THE IMPACT OF CONCORDANCE BETWEEN LIQUID AND TISSUE BIOPSY FOR ACTIONABLE MUTATIONS: INSIGHTS FROM THE ROME TRIAL

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Potential Competing Interest

AB had a role in advisory boards and Steering Committee and has been an invited speaker for Novartis, Roche, Lilly, Pfizer, Astra Zeneca, Daiichi Sankyo, Gilead, Merck Sharp & Dohme, Bristol-Myers Squibb and Gentili. CC declared no competing interests. SS was an invited speaker for Pfizer, Lilly, Novartis, Daiichi Sankyo, Gilead, Roche, Astra Zeneca. LF reports speaking honoraria from Incyte, Bristol-Myers Squibb, Lilly. He received institutional research fundings from Merck Sharp & Dohme, Bristol-Myers Squibb, Astra Zeneca, Incyte, BeiGene, Astellas, Daiichi Sankyo, Roche. He had a role in advisory board for Merck Sharp & Dohme, Astra Zeneca, Incyte, Taiho, Servier, Daiichi Sankyo, Lilly, Astellas. MB is employed in the Italian National Institute of Health (Istituto Superiore di Sanità) and is an unpayed member of the Technical and Scientific Committee of the Italian Medicine Agency (AIFA). SL was an invited speaker for Amgen, Astra Zeneca, Bristol-Myers Squibb, Incyte, GlaxoSmithKline, Lilly, Merck Serono, Merck Sharp & Dohme, Pierre Fabre, Roche, Servier and had a role in advisory board for Amgen, Astellas, Astra Zeneca, Bayer, Bristol-Myers Squibb, Daiichi Sankyo, GlaxoSmithKline, Incyte, Lilly, Merck Serono, Merck Sharp & Dohme, Servier, Takeda, Rottapharm, Beigene, Fosun pharma and Nimbus Therapeutics. LF reports speaking honoraria from

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Incyte, Bristol Myers Squibb, Lilly; institutional research funding from MSD, Bristol Myers Squibb, AstraZeneca, Incyte, BeiGene, Astellas, Daiichi Sankyo, Roche; participation in advisory board for MSD, AstraZeneca, Incyte, Taiho, Servier, Dalichi Sankyo, Lilly, Astellas. VG has role in leadership for AstraZeneca, Daiichi Sankyo, Eli Lilly, Exact Sciences, Gilead, MSD, Novartis, Pfizer, Olema Oncology, Pierre Fabre, Menarini Stemline. She had a role as a speaker for AstraZeneca, Daiichi Sankyo, Eli Lilly, Exact Sciences, Gilead, GlaxoSmithKline, Novartis, Roche, Zentiva and Menarini Stemline and was an Expert Testimony for Eli Lilly. UDG received consultation fees from Amgen, Astellas Pharma Inc., Astra Zeneca, Bayer, Bristol Myers Squibb, Esai, Ipsen, Johnson & Johnson Innovative Medicine (formerly Janssen), Merck KGaA, Merck Sharp & Dohme, Novartis, and Pfizer Inc and travel expenses for atteding symposia from AstraZeneca, Ipsen, and Pfizer Inc. PA, GB, GD, MA, PC, EC and MC declared no competing interests. SP had a role as consulting/advisor for AstraZeneca, Eli Lilly, Daiichi Sankyo, Novartis, Pfizer, Seagen, Sophos, Gilead and had travel and accommodation fees from Daiichi Sankyo, Pfizer, AstraZeneca, Eli Lilly, Menarini, Novartis and Gilead. SV had a role as speaker for Novartis. RB declared no competing interests. SB had congress travel support from Merck Sharp & Dohme, Pfizer, bayer, ipsen and had a role in advisory board and steering committee for Pfizer, Bristol-Myers Squibb, Merck Sharp & Dohme, Astellas, Roche, Johnson&Johnson, Ipsen, Bayer, Novartis, Merck, Astra Zeneca, Gilead. GB declared no competing interests. LDM received personal grant for advisory/ consultant/ speaker activities from: Agendia, Astrazeneca, Daiichi Sankyo, Eli Lilly, Eisai, Exact Sciences, Gilead, GlaxoSmithKline, Ipsen, Roche, Seagen, Menarini, Stemline, Merck Sharp & Dohme, Novartis, Olema, Pierre Fabre and Pfizer. ADC declared no competing interests. AF had financial fees from Roche, Menarini, Pfizer, Lilly, Novartis, Daiichi Sankyo, Astra Zeneca, Gentili. EF, SG, GM, AP and DP declared no competing interests. FP received honoraria for advisory boards, activities as a speaker, travel grants and research grants from Amgen, Astra Zeneca, Daiichi Sankyo, Celgene, Eisai, Eli Lilly, Exact Sciences, Gilead, Ipsen, Italfarmaco, Menarini, Merck Sharp & Dohme, Novartis, Pierre Fabre, Pfizer, Roche, Seagen, Takeda, Viatris. He received research fundings from Astra Zeneca, Eisai and Roche. ST declared no competing interests. AZ received honoraria for consultancy and advisory board from Roche, Novartis, Lilly, AstraZeneca, Pfizer, Exact sciences, Merck, Daiichi Sankyo, Gilead, Seagen, Menarini, Stemline. DM had role in advisory board for Roche, Merck Sharp & Dohme, Merck, Astra Zeneca and received fees for travels and meetings from Pierre Fabre and Amgen. EC declared no competing interests. FC received fees for membership of an advisory board or lectures from Roche, AstraZeneca, Bristol-Myers Squibb, Pfizer, Takeda, Lilly, Bayer, Amgen, Sanofi, Pharmamar, Novocure, Mirati, Galecto, OSE, Illumina, Thermofisher, Beigene and Merck Sharp & Dohme. BC had a role in advisory board for Merck Sharp & Dohme and Daiichi Sankyo. GG declared no competing interests. UM has received personal fees (as a consultant and/or speaker bureau) from Boehringer Ingelheim, Roche, Merck Sharp & Dohme, Amgen, Thermo Fisher Scientific, Eli Lilly, Diaceutics, GlaxoSmithKline, Merck, AstraZeneca, Janssen, Diatech, Novartis and Hedera. FM and MN declared no competing interests. GP had financial fees from Illumina, Roche, Lilly, Astra Zeneca, Exact Sciences, ADS Biotech. MS and LS declared no competing interests. GT had a role in advisory board for Molteni, Novartis, PharmaMar, Merck Sharp & Dohme. NM had advisory boards consultation fees for Roche, Daiichi Sankyo, Pfizer, Astra Zeneca. GC had a role in advisory board for Roche, Astra Zeneca, Daiichi Sankyo, Lilly, Novartis, Pfizer, Gilead, Menarini, Exact Sciences, BMS and Merck. PM had a consultant/advisory role for BMS, Roche, EliLilly, Novartis, AstraZeneca, Pfizer, MSD, Daiichi-Sankyo, Roche, Amgen, Accord, TomaLab. He received research fundings for Novartis, Roche, EliLilly, BMS, Pfizer, Incyte, Merk Serono, Takeda, Menarini StemCell. He is Founder & Chairman or President for AISCUP, a Patients' Association and FMP, Foundation for Personalized Medicine, a national independent health and social care charity. The Foundation holds equity shares in Drug-Pin Society.

RUNNING TITLE: Liquid-Tissue biopsy concordance in ROME trial

ABSTRACT

Purpose

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This analysis evaluated the influence of tissue and liquid biopsy concordance on outcomes in patients enrolled in the ROME Trial.

Experimental design

The ROME trial, a phase II multicenter study, enrolled 1,794 patients with advanced solid tumors. Next-generation sequencing (NGS) was performed on tissue and liquid biopsies using FoundationOne CDx and FoundationOne Liquid CDx, A centralized Molecular Tumor Board (MTB) reviewed results to identify actionable alterations, with 400 patients randomized to tailored therapy (TT) or standard-of-care (SoC).TT improved objective response rate and progression-free survival (PFS) in the Intention to Treat population. Concordance was defined as the detection of the same druggable alteration in both biopsy types; discordance indicated detection in only one.

Results

Concordance was present in 49% of cases, with alterations detected exclusively in tissue (35%) or liquid (16%) biopsies. Patients in the concordant group receiving TT experienced improved survival outcomes. Median overall survival (OS) was 11.05 vs. 7.70 months in the SoC group (HR 0.74; 95% CI: 0.51-1.07), and median PFS was 4.93 vs. 2.80 months (HR 0.55; 95% CI: 0.40-0.76). In contrast, the survival benefit of TT was less pronounced or absent in patients with discordant results. OS was higher in the T+L group (11.05 months), followed by tissue-only (9.93 months), and liquid-only groups (4.05months). PFS followed a similar pattern, with the longest PFS in T+L group (4.93 months) vs 3.06 months in tissue-only and 2.07 months in liquid-only groups.

Conclusions

The study highlights the potential value of integrating both biopsy modalities in selected clinical contexts.

