Exploiting *FA*sting-mimicking Diet and *ME*tformin to Improve the Efficacy of Platinum-pemetrexed Chemotherapy in Advanced LKB1-inactivated Lung Adenocarcinoma: The *FAME* Trial

Claudio Vernieri,[1,2] Diego Signorelli,[3] Giulia Galli,[3] Monica Ganzinelli,[3] Massimo Moro,[4] Alessandra Fabbri,[5] Elena Tamborini,[5] Mirko Marabese,[6] Elisa Caiola,[6] Massimo Broggini,[6] Lital Hollander,[7] Rosaria Gallucci,[3] Giulia Vandoni,[8] Cecilia Gavazzi,[8] Tiziana Triulzi,[9] Mario Paolo Colombo,[10] Angela Maria Rizzo,[11] Paola Antonia Corsetto,[11] Giancarlo Pruneri,[5,12] Filippo de Braud,[1,13] Gabriella Sozzi,[4] Valter Torri,[7] Marina Chiara Garassino[3]

Abstract

Advanced lung adenocarcinoma with inactive liver kinase B1 (LKB1) tumor suppressor protein is associated with poor response to immune checkpoint inhibitors and molecularly targeted agents, and with dismal patient prognosis. LKB1 is a central orchestrator of cancer cell metabolism, and halts tumor growth/proliferation during metabolic stress. Recent preclinical evidence suggests that LKB1-inactive lung adenocarcinoma is highly sensitive to metformin, a safe and low-cost antidiabetic compound that inhibits mitochondrial oxidative phosphorylation. The effects of metformin can be enhanced by nutrient deprivation (ie, glucose, amino acids), which reduces intracellular levels of ATP and anabolic precursors and can be achieved by the fasting mimicking diet (FMD). Noticeably, metformin also prevents resistance to cisplatin in preclinical in vitro and in vivo models of LKB1-inactive lung adenocarcinoma. Based on such preclinical evidence, the phase II FAME trial was designed to test the hypothesis that the addition of metformin, with or without cyclic FMD, to standard platinum-based chemotherapy improves the progression-free survival of patients with advanced, LKB-1 inactive lung adenocarcinoma. Enrolled patients will be randomized in a 1:1 ratio to receive cisplatin/carboplatin and pemetrexed with the addition of metformin alone (Arm A) or metformin plus FMD (Arm B).The FAME study will use a "pick-the-winner" design with the aim of establishing which of the 2 experimental treatments is superior in terms of antitumor efficacy and safety. The primary assumption of the study is that the combination of the 2 experimental treatments shall improve median progression-free survival from 7.6 months (historical data with

C.V., D.S., and G.G. contributed equally to this article as first authors.

[1]Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
[2]IFOM, the FIRC Institute of Molecular Oncology, Milan, Italy
[3]Unit of Thoracic Oncology, Medical Oncology Department
[4]Tumor Genomics Unit, Department of Experimental Oncology and Molecular Medicine
[5]Department of Pathology and Laboratory Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
[6]Laboratory of Molecular Pharmacology, Department of Oncology
[7]Laboratory of Methodology for Biomedical Research, Istituto di Ricerche Farmacologiche "Mario Negri" IRCCS, Milan, Italy
[8]Unit of Nutrition Therapy
[9]Molecular Targeting Unit, Department of Research
[10]Department of Experimental Oncology and Molecular Medicine, Molecular Immunology Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
[11]Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy
[12]School of Medicine
[13]Oncology and Haemato-Oncology Department, University of Milan, Milan, Italy

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Addresses for correspondence: Dr Claudio Vernieri, MD, PhD, Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Via G Venezian 1, 20133 Milan, Italy, Dr Marina Chiara Garassino, MD, Unit of Thoracic Oncology, Fondazione IRCCS Istituto Nazionale dei Tumori, Via G Venezian 1, 20133 Milan, Italy
E-mail contact: claudio.vernieri@istitutotumori.mi.it; marina.garassino@istitutotumori.mi.it

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chemotherapy alone) to 12 months. Secondary study endpoints are: objective response rate, overall survival, treatment tolerability, and compliance to the experimental treatment.

