BMJ Open

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Study)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-034393
Article Type:	Protocol
Date Submitted by the Author:	18-Sep-2019
Complete List of Authors:	Bongiovanni, Alberto; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori Srl, Osteoncology and Rare Tumor Center Liverani, Chiara; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori Srl, Osteoncology and Rare Tumor Center Pusceddu, Sara; Fondazione IRCCS Istituto Nazionale dei Tumori Leo, Silvana; ospedale civico Di Meglio, Giovanni; Bolzano Hospital Tamberi, Stefano; Medical Oncology, Ospedale degli Infermi, Faenza Santini, Daniele Gelsomino, Fabio; Azienda Ospedaliero-Universitaria di Modena Pucci, Francesca; Azienda Ospedaliero-Universitaria di Parma Berardi, Rossana; AOU-Ospedali Riuniti Umberto I - G.M. Lancisi - G. Salesi Lolli, Ivan; Istituto Nazionale di Ricovero e Cura a Carattere Scientifico Saverio de Bellis, ; IRCCS "Saverio de Bellis" Bergamo, Francesca; IOV IRCCS Ricci, Sergio; Pisa University Hospital Foca, Flavia; IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Biostatistics and Clinical Trials Unit Meldola, FC, IT Severi, Stefano; Nuclear Medicine Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS Ibrahim, Toni; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori Srl, Osteoncology and Rare Tumor Center
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

CLINICAL STUDY PROTOCOL

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Study)

Running title: Second-line CAPTEM/FOLFIRI in NECs

Alberto Bongiovanni[1], Chiara Liverani[1], Sara Pusceddu[2], Silvana Leo[3], Giovanni Di Meglio[4], Stefano Tamberi[5], Daniele Santini[6] , Fabio Gelsomino[7], Francesca Pucci[8], Rossana Berardi[9], Ivan Lolli[10], Francesca Bergamo[11], Sergio Ricci[12] , Flavia Foca [13] Stefano Severi [14] and Toni Ibrahim[1] and the SENECA Study Team Investigators**

[1]*Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [2]*Istituto Nazionale Tumori Milano IRCCS, Milan, Italy,* [3]*Oncology Unit, Ospedale Civico, Lecce, Italy,* [4]*Onocology Unit, Ospedale di Bolzano, Bolzano, Italy,* [5]*Medical Oncology, Ospedale degli Infermi, Faenza, Italy,* [6]*Università Campus Bio-Medico, Roma, Italy,* [7]*Azienda Ospedaliera-Universitaria di Modena, Modena, Italy,* [8]*Azienda Ospedaliera-Universitaria di Parma, Parma, Italy,* [9]*AOU-Ospedali Riuniti Umberto I - G.M. Lancisi - G. Salesi, Ancona, Italy,* [10]*IRCCS "Saverio De Bellis", Castellana Grotte, Italy,* [11]*Istituto Oncologico Veneto (IOV), Padua, Italy,* [12]*Ospedale S.Chiara - AOU Pisana, Pisa, Italy,* [13]*Unit of Biostatistics and Clinical Trials, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [14]*Nuclear Medicine Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy*

***Correspondence:** Alberto Bongiovanni, MD

Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Via Piero Maroncelli 40, 47014 Meldola (FC), Italy

Tel.: +39-0543-739100; Fax: +39-0543-739123

E-mail: alberto.bongiovanni@irst.emr.it

Language style: This article is formatted in British English.

Abstract

Introduction: Patients with metastatic or locally advanced, non-resectable, grade 3 poorly-differentiated gastroenteropancreatic (GEP) and lung neuroendocrine carcinomas (NECs) are usually treated with in first-line platinum-compounds. There is no standard second-line treatment upon progression. Accurate biomarkers are needed to facilitate NEC patients diagnosis and prognostic.

Methods and Analysis: The SENECA (SEcond-line therapy in NEuroendocrine CArcinomas) study is a randomized, non-comparative, multicentre phase II trial designed to evaluate the efficacy and safety of folinic acid, 5-fluorouracil and irinotecan (FOLFIRI) or capecitabine plus temozolomide (CAPTEM) regimens after failure of first-line-chemotherapy in lung and GEP-NECs patients. Secondary aims are to correlate the serum miRNA profile and primary mutational status of MEN1, DAXX, ATRX and RB-1 with prognosis and outcome and to investigate the prognostic and predictive role of Ki-67 score and FDG- or ^{68}Ga-PET/CT.

The main eligibility criteria are age ≥18 years; metastatic or locally advanced, non-resectable, grade 3 lung or GEP-NECs; progression to first-line platinum-based chemotherapy. A Bryant and Day design taking into account treatment activity and toxicity was used to estimate the sample size. All analysis will be performed separately for each group. A total of 112 patients (56/arm) will be randomly assigned (1:1) to receive FOLFIRI every 14 days or CAPTEM every 28 days until disease progression or unacceptable toxicity or for a maximum of six months . Patients undergo testing for specific biomarkers in primary tumor tissue and for miRNA in blood samples. MiRNA profile will be performed on the first 20 patients who agree to participate in the biological sub-study.

Ethics and dissemination: Seneca trial , supported by IRST, was authorized by the locals Ethics Committee and the Italian Medicines Agency (AIFA). Results will be widely disseminated via peer-reviewed manuscripts, conference presentations and reports to relevant authorities.

The study is currently open in Italy. Clinical trial registration: NCT03387592.

Keywords: neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine, temozolomide, CAPTEM

Strengths and limitations of the study

- the SENECA trial randomizes patients to receive two different treatments, FOLFIRI or CAPTEM, providing important information on the activity of both combinations in different NEC subtypes (NET G3 and NEC G3).
- The SENECA trial analyzes the role of miRNAs and other biological markers as prognostic and predictive factors. A further aim is to assess ^{68}Ga-PET/CT as a tool to improve current histological classification.

The major limitation of the study are:

- The rarity of disease and patient's prognosis. However the involvement of several centers along the Italian Country try to overcome this problem.
- Poor prognosis of NEC patients. Patients progressed to platinum chemotherapy usually have a rapidly worsening clinical conditions

Introduction

Poorly differentiated neuroendocrine carcinomas (NECs) are very rare malignancies, representing only 5%-10% of neuroendocrine neoplasias (NENs) (1-3). At the time of diagnosis, patients are generally in poor conditions due to aggressive and diffuse disease. These tumors are characterized by aggressive histological features (high Ki-67 index, extensive necrosis, and nuclear atypia) and are classified as grade (G)3 NECs according to the 2010 World Health Organization (WHO) classification (4). The 2017 WHO classification recognized a further group called G3 NETs as having intermediate features between NETs and NECs (5).

An etoposide-platinum combination is the gold standard for treatment of G3 NECs, several studies published in the 1990s reporting substantial antitumor activity and high response rates (41%-67%). However, prognosis is poor with a median progression-free survival of 9 months and a median overall survival of 15-19 months. When progression occurs after first-line chemotherapy, the disease is usually very aggressive and patients succumb rapidly (6).

Given the rarity of this disease , prospective clinical data are lacking and treatment recommendation are essentially expert-based opinions. At present, 2 phase II studies investigating the second-line treatment of GEP-NECs are registered at ClinicalTrials.gov, one focusing on the safety and tolerability of everolimus (National Clinical Trial identifier NCT02113800) and the other investigating the efficacy of avelumab (NCT03147404). A French study focusing on the identification of predictive molecular markers of response to sunitinib in GEP-NECs (NCT01215578) has now closed recruitment and results are eagerly awaited. Another French multicentre prospective phase II trial is currently ongoing to investigate the efficacy of the

bevacizumab-FOLFIRI combination after progression on platinum-etoposide (7).

Different second-line chemotherapy combinations have been evaluated but shown poor results (6, 8, 9). In a monocenter retrospective clinical trial, Hentic et al. hypothesized the potential efficacy of FOLFIRI as second-line chemotherapy in patients with G3 extra-pulmonary NECs (10). An objective response rate was obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median progression-free survival (PFS) and overall survival (OS) were 4 and 18 months, respectively.

In another retrospective study, a 71% DCR was obtained with temozolomide-based chemotherapy. A PFS of 12 months (95% CI, 5.5- 24) and OS of 22 months (95% CI, 12-31) was reported in patients who responded to treatment or showed stable disease (SD), whereas OS was only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive staining for chromogranin A (CgA). Both factors are often associated with more differentiated tumors (11).

Literature data on lung NECs in progression after first-line chemotherapy are based on small patient series (12). Moreover, there is increasing evidence of some discrepancies in the current grading of NECs, highlighting the need for more accurate biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise 2 distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived from well differentiated neuroendocrine tumors (NETs) and characterized by mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from neuroendocrine-differentiated adenocarcinomas and characterized by a mutation in RB1. Both subgroups display a distinct prognosis and different sensitivity to

chemotherapy (13-15). Micro(mi)RNAs are a class of small, non-coding, highly conserved single-stranded RNAs involved in the post-transcriptional regulation of cell proliferation, differentiation, survival, and apoptosis (16). They are often associated with resistance to therapy (17, 18). Whilst miRNAs are known to show a specific expression pattern in NETs (19), little is known about differential miRNA profiles in NEC patients. At present, no data are available on the deregulation of specific miRNAs in this setting.

In a study recently published by our group on GEP-NEC patients undergoing first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months (p <0.01) in patients with Ki-67% <50% or >50%, respectively (19). Median (m)OS was 8.1 months in the latter group but was not reached in the former group (p = 0.039). Patients with a positive ^{68}Ga-PET/CT had a longer OS than those with a negative scan (75% *vs*. 34.3%, respectively, at 18 months), but the difference was not significant (p = 0.06). Our data highlighted that ^{68}Ga-PET/CT positivity may be a discriminating factor (20,15) in predicting prognosis, especially important in the metastatic setting where histological material is not always available for evaluation. Also ^{18}fludeoxyglucose (^{18}FDG)-PET/CT could be useful to discriminate patients with different prognosis. (21)

Given the above premises, we decided to investigate the efficacy and safety of second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression after first-line platinum-based treatment. We also aimed to study the serum miRNA profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1, patient prognosis and response to therapy, and to assess the prognostic and predictive role of ^{18}FDG-PET/CT, ^{68}Ga-PET/CT and Ki-67 score.

Methods and Analysis

Study design

The SENECA study is a multicentre randomised non-comparative phase II study (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin (lung or gastroenteropancreatic) in progression after first-line treatment are randomized to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until progression or unacceptable toxicity.

