AperTO - Archivio Istituzionale Open Access dell'Università di Torino

International Tailored Chemotherapy Adjuvant (ITACA) Trial, a Phase III Multicenter Randomized Trial Comparing Adjuvant Pharmacogenomic-Driven Chemotherapy versus Standard Adjuvant Chemotherapy in completely Resected Stage II-IIIA Non-Small Cell Lung Cancer

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1814652> since 2022-05-23T11:04:55Z

Published version:

DOI:10.1016/j.annonc.2021.09.017

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

International Tailored Chemotherapy Adjuvant (ITACA) Trial, a Phase III Multicenter Randomized Trial Comparing Adjuvant Pharmacogenomic-Driven Chemotherapy versus Standard Adjuvant Chemotherapy in completely Resected Stage II-IIIa Non-Small Cell Lung Cancer

S. Novello, V. Torri, C. Grohe, S. Kurz, M. Serke, T. Wehler, A. Meyer, D. Ladage, M. Geissler, I. Colantonio, C. Cauchi, E. Stoelben, A. Ceribelli, C. Kropf-Sanchen, G. Valmadre, G. Borra, M. Schena, A. Morabito, A. Santo, V. Gregorc, R. Chiari, M. Reck, G. Schmid-Bindert, G. Folprecht, F. Griesinger, A. Follador, P. Pedrazzoli, A. Bearz, O. Caffo, N.J. Dickgreber, L. Irtelli, G. Wiest, V. Monica, L. Porcu, C. Manegold, G.V. Scagliotti

PII: S0923-7534(21)04496-3

DOI: <https://doi.org/10.1016/j.annonc.2021.09.017>

Reference: ANNONC 764

To appear in: *Annals of Oncology*

Received Date: 14 June 2021

Revised Date: 5 September 2021

Accepted Date: 26 September 2021

Please cite this article as: Novello S, Torri V, Grohe C, Kurz S, Serke M, Wehler T, Meyer A, Ladage D, Geissler M, Colantonio I, Cauchi C, Stoelben E, Ceribelli A, Kropf-Sanchen C, Valmadre G, Borra G, Schena M, Morabito A, Santo A, Gregorc V, Chiari R, Reck M, Schmid-Bindert G, Folprecht G, Griesinger F, Follador A, P. Pedrazzoli Bearz A, Caffo O, Dickgreber NJ, Irtelli L, Wiest G, Monica V, Porcu L, Manegold C, Scagliotti GV, International Tailored Chemotherapy Adjuvant (ITACA) Trial, a Phase III Multicenter Randomized Trial Comparing Adjuvant Pharmacogenomic-Driven Chemotherapy versus Standard Adjuvant Chemotherapy in completely Resected Stage II-IIIa Non-Small Cell Lung Cancer, *Annals of Oncology* (2021), doi: <https://doi.org/10.1016/j.annonc.2021.09.017>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that,

during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2021 Published by Elsevier Ltd on behalf of European Society for Medical Oncology.

International Tailored Chemotherapy Adjuvant (ITACA) Trial, a Phase III Multicenter Randomized Trial Comparing Adjuvant Pharmacogenomic-Driven Chemotherapy versus Standard Adjuvant Chemotherapy in completely Resected Stage II-IIIa Non-Small Cell Lung Cancer

S. Novello^{1*}, V. Torri^{2*}, C. Grohe³, S. Kurz⁴, M. Serke⁵, T. Wehler⁶, A. Meyer⁷, D. Ladage⁷, M. Geissler⁸, I. Colantonio⁹, C. Cauchi⁹, E. Stoelben¹⁰, A. Ceribelli¹¹, C. Kropf-Sanchen¹², G. Valmadre¹³, G. Borra¹⁴, M. Schena¹⁵, A. Morabito¹⁶, A. Santo¹⁷, V. Gregorc¹⁸, R. Chiari¹⁹, M. Reck²⁰, G. Schmid-Bindert²¹, G. Folprecht²², F. Griesinger²³, A. Follador²⁴, P. Pedrazzoli²⁵, A. Bearz²⁶, O. Caffo²⁷, N. J. Dickgreber²⁸, L. Irtelli²⁹, G. Wiest³⁰, V. Monica¹, L. Porcu^{2*}, C. Manegold^{31*}, G. V. Scagliotti^{1*}

*These authors (as co-first and co-last) equally contributed to the study.

¹ University of Torino, Department of Oncology at San Luigi Hospital, Orbassano (Torino) Italy;

² Laboratory of Methodology for Clinical Research, Oncology Department at Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan (Italy)

³ Pneumologie, Evangelische Lungenklinik Berlin, Berlin, Germany

⁴ Evangelische Lungenklinik Berlin, Berlin, Germany

⁵ Thorax-Zentrum Klinik für Hämatologie, Onkologie, Pneumologie und Palliativmedizin Evangelisches Krankenhaus Hamm, Germany

⁶ Thorax-Zentrum Klinik für Hämatologie, Onkologie, Pneumologie und Palliativmedizin Evangelisches Krankenhaus Hamm, Germany

⁷ Department of Pneumology Maria Hilf Hospital, Moenchengladbach, Germany

⁸ Esslingen Cancer Center Department of Oncology, Gastroenterology and Infectious Diseases Klinikum Esslingen, Germany

⁹ Divisione di Oncologia – Azienda Ospedaliera Santa Croce e Carle – Cuneo, Italy

¹⁰ Lungclinic Hospital of Cologne, Germany

¹¹ Divisione di Oncologia Medica A, Istituto Nazionale Regina Elena per lo Studio e la Cura dei Tumori, Roma, Italy

¹² Department of Pulmonology, Internal Medicine II, University of Ulm, Germany

- ¹³ Divisione di Oncologia Medica, Ospedale di Sondalo, Sondrio, Italy
- ¹⁴ Divisione di Oncologia Medica, AOU Maggiore della Carità, Novara, Italy
- ¹⁵ Divisione di Oncologia Medica I, AOU Città della Salute e della Scienza, Torino, Italy
- ¹⁶ Divisione di Oncologia Medica Toraco Polmonare, IRCCS Fondazione Pascale, Napoli, Italy
- ¹⁷ Unità Operativa Complessa di Oncologia – Gruppo Interdisciplinare Veronese Oncologia Polmonare (GIVOP), Verona, Italy
- ¹⁸ Divisione di Oncologia Medica, IRCCS Ospedale San Raffaele, Milano, Italy
- ¹⁹ Divisione di Oncologia Medica, Azienda Ospedaliera di Perugia, Ospedale Santa Maria della Misericordia, Perugia, Italy
- ²⁰ Onkologischer Schwerpunkt LungenClinic Grosshansdorf, Germany
- ²¹ Medical Faculty Mannheim, Heidelberg University, Germany
- ²² University Hospital Carl Gustav Carus Medical Department I Dresden, Germany
- ²³ Klinik für Hämatologie und Onkologie Medizinischer Campus Universität Oldenburg, Germany
- ²⁴ Struttura Ospedaliera Complessa di Oncologia, Presidio Ospedaliero Universitario Santa Maria della Misericordia, Azienda Sanitaria Universitaria Integrata Friuli Centrale, Udine, Italy
- ²⁵ Struttura Complessa di Oncologia, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy
- ²⁶ Divisione di Oncologia Medica, Centro di Riferimento Oncologico, Aviano, Italy
- ²⁷ Divisione di Oncologia Medica, Ospedale Santa Chiara, Trento, Italy
- ²⁸ Department for Respiratory Medicine and Thoracic Oncology Klinikum Rheine – Mathias-Spital, Rheine, Germany
- ²⁹ Clinica Oncologica, Policlinico SS. Annunziata, Chieti, Italy
- ³⁰ Department of Oncology, Hamburg Harburg, Germany
- ³¹ Department of Surgery, University Medical Center Mannheim, Heidelberg University, Mannheim, Germany