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Introduction

Lung adenocarcinoma is a highly heterogeneous disease from a biological and clinical point of view.[1] In recent years, the introduction of molecularly targeted therapies led to impressive survival improvements for patients with *EGFR*-mutated or *ALK/ROS1* rearranged neoplasms (collectively 15% of the total).[2-4] In the remaining 85% of lung adenocarcinomas, the advent of immune checkpoint inhibitors (ICIs) targeting programmed death-1 (PD-1) or PD1 ligand (PD-L1), either alone or combined with cytotoxic chemotherapy, recently prolonged patient progression-free survival (PFS) and overall survival in different treatment lines.[5,6] Unfortunately, not all neoplasms are sensitive to ICIs, and factors predictive of tumor response need to be fully elucidated. So far, high intratumor expression of PD-L1 is the only established biological variable that predicts benefit from single-agent pembrolizumab (anti-PD-1) in the first-line setting.[6]

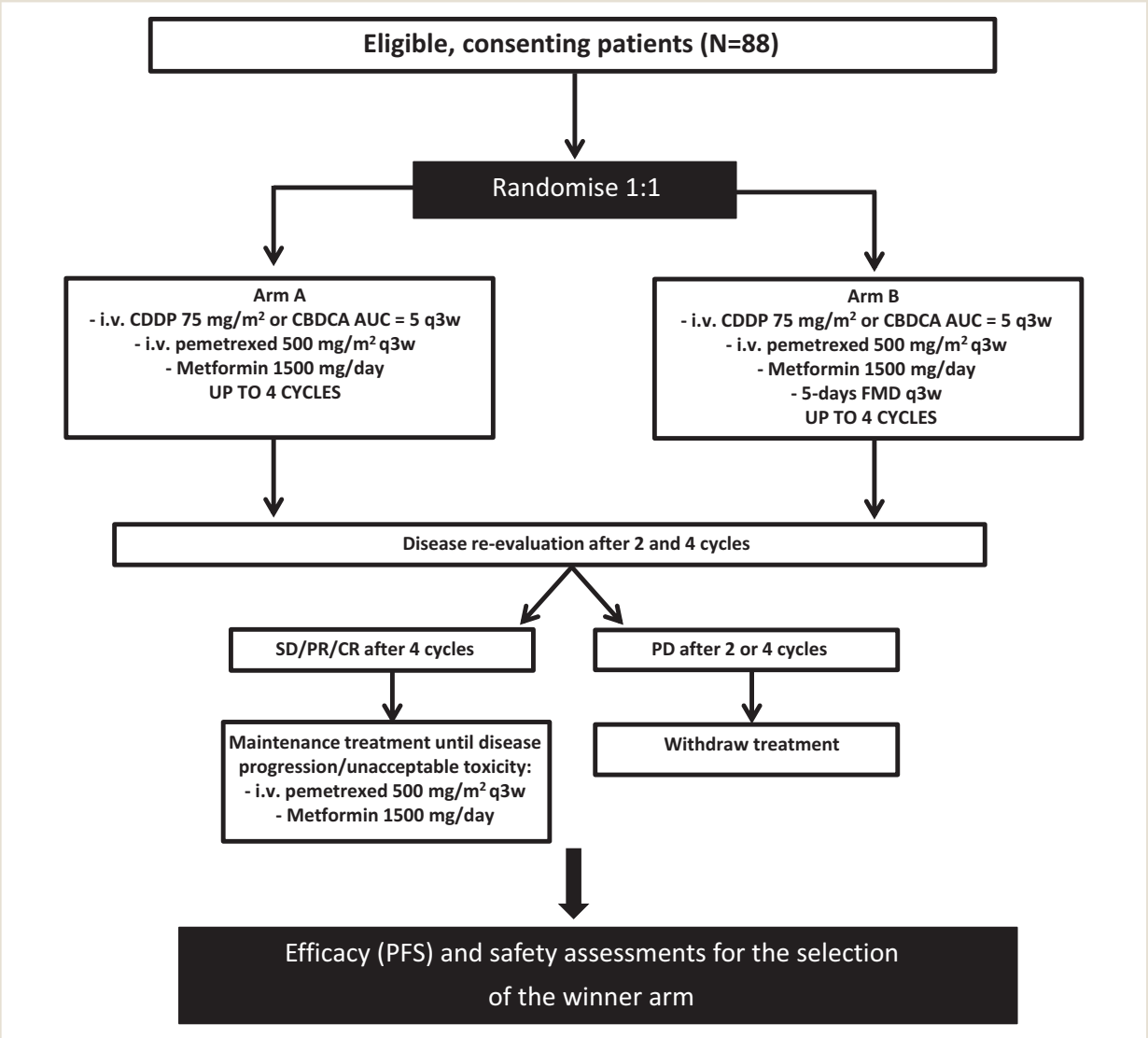
The tumor suppressor enzyme liver kinase B1 (LKB1), which orchestrates cell response to energetic intracellular status and regulates the balance between catabolic and anabolic processes, is partially or completely inactivated in about 30% of all lung adenocarcinomas through *LKB1* mutations/deletions.[7] Because *LKB1* alterations are mutually exclusive with *EGFR*, *ALK*, or *ROS1* mutations/rearrangements, patients with *LKB1*-mutated lung adenocarcinomas have been included in clinical trials with ICIs. However, recent evidence suggests that LKB1 inactivation is associated with lack of benefit from available immunotherapies.[8] As a result, LKB1-inactive lung adenocarcinoma remains orphan of effective molecularly targeted therapies, and "old" platinum-based chemotherapy probably represents the most effective treatment option in the first-line setting, with an expected median PFS of about 7.6 months.[9]