The treatments arms are as follows:

FOLFIRI regimen

- Irinotecan 180 mg/m^2, given as a 60-min. intravenous (i.v.) infusion on day 1 every 2 weeks followed by
- Leucovorin 200 mg/m^2, given as a 2-h i.v. infusion on day 1 every 2 weeks followed by
- 5-fluorouracil (5-FU) 400 mg/m^2 given as bolus, and then 5-FU 2400 mg/m^2 given as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a maximum of 12 cycles.

CAPTEM regimen

Capecitabine 750 mg/m^2 twice a day on days 1-14 in combination with temozolomide 200 mg/m^2 daily on days 10-14, every 4 weeks, until progression or for a maximum of 6 cycles.

The study includes patients aged ≥ 18 years with a histological diagnosis of G3 neuroendocrine carcinoma (GEP-NEC and lung NEC), Ki-67 >20% and measurable disease according to Response evaluation criteria in solid tumors (RECIST) 1.1 criteria. All patients must have an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 with a life expectancy > 3 months and must have already undergone first-line treatment for metastatic disease with platinum -based chemotherapy (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological, liver and renal function is required and effective contraceptive methods must be used by female patients of childbearing age. Written informed consent is obtained from all patients to take part in the study. Exclusion criteria are as follows: metastatic NECs previously treated with an irinotecan regimen, known hypersensitivity to 5-FU, calcium levofolinate, irinotecan or their recipients. All acute toxic effects of any prior therapy (including surgery, radiation therapy and chemotherapy) must have resolved to grade ≤1 according to National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.03 (CTCAE). Patients taking part in another clinical trial with any investigational agent < 30 days prior to study screening or with a history of allergic reactions attributable to compounds of similar chemical or biological composition are excluded Patients who have undergone chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study, have not recovered from adverse events caused by agents administered > 4 weeks earlier, or have known brain metastases are also not eligible for the study. Patients with other malignancies

with a disease-free interval of < 5 years (with the exception of non melanoma skin cancer or low-grade superficial bladder cancer) are excluded, as are those with any severe and/or uncontrolled medical condition or other condition that could affect their participation in the study such as:

- unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or any other clinically significant cardiac disease;
- severely impaired lung function (spirometry and DLCO 50% of the normal predicted value and/or oxygen saturation ≤ 88% at rest, in room air);
- uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of normal (ULN);
- any active (acute or chronic) or uncontrolled infections/disorders.

Tumor evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest, abdomen, pelvis, and any additional known sites of disease. These tests are performed at baseline, every three months during treatment and after therapy discontinuation in non-progressing patients until progression. When possible, ^{68}Ga-PET/CT and ^{18}FDG-PET/CT is performed at baseline.

Study endpoints

The primary endpoint of the study is the DCR of each treatment, defined as the percentage of patients who have achieved complete or partial response or stable disease for at least 12 weeks from the start of therapy. DCR will be evaluated using the new international criteria proposed by the RECIST version 1.1. Acute and late toxicity will be evaluated by CTCAE Version 4.03, the latter defined as toxicity occurring at least 30

days after the end of the last treatment cycle. Secondary endpoints are ~~the evaluation of~~ OS, calculated from the start of treatment to death from any cause and PFS, calculated from the start of treatment to the date of the first documented evidence of disease progression or of death from any cause. Patients without events at the time of analysis will be censored at their last-known-alive date for OS and at their last date of tumor evaluation for PFS. A further secondary endpoint is the evaluation of quality of life using the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). When data are available, the impact of baseline ^{68}Ga-PET/CT and ^{18}FDG-PET/CT on PFS will be analysed with exploratory intent.~~.~~ After signing the informed consent for biomarker evaluation, patients will undergo evaluation of the mutation status of MEN1, DAXX, ATRX and RB-1 in primary tumor tissue and of miRNA in blood samples. Assessment of the miRNA profile will be performed on the first 20 patients who agree to participate in the biological part of the study.

Ethical considerations

The present clinical trial (EudraCT 2016-000767-17), supported by IRST, was authorized by the local Ethics Committee and the Italian Medicines Agency (AIFA); it is also registered on the ClinicalTrials.gov website (NCT03387592). The study complies with the ethical standards laid down in the 1964 Declaration of Helsinki and the principles of Good Clinical Practice guidelines (including written informed consent).

Patient and Public Involvement

This research was done without patient involvement.

Statistical methods

The Bryant and Day design is used to estimate a sample size that takes into account both treatment activity and toxicity. Although randomisation is used to allocate patients to the 2 arms, no formal statistical comparisons between treatment regimens is planned. The purpose of randomisation is to reduce bias due to patient assignment to a specific treatment arm. The hypothesis for the control arm is based on literature data (22, 23).

An α level of 0.10 (both for toxicity and DCR) and a power of 90% were adopted. A DCR rate ≥60% and a relevant toxicity rate ≤20% are considered acceptable rates while a DCR rate ≤40% and a relevant toxicity rate ≥40% are considered inacceptable rates. Given these hypotheses, the first step of the study will require 25 patients. If ≥10 patients with a DCR are observed and ≥15 patients do not have relevant toxicity, the study will enrol patients in the next step. A total of 53 patients will be enrolled. If ≥25 patients with DCR and ≥36 patients without any relevant toxicity are observed, treatment will be considered active and not toxic. This design is used for each treatment scheme and all analyses will be performed separately. If one of the schemes does not obtain the expected proportions of the first step, the arm will be closed and patients will be enrolled in the other arm until the target is reached; if the expected proportions are not reached in any arm, the study will be prematurely closed. If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopenia, prolonged G3-G4 neutropenia (> 7 days) and drug-related hospitalizations are considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55 % *vs*. >55%) and site

of primary tumor (lung *vs*. GEP).

Complete response, partial response or stable disease for at least 12 weeks will be considered as the DCR. The proportion of patients in this category will be determined and 95% confidence intervals (95%CIs) for the DCR will be calculated. OS and PFS will be estimated using the Kaplan-Meier method (two-sided 95%CIs) (24). Appropriate statistical analyses will be performed on the basis of the data available to compare QLQ-C30 scores between baseline and subsequent follow-up visits.

When data are available, the impact of ^{68}Ga-PET/CT result on PFS will be analyzed with exploratory intent. The Shapiro-Wilk test will be used to determine the normality distribution of each biomarker and, in the event of a non-normal distribution, nonparametric statistics will be used to analyze the relationship between the serum levels of each marker, considered as continuous variables, and response to treatment. In case of normality distribution of biomarkers parametric test will be used. All endpoints will be analysed separately for each treatment group.

Discussion

There is still no truly effective second-line chemotherapy for neuroendocrine carcinoma. The overall prognosis of patients is poor, with an OS of 5 months in the metastatic setting according to the SEER (Surveillance, Epidemiology, and End Results) data (22). Only 5% of all patients are long-term survivors. There is also a marked lack of prognostic and predictive factors (5).

Three phase II studies registered at *ClinicalTrials.gov* are currently investigating the second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab

(NCT03147404). Some abstracts were presented at ESMO (European Society for Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019 on the use of immunotherapy in GEP-NECs with inconclusive results. The SENECA study uses a promising approach to the treatment of patients with metastatic NECS. First, both the activity and safety of 2 regimens are assessed in the same setting with a sizeable patient population (56 patients/arm). In addition, patients are stratified according to Ki-67 index and morphology to investigate the role of each treatment combination in both poorly differentiated and well differentiated NECs. Another aim of this study is to integrate both biological and metabolic imaging data in an effort to improve the current GEP-NEC classification.

In conclusion, there are still no FDA/EMA (Food and Drug Administration/European Medicines Agency)-approved second-line therapeutic options for patients with metastatic NECs. The SENECA trial could provide evidence of a novel therapeutic option for patients. Moreover, the integration of biological and imaging data could ~~be~~ lead to a better understanding of the natural history of the disease and help to identify potential responders.

Confidentiality

This study will be conducted in full conformity with ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) Guidelines for Good Clinical Practice , Directive 2001/20/EEC of the European Parliament and other relevant current local legislation. Participants will be allocated a unique identification (ID) number at entry. The master list linking participant personal

information and ID number will be maintained in a separate locked cabinet and password-protected hard-drive. Data will be analysed by ID number only. Patient files and other source data will for be kept a maximum of 15 years.

Dissemination

After completing the study, all data, including beneficial and adverse events, of the trial will be communicated at scientific meetings and published in indexed peer-reviewed journals. If shown to be effective, the therapy program will be made available to the general public in an appropriate manner.

Abbreviations

CAPTEM, capecitabine plus temozolomide; CgA, chromogranin A; DCR, disease control rate; GEP, gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumor; OS, overall survival; PFS, progression-free survival; SRS, somatostatin receptor scintigraphy.

Author contributions

AB, CL and TI designed the study and drafted the article. AB was responsible for data acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a clinical information data extraction method for the protocol. FF performed the statistical analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual content. All authors read and approved the present version of the manuscript for submission.

Data availability statement

The datasets used and analysed in the present study are available from the corresponding author on reasonable request.

Funding

The study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

SENECA study Team investigators: Davide Campana, Davide Pastorelli, Nicola Silvestris, Francesco Silvestris, Angela Buonadonna, Giuseppe Badalamenti

They also thank Gráinne Tierney and Cristiano Verna for editorial assistance.