Statement of significance

This exploratory analysis indicates that the combined use of tissue and liquid biopsies may enhance the detection of actionable alterations and be associated with improved survival outcomes in patients with advanced solid tumors receiving matched therapies. These findings highlight the potential relevance of integrating both biopsy modalities in the context of precision oncology.

Introduction

Genomic profiling has revolutionized cancer treatment by enabling personalized medicine approaches (1). By identifying specific genetic mutations and variations within a tumor, clinicians can tailor treatments to target these alterations, thereby increasing the efficacy of therapies and minimizing adverse effects. One significant advantage is the ability to predict patient response to certain drugs, allowing for more precise and effective treatment plans (2). Additionally, genetic profiling can identify potential resistance mechanisms, enabling the adjustment of therapeutic strategies to overcome these challenges (3). This approach improves patient outcomes and contributes to the development of novel targeted therapies, advancing the field of oncology (4).

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Recently, liquid biopsy profiling has emerged as a forefront technology in cancer diagnostics, showcasing distinct advantages compared to traditional solid biopsy profiling (5).

Genomic profiling based on solid biopsies provides a detailed histological and molecular analysis of the tumor, allowing for precise characterization and diagnosis. It is crucial for determining the tumor type, grade, and specific genetic mutations, which are critical for guiding treatment decisions and offering a comprehensive view of the tumor's cellular makeup, aiding in the identification of potential therapeutic targets (6). Although the advantages of solid biopsy are significant, it remains an invasive procedure that requires surgical extraction or biopsy of tissue, which can be associated with risks such as bleeding, infection, and patient discomfort (7). In addition, tissue biopsy may fail to capture tumor heterogeneity, as it samples only a specific area and may miss mutations present in other tumor regions or metastatic sites. Furthermore, it is limited in its ability to provide real-time information on treatment response and disease progression. (8).

On the other hand, a liquid biopsy is minimally invasive, reducing patient discomfort and the risk of complications (9). It enables real-time monitoring of treatment response and detection of molecular changes that may indicate resistance to therapy while providing a dynamic snapshot of the disease. Liquid biopsies capture genetic information from multiple tumor sites, which can be particularly useful for tracking metastatic cancer (10,11). However, liquid biopsy may not detect mutations from tumors that do not shed enough cells or DNA into the bloodstream, potentially missing critical genetic information (12). It is limited in its ability to provide detailed histological information, which is essential for certain diagnostic and treatment decisions. The technology and interpretation of liquid biopsy results are still evolving, and there may be variability in the accuracy and reliability of the tests (5,13).

Integrating liquid and solid biopsy profiling offers a comprehensive approach to tumor characterization, addressing tumor heterogeneity and enhancing the precision of cancer diagnostics and treatment. By combining both approaches, clinicians can obtain a more extensive view of the tumor's genetic makeup and its dynamic changes over time. This integrated approach enhances the ability to tailor treatments to individual patients, ultimately improving clinical outcomes and driving forward the advancements in precision oncology (14).

The complexity of interpreting genomic results from liquid and solid biopsies presents numerous challenges for clinicians, who often face difficulties in analysing such data effectively (15). In this context, molecular tumor boards (MTBs) are emerging as a robust support system for clinicians, providing valuable insights into interpreting complex genomic findings (16) within the mutational model.

The ROME trial (Clinical Trials Registry NCT04591431) was a multicentric, prospective, randomized phase II study that used the MTB discussion of extensive genomic profiling as a key milestone in driving treatment decisions based on molecular profiling of solid and liquid biopsies. In this study, 1794 patients with solid tumors who had undergone at least one but no more than two lines of therapy were screened. Patients were required to undergo genomic profiling on both solid and liquid biopsies. If alterations were found that could be discussed, the cases were brought before the MTB. If actionable alterations emerged during the MTB discussion, patients were randomized to receive either a tailored treatment (TT) or the standard of care (SoC) chosen by the clinician presenting the case (17). Representative table of the overall population is reported in Table 1 Suppl. Matching of TT with the altered pathways is illustrated in Table 2 Suppl.

In this exploratory analysis, we evaluated the concordance of genomic profiling from both solid and liquid biopsies in patients enrolled in the ROME trial. We analysed how this concordance impacted on patient outcomes. By examining the alignment between the genomic data from different biopsy types and the subsequent clinical decisions, we aimed to assess its influence on treatment efficacy.

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Methods

ROME trial

The ROME trial was a phase II, randomized, prospective, open label, and multicenter clinical study that included patients aged 18 years or older with advanced or metastatic solid tumors that were inoperable, regardless of histology. The trial assessed patients with solid neoplasms in their second or third line of treatment and required NGS profiling of tumor tissue (Foundation One CDx) and blood samples (Foundation Liquid CDx). All patients were required to have measurable or evaluable disease as defined by the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) or immune-related response criteria (irRC). Adequate renal, liver, and bone marrow function were required at baseline. Additionally, patients with exclusively bone and/or brain metastases, uncontrolled brain disease (untreated and/or symptomatic), or those whose brain metastases had not been monitored for over two months were excluded. Patients with concurrent severe and/or uncontrolled medical conditions that could compromise their participation in the study were also excluded.

Genomic information obtained from tumor tissue or liquid biopsy profiling was evaluated by an MTB panel of experts who provided guidance on TT for patients when actionable alterations were identified. Patients were then randomized 1:1 to receive either the TT as indicated by the MTB or the SoC therapy. TT encompassed targeted therapy or immunotherapy based on specific molecular targets. Crossover was permitted upon progression from the initial treatment received. Patients who were not randomized were classified as screening failures based on the MTB decision.

MTB discussion

An MTB convened weekly to review every eligible patient. The MTB consisted of medical oncologists, pathologists, geneticists, immunologists, bioinformaticians, and other relevant specialists who reviewed each case, considering comprehensive molecular profiling data alongside the patient's clinical features, such as performance status, comorbidities, and concurrent treatments. The treating clinician presented each case, highlighting potential actionable therapeutic targets.

The pathogenicity of molecular alterations was evaluated using databases such as ClinVAR (RRID:SCR_006169), OncoKB (RRID:SCR_014782), COSMIC (RRID:SCR_002260), and the ESMO ESCAT scale was used to assess clinical actionability. The board employed a Variant Allele Frequency (VAF) thresholds of 1% for tissue biopsies and 2% for liquid biopsies.

MTB recommendations included randomizing patients to receive TT, referring them to a geneticist in the presence of somatic alterations with potential germline significance, modifying SoC only when necessary, and suggesting early drug access programs, when available in Italy.

Tissue and Liquid Biopsies

A tissue sample obtained during the screening phase or within six months prior to enrollment was required for genomic testing. Samples collected within three months before the patient's informed consent form (ICF) signature were acceptable, as were samples collected within six months, with prior confirmation by the MTB. No therapeutic interventions capable of altering the genomic profile or subclonal alterations were administered between the collection of the tissue sample and the enrollment of the patients in the study. Archived tissue samples were accepted only for patients with glioblastomas and high-grade malignant gliomas to avoid another biopsy, even if there was an interval therapy. Patients with only one available biopsy—either liquid or solid—due to a failure of one method during the screening phase remained eligible for inclusion and discussion in the MTB. Conversely, if both tissue and liquid biopsy characterizations failed, patients were classified as screening failures.

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Genomic Testing

For tissue biopsies, DNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens using the DNAx extraction method and analysed with FoundationOne CDx panel (324 genes) detecting substitutions, indels, copy number alterations (CNAs), gene rearrangements, microsatellite instability (MSI) and tumor mutational burden (TMB).

For liquid biopsies, circulating cell-free DNA (cfDNA) was isolated from plasma, collected in FoundationOne Liquid CDx cfDNA blood collection tubes. and analysed with the FoundationOne Liquid CDx panel (311 genes), detecting substitutions, insertions, indels in 311 genes, rearrangements in four genes, CNAs in three genes, tumor fraction (TF), blood TMB (bTMB), and MSI-H status.

Definition of Concordance and Discordance

Definition of Subgroup Stratification and Concordance/Discordance

In this exploratory study, genomic profiling results of all randomized patient were retrospectively reviewed. A CONSORT diagram was produced to show the population flowchart and the attrition rate (Fig. 1). Patients were stratified into three groups based on the mutational data sources:

1. **T+L group**: MTB recommended TT based on concordant alterations in both biopsies.

2. **T group**: MTB recommended TT based on tissue biopsy alteration alone (liquid biopsy failed/inconclusive or judged less informative)

3. **L group**: MTB recommended TT based on liquid biopsy alterations alone (tissue biopsy failed/unavailable or judged less informative)

Concordance was defined as identical actionable alterations in both biopsies, while discordance indicated clinically relevant alterations in only one biopsy type.

Study aim and statistical analysis

This analyses aimed to evaluate the impact of biopsy concordance or discordance on patient outcomes. Kaplan Meier (KM) survival analysis was performed for overall survival (OS) and Progression free survival (PFS). Chi square tests assessed categorical variable independence. Additionally, Cox proportional hazard models were used to estimate hazard ratios (HRs) for the effect of biopsy concordance on survival outcomes within relevant subgroups, including tumor fraction (TF: low vs high with a thresholds of 1% according to the FoundationOne Liquid CDx technical infomation) and number of metastatic sites (≤ 2 vs >2) and to explore potential interaction effects. Due to the exploratory nature statistical significance thresholds and power calculations were not predefined.