Recent studies conducted by our group and other research groups have shown that LKB1 inactivation disables lung adenocarcinoma cells to halt their growth and proliferation during metabolic stress induced by nutrient or growth factor deprivation, or by compounds that decrease intracellular energy levels, thus exposing them to metabolic crisis and apoptosis.[10-13] In particular, *LKB1* deletion sensitizes lung adenocarcinoma cells to the antidiabetic compound metformin, which lowers intracellular ATP levels by inhibiting mitochondrial oxidative phosphorylation. Importantly, metformin is also capable of preventing acquired resistance to cisplatin by reducing the number of tumor-initiating cells.[13] Based on such

Table 1 Main Inclusion and Exclusion Criteria

Main Inclusion Criteria
Signed and dated informed consent, as approved by Institutional Review Board and Ethics Committee
Histologically confirmed diagnosis of lung adenocarcinoma with inactive LKB1, as defined by the absence of immunohistochemical expression of LKB1 (score = 0) AND the presence of any mutation in the *LKB1* gene sequence
Absence of *EGFR* mutations, *ALK/ROS1* gene rearrangements; PD-L1 expression < 50%
Presence of advanced disease, as defined as unresectable, locally advanced (stage IIIB) or metastatic disease (stage IV) not candidate to concomitant or sequential definitive radiotherapy on the primary tumor. Palliative radiotherapy on specific disease sites is allowed
Age ≥ 18 and ≤ 75 years
Eastern Cooperative Oncology Group performance status 0 or 1
Adequate bone marrow function
Body mass index > 20 kg/m^2 AND unintentional weight loss < 5% in the last 3 months
Main Exclusion Criteria
Prior systemic treatment for advanced lung cancer
Having completed (neo)adjuvant platinum-based chemotherapy less than 6 months before disease relapse
Diagnosis of a concurrent malignancy in the last 5 years, with the exception of adequately treated basal or squamous cell carcinoma, non-melanomatous skin cancer, or curatively resected cervical cancer
Current status of pregnancy or lactation, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test (>5 mIU/mL)
Established diagnosis of diabetes mellitus type I or diabetes mellitus type II that requires pharmacologic treatment (including, but not limited to, insulin, insulin secretagogues, and metformin)
Severe impairment of the gastrointestinal function or gastrointestinal disease that may alter the digestion and absorption of nutrients during the re-feeding phase (eg, active ulcerative diseases of the stomach or intestine, uncontrolled nausea, vomiting, diarrhea, malabsorption syndrome, or small bowel resection)
Baseline fasting plasma glucose ≤ 65 mg/dL
Severe heart, kidney, liver, chronic lung diseases