Corresponding Author:

Prof
Silvia Novello
University of Torino
Department of Oncology at S. Luigi Hospital
Regione Gonzole, 10
10043 Orbassano (Torino)
Italy
e-mail: silvia.novello@unito.it
Telephone: +390119026233
Fax: +39 011 9015184

Abstract**Background**

Several strategies have been investigated to improve the 4% survival advantage of adjuvant chemotherapy in early-stage non-small cell lung cancer (NSCLC). We aimed to evaluate in this investigator-initiated study the predictive utility of the mRNA expression levels of ERCC1 and TS as assessed in resected tumor.

Methods

Seven hundred seventy-three completely resected stage II-III NSCLC patients were enrolled and randomly assigned in each of the 4 genomic subgroups to investigator's choice of platinum-based chemotherapy (C, n=389) or tailored chemotherapy (T, n=384). All anticancer drugs were administered according to standard doses and schedules. Stratification factors included stage and smoking status. The primary end point of the study was overall survival (OS).

Results

Six hundred and ninety patients were included in the primary analysis. At a median follow-up of 45.9 months, 85 (24.6%) and 70 (20.3%) patients died in arms C and T, respectively. 5-year survival for patients in arms C and T was of 65.4% (95%CI: 58.5-71.4%) and 72.9% (95%CI: 66.5-78.3%), respectively. The estimated hazard ratio (HR) was 0.77 (95%CI: 0.56-1.06, p-value: 0.109) for arm T versus arm C. HR for recurrence-free survival was 0.89 (95%CI: 0.69-1.14, p-value: 0.341) for arm T versus arm C. Grade 3-5 toxicities were more frequently reported in arm C than in arm T.

Conclusion

In completely resected stage II-III NSCLC tailoring adjuvant chemotherapy conferred a non-statistically significant trend for OS favoring the T arm. In terms of safety, the T arm was associated with better efficacy/toxicity ratio related to the different therapeutic choices in the experimental arm.

Key words

Lung cancer, Non-small cell lung cancer, adjuvant chemotherapy, pharmacogenomics, excision repair cross-complementation 1, thymidylate synthase, messenger RNA

Highlights

- Adjuvant platinum-based chemotherapy is accepted as standard of care in stage II and III (NSCLC) patients
- Several studies addressed the question whether molecular tumor markers may serve as predictive biomarkers
- ITACA was planned to evaluate the predictive utility of ERCC1 and TS mRNA expression levels in completely resected NSCLC
- ITACA results indicate that adjuvant chemotherapy customization based on ERCC1 and TS mRNA levels did not improve efficacy
- In terms of safety, the pharmacogenomic-driven arm was associated with better efficacy/toxicity ratio

Running title

Phase III adjuvant pharmacogenomic-driven study in resected stage II-III NSCLC

Introduction

Adjuvant platinum-based chemotherapy is accepted as standard of care in stage II and III non-small cell lung cancer (NSCLC) patients (1-4).

Several studies addressed the question whether molecular tumor markers, such as DNA repair enzymes, drug transporters, cell cycle regulators, all involved in tumor growth or action of anticancer drugs, may serve as predictive biomarkers (5) in order to maximize or maintain antitumor activity and potentially spare toxicity.

In retrospective and prospective advanced NSCLC series, markers such Excision Repair Cross-Complementation1 (ERCC1), Ribonucleotide Reductase M1 (RRM1), Breast Cancer Associated gene 1 (BRCA1), Thymidylate Synthase (TS) were assessed individually or in combination to identify patients most likely to benefit from specific chemotherapy drugs (6-14). Despite promising early results (15), randomized studies in advanced NSCLC have failed to demonstrate the predictive utility of these markers (16-18).

The ITACA (International Tailored Chemotherapy Adjuvant Therapy) phase III trial was planned to evaluate the predictive utility of the mRNA expression levels of ERCC1 and TS (19) in completely resected NSCLC.

Methods

Study design and participants

This open-label, randomized, investigator-initiated study (EudraCT 2008-001764-36) was conducted in three European countries and included adult patients, ECOG performance status of 0 or 1 and completely resected pathological stage II-III NSCLC according to the 6th edition of the TNM staging system, subsequently amended when the 7th edition was implemented (20).

Types of resection included lobectomy, bi-lobectomy, sleeve lobectomy and pneumonectomy. Complete mediastinal lymph node resection or sampling was required and all surgical margins of resection were requested to be free of tumor. Patients were chemotherapy-naïve and should not have received radiation therapy prior to resection for lung cancer. An interval time from 6 weeks to maximum 9 weeks was allowed between surgery and the start of adjuvant therapy.

All patients signed and gave written informed consent and the protocol was initially approved by the Institutional Review Board of the principal investigator and subsequently by all the other participant institutions and complied with the Declaration of Helsinki.

The trial was funded by an unrestricted educational grant by Eli Lilly and Co. (Indianapolis, US) with no role in study design, data collection, data analysis, data interpretation or writing of the report.

Gene profiling analyses

After surgical resection, from each paraffin block of representative tumour areas four charged slides 5mm-thick for immunohistochemical evaluation, one 5mm-thick slide stained with hematoxylin and eosin for pathological evaluation and two unstained slides 10 μ m-thick were sent to the Pharmacogenomics Unit at San Luigi Hospital in Orbassano (Turin, Italy) for central biomarker assessment. Serial sections with a thickness of 10 μ m were stained with nuclear Fast Red (Sigma-Aldrich, St. Luis, MO). Malignant cells were identified under microscope magnification (from 5 to 10X) and dissected by a scalpel. RNA isolation and relative cDNA quantitation for ERCC1 and TS performed according to previously reported techniques (21) (see also supplementary materials).

High and low ERCC1 or TS mRNA expression was defined as the value $\geq$ or $<$ the median values for these 2 markers as assessed in a preliminary study performed at the University of Torino in 50 consecutive cases of surgically resected NSCLC. For ERCC1 the cut-off value was set at 3.90 unitless ratio and for TS at 3.50 unitless ratio. The ERCC1 and TS primers were designed to detect the entire genes and they were not detecting any specific isoforms.