References

1. Kulke MH, Shah MH, Benson AB 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, et al. Neuroendocrine tumors, version 1.2015. *J Natl Compr Canc Netw* (2015) 13(1):78-108
2. Modlin IM, Moss SF, Chung DC, Jensen RT, Snyderwine E. Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors. *J Natl Cancer Inst* (2008)100(18):1282-9. doi: 10.1093/jnci/djn275
3. Yao YC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* (2008) 26(18):3063-72
4. Fraenkel M, Kim M, Faggiano A, de Herder WW, Valk GD; Knowledge NETwork. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocr Relat Cancer* (2014) 21(3):R153-63. doi: 10.1530/ERC-13-0125
5. Lloyd RV, Osamura RY, Klöppel G, Rosai J. WHO classification of tumours of endocrine organs. 4th ed (2017). Lyon: International Agency for Research on Cancer (IARC)
6. Heetfeld M, Chougnet CN, Olsen IH, Rinke A, Borbath I, Crespo G, et al. Characteristics and treatment of patients with G3 gastroenteropancreatic neuroendocrine neoplasms. *Endocr Relat Cancer* (2015) 22(4):657-64. doi: 10.1530/ERC-15-0119
7. Walter T, Malka D, Hentic O, Lombard-Bohas C, Le Malicot K, Smith D, et al. Evaluating bevacizumab in combination with FOLFIRI after the failure of platinum-etoposide regimen in patients with advanced poorly

differentiated neuroendocrine carcinoma: The PRODIGE 41-BEVANEC randomized phase II study. *Dig Liver Dis* (2018) 50(2):195-8. doi: 10.1016/j.dld.2017.11.020

8. Hadoux J, Malka D, Planchard D, Scoazec JY, Caramella C, Guigay J, et al. Post-first-line FOLFOX chemotherapy for grade 3 neuroendocrine carcinoma. *Endocr Relat Cancer* (2015) 22(3)289-98. doi: 10.1530/ERC-15-0075
9. Olsen IH, Sørensen JB, Federspiel B, Kjaer A, Hansen CP, Knigge U, et al. Temozolomide as second or third line treatment of patients with neuroendocrine carcinomas. *Sci World J* (2012) 2012;2012:170496. doi: 10.1100/2012/170496
10. Hentic O, Hammel P, Couvelard A, Rebours V, Zappa M, Palazzo M, et al. FOLFIRI regimen: an effective second-line chemotherapy after failure of etoposide-platinum combination in patients with neuroendocrine carcinomas grade 3. *Endocr Relat Cancer* (2012) 9(6):751-7. doi: 10.1530/ERC-12-0002
11. Welin S, Sorbye H, Sebjornsen S, Knappskog S, Busch C, Oberg K. Clinical effect of temozolomide-based chemotherapy in poorly differentiated endocrine carcinoma after progression on first-line chemotherapy. *Cancer* (2011) 117(20):4617-22. doi: 10.1002/cncr.26124
12. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* (2002) 346(2):85-91. doi: 10.1056/NEJMoa003034
13. Derks JL, Leblay N, Thunnissen E, van Suylen RJ, den Bakker M, Groen HJM, et al. Molecular subtypes of pulmonary large-cell neuroendocrine carcinoma predict chemotherapy treatment outcome. *Clin Cancer Res* (2018) 24(1):33-42. doi: 10.1158/1078-0432.CCR-17-1921

14. Tang LH, Untch BR, Reidy DL, O'Reilly E, Dhall D, Jih L, et al. Well-differentiated neuroendocrine tumors with a morphologically apparent high-grade component: a pathway distinct from poorly differentiated neuroendocrine carcinomas. *Clin Cancer Res* (2016) 22(4):1011-7. doi: 10.1158/1078-0432.CCR-15-0548

15. Liverani C, Bongiovanni A, Mercatali L, Foca F, Pieri F, De Vita A, Spadazzi C, Miserocchi G, Recine F, Riva N, Nicolini S, Severi S, Martinelli G, Ibrahim T. Grading of Neuroendocrine Carcinomas: Correlation of 68Ga-PET/CT Scan with Tissue Biomarkers. *Dis Markers.* 2018 Dec 2;2018:6878409.

16. Grolmusz VK, Kövesdi A, Borks K, Igaz P, Patócs A. Prognostic relevance of proliferation-related miRNAs in pancreatic neuroendocrine neoplasms Eur J Endocrinol (2018) 179(4):219-28. doi: 10.1530/EJE-18-0305

17. Gill P, Kim E, Chua TC, Clifton-Bligh RJ, Nahm CB, Mittal A, et al. MiRNA-3653 is a potential tissue biomarker for increased metastatic risk in pancreatic neuroendocrine tumours. *Endocr Pathol* (2019). doi: 10.1007/s12022-019-9570-y [Epub ahead of print]

18. Panarelli N, Tyryshkin K, Wong JJM, Majewski A, Yang X, Scognamiglio T, et al. Evaluating gastroenteropancreatic neuroendocrine tumors through microRNA sequencing. *Endocr Relat Cancer* (2019) 26(1):47-57. doi: 10.1530/ERC-18-0244

19. Malczewska A, Kidd M, Matar S, Kos-Kudla B, Modlin IM. A comprehensive assessment of the role of mirnas as biomarkers in gastroenteropancreatic neuroendocrine tumors. *Neuroendocrinology* (2018)107(1):73-90. doi: 10.1159/000487326

20. Bongiovanni A, Riva N, Ricci M, Liverani C, La Manna F, De Vita A, et al. First-

line chemotherapy in patients with metastatic gastroenteropancreatic neuroendocrinecarcinoma. *Onco Targets Ther* (2015) 8:3613-9. doi: 10.2147/OTT.S91971

21. Sansovini M, Severi S, Ianniello A, Nicolini S, Fantini L, Mezzenga E, Ferroni F, Scarpi E, Monti M, Bongiovanni A, Cingarlini S, Grana CM, Bodei L, Paganelli G. Long-term follow-up and role of FDG PET in advanced pancreatic neuroendocrine patients treated with 177Lu-DOTATATE. *Eur J Nucl Med Mol Imaging.* 2017 Mar;44(3):490-499.

22. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced Gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60. doi: 10.1093/annonc/mds276

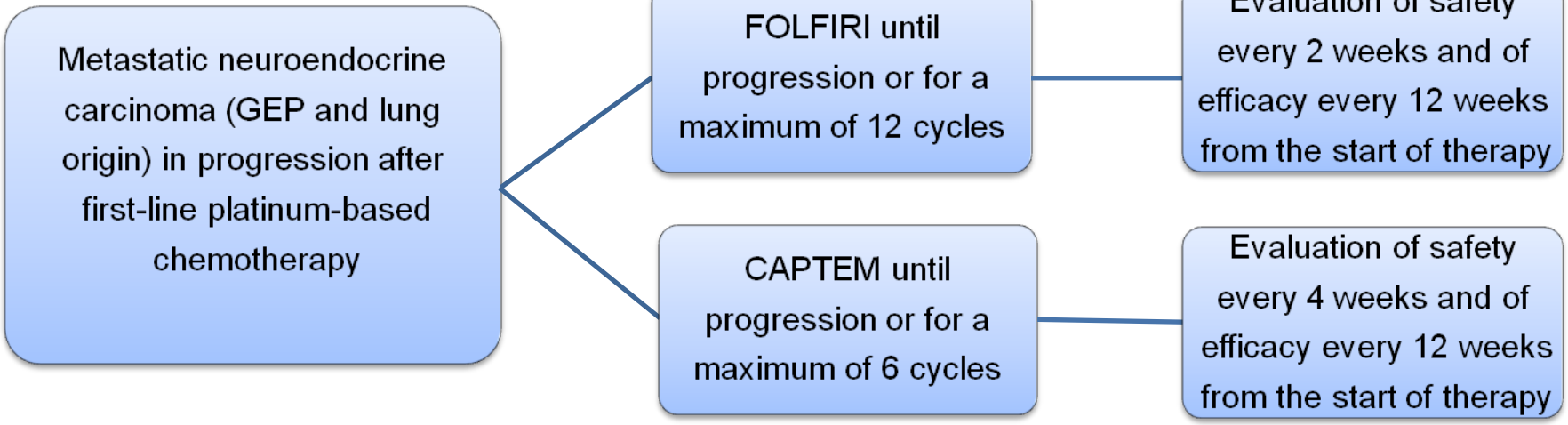
23. Perren A, Couvelard A, Scoazec JY, Costa F, Borbath I, Delle Fave G, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: pathology: diagnosis and prognostic stratification. *Neuroendocrinology* (2017) 105(3):196-200. doi: 10.1159/000457956

24. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J. Amer. *Statist Assoc* (1958) 53:457-81.

Figure legend

FIGURE 1 | SENECA study design.

Fig. 1


Metastatic neuroendocrine carcinoma (GEP and lung origin) in progression after first-line platinum-based chemotherapy
FOLFIRI until progression or for a maximum of 12 cycles
CAPTEM until progression or for a maximum of 6 cycles
Evaluation of safety every 2 weeks and of efficacy every 12 weeks from the start of therapy
Evaluation of safety every 4 weeks and of efficacy every 12 weeks from the start of therapy

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5-7
	2b	Specific objectives or hypotheses	7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8-11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	not Applicable
Participants	4a	Eligibility criteria for participants	9
	4b	Settings and locations where the data were collected	14-15.
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8,9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10, 11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	12
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	12
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	12
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	12
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	12
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	N/A

		assessing outcomes) and how	N/A
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	12, 13
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	12
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	8, 12
	13b	For each group, losses and exclusions after randomisation, together with reasons	12
Recruitment	14a	Dates defining the periods of recruitment and follow-up	12
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	N/A
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	N/A
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	12
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	4
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13, 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13, 14
Other information			
Registration	23	Registration number and name of trial registry	13
Protocol	24	Where the full trial protocol can be accessed, if available	14, 16
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

The above items were been addressed when applicable.
the corresponding author.

BMJ Open

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Study)

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-034393.R1
Article Type:	Protocol
Date Submitted by the Author:	24-Jan-2020
Complete List of Authors:	Bongiovanni, Alberto; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori Srl, Osteoncology and Rare Tumor Center Liverani, Chiara; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori Srl, Osteoncology and Rare Tumor Center Pusceddu, Sara; Fondazione IRCCS Istituto Nazionale dei Tumori Leo, Silvana; ospedale civico Di Meglio, Giovanni; Bolzano Hospital Tamberi, Stefano; Medical Oncology, Ospedale degli Infermi, Faenza Santini, Daniele Gelsomino, Fabio; Azienda Ospedaliero-Universitaria di Modena Pucci, Francesca; Azienda Ospedaliero-Universitaria di Parma Berardi, Rossana; AOU-Ospedali Riuniti Umberto I - G.M. Lancisi - G. Salesi Lolli, Ivan; Istituto Nazionale di Ricovero e Cura a Carattere Scientifico Saverio de Bellis, ; IRCCS "Saverio de Bellis" Bergamo, Francesca; IOV IRCCS Ricci, Sergio; Pisa University Hospital Foca, Flavia; IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Biostatistics and Clinical Trials Unit Meldola, FC, IT Severi, Stefano; Nuclear Medicine Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS Ibrahim, Toni; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori Srl, Osteoncology and Rare Tumor Center
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Oncology
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

CLINICAL STUDY PROTOCOL

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Study)