Ethical Considerations

The trial adhered to the principles of the Declaration of Helsinki regarding research involving human subjects, received institutional review board approval, and obtained written informed consent from all participants.

Protocol information: Clinical Trials Registry NCT04591431

Data Availability

The authors confirm that the data supporting the findings of this study are accessible within the article and its Supplementary material. The results of FoundationOne CDx and FoundationOne Liquid CDx analyses supporting the findings of this study can be obtained from the corresponding author upon reasonable request.

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Results

This exploratory analysis evaluated how MTB decisions based on tissue and liquid biopsy concordance/discordance influenced clinical outcomes in the ROME trial. Patient selection is depicted in the CONSORT diagram (Fig. 1), with baseline characteristics of concordant group summarized in Table 1 and concordant versus discordant in Table 2. The definitions of concordance groups (T+L) and discordance groups (T only or L only) are provided in the Methods section.

The overall concordance rate between T and L biopsies in MTB-indicated alterations, was 49.2% (197 patients, T+L group), with actionable alterations found exclusively in tissue biopsies in 34.8% of cases (139 patients, T group) and exclusively in liquid biopsies in 16.0% (64 patients, L group). Among the 203 discordant cases, contributors included molecular alteration discrepancies (43%), discordant high tumor mutational burden (hTMB) detection (35%), test failures (21%), and microsatellite instability (MSI) mismatches (1%). Test failures occurred in 22 tissue and 20 liquid biopsies due to: insufficient DNA (4 cases), unsuccessful DNA extraction (1 case), sample failure (2 cases), biopsy failure (3 cases), inadequate material (5 cases), insufficient ctDNA (7 cases), brain primary tumors (8 cases), and other technical issues (12 cases).

Among the 91 molecularly discordant cases, alterations involved: PTEN/PI3C/AKT/MTOR pathway (50.5%), FGF/FGFR pathway (15.4%), ERBB (13.2%), and other pathways (20.9%). In concordant cases, prevalent alterations included: TMB (23.9%), PTEN/PI3C/AKT/MTOR pathway (20.8%) and ERBB2 pathway (19.8%), FGF/FGFR pathway (9.1%), MSI (8.1%) and other pathways (18.3 %).

Patients with both T+L findings who received TT demonstrated improved survival outcomes compared to those receiving SoC. In this group, 95 patients were randomized to TT and 102 to SoC. In the TT arm, median OS was 10.89 months, versus 7.70 months in the SoC arm (HR 0.76; 95% CI: 0.53–1.09; Fig 2A), and median PFS was 4.90 months versus 2.80 months (HR 0.56; 95% CI: 0.41–0.77; Fig 2B). Additionally, the 9-month OS rate was 54.50% in the TT group and 46.80% in the SoC group, while the 12-month OS rate was 47.10% and 38.80%, respectively. For PFS, the 9-month and 12-month rates were 33.90% and 26.60% in the TT arm compared to 13.70% and 9.10% in the SoC arm. Among T+L patients, ORR was 22.1% in TT arm versus 12.8% in SoC arm.

Distinctively, among patients whose treatment decisions were guided exclusively by tissue biopsy findings (T group), 71 were randomized to TT and 68 to SoC. Median OS was 9.93 months in the TT arm and 10.03 months in the SoC arm (HR 1.14; 95% CI: 0.73–1.78; Suppl. Fig. 1). In this group, the 12-month OS rates were 41.1% for TT and 47.6% for SoC. Median PFS was 3.06 months in the TT group versus 2.99 months in the SoC group (HR 0.81; 95% CI: 0.56–1.17; Suppl. Fig. 2), with 12-month PFS rates of 19.70% and 10.10%, respectively. The ORR in T only group was 12.7% in TT arm versus 5.9% in SoC arm.

Finally, 34 and 30 patients in the L group were randomized to receive TT and SoC, respectively. Median OS was 4.05 months for TT versus 3.49 months for SoC (HR 1.10; 95% CI: 0.63–1.92; Suppl. Fig. 3), and median PFS was 2.07 months versus 2.32 months (HR 0.78; 95% CI: 0.46–1.32; Suppl. Fig. 4). The 9-month OS rate was 36.60% in the TT group versus 30.00% in the SoC group, while the 12-month OS rate was 16.50% versus 26.70%, respectively. The 9-month PFS rate was 17.50% in the TT arm compared to 3.30% in the SoC arm, while the 12-month PFS rate was 14.00% in the TT arm, and all patients treated with standard progressed (0%). In this group ORR was 14.7% in TT group versus 10% in SoC group.

To specifically assess the magnitude of benefit conferred by the TT relative to the modality through which actionable genomic alterations were detected, we report separately a comparative analysis among T+L, T and L groups focusing exclusively on patients in the TT arm. By isolating the TT arm, we aimed to directly evaluate how the comprehensive or limited detection of actionable alterations through these different biopsy approaches influenced survival outcomes. Median OS was 10.89 months in the T+L group, compared

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to 9.93 months in the T group and 4.05 months in the L group (Fig. 3A). Similarly, median PFS was 4.90 months in the T+L group, versus 3.06 months in the T group and 2.07 months in the L group (Fig. 3B). The 12-month OS rates were 47.10% for T+L, 41.10% for T, and 16.50% for L, while the 12-month PFS rates were 26.60%, 19.70%, and 14.00%, respectively.

Survival outcomes were further examined by categorizing patients based on whether tissue and liquid biopsies provided concordant (T+L) or discordant (T or L) results for the specific genomic alteration that informed the therapeutic target proposed for TT. One hundred ninety-seven patients (49.2%) had concordant tests. In comparison, 161 patients (40.3%) were discordant due to the absence of the alteration in one of the samples, and 42 patients (10.5%) were discordant due to tissue or liquid test failure. Among patients with concordant findings (n = 95 in the TT arm, n = 102 in the SoC arm), those receiving TT showed improved outcomes compared to those receiving SoC as already shown in Fig. 2. In contrast, patients with available but discordant molecular results (n = 88 in the TT arm, n = 73 in the SoC arm) experienced less benefit from the TT. In the TT arm, the median OS for discordant cases was 7.34 months, compared to 8.49 months in the SoC arm (HR 1.21; 95% CI: 0.81–1.79; Suppl. Fig. 5). Similarly, the median PFS in the TT arm was 2.86 months, compared to 2.93 months in the SoC arm (HR 0.87; 95% CI: 0.62–1.23; Suppl. Fig. 6). The 9-month OS rate in discordant TT patients was 44.90%, and the 12-month OS rate was 32.00%, compared to 47.10% and 41.70%, respectively, in the SoC group. For PFS, the 9-month and 12-month rates were 23.20% and 17.70% in the TT arm, compared to 16.90% and 9.40% in the SoC arm. In discordant group ORR was 12.5% in TT arm versus 6.9% in SoC arm.

The comparative analysis of concordant, true discordant, and discordant due to failure groups, conducted exclusively in patients randomized to the TT as recommended by the MTB, demonstrated that those with concordant biopsy results experienced the most considerable benefit, achieving notably longer survival outcomes. In the TT arm, median OS was highest in the concordant group (10.89 months), followed by the failure discordant group (10.39 months) and the true discordant group (7.34 months) (Fig. 4A). Median PFS was longest in the concordant group (4.90 months), followed by the true discordant group (2.86 months) and failure discordant group (2.53 months) (Fig. 4B). The 12-month OS rates for the concordant, failure discordant, and true discordant groups were 47.10%, 41.20%, and 32.00%, respectively, while the 12-month PFS rates were 26.60%, 17.60%, and 17.70%, respectively.

To further investigate concordance rates, an analysis was conducted based on tumor fraction (TF), the number of metastatic sites, and the location of both the site of genomic profilation and the primary tumor.

A total of 317 patients with detectable TF were identified, revealing a higher concordance rate when TF was high compared to non-high (62.4% vs. 43%; Chi square p value < 0.001). By excluding patients whose profiling tests failed and those with primary brain tumors, the concordance rate (T+L) increased to 70.1% for high TF compared to 44.6% for non-high TF (Chi square p value < 0.0001). Additionally, a sub-analysis was performed, excluding patients whose MTB decision was based on TMB values or microsatellite stability. This refined analysis focused solely on patients for whom treatment decisions relied on molecular alterations. Under these conditions, concordance rates further increased to 88.6% for high TF and 58.6% for non-high TF (Chi square p value < 0.0001). Interaction analysis of OS among concordant patients treated with TT vs. SoC, stratified by TF, demonstrated HRs of 0.64 (95% CI, 0.31–1.29) for patients with low TF and 0.89 (95% CI, 0.56–1.43) for those with high TF. Similarly, PFS benefits in this subgroup were confirmed through interaction analysis, yielding HRs of 0.50 (95% CI, 0.28–0.88) for patients with low TF and 0.60 (95% CI, 0.39–0.91) for those with high TF. In T and L only subgroups HRs were in line with HR evaluated without interaction stratification, not showing differences on outcomes across TF strata.