Randomization

Eligible patients were stratified by disease stage (stage II *versus* IIIA), smoking status (never/former *versus* current), genomic profile and allocated 1:1 to tailored or standard adjuvant chemotherapy (Figure 1). Patients were randomly assigned to investigator's choice of platinum-based chemotherapy or chemotherapy defined by the molecular markers. In the tailored arm patients with tumors that had high ERCC1 and high TS (highERCC1/highTS) received single-agent paclitaxel; patients with high ERCC1 and low TS (highERCC1/lowTS) received single-agent pemetrexed;

patients with low ERCC1 and high TS (lowERCC1/highTS) received cisplatin and gemcitabine; patients with low ERCC1 and low TS (lowERCC1/lowTS) received cisplatin and pemetrexed.

Procedures

Prior to any study-related procedures, signed informed consent was obtained and the pathology report and slide preparation completed. Within 35 days prior to the start of adjuvant treatment the patients were assessed for inclusion/exclusion criteria, vital signs, concomitant medications, computer tomography (CT) scan of thorax and upper abdomen, brain imaging (magnetic resonance imaging or CT) and recording of adverse events. In the control arm patients received cisplatin-based therapy according to investigator choice. Chemotherapy was either vinorelbine 30 mg/m² IV over 10 minutes, days 1 and 8, or docetaxel 75 mg/m² intravenously over 60 minutes on day 1 and cisplatin 75 mg/m² IV over 60 minutes, day 1, immediately following vinorelbine or docetaxel. Gemcitabine 1200 mg/m² IV over 30 minutes was administered on days 1 and 8 and cisplatin 75 mg/m² IV over 60 minutes day 1, immediately following gemcitabine.

In the experimental arm patients in the highERCC1/highTS group received 4 cycles of single-agent paclitaxel on day 1 of a 3-week cycle, in highERCC1/lowTS received 4 cycles of single-agent pemetrexed, in lowERCC1/highTS four cycles of cisplatin/gemcitabine and in lowERCC1/lowTS four cycles of cisplatin/pemetrexed.

Paclitaxel was administered at a dose of 200 mg/m² intravenously over 3 hours on day 1 and then every 21 days. Prior to receiving paclitaxel, all patients received standard premedication.

Pemetrexed was administered at 500 mg/m² intravenously on Day 1 of each 21-day cycle with standard premedication. Cisplatin and gemcitabine were administered at doses and schedule as in the control arm.

All chemotherapy regimens were administered for a total of 4 cycles on a 21-day basis. All patients were followed for toxicity and response with dose modifications made as needed.

Patients discontinued protocol therapy if there was evidence of recurrent NSCLC or in case of extraordinary medical circumstances or if the patient had withdrawn consent. All patients have to be followed for at least ten years or until death, whichever came first. Treatment at the time of relapse was left at the discretion of each investigator participating in the study.

Outcomes

The primary end point was overall survival (OS) defined as the time from randomization to death from any cause. Secondary end points were recurrence-free survival (RFS), defined as the time from randomization to tumor recurrence or death from any cause, whichever came first, the toxicity of the two treatment arms, and the therapeutic compliance. Dose intensity was defined as the total amount of drug given (mg/m^2) divided by the total number of weeks on treatment.

Statistical analysis

Primary statistical analysis was performed on a modified intent-to-treat (ITT) population, analyzing the patients according to the randomization scheme, independently from compliance to the allocated treatment. Randomized patients were excluded from the modified ITT population if one of the following conditions was present: patients' or physicians' refusal after randomization to take part in the clinical trial, one major eligible criterion not satisfied, absence of efficacy and safety endpoint data at the database lock on February 2019. In the per protocol (PP) analysis only those patients included in the modified ITT population that received at least one dose of the treatment allocated by randomization were analyzed. PP population was used for the safety analysis.

It was assumed that the 5-year survival for the patients treated with standard adjuvant chemotherapy was 45% and the hazard reduction associated with the experimental treatment was 30%. Requiring a power of 90% to detect the estimated effect, with log-rank test a significant level of 5% (2 tailed), 336 deaths had to be observed. Since the accrual was initially expected to last 24 months and cut-off time for analysis was planned at 60

months of follow-up after randomization closure, the expected total number of patients was 684, which was rounded up to 700 pts (350 per arm) in order to take account of possible dropouts. Further details of the statistical analysis are reported in Supplementary statistical methods.

Results

Between December 2008 and August 2014, 773 patients were screened and randomized by 31 participating centers. Three hundred eighty-nine participants were randomly allocated to control arm and 384 to tailored arm. Figure 1 summarizes the distribution of patients assigned to control arm or tailored arm in each genomic profile. Eighty-three (10.7%) patients were excluded before statistical analysis was performed.

Patients demographics and disease characteristics (Table 1) were similar between the control arm and the tailored arm. Lobectomy was, by far, the commonest surgical procedure (n=475, 75.4%) followed by pneumonectomy (n=97, 15.4%) with missing information in 60 (8.7%) patients. Node sampling or systematic nodal dissection was left to the standard surgical procedures of each participating centre and was not recorded in the case report form.

In the control arm, 159 patients (50.0%) were treated with cisplatin and gemcitabine, 123 (38.7%) received cisplatin and vinorelbine, 23 (7.2%) received cisplatin and docetaxel, 5 cisplatin and pemetrexed (1.5%) with incomplete information in 28 (8.1%) patients. Eight patients had a protocol violation because the investigator did not follow the recommended treatment. In the experimental arm 137 patients (39.8%) were allocated to the treatment according to highERCC1/highTS, 35 (10.2%) to highERCC1/lowTS, 85 (24.7%) to lowERCC1/highTS and 87 (25.3%) to lowERCC1/lowTS. One hundred thirty-four (97.8%), 33 (94.3%), 83 (97.6%) and 87 (100%) patients in the tailored arm were treated according to study design based on the 4 different genetic profiles, respectively.

In the control and tailored arm, the median number of treatment cycles was four (range 1-4) cycles. Detailed information about dose intensity in the control and tailored arm are reported in Supplementary table 1.

After a median follow-up of 45.9 months (IQR: 13.3-84.9 months) less than half of expected OS events have been observed. Five-year survival for patients in the control and tailored arm was underestimated by design: expected 5-year survival of 45% and 57% versus observed 5-year survival of 65.4% (95%CI: 58.5-71.4%) and 72.9% (95%CI: 66.5-78.3%), respectively. For this reason, we decided to prematurely close study follow-up and to perform the final analysis. In the modified ITT analysis, the estimated median OS was not yet defined in both arms for an estimated Hazard Ratio (HR) of 0.77 for tailored arm versus control arm (95%CI: 0.56-1.06; unstratified p-value: 0.109; stratified p-value: 0.124). In the multivariate analysis, the estimated HR was 0.78 (95%CI: 0.56-1.07; p-value: 0.120). In the PP analysis, 5-year survival for patients in the tailored and control arm was of 65.3% (95%CI: 58.4-71.4) and 73.3% (95%CI: 66.8-78.7%), respectively, with an estimated HR of 0.76 (95%CI: 0.55-1.04; p-value: 0.089). In the multivariate analysis, the estimated HR was 0.77 (95%CI: 0.56-1.06; p-value: 0.111). Figure 2a and Supplementary figure 2a show OS by treatment arm for the modified ITT and PP population, respectively. There was no evidence of heterogeneity ($I^2=0\%$) between the four genomic profiles both in the modified ITT and PP population (Supplementary figure 1a and figure 1b).