Figure 1 Trial Schema

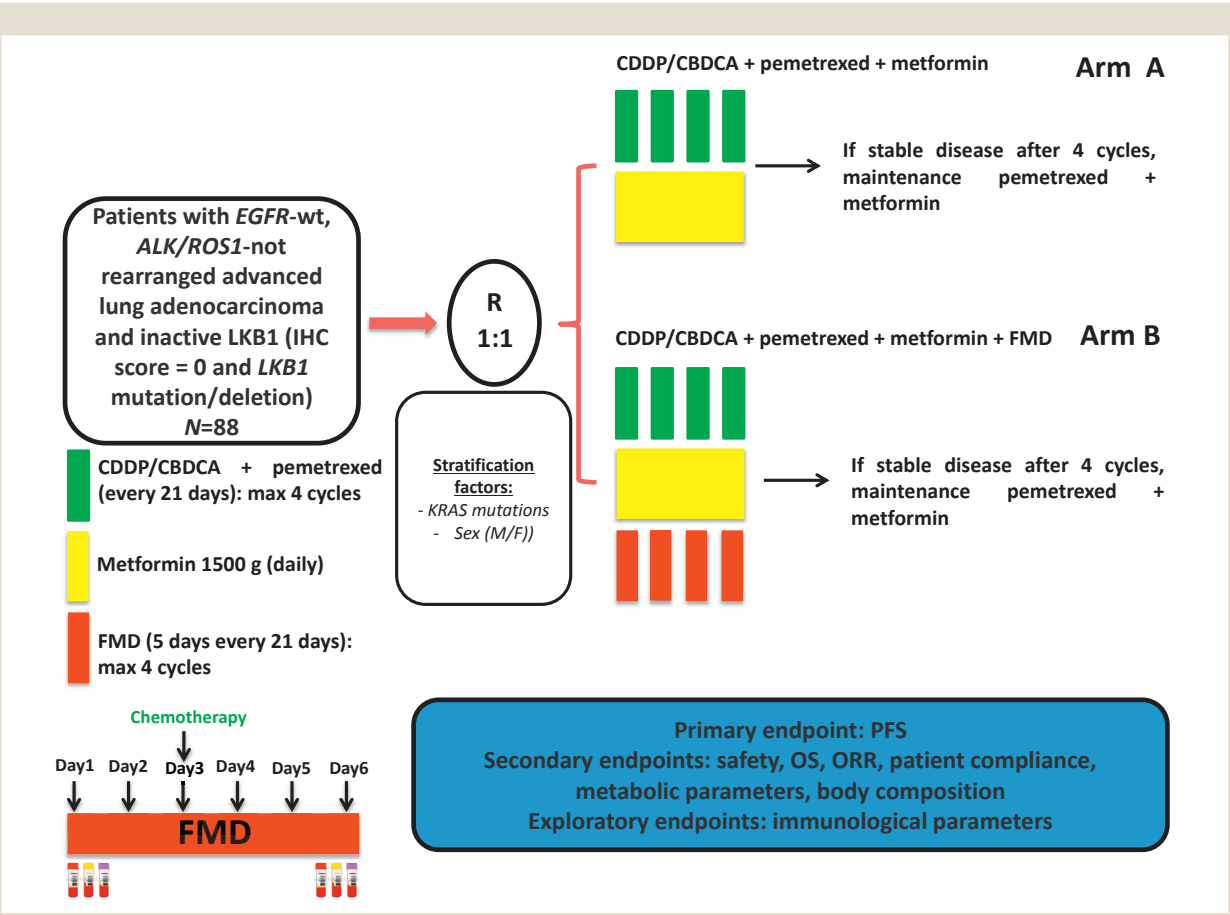


Abbreviations: AUC = area under the curve; CBDCA = carboplatin; CDDP = cisplatin; CR = complete response; i.v. = intravenously; q3w = every 3 weeks; SD = stable disease; PD = progressive disease; PFS = progression-free survival; PR = partial response.

evidence, the addition of metformin to platinum-based chemotherapy may target the metabolic vulnerabilities of *LKB1*-mutated lung adenocarcinomas, possibly improving treatment effectiveness and, consequently, patient prognosis.