For the number of metastatic sites, patients were divided into two groups (≤2 sites vs. >2 sites). The concordance rate was higher in patients with one or two sites (55.4%) than in those with more than two

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sites (39.6%). In the combined T+L subgroup, the comparison of TT vs. SoC yielded OS HRs of 0.81 (95% CI, 0.50–1.31) for patients with ≤2 sites and 0.68 (95% CI, 0.39–1.20) for those with >2 sites. Similarly, PFS HRs were 0.52 (95% CI, 0.34–0.79) for patients with ≤2 sites and 0.63 (95% CI, 0.39–1.01) for those with >2 sites. Interaction analysis of OS by metastatic site count in the 'T only' and 'L only' subgroups did not differ significantly from the HR evaluated without interaction strata. Regarding the site of primary tumor and the site of the solid tissue samples used for genomic profiling, concordance rate was assessed using the stratified chi-square test. (Suppl. Fig. 7) (Suppl. Fig. 8).

To assess whether some discordant results were due to differences in variant coverage between the two tests, we generated a Venn diagram (Suppl. Fig. 9) illustrating the alterations covered by both assays or by only one of them. While all therapeutic target genes with full exonic coverage were included in both panels, the FoundationOne CDx panel encompassed a greater number of genes with select intronic region coverage, thereby enabling more accurate detection of rearrangements. Nevertheless, the proportion of patients who received a treatment indication based on rearrangements in genes with intronic coverage by FoundationOne CDx was low (19/400, 4.8%, 12 pts assigned to TT arm and 7 to SoC). In 17 out of 18 patients who underwent both tests, the rearrangement was detected by both assays, while in only one case it was identified in the tissue sample but not in the liquid biopsy.

Discussion

This exploratory analysis of the ROME trial highlights the complementary roles of tissue and liquid biopsy profiling in guiding molecularly tailored treatments and underscores the impact of concordance between these modalities on clinical outcomes. The study provides insights into the predictive value of integrating these diagnostic approaches in advanced solid tumors by evaluating concordant and discordant biopsy results.

The ROME trial, which utilized extensive NGS on tissue samples, collected primarily at progression, and circulating tumor DNA (ctDNA) from liquid biopsies, provided a unique opportunity to evaluate dual-source genomic information's impact on MTB decisions and clinical outcomes. During the MTB discussion, TT was proposed based on alterations detected in both biopsies in 49.2% of cases, while actionable alterations were identified exclusively in tissue biopsies in 35% of cases and in liquid biopsies in 16%. This indicates that nearly half of therapeutic decisions relied on concordant data from both modalities, while in the remained depended on one test alone. Concordance and discordance were assessed basing only on mutations considered actionable after MTB review – a novel approach to defining these terms.

The observed concordance rate (49.2%) is lower than literature reports, attributable to multiple factors (18). First, certain genomic alterations are more detectable in tissue or liquid biopsies due to biological properties. Low ctDNA shedding in some tumors, reduces liquid biopsy sensitivity, while liquid biopsies better capture tumor heterogeneity (19,20). TMB concordance is generally lower than for individual molecular alterations, with liquid biopsies often reporting higher TMB levels. Amplifications, for example, are more reliably detected in tissue samples (21,22). Test failure (approximately 20% of cases), further contributed to the observed discordance.

When revising differences in variant coverage between the liquid and tissue profiling by Venn diagram (Suppl. Fig. 10), we can assume that differences in coverage did not significantly impact our concordance analysis.

In our study, concordance rates between liquid and tissue biopsies were markedly higher in cases with elevated ctDNATF, especially after excluding technical failures and primary brain tumors. Under these conditions – and when considering specific genomic alterations rather than broad biomarkers like tumor mutational burden or microsatellite instability – our concordance rates approach the values reported in

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literature (23), although they remain slightly lower. We believe this discrepancy stems from our stricter definition of concordance: whereas most studies count any shared mutation between tissue and plasma as concordant, we only score concordance when the identical actionable mutation is present in both sample types. Notably, primary brain tumors (≈10% of our cohort) showed especially high discordance, which further reduced the overall concordance rate (see Suppl. Fig. 8).

These findings emphasize the utility of dual biopsy data to propose a TT, even when actionable information derives from one modality, underscoring their integration's value in precision oncology. An aspect not evaluated in this study concerns the possibility of performing liquid biopsy not only on blood but also on other biological fluids, such as cerebrospinal fluid (for brain tumors), ascitic fluid, or pleural fluid for specific disease localizations, where blood might prove to be an unreliable source of relevant genomic alterations.

Although recent studies suggest that ctDNA positivity is significantly correlated with poorer outcomes across various cancer types, confirming its prognostic significance (24,25), its ability to predict response to a targeted therapy in patients harboring specific actionable genomic alterations in both tissue and liquid biopsies remains less established.

In our study the most meaningful clinical benefit of TT compared to SoC was observed in the T+L group. The 12-month OS rate in this group approached 50% (47.10%), further emphasizing the robust benefit derived from concordant findings. The presence of actionable alterations in both tissue and liquid biopsies may indicate a central role for these alterations in driving tumor progression, making them particularly effective therapeutic targets. This finding could also reflect the biological dominance of these alterations, representing critical driver events that are consistently detectable across multiple biopsy modalities. Furthermore, the concordance of results may suggest a more stable and homogeneous tumor biology in these patients, which might enhance the efficacy of TT. Finally, the availability of dual-source data likely enhanced the confidence of the MTB in identifying actionable targets, resulting in more precise therapeutic recommendations.

The uncertain benefit observed in the T-only group (median OS of 9.93 months with TT vs. 10.03 months with SoC; HR 1.14; median PFS of 3.06 months with TT group vs 2.99 months with SoC; HR 0.81) underscores the challenges of relying exclusively on tissue biopsies. Tissue sampling, while providing detailed histological and molecular information, is inherently limited in capturing intratumoral heterogeneity and dynamic molecular changes over time. These findings suggest that additional molecular data from liquid biopsies may enhance treatment precision in this subgroup.

The results of the L subgroup, given the limited number of patients, should be interpreted with caution. The poor outcomes in this subgroup could reflect a higher tumor burden, as indicated by the detectability of circulating tumor DNA (ctDNA) in liquid biopsies. Additionally, the generally lower OS and PFS observed in this subgroup compared to others supports the need for further exploration of these patients' unique molecular and clinical characteristics. Alternatively, it may point to technical limitations of current liquid biopsy technologies, such as reduced sensitivity in detecting alterations from non-shedding tumors. These observations highlight the importance of refining liquid biopsy methodologies to improve their sensitivity and reliability. Additionally, interpreting liquid biopsy findings within the broader clinical and molecular context is essential to ensure accurate therapeutic decisions, particularly in cases where actionable alterations are detected exclusively in liquid biopsies.

Furthermore, the comparative analysis of concordant versus discordant biopsy results revealed interesting differences in survival outcomes.Patients with concordant findings demonstrated better OS and PFS with TT compared to SoC, while those with discordant results showed less pronounced benefits from TT. The concordant group's 12-month OS rate of 47.1% compared to 32.0% in the true discordant group reinforces the potential of concordance as a predictive biomarker for therapeutic efficacy. This suggests that

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integrating both tissue and liquid biopsy results may help identify patients most likely to benefit from tailored therapies.

Subgroup analyses were conducted to assess whether the prognostic impact of tissue–liquid biopsy concordance varied by tumor fraction (TF: low vs. high) and metastatic burden (≤2 vs. >2 sites). A consistent survival benefit was observed for patients with concordant profiling (T+L) compared to discordant profilng (T only or L only) across both TF strata and levels of metastatic involvement. In both TF-low and TF-high subgroups, hazard ratios (HRs) for OS and PFS favored the concordant group, with overlapping confidence intervals and no significant interaction effects. Likewise, the survival advantage of the T+L group was maintained irrespective of the number of metastatic sites, with no evidence of effect modification. These findings indicate that the predictive value of tissue–liquid biopsy concordance is robust and not affected by tumor DNA shedding or disease extent.Additionally, we observed that high discordance rates in actionable alterations involving the PI3K/PTEN/AKT/mTOR and ERBB2 pathways highlight the molecular complexity and spatial heterogeneity of these signalling networks. For ERBB2, this may partly stem from the technical limitations of liquid biopsies in detecting gene amplifications, which are more reliably identified in tissue samples (22). Additionally, the dynamic nature of signalling in these pathways and their frequent involvement in tumor heterogeneity may contribute to discordance (26,27). This underscores the need for combining tissue and liquid biopsies to obtain a more comprehensive molecular profile, particularly when targeting these pathways. Additional strategies, such as repeat biopsies, advanced bioinformatics tools, or complementary diagnostic modalities, may be required for patients with discordant results to refine treatment selection and improve outcomes.Our findings have several important implications for clinical practice and future research. They support the integration of both tissue and liquid biopsy profiling as a viable approach in molecular diagnostics and highlight the potential of concordance between biopsy modalities as a stratification factor in clinical trials evaluating molecularly guided therapies. Finally, they underscore the need for prospective validation of these findings and the development of strategies to address discordance, such as incorporating additional molecular profiling methods or enhancing the sensitivity and specificity of existing technologies.