Overall 252 (36.5%) patients had a recurrence event, 129 (37.3%) in the control arm and 123 (35.8%) in the tailored arm. In the modified ITT analysis, the estimated median RFS was 40.4 months (95%CI: 29.2-55.9 months) in the control arm and 64.4 months (95%CI: 39.8-89.7 months) in the tailored arm with an estimated HR of 0.89 (95%CI: 0.69-1.14; unstratified p-value: 0.341; stratified p-value: 0.298). At the multivariate analysis, the estimated HR was 0.88 (95%CI: 0.69-1.13; p-value: 0.328). In the PP analysis, the estimated median RFS were unchanged in both arms with an estimated HR of 0.89 (95%CI: 0.69-1.14; p-value: 0.344). In the multivariate analysis, the estimated HR was 0.88 (95%CI: 0.69-1.14; p-value: 0.336). Figure 2b and Supplementary figure 2b show RFS by treatment arm for the modified ITT and PP population, respectively. As shown in Supplementary figure 1c and Figure 1d, no heterogeneity ($I^2=0\%$) was detected between the four genomic profiles both in the modified ITT and PP population.

OS and RFS across the two stratification factors and other patient and disease characteristics were consistent with the overall outcomes of the study (Figure 3). The tailored arm showed a non-statistically significant trend to improved OS, and less relevantly for RFS, in several of the considered subgroups, being more evident for stage II disease (HR of death:0.64; 95%CI: 0.42-0.97). No heterogeneity of treatment effect was detected across

different center (OS: p-value:0.89, I^2 : 0%; RFS: p-value:0.96, I^2 : 0%). (HR of death:0.64; 95%CI: 0.42-0.97). No heterogeneity of treatment effect was detected across different center (OS: p-value:0.89, I^2 : 0%; RFS: p-value:0.96, I^2 : 0%).

Using the Cox regression model we estimated the risk of relapse at increasing values of ERCC1 for patients in the control and tailored arm resulting in an HR of 1.00 (p-value:0.33) and HR of 1.00 (p-value:0.76), respectively. Similarly, we estimated the risk of relapse at increasing values of TS for patients in the control and in the tailored arm resulting in an HR of 1.00 (p-value:0.12) and HR of 1.00 (p-value:0.39), respectively. Finally, we tested an interaction effect for RFS endpoint between ERCC1 and TS in the control and in the tailored arm: p-value of 0.82 and 0.36, respectively. In every Cox regression model, biomarkers were evaluated as continuous predictors. Analysis of site of recurrences and therapy after recurrence are reported in Supplementary table 3.

Distribution of any attribution- related adverse events according to both grade 1-2 or 3 to 5 and to each genomic profile in the 2 arms of the study are reported in Table 2. Data shown indicate a reduced toxicity favouring the tailored arm, more pronounced for haematological toxicity. Grade 3-5 toxicity was observed in 32.6% of the patients in the tailored arm and 45.9% of the patients in the control arm, respectively. The estimated risk of grade 3-5 AEs in the tailored arm versus the control arm was 0.57 in terms of odds ratio (95%CI:0.42-0.78; p value<0.001). Adverse events regardless of relationship, worst grade by patient safety population grouped by category/MedDRA code are summarized in Supplementary table 2.

Discussion

In completely resected NSCLC patients, adjuvant strategies aimed to improve efficacy of cytotoxic chemotherapy have been extensively investigated and include integration of immunotherapy (22-23), targeted therapies (24-26), and chemotherapy customization using molecular markers. This study results indicate that adjuvant chemotherapy customization based on the primary tumor tissue mRNA expression of two molecular markers associated with cytotoxic drug metabolism did not significantly improve efficacy outcomes in terms of OS and RFS. As expected, treatment

customization significantly improved toxicity profile of the adjuvant therapy without compromising the activity. Unfortunately, at increasing tissue level of both ERCC1 and TS we failed to show a differential clinical activity.

It should be pointed out that at the time of the analysis only 155 events of the 336 (46.1%) expected events were reported. Therefore, the failure to reach the statistical significance can clearly be attributed to the lack of power of the study (i.e. real statistical power by Freedman method: 59%), depending upon reasons related to better prognosis of patients (possibly due to stage migration and to the efficacy of second-line therapies), not expected at the time when the study was planned.

OS data in the control arm of the present study were significantly better than those previously reported in the Italian ALPI adjuvant study which compared MVP versus control in completely resected NSCLC stage IB-II-IIIa (27) and this improvement may be partially attributed to a significant stage migration and optimization of surgical procedures over the last 20 years.

Treatment effect on RFS endpoint (HR: 0.89) was even less significant than that on the OS endpoint (HR: 0.77). This result could be explained: 1) ITACA was an investigator-initiated multi-center randomized trial and the assessment of disease relapse was not standardized by protocol. Therefore, it was left to each investigational center to define the frequency of disease assessment for each patient; 2) broad random error was present in OS benefit (i.e. HR, 95%CI: 0.56-1.06) and RFS benefit (i.e. HR, 95%CI: 0.69-1.14); 3) tumor biology and second-line treatment could have modified post-relapse survival.

The lack of data about ERCC1 and TS mRNA expression in early stage NSCLC drove the pre-study feasibility to investigate the level of expression and to define a median value of mRNA expression in order to segregate patients into high and low expressors. The choice to select the median value of expression instead of quartile or tertile distribution was influenced by practical reasons in analyzing the final dataset and by the impossibility to predict the tumor proportions in each genomic subgroup.

Although the study was not designed to analyze data based on subgroup genomic profiles, because of different patient numbers in each profile and for the lack of power, it is worth noting that single-agent adjuvant therapy in high ERCC1 subgroups was not detrimental and the data were in agreement with a previously reported study (28). Conversely, the highest benefit was observed in tumors with low ERCC1 and TS (see Supplementary figure 1)

Limitations of our trial include the long time to recruit patients against a background of competing studies, the relative high proportion of ineligible patients and the disappointing results from advanced disease studies addressing the same type of question. The multicenter nature of the study may also generate biases related to specimen processing and handling procedures at the collection sites. The extremely long enrolment time may also have contributed to the considerable inter-assay variability for ERCC1 and TS. Further challenge consisted in the academic nature of the study ruling some pragmatic but limiting choices. For instance, in addition to thymidylate synthase pemetrexed inhibits at least two other enzymes involved in the folate pathway; dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). Furthermore, folate receptor alpha (FRA) regulates cellular uptake of folates and antifolates and may be involved in pemetrexed efficacy when assessed by immunohistochemistry (29).