Another strategy to affect cell bioenergetics and metabolic pathways in cancer cells consists in restrictive dietary approaches that reduce the blood concentration of essential metabolites, such as glucose and amino acids, which play a role in the production of energy units (ATP) and anabolic precursors of protein and lipid molecules.[14] In particular, fasting or a plant-based, low-calorie, carbohydrate- and protein-restricted diet, also known as fasting mimicking diet (FMD), have both demonstrated synergistic anticancer activity with cytotoxic agents, while protecting normal cells from treatment-induced adverse events, in several preclinical models of human cancer, including lung adenocarcinoma.[14-16] The main mechanism at the basis of the anticancer effects of fasting/FMD consists in the reduction of plasma levels of glucose, insulin, and insulin-like growth factor 1 (IGF-1). Other preclinical evidence indicates that FMD stimulates antitumor immunity by increasing tumor infiltration by CD8+ lymphocytes, while reducing immunosuppressive, regulatory T cells.[15] Compared with complete fasting, cyclic FMD is likely to be better accepted and tolerated, and is not associated with the loss of lean mass.[17] Several studies are currently ongoing to test the safety, feasibility, and metabolic effects of FMD in patients with cancer (NCT03340935, NCT03595540, NCT02126449), of which the NCT03340935 trial, conducted at our institution, has just completed patient enrollment.

Figure 2 FAME trial Design



Abbrevations: CBDCA = carboplatin; CDDP = cisplatin; F = female; FMD = fasting-mimicking diet; IHC = immunohistochemical; LKB1 = Liver Kinase B1; M = male; ORR = overall response rate; OS = overall survival; PFS = progression-free survival; R = randomized.

Because the synergistic anticancer effects of both metformin and fasting/FMD are mediated by inhibition of glycolysis and mitochondrial metabolism (ie, the 2 most important sources of energy units and anabolic precursors for cancer cells), their combination could be particularly effective in targeting the metabolic vulnerabilities of the LKB1-inactive subtype of lung adenocarcinoma.

In summary, considering: (1) the strong sensitivity of LKB1-inactive lung adenocarcinomas to metformin and metabolic stress; (2) the ability of metformin to prevent cisplatin resistance; and (3) the synergistic activity between metformin and fasting/FMD in many preclinical models, we designed a prospective trial to test the anticancer activity of standard, platinum-pemetrexed chemotherapy combined with metformin with or without cyclic FMD in patients with advanced, LKB1-inactive lung adenocarcinoma candidate to receive first-line systemic treatment.

Patients and Methods

Study Design

FAME is a monocentric, prospective, open-label, randomized, phase II trial that will use a "pick-the-winner" design to compare 2 experimental arms, both consisting of the combination of metabolic treatments and standard-of-care platinum-based chemotherapy, in patients with advanced LKB1-inactive lung adenocarcinoma. The main inclusion and exclusion criteria are summarized in Table 1. LKB1-inactivation will be defined by the absence of immunohistochemical LKB1 staining (score of 0 according to a scoring system that takes into account both protein expression and staining intensity) and the concurrent presence of a mutation/deletion in *LKB1* gene.

The trial schema is illustrated in Figure 1. Eligible patients will be randomized in a 1:1 ratio to receive cisplatin 75 mg/m^2 (or carboplatin at an area under the curve [AUC] of 5) plus pemetrexed 500 mg/m^2 every 21 days plus: (1) daily metformin up to a maximum dosage of 1500 mg (Arm A), OR (2) daily metformin up to a maximum dosage of 1500 mg plus up to four 5-day FMD diet, repeated in parallel to the chemotherapy 21 day cycles (Arm B) (Figures 1, 2).