Running title: Second-line CAPTEM/FOLFIRI in NECs

Alberto Bongiovanni[1], Chiara Liverani[1], Sara Pusceddu[2], Silvana Leo[3], Giovanni Di Meglio[4], Stefano Tamberi[5], Daniele Santini[6] , Fabio Gelsomino[7], Francesca Pucci[8], Rossana Berardi[9], Ivan Lolli[10], Francesca Bergamo[11], Sergio Ricci[12] , Flavia Foca [13] Stefano Severi [14], Toni Ibrahim[1], and the SENECA Study Team Investigators**

[1]*Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [2]*Istituto Nazionale Tumori Milano IRCCS, Milan, Italy,* [3]*Oncology Unit, Ospedale Civico, Lecce, Italy,* [4]*Onocology Unit, Ospedale di Bolzano, Bolzano, Italy,* [5]*Medical Oncology, Ospedale degli Infermi, Faenza, Italy,* [6]*Università Campus Bio-Medico, Roma, Italy,* [7]*Azienda Ospedaliera-Universitaria di Modena, Modena, Italy,* [8]*Azienda Ospedaliera-Universitaria di Parma, Parma, Italy,* [9]*AOU-Ospedali Riuniti Umberto I - G.M. Lancisi - G. Salesi, Ancona, Italy,* [10]*IRCCS "Saverio De Bellis", Castellana Grotte, Italy,* [11]*Istituto Oncologico Veneto (IOV), Padua, Italy,* [12]*Ospedale S.Chiara - AOU Pisana, Pisa, Italy,* [13]*Unit of Biostatistics and Clinical Trials, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [14]*Nuclear Medicine Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy*

***Correspondence:** Alberto Bongiovanni, MD

Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Via Piero Maroncelli 40, 47014 Meldola (FC), Italy

Tel.: +39-0543-739100; Fax: +39-0543-739123

E-mail: alberto.bongiovanni@irst.emr.it

Language style: This article is formatted in British English.

Abstract

Introduction: Patients with metastatic or locally advanced, non-resectable, grade 3 poorly-differentiated gastroenteropancreatic (GEP) and lung neuroendocrine carcinomas (NECs) are usually treated with in first-line platinum compounds. There is no standard second-line treatment upon progression. Accurate biomarkers are needed to facilitate diagnosis and prognostic assessment of NEC patients.

Methods and Analysis: The SENECA (SEcond-line therapy in NEuroendocrine CArcinomas) study is a randomised, non-comparative, multicentre phase II trial designed to evaluate the efficacy and safety of folinic acid, 5-fluorouracil and irinotecan (FOLFIRI) or capecitabine plus temozolomide (CAPTEM) regimens after failure of first-line-chemotherapy in lung NEC and GEP-NEC patients. Secondary aims are to correlate the serum miRNA profile and primary mutational status of MEN1, DAXX, ATRX and RB-1 with prognosis and outcome and to investigate the prognostic and predictive role of the Ki-67 score and FDG- or ^{68}Ga-PET/CT. The main eligibility criteria are age ≥18 years; metastatic or locally advanced, non-resectable, grade 3 lung or GEP-NECs; progression to first-line platinum-based chemotherapy. A Bryant and Day design taking into account treatment activity and toxicity was used to estimate the sample size. All analysis will be performed separately for each treatment group in the intention-to-treat population. A total of 112 patients (56/arm) will be randomly assigned (1:1) to receive FOLFIRI every 14 days or CAPTEM every 28 days until disease progression or unacceptable toxicity or for a maximum of six months. Patients undergo testing for specific biomarkers in primary tumour tissue and for miRNA in blood samples. MiRNA profiling will be performed in the first 20 patients who agree to

participate in the biological sub-study.

Ethics and dissemination: The SENECA trial, supported by IRST, was authorised by the locals Ethics Committee and the Italian Medicines Agency (AIFA). Results will be widely disseminated via peer-reviewed manuscripts, conference presentations and reports to relevant authorities.

The study is currently open in Italy. Clinical trial registration: NCT03387592.

Keywords: neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine, temozolomide, CAPTEM

Strengths and limitations of the study

- the SENECA trial randomises patients to receive two different treatments, FOLFIRI or CAPTEM, providing important information on the activity of both combinations in different NEC subtypes (NET grade (G) 3 and NEC G3).
- The SENECA trial analyses the role of miRNAs and other biological markers as prognostic and predictive factors. A further aim is to assess ^{68}Ga-PET/CT as a tool to improve current histological classification.

The major limitations of the study are:

- The rarity of the disease and patient prognosis. However, the involvement of several Italian centres will hopefully help to overcome this problem.
- Poor prognosis of NEC patients. Patients progressing on platinum chemotherapy usually have rapid deterioration of clinical conditions

Introduction

Poorly differentiated neuroendocrine carcinomas (NECs) are very rare malignancies, representing only 5%-10% of neuroendocrine neoplasias (NENs) (1-3). At the time of diagnosis, patients are generally in poor conditions due to aggressive and diffuse disease. These tumors are characterised by aggressive histological features (high Ki-67 index, extensive necrosis, and nuclear atypia) and are classified as grade (G) 3 NECs according to the 2010 World Health Organization (WHO) classification (4). The 2017 WHO classification recognized a further group called G3 NETs as having intermediate features between NETs and NECs (5).

An etoposide-platinum combination is the gold standard for the treatment of G3 NECs, several studies published in the 1990s reporting substantial anti-tumor activity and high response rates (41%-67%) (6). However, prognosis is generally poor with a median progression-free survival (PFS) of 9 months and a median overall survival (OS) of 15-19 months. When progression occurs after first-line chemotherapy, the disease is usually very aggressive and patients succumb rapidly (7).

Given the rarity of this disease, prospective clinical data are lacking and treatment recommendations are essentially expert-based opinions. Two phase II studies investigating the second-line treatment of GEP-NECs are currently registered at *ClinicalTrials.gov*, one evaluating the safety and tolerability of everolimus in 40 patients with G3 NEC/NET or G1/G2 NET switching to G3 NEN (National Clinical Trial identifier NCT02113800) and the other investigating the efficacy of avelumab (NCT03147404). A French study focusing on the identification of predictive molecular markers of response to sunitinib in poorly differentiated digestive NETs

(NCT01215578) has now closed recruitment and results are eagerly awaited. Another French multicentre prospective phase II trial is currently ongoing to investigate the efficacy of the bevacizumab-FOLFIRI combination after progression on a platinum/etoposide combination (7).

Different second-line chemotherapy combinations have been evaluated but shown poor results (8-10). In a monocentre retrospective clinical trial, Hentic et al. hypothesised the potential efficacy of FOLFIRI as second-line chemotherapy in 19 patients with G3 extra-pulmonary NECs (11). An objective response rate was obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median PFS and OS were 4 and 18 months, respectively.

In another retrospective study, a 71% DCR was obtained with temozolomide-based chemotherapy in 25 patients with metastatic poorly differentiated endocrine carcinoma of different sites and atypical bronchial carcinoid with Ki-67 > 20%. Small cell lung carcinoma and large cell lung carcinoma were excluded. A PFS of 12 months (95% confidence interval [CI], 5.5- 24) and OS of 22 months (95% CI, 12-31) were reported in patients who responded to treatment or showed stable disease (SD), whereas OS was only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive staining for chromogranin A. Both factors are often associated with more highly differentiated tumours (12).

Literature data on lung NECs in progression after first-line chemotherapy are based on small patient series (13). Moreover, there is increasing evidence of some discrepancies in the current grading of NECs, highlighting the need for more accurate

biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise two distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived from well differentiated neuroendocrine tumours (NETs) and characterised by mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from neuroendocrine-differentiated adenocarcinomas and characterised by a mutation in RB1. Both subgroups display a distinct prognosis and different sensitivity to chemotherapy (14-16). Micro(mi)RNAs are a class of small, non-coding, highly conserved single-stranded RNAs involved in the post-transcriptional regulation of cell proliferation, differentiation, survival, and apoptosis (17). They are often associated with resistance to therapy (18, 19). Whilst miRNAs are known to show a specific expression pattern in NETs (20), little is known about differential miRNA profiles in NEC patients. At present, no data are available on the deregulation of specific miRNAs in this setting.

In a study recently published by our group on GEP-NEC patients undergoing first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months (p <0.01) in patients with Ki-67 value between 20% and 50% or >50%, respectively (19). Median (m)OS was 8.1 months in the latter group but was not reached in the former group (p = 0.039). Patients with a positive ^{68}Ga-PET/CT had a higher 18-month OS rate than those with a negative scan (75% *vs*. 34.3%, respectively), but the difference was not statistically significant (p = 0.06). Our data highlighted that ^{68}Ga-PET/CT positivity may be a discriminating factor (16, 21) in predicting prognosis, especially ~~important~~ in the metastatic setting where histological material is not always available for evaluation. Furthermore, ^{18}fludeoxyglucose (^{18}FDG)-PET/CT may be useful to discriminate between patients with different prognosis (22).

Given the above premises, we decided to investigate the efficacy and safety of second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression after first-line platinum-based treatment. We also aimed to study the serum miRNA profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1, patient prognosis and response to therapy, and to assess the prognostic and predictive role of ^{18}FDG-PET/CT, ^{68}Ga-PET/CT and Ki-67 score.

Methods and Analysis

Study design

The SENECA study is a multicentre randomised non-comparative phase II study (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin (lung or gastroenteropancreatic) in progression after first-line treatment are randomized to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until progression or unacceptable toxicity.

The treatment arms are as follows:

FOLFIRI regimen

- Irinotecan 180 mg/m^2, given as a 60-min. intravenous (i.v.) infusion on day 1 every 2 weeks followed by
- Leucovorin 200 mg/m^2, given as a 2-h i.v. infusion on day 1 every 2 weeks followed by

- 5-fluorouracil (5-FU) 400 mg/m^2 given as bolus, and then 5-FU 2400 mg/m^2 given as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a maximum of 12 cycles.

CAPTEM regimen

Capecitabine 750 mg/m^2 twice a day on days 1-14 in combination with temozolomide 200 mg/m^2 daily on days 10-14, every 4 weeks, until progression or for a maximum of 6 cycles.