Several limitations of this study should be acknowledged. The exploratory nature of the analysis and the absence of predefined statistical power for subgroup comparisons limit the generalizability of the findings. High rate of test failures (~20% of discordant cases) and small sample size in some subgroups (especially liquid-only) could affect robustness of subgroup analyses. Discordance due to tumor heterogeneity, technical limitations in detecting of amplifications, low ctDNA shedding, and challenges in specific tumor types (notably brain tumors) need to be interpretated. The temporal proximity of tissue and liquid biopsy collection in this study minimizes the likelihood that discordance is attributable to tumor evolution over time, suggesting that other factors, such as biological and technical differences, may play a more significant role.

Future studies should focus on prospectively validating concordance as a biomarker for therapeutic success and exploring the integration of additional diagnostic modalities to address discordance. Advances in liquid biopsy technologies and bioinformatics are likely to further enhance the precision of molecular profiling, ultimately improving outcomes for patients with advanced solid tumors. By addressing the challenges of discordance and leveraging the strengths of both biopsy modalities, future strategies can refine precision oncology algorithms and enhance clinical outcomes for patients with advanced cancers.

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Funder

Erlotinib, Pertuzumab, Vemurafenib, Trastuzumab Emtansine, Alectinib, Vismodegib, Cobimetinib, Atezolizumab, Trastuzumab, Ipatasertib (GDC-0068), Entrectinib and Pralsetinib were provided by Roche; Everolimus, Lapatinib, Alpelisib were provided by Novartis, Palbociclib and Talazoparib were provided by Pfizer, Ipilimumab and Nivolumab were provided by Bristol-Myers Squibb (BMS); Brigatinib was provided by Takeda Pharmaceutical Co.; Ponatinib, Itacitinib (INCB039110), Pemigatinib (INCB054828) were provided by Incyte; Selpercatinib was provided by Eli-Lilly; Tepotinib was provided by the healthcare business of Merck KGaA, Darmstadt, Germany (CrossRef Funder ID: 10.13039/100009945). NGS test FoundationOne CDx and FoundationOne Liquid CDx were provided by Foundation Medicine, Inc. This study was supported through unrestricted grants provided by Roche, Takeda, Bristol-Myers Squibb (BMS), Pfizer, Incyte, and Eli Lilly. These contributions were regulated by contractual agreements with the study sponsor, the Fondazione per la Medicina Personalizzata (FMP). The funding entities had no role in the study design, data collection, analysis, interpretation, or manuscript preparation.

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TABLES

Table 1 Patients' characteristics in concordant group

Characteristics	**Overall** N=197(%)	**SoC** N=102 (%)	**TT** N=95 (%)
Age (years)			
Median (range)	61 (36-85)	60 (37-84)	63 (36-85)
Gender			
Male	83 (42.1%)	48 (47.1%)	35 (36.8%)
Female	114 (57.9%)	54 (52.9%)	60 (63.2%)
Ethnicity			
Caucasian	193 (98%)	100 (98%)	93 (97.8%)
Oriental	1 (0.5%)	0	1 (1.1%)
Hispanic	1 (0.5%)	1 (1%)	0
Afro-American	0	0	0
Other	1 (0.5%)	0	1 (1.1%)
NA	1 (0.5%)	1 (1%)	0
Primary tumor			
Breast	24 (12.2%)	10 (9.8%)	14 (14.7%)
Gastrointestinal	59 (30%)	35 (34.3%)	24 (25.3%)
NSCLC	17 (8.6%)	8 (7.8%)	9 (9.5%)
Other	97 (49.2%)	49 (48%)	48 (50.5%)
ECOG PS			
0	120 (60.9%)	63 (61.8%)	57 (60%)
1	77 (39.1%)	39 (38.2%)	38 (40%)
2	0	0	0
No. of metastatic sites			
≤ 2	109 (55.3%)	54 (53.0%)	55 (57.9%)
>2	78 (39.6%)	40 (39.2%)	38 (40.0%)
NA	10 (5.1%)	8 (7.8%)	2 (2.1%)

Table 2 Patients' characteristics in concordant versus discordant groups

Characteristics	**Overall** N=400 (%)	**Concordant (T+L)** N=197 (%)	**Discordant (T or L)** N=203 (%)
Age (years)			
Median (range)	61 (22-85)	61 (36-85)	61 (22-83)
Gender			

Male	192 (48.0%)	83 (42.1%)	109 (53.7%)
Female	208 (52.0%)	114 (57.9%)	94 (46.3%)
Ethnicity			
Caucasian	392 (98.0%)	193 (98.0%)	199 (98.0%)
Oriental	2 (0.5%)	1 (0.5%)	1 (0.5%)
Hispanic	3 (0.7%)	1 (0.5%)	2 (1.0%)
Afro-American	1 (0.3%)	0	1 (0.5%)
Other	1 (0.3%)	1 (0.5%)	0
NA	1 (0.3%)	1 (0.5%)	0
Primary tumor			
Breast	40 (10.0%)	24 (12.2%)	16 (7.9%)
Gastrointestinal	109 (27.2%)	59 (30.0%)	50 (24.6%)
NSCLC	31 (7.8%)	17 (8.6%)	14 (6.9%)
Other	220 (55.0%)	97 (49.2%)	123 (60.6%)
ECOG PS			
0	237 (59.2%)	120 (60.9%)	117 (57.6%)
1	162 (40.5%)	77 (39.1%)	85 (41.9%)
2	1 (0.3%)	0	1 (0.5%)
No. of metastatic sites			
≤ 2	255 (63.7%)	109 (55.4%)	146 (71.9%)
>2	128 (32.0%)	78 (39.6%)	50 (24.6%)
NA	17 (4.3%)	10 (5.0%)	7 (3.5%)

TABLES LEGENDS

Table 1 shows patient population characteristics in concordant group

Table 2 shows patient population in concordant compared to discordant groups

FIGURES legends

Fig. 1 Consort diagram shows flow of patients' selection for this exploratory analysis

Fig.2 Overall survival (OS) and progression free survival (PFS) probability in patients with concordant tissue and liquid biopsy results (T+L group). The blue line represents patients receiving SoC (Arm A, n=102), while the red line represents patients receiving TT (Arm B, n=85). **A)** Median OS was 7.70 months in Arm A versus 10.89 months in Arm B (HR 0.76, 95% CI 0.53–1.09). **B)** Median PFS was 2.80 months vs 4.90 months in arm A vs B (HR 0.56; 95% CI: 0.41–0.77).

Fig. 3 Kaplan-Meier curves showing overall survival (OS) and progression free survival (PFS) probability stratified by concordance status in the TT arm. Blue line: patients with concordant tissue and liquid biopsy (T+L group, n=95). Red line: patients with tissue biopsy only (T group, n=71), green line: patients with liquid biopsy only (L group, n=34). **A)** Median OS was 10.89 months in the T+L group, compared to 9.93 months in

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the T group and 4.05 months in the L group. The 12-month OS rates were 47.10% for T+L, 41.10% for T, and 16.50% for L, respectively **B)** Median PFS was 4.90 months in the T+L group vs 3.06 months in the T group and 2.07 months in the L group. The 12-month PFS rates were 26.60%, 19.70%, and 14.00%.

Fig. 4 Kaplan-Meier curves showing (A) overall survival (OS) and (B) progression-free survival (PFS) in patients receiving TT stratified by specific genomic alteration concordance status. The blue line represents patients with discordant results due to test failure (group 1, n=17), the red line patients with true discordant results (group 2, n=88), and the green line represents patients with concordant results (group 3, n=95). Median OS: 10.89 months in group 3 vs 10.39 in group 1 and 7.34 in group 2. Median PFS: 4.90 months in the concordant group, followed by the true discordant group (2.86 months) and failure discordant group (2.53 months).