At randomization, patients will be stratified according to the presence of tumor *KRAS* mutations (yes/no) and gender (M/F).

The FMD will consist of an every-3-weeks, 5-days, plant-based, calorie-restricted (700 Kcal on day 1; 300 Kcal on days 2, 3, and 4; 450 Kcal on day 5), sucrose-free, low-carbohydrate, low-protein dietary scheme that will be repeated every 3 weeks. It is the same dietary regimen used in the NCT03340935 and NCT03454282

trials. During the 5 days of FMD (Arm B), patients randomized to Arm A will receive generic dietary recommendations that are based on International Guidelines for survivors of cancer, without severe restrictions in terms of total calorie intake and macronutrient composition. Disease response will be evaluated every 2 treatment cycles (once every 6 weeks). Patients with stable disease at the end of the 4 treatment cycles will receive maintenance pemetrexed plus metformin treatment until disease progression, patient death, or unacceptable toxicities, whichever occurs first (Figure 1).

Study Objectives and Endpoints

The main objective of the study is to demonstrate that both experimental treatments improve median PFS from 7.6 months (historical data with chemotherapy alone) to 12 months. The primary endpoint of the study is PFS, which will be defined as the time between treatment initiation and disease progression or patient death from any cause, whichever comes first. Secondary endpoints will consist in: treatment tolerability (incidence of grade 3/4 adverse events and of serious adverse events), patient compliance to the experimental treatments (metformin in Arm A and metformin plus FMD in Arm B), tumor objective response rate, overall survival, short- and long-term systemic metabolism modifications with the experimental treatments, and modifications of body weight and composition (Figure 2). Body composition will be assessed through bioelectrical impedance analysis and the evaluation of body fat/lean mass through quantitative analyses of computed tomography images. Patients will be followed by an expert nutritionist and a dietician to assess changes in nutritional status and body composition.

Statistical Plan

This study is based on a "pick the winner" design aiming to identify, among the 2 experimental treatments, the best one (winner) in terms of treatment activity (PFS) and safety (adverse events leading to treatment discontinuation). Forty-one patients per arm will be necessary to identify an absolute increase in median PFS of 4.4 months (from 7.6 to 12 months). The Brookmeyer-Crowley test will be used with an α tail of 0.15 and a statistical power of 0.80. Assuming a dropout rate of 5%, a total number of 88 patients will need to be enrolled to ensure 82 patients are evaluable for the primary endpoint.

The following screening criteria of efficacy and safety will be applied to each treatment arm: (1) 85% 1-tail lower confidence limit for median PFS $>$ 9.76 months; (2) $<$ 12 patients in the first evaluable 41 with at least 1 adverse event leading to treatment discontinuation.

If none of the 2 treatment arms satisfies both criteria at the same time, the experimental treatments will not be investigated further. If 1 treatment arm does not satisfy both criteria, it will be stopped. On the contrary, if both treatment arms satisfy these screening criteria, the following sequential selection rules will be applied to identify the winner arm: (1) difference $\geq$ 5 in the number of patients without at least 1 adverse event leading to treatment discontinuation in favor of the winner arm; (2) difference $\geq$ 4 in the number of patients alive and free from progression after 1 year in favor of the winner arm.

The "winner" will be selected for the design of a subsequent, larger, phase III trial.

Conclusion

The FAME trial will investigate if the combination of standard, platinum-based chemotherapy with 2 metabolic strategies—metformin alone or metformin plus FMD—is able to target the metabolic vulnerabilities of LKB1-inactive advanced lung adenocarcinoma, thus improving patient PFS when compared with historical data with chemotherapy alone. By selecting the experimental treatment that is the most active and better tolerated in patients with LKB1-inactive lung adenocarcinomas, this study will pave the way for performing a large, randomized phase III in this patient population.

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Disclosure

The authors have stated that they have no conflicts of interest.

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