The study includes patients aged ≥ 18 years with a histological diagnosis of G3 neuroendocrine carcinoma (GEP-NEC and lung NEC) according to the 2010 and 2015 GEP and Lung NEN WHO classifications, respectively, Ki-67 >20% and measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria. All patients must have an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 with a life expectancy > 3 months and must have already undergone first-line treatment for metastatic disease with platinum -based chemotherapy (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological, liver and renal function is required and effective contraceptive methods must be used by female patients of childbearing age. Written informed consent is obtained from all patients to take part in the study. Exclusion criteria are as follows: metastatic NECs previously treated with an irinotecan or temozolomide regimen, known hypersensitivity to 5-FU/capecitabine, calcium levofolinate, irinotecan or their recipients. All acute toxic effects of any prior therapy (including surgery, radiation therapy and chemotherapy) must have resolved to grade ≤1 according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (CTCAE). Patients

taking part in another clinical trial with any investigational agent < 30 days prior to study screening or with a history of allergic reactions attributable to compounds of similar chemical or biological composition are excluded. Patients who have undergone chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study, have not recovered from adverse events caused by agents administered > 4 weeks earlier, or have known brain metastases are ~~also~~ not eligible for the study. Patients with other malignancies with a disease-free interval of < 5 years (with the exception of non melanoma skin cancer or low-grade superficial bladder cancer) are excluded, as are those with any severe and/or uncontrolled medical condition or other condition that could affect their participation in the study such as:

- unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or any other clinically significant cardiac disease;
- severely impaired lung function (spirometry and DLCO 50% of the normal predicted value and/or oxygen saturation ≤ 88% at rest, in room air);
- uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of normal;
- any active (acute or chronic) or uncontrolled infections/disorders.

Tumour evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest, abdomen, pelvis, and any additional known sites of disease. These tests are performed at baseline, every three months during treatment and after the end of treatment in non-progressing patients until progression. When possible, ^{68}Ga-PET/CT and ^{18}FDG-PET/CT are performed at baseline. An EORTC quality of life questionnaire is administered at baseline and every three months thereafter during the treatment period.

Study endpoints

The primary endpoint of the study is the DCR of each treatment, defined as the percentage of patients who have achieved complete or partial response or stable disease for ≥12 weeks from the start of therapy. DCR will be evaluated using the new international criteria proposed by the RECIST version 1.1. Acute and late toxicity will be assessed by CTCAE Version 4.03, the latter defined as toxicity occurring at least 30 days after the end of the last treatment cycle. Secondary endpoints are OS, calculated from the start of treatment to death from any cause, and PFS, calculated from the start of treatment to the date of the first documented evidence of disease progression or of death from any cause. All the analyses will be performed in the intention-to-treat population. Patients without events at the time of analysis will be censored at their last-known-alive date for OS and at their last date of tumour evaluation for PFS. A further secondary endpoint is the evaluation of quality of life using the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). When data are available, the impact of baseline ^{68}Ga-PET/CT and ^{18}FDG-PET/CT on PFS will be analysed with exploratory intent. After signing the informed consent for biomarker assessment, patients will undergo evaluation of the mutation status of MEN1, DAXX, ATRX and RB-1 in primary tumour tissue and of miRNA in blood samples. Assessment of the miRNA profile will be performed on the first 20 patients who agree to participate in the biological part of the study.

Ethical considerations

The present clinical trial (EudraCT 2016-000767-17), supported by IRST, was authorised by the local Ethics Committee and by the Italian Medicines Agency (AIFA). The study is also registered on the *ClinicalTrials.gov* website (NCT03387592). The study complies with the ethical standards laid down in the 1964 Declaration of Helsinki and the principles of Good Clinical Practice guidelines (including written informed consent).

Patient and public involvement

This research work was performed without patient involvement in the study design, execution or outcome measures.

Statistical methods

The Bryant and Day design is used to estimate a sample size that takes into account both treatment activity and toxicity. Although randomisation is used to allocate patients to the two arms, no formal statistical comparisons between treatment regimens are planned. The purpose of randomisation is to reduce bias due to patient assignment to a specific treatment arm. The hypothesis for the control arm is based on literature data (23, 24).

An α level of 0.10 (both for toxicity and DCR) and a power of 90% have been adopted. A DCR rate ≥60% and a relevant toxicity rate ≤20% are considered acceptable rates, while a DCR rate ≤40% and a relevant toxicity rate ≥40% are considered inacceptable rates. Given these hypotheses, the first step of the study will require 25 patients. If ≥10 patients with a DCR are observed and ≥15 patients do not have significant toxicity, the study will enrol patients in the next step. A total of 53 patients

will be enrolled. If ≥25 patients with DCR and ≥36 patients without any relevant toxicity are observed, treatment will be considered active and not toxic. This design is used for each treatment scheme and all analyses will be performed separately. If one of the schemes does not obtain the expected proportions of the first step, the arm will be closed and patients will be enrolled in the other arm until the target is reached; if the expected proportions are not reached in any arm, the study will be prematurely closed. If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopoenia, prolonged G3-G4 neutropoenia (> 7 days) and drug-related hospitalisations are considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55 % *vs*. >55%) and site of primary tumour (lung *vs*. GEP).

Complete response, partial response or stable disease for at least 12 weeks will be considered as the DCR. The proportion of patients in this category will be determined and 95% CIs for the DCR will be calculated. OS and PFS will be estimated using the Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate statistical analyses will be performed on the basis of the data available to compare QLQ-C30 scores between baseline and subsequent follow-up visits.

When data are available, the impact of ^{68}Ga-PET/CT result on PFS will be analysed with exploratory intent. The Shapiro-Wilk test will be used to determine the normality distribution of each clinical, demographic and biological biomarker (25). In the event of a non-normal distribution, nonparametric statistics will be used to analyse the relationship between the serum levels of each marker, considered as continuous variables, and response to treatment. In the event of normal biomarker distribution,

a parametric test will be used. All endpoints will be analysed separately for each treatment group.

Discussion

There is still no truly effective second-line chemotherapy for neuroendocrine carcinoma. Overall prognosis of patients is poor, with an OS of 5 months in the metastatic setting according to SEER (Surveillance, Epidemiology, and End Results) data (26). Only 5% of all patients are long-term survivors. There is also a marked lack of prognostic and predictive factors (5).

Three phase II studies registered at *ClinicalTrials.gov* are currently investigating second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab (NCT03147404). Some abstracts were presented at ESMO (European Society for Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019 on the use of immunotherapy in GEP-NECs, all showing inconclusive results. The SENECA study uses a promising approach to the treatment of patients with metastatic NECs. First, both the activity and safety of 2 regimens are assessed in the same setting with a sizeable patient population (56 patients/arm). In addition, patients are stratified according to Ki-67 index and morphology to investigate the role of each treatment combination in both poorly differentiated and well differentiated NECs. Another aim of this study is to integrate both biological and metabolic imaging data in an effort to improve the current GEP-NEC classification.

In conclusion, there are still no FDA/EMA (Food and Drug Administration/European Medicines Agency)-approved second-line therapeutic options for patients with metastatic NECs, and the SENECA trial could represent a step forward in finding novel

therapies to prolong survival and maintain quality of life. Moreover, the integration of biological and imaging data could -lead to a better understanding of the natural history of the disease and help to identify potential responders.

Confidentiality

This study will be conducted in full conformity with ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) Guidelines for Good Clinical Practice, Directive 2001/20/EEC of the European Parliament and other relevant current local legislation. Participants will be allocated a unique identification (ID) number at entry. The master list linking participant personal information and ID number will be maintained in a separate locked cabinet and password-protected hard-drive. Data will be analysed by ID number only. Patient files and other source data will for be kept a maximum of 15 years.

Dissemination

After completing the study, all data, including beneficial and adverse events, of the trial will be communicated at scientific meetings and published in indexed peer-reviewed journals. If shown to be effective, the therapy program will be made available to the general public in an appropriate manner.

Abbreviations

CAPTEM, capecitabine plus temozolomide; DCR, disease control rate; GEP, gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour; OS, overall survival; PFS, progression-free survival

Author contributions

AB, CL and TI designed the study and drafted the article. AB was responsible for data acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a clinical information data extraction method for the protocol. FF performed the statistical analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual content. All authors read and approved the present version of the manuscript for submission.

Data availability statement

The datasets used and analysed in the present study are available from the corresponding author on reasonable request.

Funding

The study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

SENECA study Team investigators: Davide Campana, Davide Pastorelli, Nicola Silvestris, Francesco Silvestris, Angela Buonadonna and Giuseppe Badalamenti.

The authors thank Gráinne Tierney and Cristiano Verna for editorial assistance.

References

1. Kulke MH, Shah MH, Benson AB 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, et al. Neuroendocrine tumors, version 1.2015. *J Natl Compr Canc Netw* (2015) 13(1):78-108
2. Modlin IM, Moss SF, Chung DC, Jensen RT, Snyderwine E. Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors. *J Natl Cancer Inst* (2008)100(18):1282-9. doi: 10.1093/jnci/djn275
3. Yao YC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* (2008) 26(18):3063-72
4. Fraenkel M, Kim M, Faggiano A, de Herder WW, Valk GD; Knowledge NETwork. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocr Relat Cancer* (2014) 21(3):R153-63. doi: 10.1530/ERC-13-0125
5. Lloyd RV, Osamura RY, Klöppel G, Rosai J. WHO classification of tumours of endocrine organs. 4th ed (2017). Lyon: International Agency for Research on Cancer (IARC)
6. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with

advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60.