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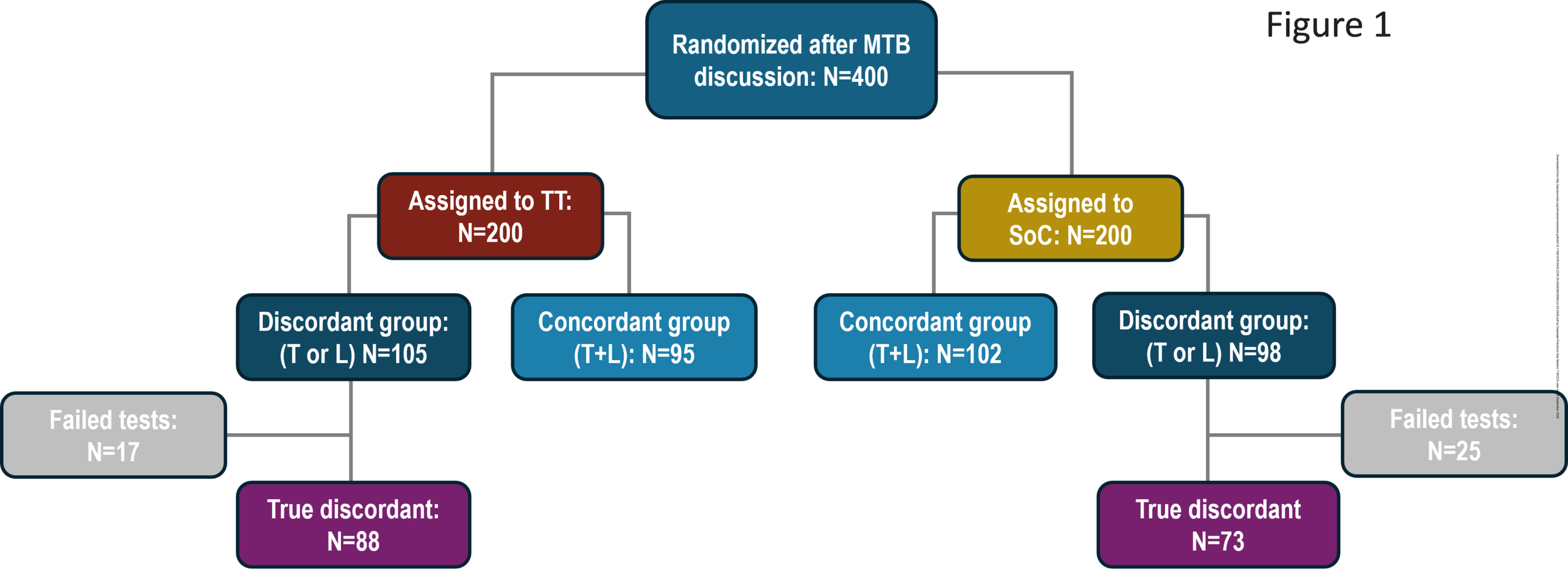
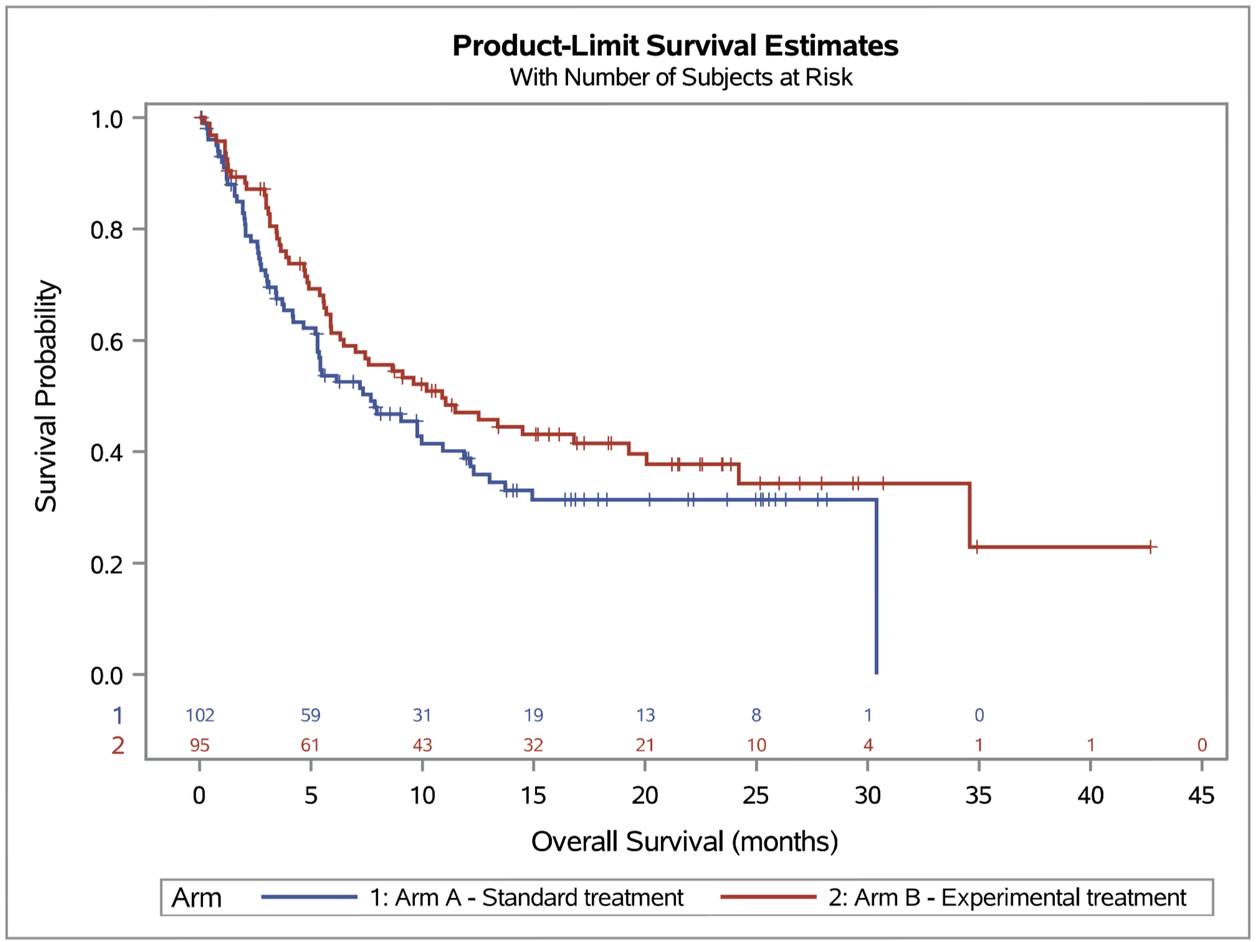
Figure 1

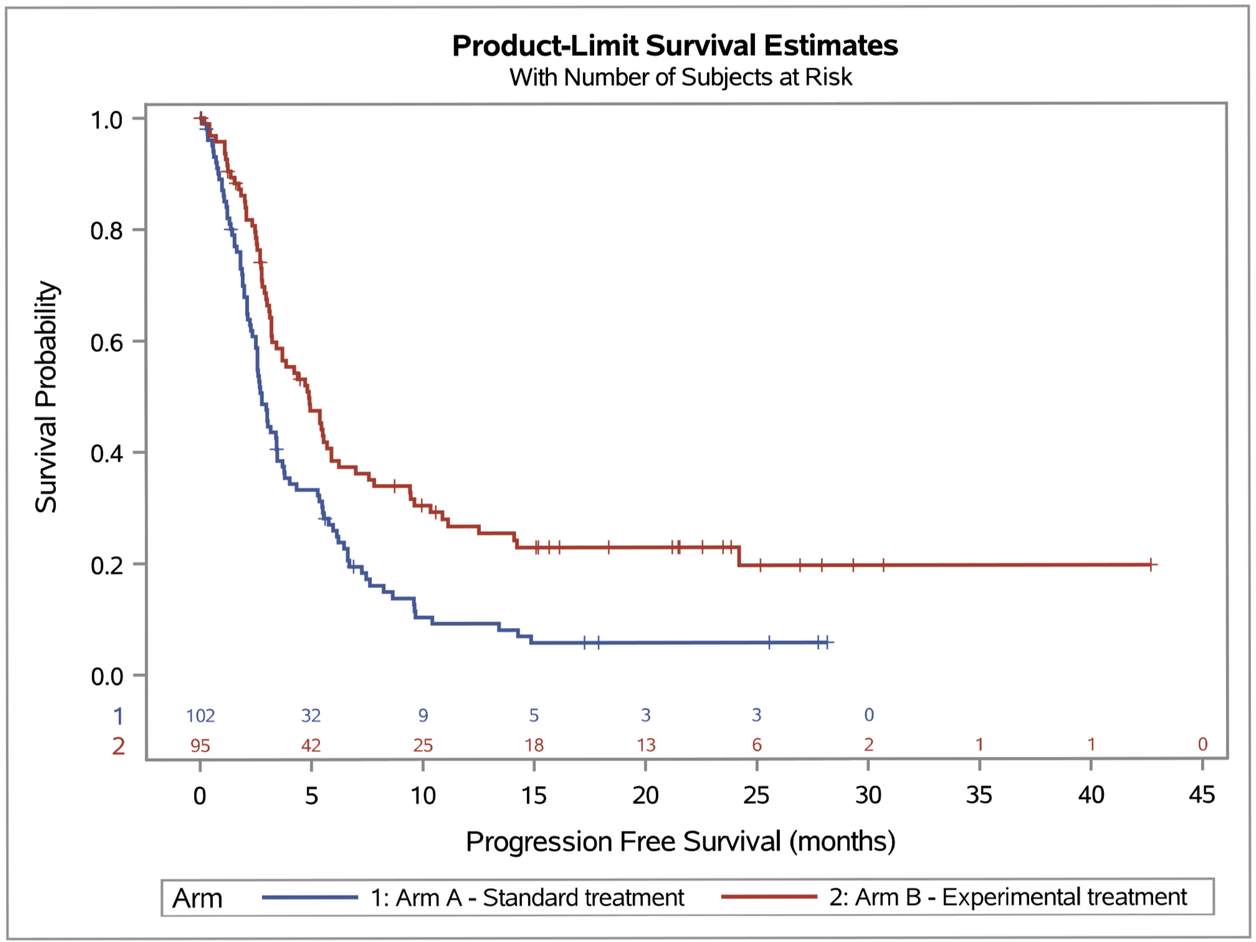

Randomized after MTB discussion: N=400
Assigned to TT: N=200
Assigned to SoC: N=200
Discordant group: (T or L) N=105
Concordant group (T+L): N=95
Concordant group (T+L): N=102
Discordant group: (T or L) N=98
Failed tests: N=17
True discordant: N=88
Failed tests: N=25
True discordant N=73