More recently it was shown that the ERCC1 gene generates four isoforms (designated 201, 202, 203, and 204) by alternative splicing. In a study the relative levels of isoform expression differed among tumors. The authors also demonstrated that ERCC1 isoforms were not equal in function; only ERCC1-202 isoform was found to repair platinum-DNA adducts (30). Thus, ample expression of one or several non-functional isoforms may lead to a false classification of the tumor as ERCC1-positive. However, the development of such an assay that specifically distinguishes and measures the functional ERCC1 protein isoform is a challenge because of strong homology among the four protein isoforms.

Another intrinsic challenge is related to the lack of a clear prognostic and predictive role of these molecular markers as previously reported (31-33). Consequently, we implemented a completely different study design where patients were randomized to control or experimental treatment in each of the 4 identified genomic profiles maximizing the separation between potential prognostic from the potential predictive information. Furthermore, despite the critical role of ERCC1 and TS in drug metabolism the complexity of drug interactions and multiple resistance mechanisms forestall the application of these two molecular markers and their simple separation into high and low categories. Beyond any other consideration the lack of standardization for determining the levels of markers is making the interpretation of results across trials difficult and hinders the future development of additional studies.

The available results of all the pharmacogenomic studies performed in the setting of advanced (16,34-35) and early stage NSCLC (36-38) as well as the outcome of the present study indicate that pharmacogenomic strategies based on single/few genes without alterations that imply oncogene addiction do not warrant use in the clinical practice.

The growing evidence of intratumor heterogeneity and the availability of next-generation sequencing and high-throughput diagnostic techniques are preferred in the attempt to tailor adjuvant chemotherapy in completely resected NSCLC. In a future therapeutic landscape, where immune-checkpoint inhibitors and targeted therapies will play a relevant role also in the early disease therapeutic strategy, the role of chemotherapy will remain critical in maximizing the therapeutic benefit as already demonstrated in the advanced disease setting and, consequently, any attempt to improve its therapeutic ratio should be carefully considered.

Contributors

SN, GVS, VT were involved in the conception, design and planning of the study; all the other authors presented in the front page of the manuscript as well as those listed among contributors collected the data. VM performed the molecular analyses. LP and VT performed the statistical analyses. All the authors had the opportunity to interpret the results and reviewed the manuscript in its final version. GVS and SN drafted the manuscript that was critically reviewed, revised and approved for submission by all the authors.

List of additional contributors

Francesco Cognetti, Michele Milella Divisione di Oncologia Medica A, Istituto Nazionale Regina Elena per lo Studio e la Cura dei Tumori, Roma, Italy

Alessandra Ferrari; Emma Pozzi; Francesco Agustoni SC Oncologia, Fondazione IRCCS Policlinico S. Matteo – Pavia, Italy

Simone Busso; Francesco Ardisson, Mauro Papotti, Luisella Righi, Dipartimento di Oncologia, AOU San Luigi Orbassano, Università di Torino, Italy

Gaetano Rocco, Carmine La Manna, Anna Manzo, Pasqualina Giordano IRCCS, Fondazione Pascale, Napoli, Italy

Giulio Metro, Oncologia Medica, Azienda Ospedaliera di Perugia, Italy

Mario Spatafora, Divisione di Pneumologia Ospedale Cervello, Palermo, Italy

Hector Sotoparra, UOC di Oncologia Medica AOU "Policlinico – Vittorio Emanuele" Catania, Italy

Lothar R. Pilz, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Peter Hohenberger, Department of Surgery, University Medical Center Mannheim, Heidelberg University, Mannheim, Germany,

Walburga Engel-Riedel; Jessica Juergens; Gudrun Klauke: Lungclinic Hospital of Cologne, Germany

Beate Polte, Sylvia Dippman, Hieronima Daiber, Andrea Igel, Department of Pulmonology, Internal Medicine II, University of Ulm, Germany

Rodrigo Ramlau Oncology Department Poznan University of Medical Sciences, Poland.

Acknowledgement

The Authors are grateful to Prof. Paolo Bertinetti for the editing of the manuscript

Funding

The trial was funded by an unrestricted educational grant by Eli Lilly and Co. (Indianapolis, US) with no role in study design, data collection, data analysis, data interpretation or writing of the report. No grant number applicable.

Conflict of Interest

Giorgio Scagliotti received honoraria from AstraZeneca, Eli Lilly, MSD, Pfizer, Roche, Johnson & Johnson, Takeda; consulting or advisory role for Eli Lilly, Beigene and AstraZeneca, received institutional research funding from Eli Lilly and MSD and received travel, accommodations from Bayer. Silvia Novello received honoraria or consulted for Eli Lilly, Amgen, Beigene, AstraZeneca, Bristol Myers Squibb, Abbvie, Boehringer Ingelheim, MSD, Roche, Pfizer and Takeda

All the other Authors declared the absence of any conflicts of interest related to the present study.

Figure Legends

Figure 1. Study participants flow-chart

Figure 2. Overall and recurrence-free survival

Figure 3. Subgroup analysis by patients' demographics and disease characteristics

References

1. Pignon JP, Tribodet H, Scagliotti GV, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. *J Clin Oncol.* 2008; 26:3552-9.
2. Butts CA, Ding K, Seymour L et al. Randomized phase III trial of vinorelbine plus cisplatin compared with observation in completely resected stage IB and II non-small-cell lung cancer: updated survival analysis of JBR-10. *J Clin Oncol.* 2010 28:29-34
3. NSCLC Meta-analyses Collaborative Group Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data. *Lancet.* 2010; 375:1267-77
4. Burdett S, Pignon JP, Tierney J, et al. Adjuvant chemotherapy for resected early-stage non-small cell lung cancer *Cochrane Database Syst Rev.* 2015; 2;3:CD011430.
5. Simon GR, Rosell Costa R, Gandara DR. Pharmacogenomics in Lung Cancer: Predictive Biomarkers for Chemotherapy in "The IASLC Multidisciplinary Approach to Thoracic Oncology" IASLC press. 2014; pag. 647-658
6. Li Q., Yu JJ, Mu C, et al. Association between the level of ERCC-1 expression and the repair of cisplatin-induced DNA damage in human ovarian cancer cells. *Anticancer Res* 2000; 20:645-652.
7. Metzger R, Leichman CG, Danenberg KD et al. ERCC1 mRNA levels complement thymidylate synthase mRNA levels in predicting response and survival for gastric cancer patients receiving combination cisplatin and fluorouracil chemotherapy. *J Clin Oncol* 1998; 16:309-316.
8. Shirota Y, Stoecklricher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. *J Clin. Oncol* 2001;19;4298-304.
9. Joshi MB, Shirota Y, Danenberg KD et al. High gene expression of TS1, GSTP1, and ERCC1 are risk factors for survival in patients treated with trimodality therapy for esophageal cancer. *Clin Cancer Res.* 2005;11(6):2215-21.
10. Dabholkar M, Vionnet J, Bostick-Bruton F et al. Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. *J Clin Invest.* 1994;94(2):703-8

