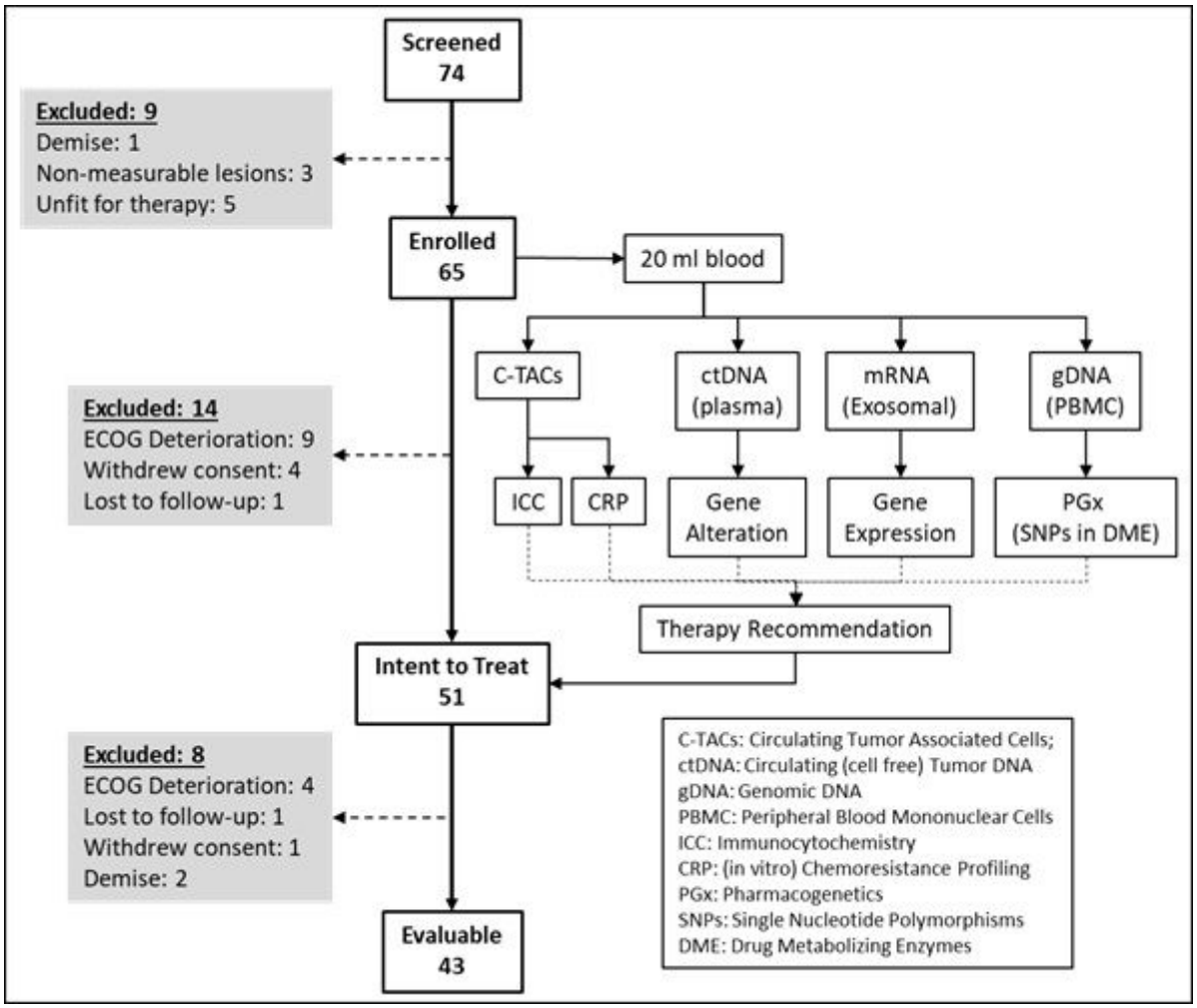

Figure 1

CONSORT Diagram. Among the 74 patients who were screened, 51 eventually started treatment and 43 were finally evaluable based on study criteria. Patients provided blood samples at baseline which was used for multi-analyte cancer profiling (eLBx: Encyclopedic Liquid Biopsy).