A

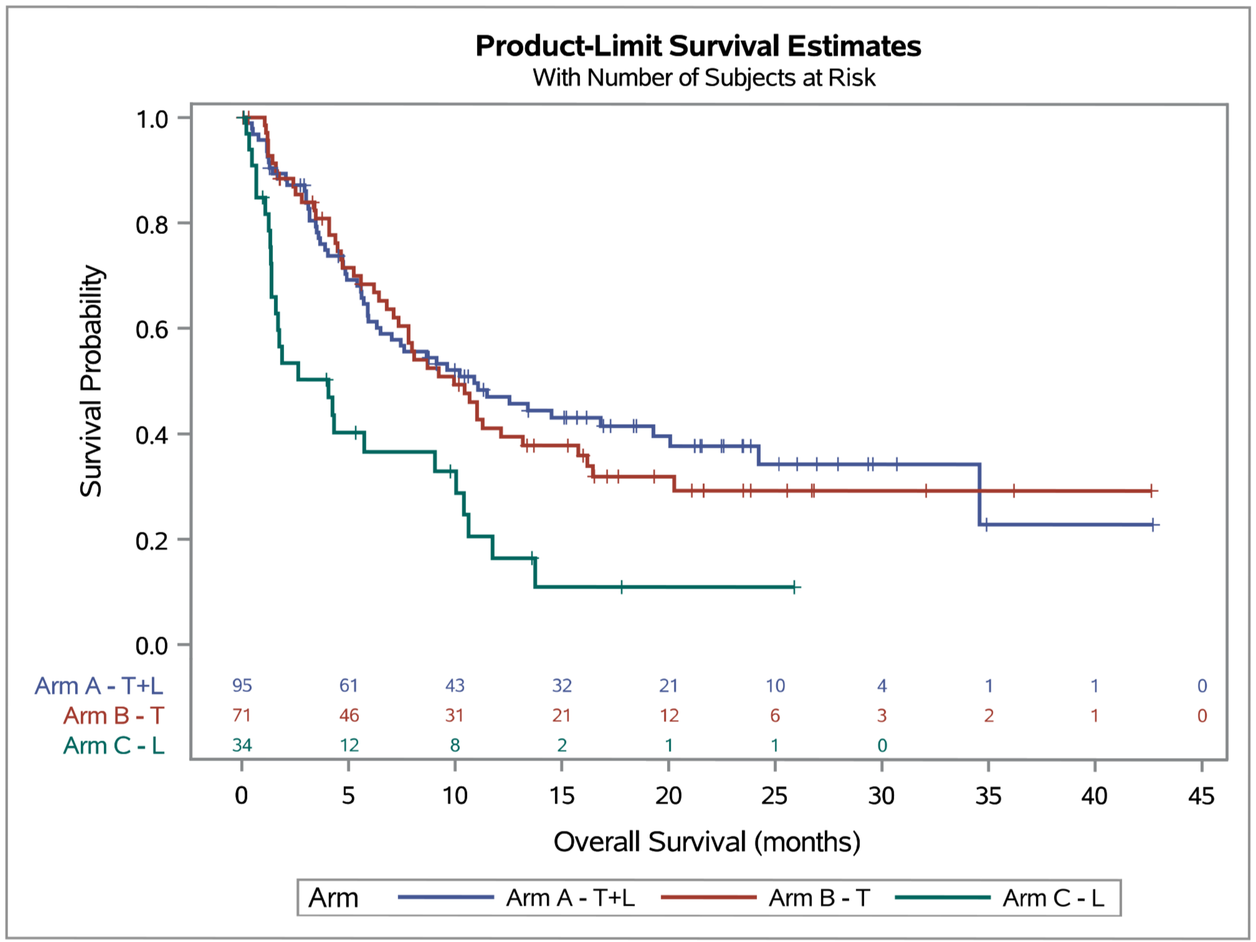
B

Fig. 2


Product-Limit Survival Estimates
With Number of Subjects at Risk
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
1 102 59 31 19 13 8 1 0
2 95 61 43 32 21 10 4 1 1 0
0 5 10 15 20 25 30 35 40 45
Overall Survival (months)
Arm
1: Arm A - Standard treatment
2: Arm B - Experimental treatment


Product-Limit Survival Estimates
With Number of Subjects at Risk
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
1 102 32 9 5 3 3 0
2 95 42 25 18 13 6 2 1 1 0
0 5 10 15 20 25 30 35 40 45
Progression Free Survival (months)
Arm
1: Arm A - Standard treatment
2: Arm B - Experimental treatment

A


Product-Limit Survival Estimates
With Number of Subjects at Risk
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
Arm A - T+L 95 61 43 32 21 10 4 1 1 0
Arm B - T 71 46 31 21 12 6 3 2 1 0
Arm C - L 34 12 8 2 1 1 0
0 5 10 15 20 25 30 35 40 45
Overall Survival (months)
Arm Arm A - T+L Arm B - T Arm C - L