11. Ceppi P, Volante M, Saviozzi S, et al: Squamous cell carcinoma of the lung compared with other histotypes shows higher mRNA and protein levels for thymidylate synthase *Cancer* 2006; 107: 1589– 1596
12. Maus MK, Mack PC, Astrow SH et al. Histology-related associations of ERCC1, RRM1, and TS biomarkers in patients with non-small-cell lung cancer: implications for therapy. *J Thorac Oncol.* 2013;8(5):582-6
13. Scagliotti GV, Parikh P, von Pawel J, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naïve patients with advanced-stage non-small-cell lung cancer. *J Clin Oncol* 2008;26:3543-51
14. Ceppi P, Volante M, Ferrero A, Thymidylate synthase expression in gastroenteropancreatic and pulmonary neuroendocrine tumors. *Clin Cancer Res.* 2008; 14:1059-64
15. Simon GR, Schell MJ, Begum M et al. Preliminary indication of survival benefit from ERCC1 and RRM1-tailored chemotherapy in patients with advanced non-small cell lung cancer: evidence from an individual patient analysis. *Cancer* 2012 118(9):2525-31.
16. Cobo M, Isla D, Massuti B, et al. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. *J Clin Oncol.* 2007;25(19):2747-54
17. Reynolds C, Obasaju C, Schell MJ, et al. Randomized phase III trial of gemcitabine-based chemotherapy with in situ RRM1 and ERCC1 protein levels for response prediction in non-small-cell lung cancer. *J Clin Oncol* 2009; 27:5808-5815
18. Bepler G, Williams C, Schell MJ, et al. Randomized international phase III trial of ERCC1 and RRM1 expression-based chemotherapy versus gemcitabine/carboplatin in advanced non-small cell lung cancer. *J Clin Oncol.* 2013; 31:2404-2412.
19. Buffoni L, Vavalà T, Novello S. Adjuvant Therapy of Resected Non-small Cell Lung Cancer: can We Move Forward? *Curr Treat Options Oncol.* 2016; 17:54
20. Goldstraw P, Crowley J, Chansky K et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol.* 2007; 2:706-14

21. Ceppi P, Volante M, Saviozzi S, et al: Squamous cell carcinoma of the lung compared with other histotypes shows higher messenger RNA and protein levels for thymidylate synthase *Cancer* 2006; 107: 1589– 1596
22. Vansteenkiste JF, Cho BC, Vanakesa T, et al. Efficacy of the MAGE-A3 cancer immunotherapeutic as adjuvant therapy in patients with resected MAGE-A3 positive non-small-cell lung cancer (MAGRIT): a randomized, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2016; 17(6):822-35
23. Bunn PA Jr, Schenk E, Pacheco J, et al. New Developments in Neoadjuvant Therapy for Lung Cancer. *Oncology (Williston Park)*. 2019; 33:101-106, 109
24. Wakelee HA, Dahlberg SE, Keller SM, et al. Adjuvant chemotherapy with or without bevacizumab in patients with resected non-small-cell lung cancer (E1505): an open-label, multicentre, randomised, phase 3 trial. *Lancet Oncol*. 2017; 18:1610-1623
25. Kelly K, Altorki NK, Eberhardt WE, et al. Adjuvant erlotinib versus placebo in patients with stage IB-IIIA non-small-cell lung cancer (RADIANT): a randomized, double-blind, phase III trial. *J Clin Oncol* 2015; 33:4007-14
26. Wu YL et al. Osimertinib in Resected EGFR-Mutated Non-Small-Cell Lung Cancer. *N Engl J Med*. 2020 Sep 19. doi: 10.1056/NEJMoa2027071. Online ahead of print
27. Scagliotti GV, Fossati R, Torri V, et al. Randomized study of adjuvant chemotherapy for completely resected stage I, II, or IIIA non-small-cell Lung cancer. *J Natl Cancer Inst*. 2003; 95:1453-61
28. Cobo M, Massuti B., Moran, T. et al. Spanish customized adjuvant trial (SCAT) based on BRCA1 mRNA levels. *ASCO Meeting Abstracts* 26: 7533. 2008
29. Christoph DC, Reyna Asuncion B, Hassan B et al. Significance of folate receptor alpha and thymidylate synthase protein expression in patients with non-small-cell lung cancer treated with pemetrexed. *J Thorac Oncol* 2013; 8:19-30
30. Friboulet L, Olaussen KA, Pignon J-P et al. ERCC1 isoform expression and DNA repair in non-small-cell lung cancer. *N Engl J Med* 2013; 328:1101-1110

31. Rosell R, Skrzypski M, Jassem E, et al. BRCA1: a novel prognostic factor in resected non-small-cell lung cancer. *PLoS One*. 2007;2(11):e1129
32. Zheng Z, Li X, Schell MJ, et al. Thymidylate synthase in situ protein expression and survival in stage I non-small cell lung cancer. *Cancer*. 2008; 112:2765-73
33. Zheng Z, Chen T, Li X, Haura E, et al. DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. *N Engl J Med*. 2007; 356:800-8. *J Clin Oncol*. 2007; 25:2747-54
34. Reynolds C, Obasaju C, Schell MJ, et al. Randomized phase III trial of gemcitabine-based chemotherapy with in situ RRM1 and ERCC1 protein levels for response prediction in non-small-cell lung cancer. *J Clin Oncol* 2009; 27:5808-5815
35. Bepler G, Williams C, Schell MJ, et al. Randomized international phase III trial of ERCC1 and RRM1 expression-based chemotherapy versus gemcitabine/carboplatin in advanced non-small-cell lung cancer. *J Clin. Oncol*. 2013; 31:2404-12
36. Bepler G, Zinner RG, Moon J, et al. A phase 2 cooperative group adjuvant trial using a biomarker-based decision algorithm in patients with stage I non-small cell lung cancer (SWOG-0720, NCT00792701). *Cancer*. 2014; 120:2343-5
37. Massuti B, Rodriguez-Paniagua JM, Cobo Dols M, et al. Results phase III trial customized adjuvant CT after resection of NSCLC with lymph node metastases SCAT: a Spanish lung cancer group trial. *J Thorac Oncol*. 2015;10:s180.
38. Wislez M, Barlesi F, Besse B, et al. Customized adjuvant phase II trial in patients with non-small-cell lung cancer: IFCT-0801 TASTE. *J Clin. Oncol*. 2014; 32:1256-61