7. Heetfeld M, Chougnet CN, Olsen IH, Rinke A, Borbath I, Crespo G, et al. Characteristics and treatment of patients with G3 gastroenteropancreatic neuroendocrine neoplasms. *Endocr Relat Cancer* (2015) 22(4):657-64. doi: 10.1530/ERC-15-0119

8. Walter T, Malka D, Hentic O, Lombard-Bohas C, Le Malicot K, Smith D, et al. Evaluating bevacizumab in combination with FOLFIRI after the failure of platinum-etoposide regimen in patients with advanced poorly differentiated neuroendocrine carcinoma: The PRODIGE 41-BEVANEC randomized phase II study. *Dig Liver Dis* (2018) 50(2):195-8. doi: 10.1016/j.dld.2017.11.020

9. Hadoux J, Malka D, Planchard D, Scoazec JY, Caramella C, Guigay J, et al. Post-first-line FOLFOX chemotherapy for grade 3 neuroendocrine carcinoma. *Endocr Relat Cancer* (2015) 22(3)289-98. doi: 10.1530/ERC-15-0075

10. Olsen IH, Sørensen JB, Federspiel B, Kjaer A, Hansen CP, Knigge U, et al. Temozolomide as second or third line treatment of patients with neuroendocrine carcinomas. *Sci World J* (2012) 2012;2012:170496. doi: 10.1100/2012/170496

11. Hentic O, Hammel P, Couvelard A, Rebours V, Zappa M, Palazzo M, et al. FOLFIRI regimen: an effective second-line chemotherapy after failure of etoposide-platinum combination in patients with neuroendocrine carcinomas grade 3. *Endocr Relat Cancer* (2012) 9(6):751-7. doi: 10.1530/ERC-12-0002

12. Welin S, Sorbye H, Sebjornsen S, Knappskog S, Busch C, Oberg K. Clinical effect of temozolomide-based chemotherapy in poorly differentiated endocrine

carcinoma after progression on first-line chemotherapy. *Cancer* (2011) 117(20):4617-22. doi: 10.1002/cncr.26124

13. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* (2002) 346(2):85-91. doi: 10.1056/NEJMoa003034

14. Derks JL, Leblay N, Thunnissen E, van Suylen RJ, den Bakker M, Groen HJM, et al. Molecular subtypes of pulmonary large-cell neuroendocrine carcinoma predict chemotherapy treatment outcome. *Clin Cancer Res* (2018) 24(1):33-42. doi: 10.1158/1078-0432.CCR-17-1921

15. Tang LH, Untch BR, Reidy DL, O'Reilly E, Dhall D, Jih L, et al. Well-differentiated neuroendocrine tumors with a morphologically apparent high-grade component: a pathway distinct from poorly differentiated neuroendocrine carcinomas. *Clin Cancer Res* (2016) 22(4):1011-7. doi: 10.1158/1078-0432.CCR-15-0548

16. Liverani C, Bongiovanni A, Mercatali L, Foca F, Pieri F, De Vita A, Spadazzi C, Miserocchi G, Recine F, Riva N, Nicolini S, Severi S, Martinelli G, Ibrahim T. Grading of Neuroendocrine Carcinomas: Correlation of 68Ga-PET/CT Scan with Tissue Biomarkers. *Dis Markers.* 2018 Dec 2;2018:6878409.

17. Grolmusz VK, Kövesdi A, Borks K, Igaz P, Patócs A. Prognostic relevance of proliferation-related miRNAs in pancreatic neuroendocrine neoplasms Eur J Endocrinol (2018) 179(4):219-28. doi: 10.1530/EJE-18-0305

18. Gill P, Kim E, Chua TC, Clifton-Bligh RJ, Nahm CB, Mittal A, et al. MiRNA-3653 is a potential tissue biomarker for increased metastatic risk in pancreatic neuroendocrine tumours. *Endocr Pathol* (2019). doi: 10.1007/s12022-019-9570-y

[Epub ahead of print]

19. Panarelli N, Tyryshkin K, Wong JJM, Majewski A, Yang X, Scognamiglio T, et al. Evaluating gastroenteropancreatic neuroendocrine tumors through microRNA sequencing. *Endocr Relat Cancer* (2019) 26(1):47-57. doi: 10.1530/ERC-18-0244
20. Malczewska A, Kidd M, Matar S, Kos-Kudla B, Modlin IM. A comprehensive assessment of the role of mirnas as biomarkers in gastroenteropancreatic neuroendocrine tumors. *Neuroendocrinology* (2018)107(1):73-90. doi: 10.1159/000487326
21. Bongiovanni A, Riva N, Ricci M, Liverani C, La Manna F, De Vita A, et al. First-line chemotherapy in patients with metastatic gastroenteropancreatic neuroendocrinecarcinoma. *Onco Targets Ther* (2015) 8:3613-9. doi: 10.2147/OTT.S91971
22. Sansovini M, Severi S, Ianniello A, Nicolini S, Fantini L, Mezzenga E, Ferroni F, Scarpi E, Monti M, Bongiovanni A, Cingarlini S, Grana CM, Bodei L, Paganelli G. Long-term follow-up and role of FDG PET in advanced pancreatic neuroendocrine patients treated with 177Lu-D OTATATE. *Eur J Nucl Med Mol Imaging.* 2017 Mar;44(3):490-499.
23. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced Gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60. doi: 10.1093/annonc/mds276
24. Perren A, Couvelard A, Scoazec JY, Costa F, Borbath I, Delle Fave G, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors:

pathology: diagnosis and prognostic stratification. *Neuroendocrinology* (2017) 105(3):196-200. doi: 10.1159/000457956

25. Sorbye H, Köhne CH, Sargent DJ, Glimelius B. Patient characteristics and stratification in medical treatment studies for metastatic colorectal cancer: a proposal for standardization of patient characteristic reporting and stratification. *Ann Oncol* (2007) 18(10):1666-72.

26. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J. Amer. *Statist Assoc* (1958) 53:457-81.

Figure legend

FIGURE 1 | SENECA study design.

Fig. 1

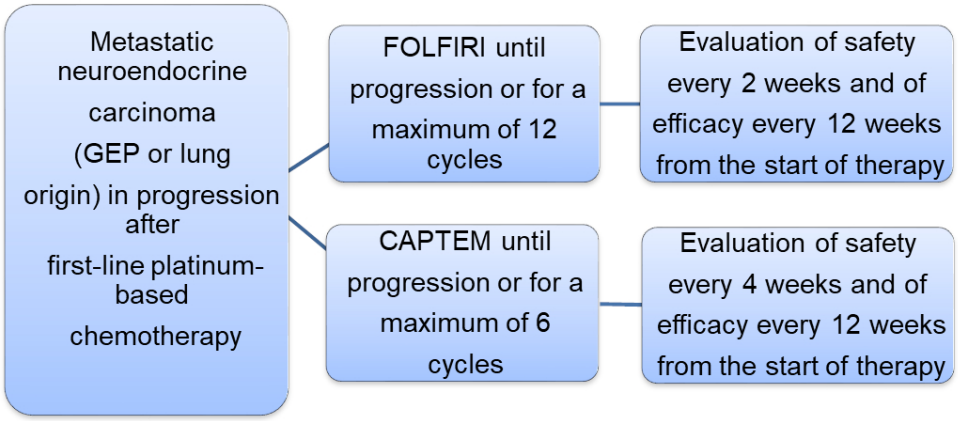


FIGURE 1 | SENECA study design.

90x91mm (300 x 300 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5-7
	2b	Specific objectives or hypotheses	7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8-11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	not Applicable
Participants	4a	Eligibility criteria for participants	9
	4b	Settings and locations where the data were collected	14-15.
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8,9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10, 11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	12
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	12
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	12
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	12
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	12
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	N/A

		assessing outcomes) and how	N/A
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	12, 13
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	12
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	8, 12
	13b	For each group, losses and exclusions after randomisation, together with reasons	12
Recruitment	14a	Dates defining the periods of recruitment and follow-up	12
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	N/A
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	N/A
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	12
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	4
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13, 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13, 14
Other information			
Registration	23	Registration number and name of trial registry	13
Protocol	24	Where the full trial protocol can be accessed, if available	14, 16
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

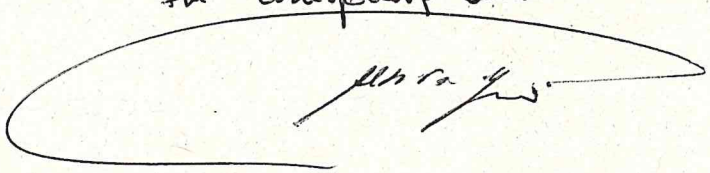
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

The above items were been addressed when applicable.
the corresponding author.



BMJ Open

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Trial): A Study Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-034393.R2
Article Type:	Protocol
Date Submitted by the Author:	27-Mar-2020
Complete List of Authors:	Bongiovanni, Alberto; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center Liverani, Chiara; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center Pusceddu, Sara; Istituto Nazionale Tumori Milano IRCCS , Department of Medical Oncology Leo, Silvana; Vito Fazzi Hospital, Oncology Unit Di Meglio, Giovanni; Ospedale di Bolzano, Oncology Unit Tamberi, Stefano; Medical Oncology, Ospedale degli Infermi, Faenza Santini, Daniele; Università Campus Bio-Medico, Department of Medical Oncology Gelsomino, Fabio; Azienda Ospedaliero-Universitaria di Modena, Department of Oncology and Hematology Pucci, Francesca; Azienda Ospedaliero-Universitaria di Parma, Medical Oncology Unit Berardi, Rossana; AOU-Ospedali Riuniti Umberto I - G.M. Lancisi - G. Salesi, Oncology Clinic Lolli, Ivan; IRCCS 'Saverio Belli', Department of Oncology Bergamo, Francesca; Istituto Oncologico Veneto (IOV), Department of Clinical and Experimental Oncology Ricci, Sergio; Ospedale S. Chiara, Internal Medicine and Medical Oncology Foca, Flavia; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Unit of Biostatistics and Clinical Trials Severi, Stefano; Nuclear Medicine Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS Ibrahim, Toni; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Oncology
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

CLINICAL STUDY PROTOCOL

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Trial): A Study Protocol

Running title: Second-line CAPTEM/FOLFIRI in NECs

Alberto Bongiovanni[1], Chiara Liverani[1], Sara Pusceddu[2], Silvana Leo[3], Giovanni Di Meglio[4], Stefano Tamberi[5], Daniele Santini[6] , Fabio Gelsomino[7], Francesca Pucci[8], Rossana Berardi[9], Ivan Lolli[10], Francesca Bergamo[11], Sergio Ricci[12] , Flavia Foca [13] Stefano Severi [14], Toni Ibrahim[1], and the SENECA Study Team Investigators**

[1]*Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [2] *Department of Medical Oncology, Istituto Nazionale Tumori Milano IRCCS, Milan, Italy,* [3]*Oncology Unit, Vito Fazzi Hospital, Lecce, Italy,* [4]*Onocology Unit, Ospedale di Bolzano, Bolzano, Italy,* [5]*Medical Oncology, Ospedale degli Infermi, Faenza, Italy,* [6] *Department of Medical Oncology, Università Campus Bio-Medico, Rome, Italy,* [7] *Department of Oncology and Hematology, Azienda Ospedaliera-Universitaria di Modena, Modena, Italy,* [8] *Medical Oncology Unit, Azienda Ospedaliera-Universitaria di Parma, Parma, Italy,* [9] *Oncology Clinic, AOU-Ospedali Riuniti Umberto I - G.M. Lancisi - G. Salesi, Ancona, Italy,* [10] *Department of Oncology, IRCCS "Saverio De Bellis", Castellana Grotte, Italy,* [11] *Department of Clinical and Experimental Oncology, Istituto Oncologico Veneto (IOV), Padua, Italy,* [12] *Internal Medicine and Medical Oncology, Ospedale S.Chiara -*

AOU Pisana, Pisa, Italy, [13]*Unit of Biostatistics and Clinical Trials, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [14]*Nuclear Medicine Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy*

***Correspondence:** Alberto Bongiovanni, MD

Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Via Piero Maroncelli 40, 47014 Meldola (FC), Italy

Tel.: +39-0543-739100; Fax: +39-0543-739123

E-mail: alberto.bongiovanni@irst.emr.it

Language style: This article is formatted in British English.