B

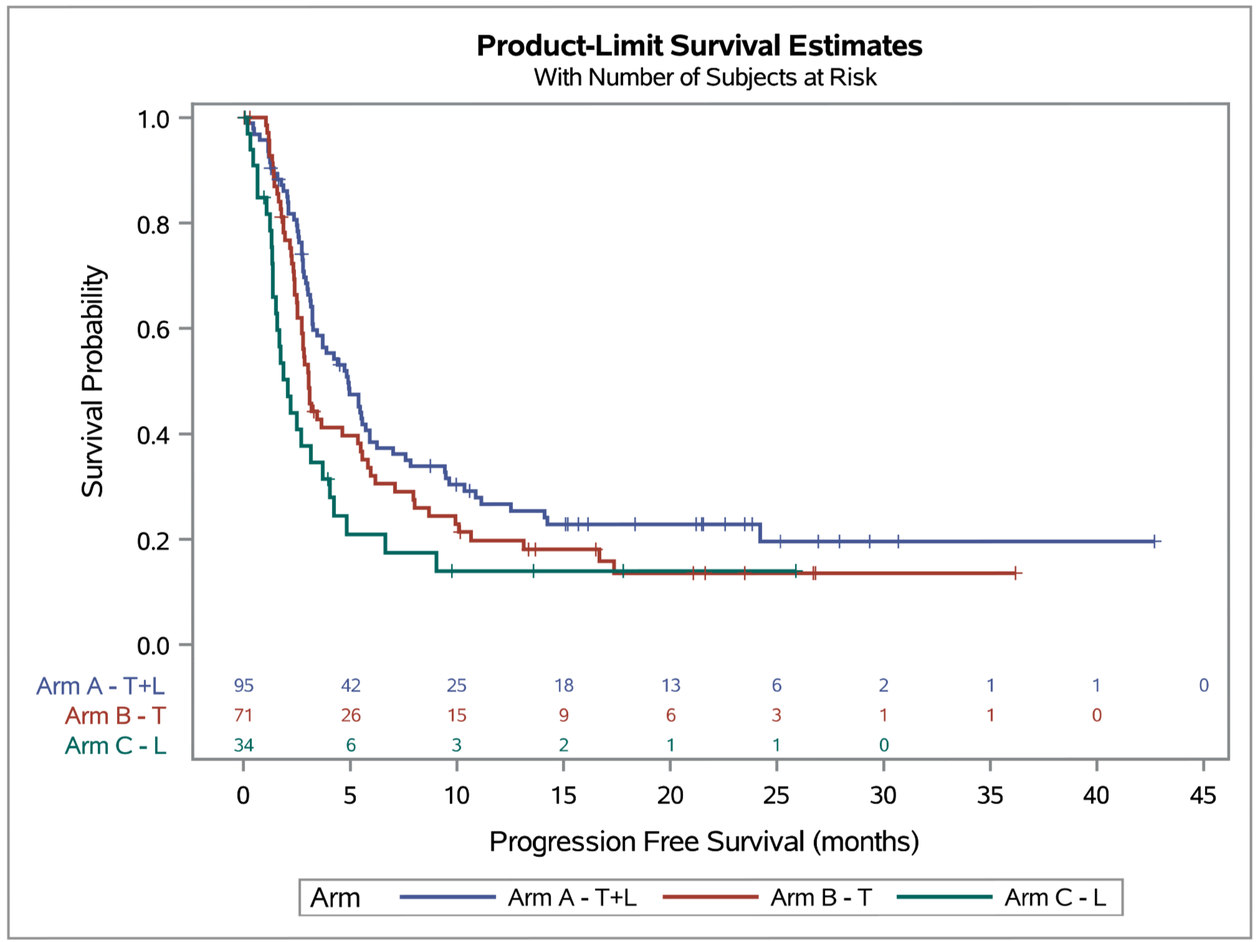
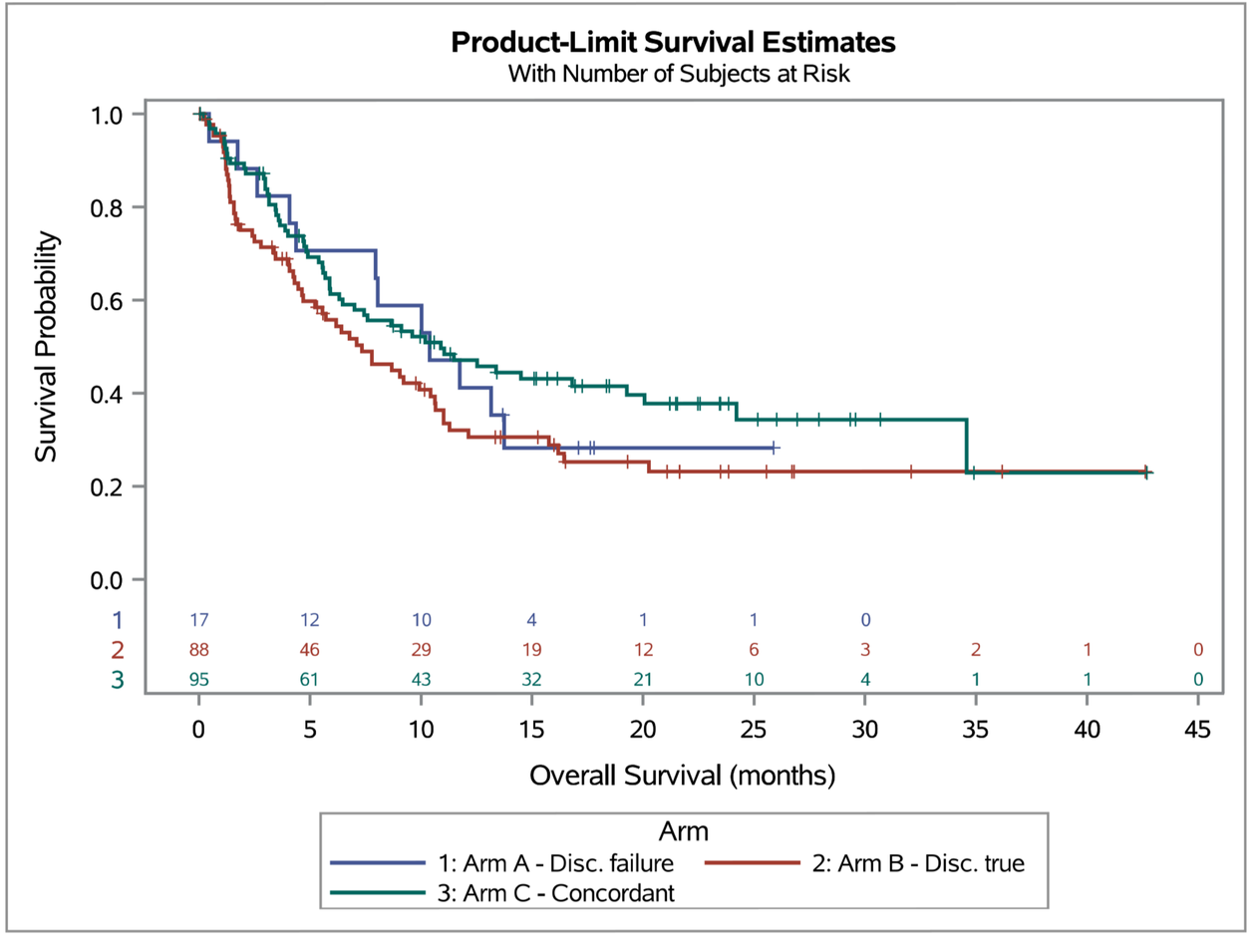

Product-Limit Survival Estimates
With Number of Subjects at Risk
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
Arm A - T+L 95 42 25 18 13 6 2 1 1 0
Arm B - T 71 26 15 9 6 3 1 1 0
Arm C - L 34 6 3 2 1 1 0
0 5 10 15 20 25 30 35 40 45
Progression Free Survival (months)
Arm Arm A - T+L Arm B - T Arm C - L

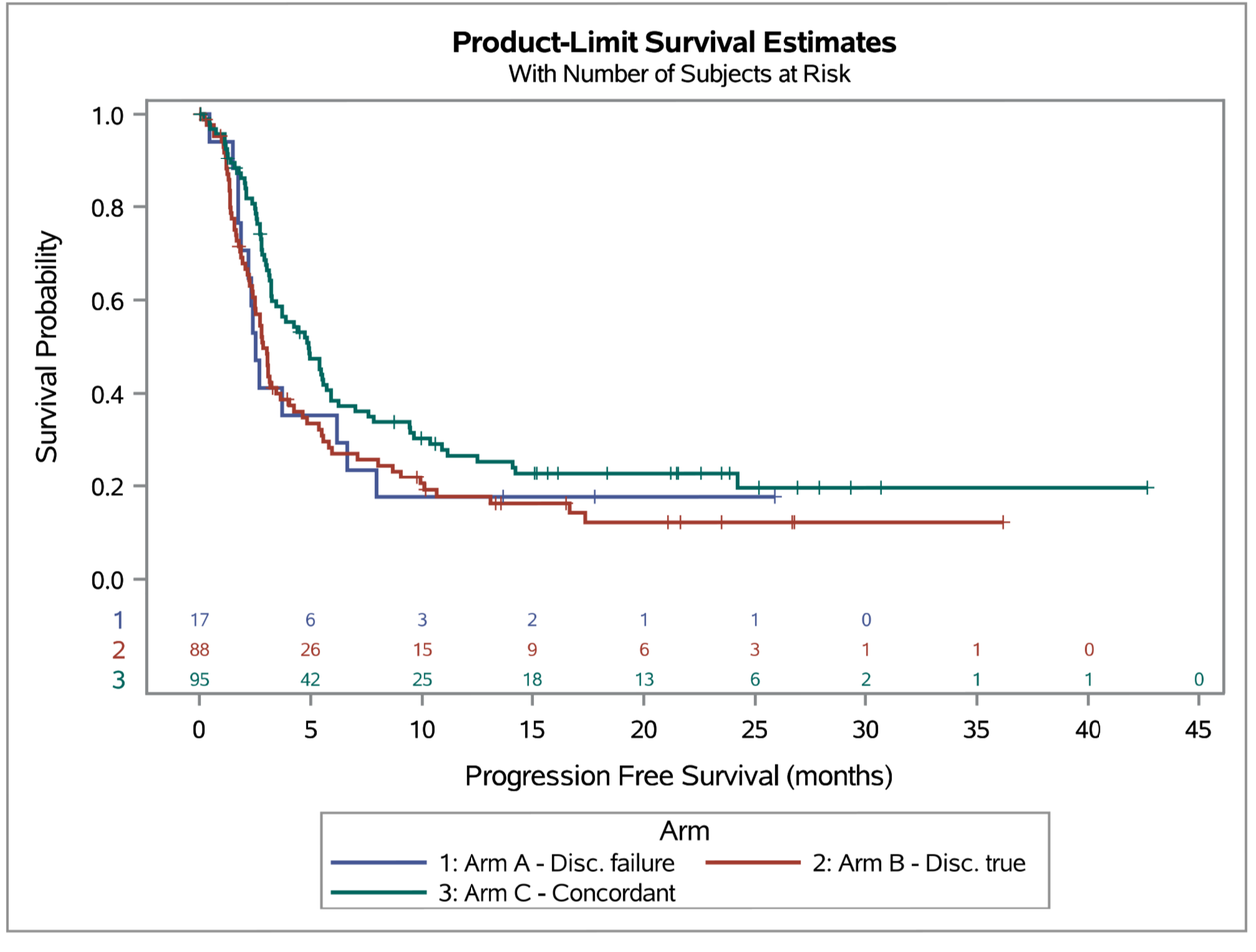
Fig. 3

A

B

Fig. 4


Product-Limit Survival Estimates
With Number of Subjects at Risk
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
1 17 12 10 4 1 1 0
2 88 46 29 19 12 6 3 2 1 0
3 95 61 43 32 21 10 4 1 1 0
0 5 10 15 20 25 30 35 40 45
Overall Survival (months)
Arm
1: Arm A - Disc. failure
2: Arm B - Disc. true
3: Arm C - Concordant


Product-Limit Survival Estimates
With Number of Subjects at Risk
Survival Probability
1.0
0.8
0.6
0.4
0.2
0.0
1 17 6 3 2 1 1 0
2 88 26 15 9 6 3 1 1 0
3 95 42 25 18 13 6 2 1 1 0
0 5 10 15 20 25 30 35 40 45
Progression Free Survival (months)
Arm
1: Arm A - Disc. failure
2: Arm B - Disc. true
3: Arm C - Concordant