Table 1. Baseline characteristics

	Overall		High ERCC1/High TS		High ERCC1/Low TS		Low ERCC1/High TS		Low ERCC1/Low TS	
	Tailored N=344	Control N=346	Tailored N=137	Control N=127	Tailored N=35	Control N=41	Tailored N=85	Control N=98	Tailored N=87	Control N=80
Age, median (min-max)	64 (30-81)	64 (34-82)	65 (38-81)	64 (34-77)	67 (52-78)	67 (42-82)	63 (31-78)	64 (43-77)	62 (30-80)	64 (39-81)
Sex										
Male	238 (70%)	236 (68%)	100 (73%)	94 (74%)	23 (66%)	27 (66%)	59 (70%)	72 (73%)	56 (64%)	43 (54%)
Female	104 (30%)	110 (32%)	37 (27%)	33 (26%)	12 (34)	14 (34%)	24 (28%)	26 (27%)	31 (37%)	37 (46%)
<i>Missing data</i>	2 (1%)	0	0	0	0	0	2 (2%)	0	0	0
Race										
Caucasian/white	336 (99%)	337 (100%)	134 (99%)	122 (100%)	34 (100%)	41 (100%)	83 (99%)	96 (100%)	85 (100%)	78 (100%)
Other	2 (1%)	0	1 (1%)	0	0	0	1 (1%)	0	0	0
<i>Missing data</i>	6 (2%)	9 (3%)	2 (1.5%)	5 (4%)	1 (3%)	0	1 (1%)	2 (2%)	2 (2%)	2 (2%)
Smoking history										
Never smoker	42 (12%)	37 (11%)	7 (5%)	5 (4%)	8 (23%)	10 (24%)	7 (8%)	8 (8%)	20 (23%)	14 (18%)
Former smoker	118 (35%)	144 (41%)	48 (35%)	52 (41%)	15 (43%)	19 (47%)	33 (39%)	44 (45%)	22 (25%)	29 (36%)
Current smoker	184 (53%)	165 (48%)	82 (60%)	70 (55%)	12 (34%)	12 (29%)	45 (53%)	46 (47%)	45 (52%)	37 (46%)
ECOG performance status -										
Grade 0	255 (76%)	260 (78%)	89 (66%)	90 (74%)	23 (68%)	25 (61%)	67 (81%)	79 (83%)	76 (90%)	66 (86%)
Grade 1	81 (24%)	75 (22%)	46 (34%)	32 (26%)	11 (32%)	16 (39%)	16 (19%)	16 (17%)	8 (10%)	11 (14%)
<i>Missing data</i>	8 (2%)	11 (3%)	2 (1%)	5 (4%)	1 (3%)	0 (0%)	2 (2%)	3 (3%)	3 (3%)	3 (4%)
Stage										
II	206 (60%)	200 (58%)	81 (59%)	75 (59%)	24 (69%)	25 (61%)	52 (62%)	55 (56%)	49 (56%)	45 (56%)
	137 (40%)	145 (42%)	56 (41%)	51 (41%)	11 (31%)	16 (39%)	32 (38%)	43 (44%)	38 (44%)	35 (44%)

	Overall		High ERCC1/High TS		High ERCC1/Low TS		Low ERCC1/High TS		Low ERCC1/Low TS	
	Tailored N=344	Control N=346	Tailored N=137	Control N=127	Tailored N=35	Control N=41	Tailored N=85	Control N=98	Tailored N=87	Control N=80
III A										
<i>Missing data</i>	1 (0.3%)	1 (0.3%)	0	1 (0.8%)	0	0	1 (1%)	0	0	0
Histotype										
Adenocarcinoma	201 (58%)	206 (59%)	70 (51%)	65 (51%)	22 (63%)	32 (78%)	45 (53%)	54 (55%)	64 (74%)	55 (69%)
Squamous cell carcinoma	121 (35%)	118 (34%)	60 (44%)	56 (44%)	8 (23%)	9 (22%)	35 (41%)	36 (37%)	18 (21%)	17 (21%)
Large cell carcinoma	10 (3%)	16 (5%)	5 (4%)	4 (3%)	1 (3%)	0	2 (3%)	7 (7%)	2(2%)	5 (6%)
Other*	11 (3%)	6 (2%)	2 (1%)	2 (2%)	4 (11%)	0	2 (3%)	1 (1%)	3	3 (4)
<i>Missing data</i>	1 (0.3%)	0	0	0	0	0	1 (1.2)	0	0	0
Type of surgery										
Lobectomy	234 (75%)	241 (76%)	86 (71%)	88 (76%)	27 (82%)	27 (73%)	58 (77%)	66 (72%)	63 (76%)	60 (81%)
Bi-lobectomy	18 (6%)	26 (8%)	8 (7%)	10 (9%)	2 (6%)	4 (11%)	4 (5%)	8 (9%)	4 (5%)	4 (5%)
Sleeve Lobectomy	8 (3%)	6 (2%)	5 (4%)	3 (3%)	0	0	0	1 (1%)	3 (4%)	2 (2%)
Pneumonectomy	52 (16%)	45 (14%)	22 (18%)	15 (12%)	4 (12%)	6 (16%)	13 (17%)	16 (18%)	13 (16%)	8 (9%)
<i>Missing data</i>	32 (9%)	28 (9%)	16 (12%)	11 (9%)	2 (6%)	4 (10%)	10 (12%)	7 (7%)	4 (5%)	6 (7%)

*Adeno-squamous n=7, squamous and large cell n=1,mucoepidermoid n=4,neuroendocrine n=1, pleomorphic n=2, sarcomatoid n=1,

Table 2. Safety analysis

	Overall				High ERCC1/High TS				High ERCC1/Low TS				Low ERCC1/High TS				Low ERCC1/Low TS			
	Tailored		Control		Tailored		Control		Tailored		Control		Tailored		Control		Tailored		Control	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Anemia																				
1-2	61	18.1	85	25.1	16	11.9	31	25.8	8	24.2	14	35.0	22	26.5	19	19.4	15	17.2	21	26.3
3-4	3	0.9	9	2.7	1	0.7	4	3.3	0	0	3	7.5	2	2.4	1	1.0	0	0	1	1.3
Neutropenia																				
1-2	29	8.6	21	6.2	8	6.0	12	10.0	2	6.1	0	0	9	10.8	4	4.1	10	11.5	5	6.3
3-4	45	13.4	64	18.9	11	8.2	24	20.0	4	12.1	9	22.5	20	24.1	18	18.4	10	11.5	13	16.3
Leucopenia																				
1-2	31	9.2	43	12.7	5	3.7	17	14.2	3	9.1	5	12.5	15	18.1	8	8.2	8	9.2	13	16.3
3-4	13	3.9	45	13.3	1	0.7	21	17.5	3	9.1	9	22.5	7	8.4	11	11.2	2	2.3	4	5.0
Thrombocytopenia																				
1-2	30	8.9	40	11.8	0	0	18	15.0	5	15.2	4	10.0	18	21.7	9	9.2	7	8.0	9	11.3
3-4	11	3.3	26	7.7	0	0	11	9.2	0	0	7	17.5	11	13.3	2	2.0	0	0	6	7.5
Fatigue																				
1-2	50	14.8	56	16.6	16	11.9	23	19.2	3	9.1	5	12.5	15	18.1	15	15.3	16	18.4	13	16.3
3-4	9	2.7	5	1.5	1	0.7	1	0.8	0	0	0	0	4	4.8	2	2.0	4	4.6	2	2.5
Nausea																				
1-2	70	20.8	96	28.4	20	14.9	37	30.8	5	15.2	11	27.5	17	20.5	25	25.5	28	32.2	23	28.8
3-4	13	3.9	15	4.4	0	0	7	5.8	1	3.0	2	5.0	6	7.2	4	4.1	6	6.9	2	2.5
Vomiting																				
1-2	15	4.5	45	13.3	5	3.7	21	17.5	0	0	3	7.5	5	6.0	11	11.2	5	5.7	10	12.5
3-4	7	2.1	6	1.8	0	0	4	3.3	0	0	1	2.5	3	3.6	1	1.0	4	4.6	0	0
Diarrhea																				
1-2	18	5.3	25	7.4	10	7.5	13	10.8	3	9.1	1	2.5	2	2.4	7	7.1	3	3.4	4	5.0