Abstract

Introduction: Patients with metastatic or locally advanced, non-resectable, grade 3 poorly-differentiated gastroenteropancreatic (GEP) and lung neuroendocrine carcinomas (NECs) are usually treated with in first-line platinum compounds. There is no standard second-line treatment upon progression. Accurate biomarkers are needed to facilitate diagnosis and prognostic assessment of NEC patients.

Methods and Analysis: The SENECA (SEcond-line therapy in NEuroendocrine CArcinomas) study is a randomised, non-comparative, multicentre phase II trial designed to evaluate the efficacy and safety of folinic acid, 5-fluorouracil and irinotecan (FOLFIRI) or capecitabine plus temozolomide (CAPTEM) regimens after failure of first-line-chemotherapy in lung NEC and GEP-NEC patients. Secondary aims are to correlate the serum miRNA profile and primary mutational status of MEN1, DAXX, ATRX and RB-1 with prognosis and outcome and to investigate the prognostic and predictive role of the Ki-67 score and FDG- or ^{68}Ga-PET/CT. The main eligibility criteria are age ≥18 years; metastatic or locally advanced, non-resectable, grade 3 lung or GEP-NECs; progression to first-line platinum-based chemotherapy. A Bryant and Day design taking into account treatment activity and toxicity was used to estimate the sample size. All analyses will be performed separately for each treatment group in the intention-to-treat population. A total of 112 patients (56/arm) will be randomly assigned (1:1) to receive FOLFIRI every 14 days or CAPTEM every 28 days until disease progression or unacceptable toxicity or for a maximum of six months. Patients undergo testing for specific biomarkers in primary tumour tissue and for miRNA in blood samples. MiRNA profiling will be performed in the first 20 patients who agree to participate in the biological sub-study.

Ethics and dissemination: The SENECA trial, supported by IRST, was authorised by the locals Ethics Committee and the Italian Medicines Agency (AIFA). Results will be widely disseminated via peer-reviewed manuscripts, conference presentations and reports to relevant authorities.

The study is currently open in Italy. Clinical trial registration: NCT03387592.

Keywords: neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine, temozolomide, CAPTEM

Strengths and limitations of the study

- the SENECA trial randomises patients to receive two different treatments, FOLFIRI or CAPTEM, providing important information on the activity of both combinations in different NEC subtypes (NET grade (G) 3 and NEC G3).
- The SENECA trial analyses the role of miRNAs and other biological markers as prognostic and predictive factors. A further aim is to assess ^{68}Ga-PET/CT as a tool to improve current histological classification.

The major limitations of the study are:

- The rarity of the disease and patient prognosis. However, the involvement of several Italian centres will hopefully help to overcome this problem.
- Poor prognosis of NEC patients. Patients progressing on platinum chemotherapy usually have rapid deterioration of clinical conditions

Introduction

Poorly differentiated neuroendocrine carcinomas (NECs) are very rare malignancies, representing only 5%-10% of neuroendocrine neoplasias (NENs) (1-3). At the time of diagnosis, patients are generally in poor conditions due to aggressive and diffuse disease. These tumors are characterised by aggressive histological features (high Ki-67 index, extensive necrosis, and nuclear atypia) and are classified as grade (G) 3 NECs according to the 2010 World Health Organization (WHO) classification (4). The 2017 WHO classification recognized a further group called G3 NETs as having intermediate features between NETs and NECs (5).

An etoposide-platinum combination is the gold standard for the treatment of G3 NECs, several studies published in the 1990s reporting substantial anti-tumor activity and high response rates (41%-67%) (6). However, prognosis is generally poor with a median progression-free survival (PFS) of 9 months and a median overall survival (OS) of 15-19 months. When progression occurs after first-line chemotherapy, the disease is usually very aggressive and patients succumb rapidly (7).

Given the rarity of this disease, prospective clinical data are lacking and treatment recommendations are essentially expert-based opinions. Two phase II studies investigating the second-line treatment of GEP-NECs are currently registered at *ClinicalTrials.gov*, one evaluating the safety and tolerability of everolimus in 40 patients with G3 NEC/NET or G1/G2 NET switching to G3 NEN (National Clinical Trial identifier NCT02113800) and the other investigating the efficacy of avelumab (NCT03147404). A French study focusing on the identification of predictive molecular markers of response to sunitinib in poorly differentiated digestive NETs

(NCT01215578) has now closed recruitment and results are eagerly awaited. Another French multicentre prospective phase II trial is currently ongoing to investigate the efficacy of the bevacizumab-FOLFIRI combination after progression on a platinum/etoposide combination (7).

Different second-line chemotherapy combinations have been evaluated but shown poor results (8-10). In a monocentre retrospective clinical trial, Hentic et al. hypothesised the potential efficacy of FOLFIRI as second-line chemotherapy in 19 patients with G3 extra-pulmonary NECs (11). An objective response rate was obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median PFS and OS were 4 and 18 months, respectively.

In another retrospective study, a 71% DCR was obtained with temozolomide-based chemotherapy in 25 patients with metastatic poorly differentiated endocrine carcinoma of different sites and atypical bronchial carcinoid with Ki-67 > 20%. Small cell lung carcinoma and large cell lung carcinoma were excluded. A PFS of 12 months (95% confidence interval [CI], 5.5- 24) and OS of 22 months (95% CI, 12-31) were reported in patients who responded to treatment or showed stable disease (SD), whereas OS was only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive staining for chromogranin A. Both factors are often associated with more highly differentiated tumours (12).

Literature data on lung NECs in progression after first-line chemotherapy are based on small patient series (13). Moreover, there is increasing evidence of some discrepancies in the current grading of NECs, highlighting the need for more accurate

biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise two distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived from well differentiated neuroendocrine tumours (NETs) and characterised by mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from neuroendocrine-differentiated adenocarcinomas and characterised by a mutation in RB1. Both subgroups display a distinct prognosis and different sensitivity to chemotherapy (14-16). Micro(mi)RNAs are a class of small, non-coding, highly conserved single-stranded RNAs involved in the post-transcriptional regulation of cell proliferation, differentiation, survival, and apoptosis (17). They are often associated with resistance to therapy (18, 19). Whilst miRNAs are known to show a specific expression pattern in NETs (20), little is known about differential miRNA profiles in NEC patients. At present, no data are available on the deregulation of specific miRNAs in this setting.

In a study recently published by our group on GEP-NEC patients undergoing first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months (p <0.01) in patients with Ki-67 value between 20% and 50% or >50%, respectively (19). Median (m)OS was 8.1 months in the latter group but was not reached in the former group (p = 0.039). Patients with a positive ^{68}Ga-PET/CT had a higher 18-month OS rate than those with a negative scan (75% *vs*. 34.3%, respectively), but the difference was not statistically significant (p = 0.06). Our data highlighted that ^{68}Ga-PET/CT positivity may be a discriminating factor (16, 21) in predicting prognosis, especially in the metastatic setting where histological material is not always available for evaluation. Furthermore, ^{18}fludeoxyglucose (^{18}FDG)-PET/CT may be useful to discriminate between patients with different prognosis (22).

Given the above premises, we decided to investigate the efficacy and safety of second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression after first-line platinum-based treatment. We also aimed to study the serum miRNA profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1, patient prognosis and response to therapy, and to assess the prognostic and predictive role of ^{18}FDG-PET/CT, ^{68}Ga-PET/CT and Ki-67 score.

Methods and Analysis

Study design

The SENECA study is a multicentre randomised non-comparative phase II study (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin (lung or gastroenteropancreatic) in progression after first-line treatment are randomized to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until progression or unacceptable toxicity.

The treatment arms are as follows:

FOLFIRI regimen

- Irinotecan 180 mg/m^2, given as a 60-min. intravenous (i.v.) infusion on day 1 every 2 weeks followed by
- Leucovorin 200 mg/m^2, given as a 2-h i.v. infusion on day 1 every 2 weeks followed by

- 5-fluorouracil (5-FU) 400 mg/m^2 given as bolus, and then 5-FU 2400 mg/m^2 given as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a maximum of 12 cycles.

CAPTEM regimen

Capecitabine 750 mg/m^2 twice a day on days 1-14 in combination with temozolomide 200 mg/m^2 daily on days 10-14, every 4 weeks, until progression or for a maximum of 6 cycles.

The study includes patients aged ≥ 18 years with a histological diagnosis of G3 neuroendocrine carcinoma (GEP-NEC and lung NEC) according to the 2010 and 2015 GEP and Lung NEN WHO classifications, respectively, Ki-67 >20% and measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria. All patients must have an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 with a life expectancy > 3 months and must have already undergone first-line treatment for metastatic disease with platinum -based chemotherapy (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological, liver and renal function is required and effective contraceptive methods must be used by female patients of childbearing age. Written informed consent is obtained from all patients to take part in the study. Exclusion criteria are as follows: metastatic NECs previously treated with an irinotecan or temozolomide regimen, known hypersensitivity to 5-FU/capecitabine, calcium levofolinate, irinotecan or their recipients. All acute toxic effects of any prior therapy (including surgery, radiation therapy and chemotherapy) must have resolved to grade ≤1 according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (CTCAE). Patients

taking part in another clinical trial with any investigational agent < 30 days prior to study screening or with a history of allergic reactions attributable to compounds of similar chemical or biological composition are excluded. Patients who have undergone chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study, have not recovered from adverse events caused by agents administered > 4 weeks earlier, or have known brain metastases are not eligible for the study. Patients with other malignancies with a disease-free interval of < 5 years (with the exception of non melanoma skin cancer or low-grade superficial bladder cancer) are excluded, as are those with any severe and/or uncontrolled medical condition or other condition that could affect their participation in the study such as:

- unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or any other clinically significant cardiac disease;
- severely impaired lung function (spirometry and DLCO 50% of the normal predicted value and/or oxygen saturation ≤ 88% at rest, in room air);
- uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of normal;
- any active (acute or chronic) or uncontrolled infections/disorders.