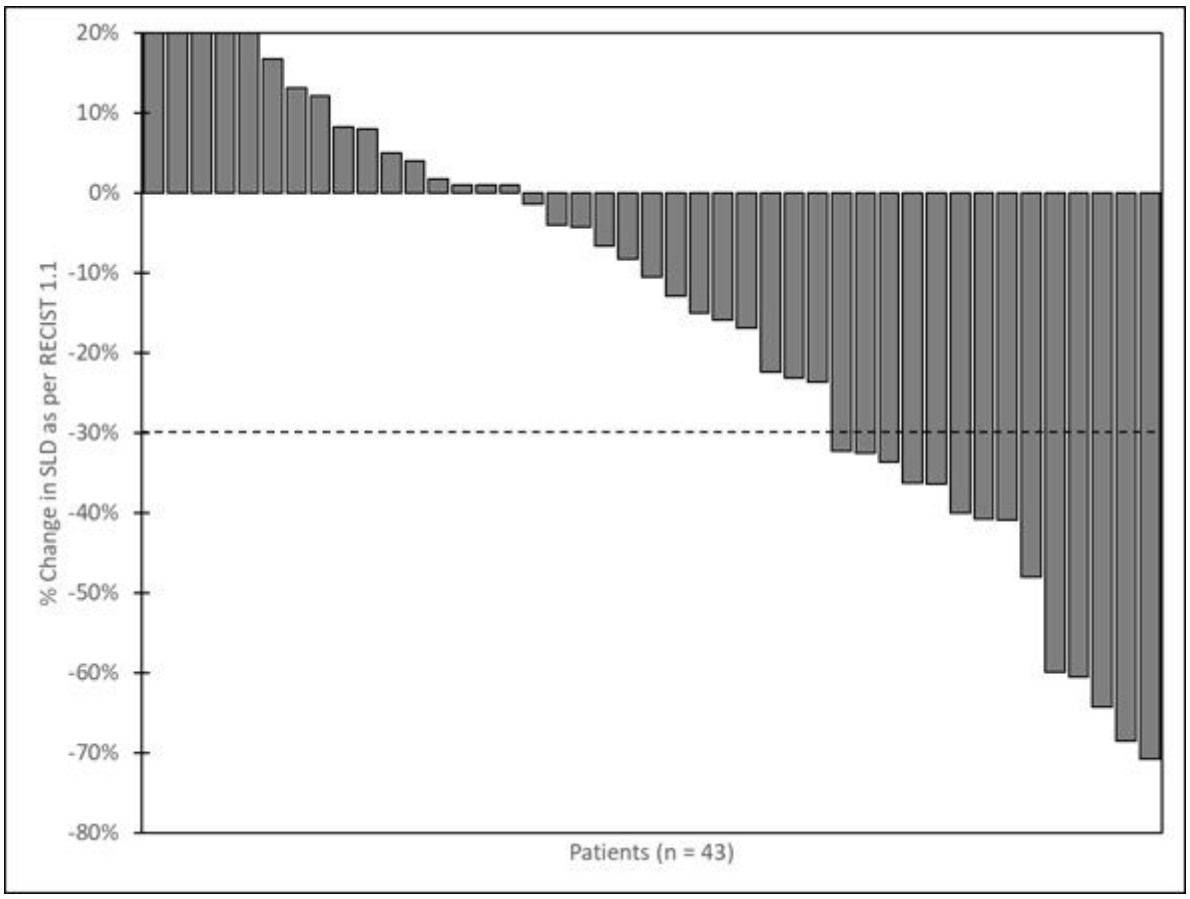

Figure 2

Summary of Outcomes. 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 best response are represented. Patients are arranged in descending order of change (%) in SLD.

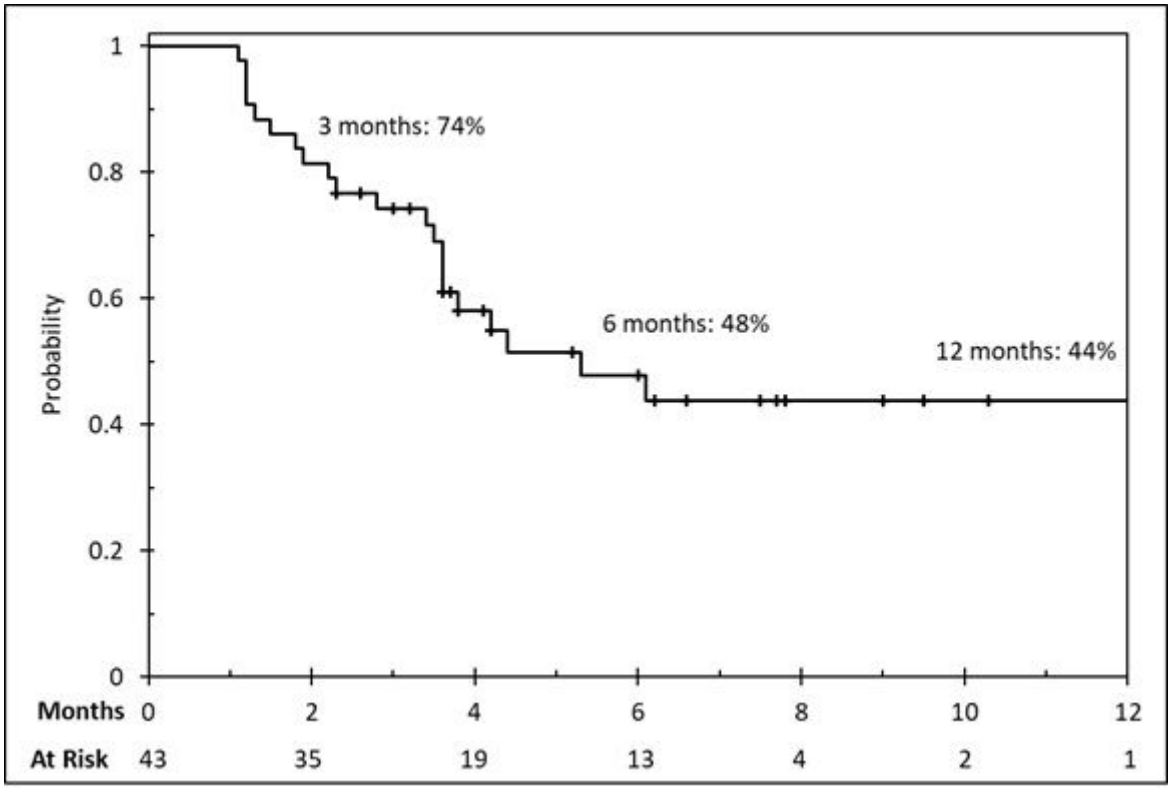

Figure 3

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation.xlsx