	Overall				High ERCC1/High TS				High ERCC1/Low TS				Low ERCC1/High TS				Low ERCC1/Low TS			
	Tailored		Control		Tailored		Control		Tailored		Control		Tailored		Control		Tailored		Control	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
3-4	5	1.5	1	0.3	0	0	0	0	1	3.0	0	0	1	1.2	1	1.0	3	3.4	0	0
Constipation																				
1-2	34	10.1	49	14.5	7	5.2	17	14.2	3	9.1	4	10.0	12	14.5	15	15.3	12	13.8	13	16.3
3-4	1	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.1	0	0
Abdominal pain																				
1-2	13	3.9	26	7.7	6	4.5	12	10.0	2	6.1	3	7.5	0	0	5	5.1	5	5.7	6	7.5
3-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomatitis																				
1-2	16	4.7	13	3.8	4	3.0	4	3.3	1	3.0	1	2.5	3	3.6	2	2.0	8	9.2	6	7.5
3-4	1	0.3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.1	0	0
Tinnitus/vertigo																				
1-2	11	3.3	15	4.4	4	3.0	6	5.0	0	0	1	2.5	3	3.6	6	6.1	4	4.6	2	2.5
3-4	0	0	1	0.3	0	0	1	0.8	0	0	0	0	0	0	0	0	0	0	0	0
Asthenia																				
1-2	20	5.9	27	8.0	5	3.7	15	12.5	1	3.0	3	7.5	4	4.8	5	5.1	10	11.5	4	5.0
3-4	1	0.3	5	1.5	0	0	4	3.3	1	3.0	0	0	0	0	1	1.0	0	0	0	0
Mucositis																				
1-2	12	3.6	13	3.8	5	3.7	5	4.2	1	3.0	2	5.0	1	1.2	3	3.1	5	5.7	3	3.8
3-4	2	0.6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2.3	0	0
Pyrexia																				
1-2	13	3.9	12	3.6	5	3.7	6	5.0	5	15.2	1	2.5	2	2.4	4	4.1	1	1.1	1	1.3
3-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Decreased appetite																				
1-2	18	5.3	36	10.7	5	3.7	16	13.3	3	9.1	3	7.5	5	6.0	10	10.2	5	5.7	7	8.8

	Overall				High ERCC1/High TS				High ERCC1/Low TS				Low ERCC1/High TS				Low ERCC1/Low TS			
	Tailored		Control		Tailored		Control		Tailored		Control		Tailored		Control		Tailored		Control	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
3-4	2	0.6	1	0.3	0	0	0	0	0	0	0	0	2	2.4	1	1.0	0	0	0	0
Hyperglycaemia																				
1-2	8	2.4	2	0.6	4	3.0	0	0	0	0	1	2.5	3	3.6	0	0	1	1.1	1	1.3
3-4	3	0.9	1	0.3	0	0	1	0.8	0	0	0	0	0	0	0	0	3	3.4	0	0
Myalgia																				
1-2	12	3.6	11	3.3	10	7.5	5	4.2	0	0	1	2.5	2	2.4	3	3.1	0	0	2	2.5
3-4	3	0.9	2	0.6	1	0.7	1	0.8	0	0	0	0	1	1.2	1	1.0	1	1.1	0	0
Paraesthesia																				
1-2	19	5.6	7	2.1	16	11.9	3	2.5	0	0	4	10.0	1	1.2	0	0	2	2.3	0	0
3-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dyspnea																				
1-2	16	4.7	23	6.8	7	5.2	7	5.8	2	6.1	4	10.0	4	4.8	6	6.1	3	3.4	6	7.5
3-4	2	0.6	1	0.3	1	0.7	1	0.8	1	3.0	0	0	0	0	0	0	0	0	0	0
Alopecia																				
1-2	40	11.9	13	3.8	33	24.6	2	1.7	1	3.0	4	10.0	6	7.2	5	5.1	0	0	2	2.5
3-4	7	2.1	0	0	7	5.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rash																				
1-2	13	3.9	11	3.3	5	3.7	2	1.7	4	12.1	1	2.5	1	1.2	4	4.1	3	3.4	4	5.0
3-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Worst degree (all grade toxicities were evaluated)																				
1-2	130	38.6	102	30.2	59	44.0	36	30.0	16	48.5	7	17.5	21	25.3	32	32.7	34	39.1	27	33.7
3-5 ■	110	32.6	155	45.9	32	23.9	65	54.2	10	30.3	22	55.0	41	49.4	38	38.8	27	31.0	30	37.5

Note: the per protocol population was used for the analysis of safety. The estimated risk of grade 3-5 AEs by genomic profile was as follows: a) high ERCC1/high TS: 0.27 (95%CI: 0.16-0.45); b) high ERCC1/low TS: 0.36 (95%CI: 0.14-0.94); c) low ERCC1/high TS: 1.54 (95%CI: 0.85-2.79); d) low ERCC1/low TS: 0.75 (95%CI: 0.39-1.42); p-value for heterogeneity across genetic profiles: < 0.001; I²: 85%.

- CTCAE grade 5: one case of death related to acute myocardial infarction. This case, with genetic profile “low ERCC1/high TS”, was assigned to control arm

Figure 1. Study participants flow-chart

Figure 2. Overall and recurrence-free survival

Note: the modified intent to treat population was used for the survival analysis. O: observed events. E: expected events. For overall survival the range, IQR, and median follow-up of alive patients were 0.03-136.0, 10.8-78.5 and 31.9 months respectively, for patients in the tailored arm. The range, IQR and median follow-up of alive patients were 0.03-129.1, 6.1-75.5 and 28.5 months respectively, for patients in the control arm.

Figure 3. Subgroup analysis by patients' demographics and disease characteristics

Note: the modified intent to treat population was used for the subgroup analysis. OS: overall survival. RFS: recurrence-free survival. P-value: p-value for interaction