Tumour evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest, abdomen, pelvis, and any additional known sites of disease. These tests are performed at baseline, every three months during treatment as per national regulatory agency indications, and after the end of treatment in non-progressing patients until progression. It is recommended that ^{68}Ga-PET/CT and ^{18}FDG-PET/CT scans be performed at baseline or a maximum of 90 days before study enrollment. An EORTC quality of life

questionnaire is administered at baseline and every three months thereafter during the treatment period.

Study endpoints

The primary endpoint of the study is the DCR of each treatment, defined as the percentage of patients who have achieved complete or partial response or stable disease for ≥12 weeks from the start of therapy. DCR will be evaluated using the new international criteria proposed by the RECIST version 1.1. Acute and late toxicity will be assessed by CTCAE Version 4.03, the latter defined as toxicity occurring at least 30 days after the end of the last treatment cycle. Secondary endpoints are OS, calculated from the start of treatment to death from any cause, and PFS, calculated from the start of treatment to the date of the first documented evidence of disease progression or of death from any cause. All the analyses will be performed in the intention-to-treat population. Patients without events at the time of analysis will be censored at their last-known-alive date for OS and at their last date of tumour evaluation for PFS. A further secondary endpoint is the evaluation of quality of life using the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). When data are available, the impact of baseline ^{68}Ga-PET/CT and ^{18}FDG-PET/CT on PFS will be analysed with exploratory intent. After signing the informed consent for biomarker assessment, patients will undergo evaluation of the mutation status of MEN1, DAXX, ATRX and RB-1 in primary tumour tissue and of miRNA in blood samples. Assessment of the miRNA profile will be performed on the first 20 patients who agree to participate in the biological part of the study.

Ethical considerations

The present clinical trial (EudraCT 2016-000767-17), supported by IRST, was authorised by the local Ethics Committee and by the Italian Medicines Agency (AIFA). The study is also registered on the *ClinicalTrials.gov* website (NCT03387592). The study complies with the ethical standards laid down in the 1964 Declaration of Helsinki and the principles of Good Clinical Practice guidelines (including written informed consent).

Patient and public involvement

This research work was performed without patient involvement in the study design, execution or outcome measures.

Statistical methods

The Bryant and Day design is used to estimate a sample size that takes into account both treatment activity and toxicity. Although randomisation is used to allocate patients to the two arms, no formal statistical comparisons between treatment regimens are planned. The purpose of randomisation is to reduce bias due to patient assignment to a specific treatment arm. The hypothesis for the control arm is based on literature data (23, 24).

An α level of 0.10 (both for toxicity and DCR) and a power of 90% have been adopted. A DCR rate ≥60% and a relevant toxicity rate ≤20% are considered acceptable rates, while a DCR rate ≤40% and a relevant toxicity rate ≥40% are considered inacceptable rates. Given these hypotheses, the first step of the study will require 25 patients. If ≥10 patients with a DCR are observed and ≥15 patients do not have

significant toxicity, the study will enrol patients in the next step. A total of 53 patients will be enrolled. If ≥25 patients with DCR and ≥36 patients without any relevant toxicity are observed, treatment will be considered active and not toxic. This design is used for each treatment scheme and all analyses will be performed separately. If one of the schemes does not obtain the expected proportions of the first step, the arm will be closed and patients will be enrolled in the other arm until the target is reached; if the expected proportions are not reached in any arm, the study will be prematurely closed. If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopoenia, prolonged G3-G4 neutropoenia (> 7 days) and drug-related hospitalisations are considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55 % *vs*. >55%) and site of primary tumour (lung *vs*. GEP). A subgroup analysis of the efficacy of both treatments according to these stratification factors has been planned.

Complete response, partial response or stable disease for at least 12 weeks will be considered as the DCR. The proportion of patients in this category will be determined and 95% CIs for the DCR will be calculated. OS and PFS will be estimated using the Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate statistical analyses will be performed on the basis of the data available to compare QLQ-C30 scores between baseline and subsequent follow-up visits.

When data are available, the impact of ^{68}Ga-PET/CT result on PFS will be analysed with exploratory intent. The Shapiro-Wilk test will be used to determine the normality distribution of each clinical, demographic and biological biomarker (25). In the event of a non-normal distribution, nonparametric statistics will be used to analyse the

relationship between the serum levels of each marker, considered as continuous variables, and response to treatment. In the event of normal biomarker distribution, a parametric test will be used. All endpoints will be analysed separately for each treatment group.

Discussion

There is still no truly effective second-line chemotherapy for neuroendocrine carcinoma. Overall prognosis of patients is poor, with an OS of 5 months in the metastatic setting according to SEER (Surveillance, Epidemiology, and End Results) data (26). Only 5% of all patients are long-term survivors. There is also a marked lack of prognostic and predictive factors (5).

Three phase II studies registered at *ClinicalTrials.gov* are currently investigating second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab (NCT03147404). Some abstracts were presented at ESMO (European Society for Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019 on the use of immunotherapy in GEP-NECs, all showing inconclusive results. The SENECA study uses a promising approach to the treatment of patients with metastatic NECs. First, both the activity and safety of 2 regimens are assessed in the same setting with a sizeable patient population (56 patients/arm). In addition, patients are stratified according to Ki-67 index and morphology to investigate the role of each treatment combination in both poorly differentiated and well differentiated NECs. Another aim of this study is to integrate both biological and metabolic imaging data in an effort to improve the current GEP-NEC classification.

The duration of treatments in the metastatic setting is a dilemma in NENs and especially in neuroendocrine carcinomas. Given the lack of evidence-based recommendations on treatment duration of second-line chemotherapy in NECs, we decided to opt for a fixed duration of treatments to avoid unnecessary exposure to cytotoxic agents and consequent bone marrow reserve depletion (27).

In conclusion, there are still no FDA/EMA (Food and Drug Administration/European Medicines Agency)-approved second-line therapeutic options for patients with metastatic NECs, and the SENECA trial could represent a step forward in finding novel therapies to prolong survival and maintain quality of life. Moreover, the integration of biological and imaging data could -lead to a better understanding of the natural history of the disease and help to identify potential responders.

Confidentiality

This study will be conducted in full conformity with ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) Guidelines for Good Clinical Practice, Directive 2001/20/EEC of the European Parliament and other relevant current local legislation. Participants will be allocated a unique identification (ID) number at entry. The master list linking participant personal information and ID number will be maintained in a separate locked cabinet and password-protected hard-drive. Data will be analysed by ID number only. Patient files and other source data will for be kept a maximum of 15 years.

Ethics and Dissemination

The SENECA trial, supported by IRST, involves several Italian centres and was authorised by the local Ethics Committees of the centres taking part and by the Italian Medicines Agency (AIFA). After completing the study, all data, including beneficial and adverse events, of the trial will be communicated at scientific meetings and published in indexed peer-reviewed journals. If shown to be effective, the therapy program will be made available to the general public in an appropriate manner.

Abbreviations

CAPTEM, capecitabine plus temozolomide; DCR, disease control rate; GEP, gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour; OS, overall survival; PFS, progression-free survival

Author contributions

AB, CL and TI designed the study and drafted the article. AB was responsible for data acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a clinical information data extraction method for the protocol. FF performed the statistical analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual content. All authors read and approved the present version of the manuscript for submission.

Data availability statement

The datasets used and analysed in the present study are available from the corresponding author on reasonable request.

Funding

The study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

SENECA study Team investigators: Davide Campana, Davide Pastorelli, Nicola Silvestris, Francesco Silvestris, Angela Buonadonna and Giuseppe Badalamenti.

The authors thank Gráinne Tierney and Cristiano Verna for editorial assistance.

References

1. Kulke MH, Shah MH, Benson AB 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, et al. Neuroendocrine tumors, version 1.2015. *J Natl Compr Canc Netw* (2015) 13(1):78-108
2. Modlin IM, Moss SF, Chung DC, Jensen RT, Snyderwine E. Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors. *J Natl Cancer Inst* (2008)100(18):1282-9. doi: 10.1093/jnci/djn275
3. Yao YC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* (2008) 26(18):3063-72
4. Fraenkel M, Kim M, Faggiano A, de Herder WW, Valk GD; Knowledge NETwork. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocr Relat Cancer* (2014) 21(3):R153-63. doi: 10.1530/ERC-13-0125
5. Lloyd RV, Osamura RY, Klöppel G, Rosai J. WHO classification of tumours of endocrine organs. 4th ed (2017). Lyon: International Agency for Research on Cancer (IARC)
6. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60.
7. Heetfeld M, Chougnet CN, Olsen IH, Rinke A, Borbath I, Crespo G, et al. Characteristics and treatment of patients with G3 gastroenteropancreatic neuroendocrine neoplasms. *Endocr Relat Cancer* (2015) 22(4):657-64. doi:

10.1530/ERC-15-0119

8. Walter T, Malka D, Hentic O, Lombard-Bohas C, Le Malicot K, Smith D, et al. Evaluating bevacizumab in combination with FOLFIRI after the failure of platinum-etoposide regimen in patients with advanced poorly differentiated neuroendocrine carcinoma: The PRODIGE 41-BEVANEC randomized phase II study. *Dig Liver Dis* (2018) 50(2):195-8. doi: 10.1016/j.dld.2017.11.020
9. Hadoux J, Malka D, Planchard D, Scoazec JY, Caramella C, Guigay J, et al. Post-first-line FOLFOX chemotherapy for grade 3 neuroendocrine carcinoma. *Endocr Relat Cancer* (2015) 22(3)289-98. doi: 10.1530/ERC-15-0075
10. Olsen IH, Sørensen JB, Federspiel B, Kjaer A, Hansen CP, Knigge U, et al. Temozolomide as second or third line treatment of patients with neuroendocrine carcinomas. *Sci World J* (2012) 2012;2012:170496. doi: 10.1100/2012/170496
11. Hentic O, Hammel P, Couvelard A, Rebours V, Zappa M, Palazzo M, et al. FOLFIRI regimen: an effective second-line chemotherapy after failure of etoposide-platinum combination in patients with neuroendocrine carcinomas grade 3. *Endocr Relat Cancer* (2012) 9(6):751-7. doi: 10.1530/ERC-12-0002
12. Welin S, Sorbye H, Sebjornsen S, Knappskog S, Busch C, Oberg K. Clinical effect of temozolomide-based chemotherapy in poorly differentiated endocrine carcinoma after progression on first-line chemotherapy. *Cancer* (2011) 117(20):4617-22. doi: 10.1002/cncr.26124
13. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* (2002) 346(2):85-91. doi: 10.1056/NEJMoa003034

14. Derks JL, Leblay N, Thunnissen E, van Suylen RJ, den Bakker M, Groen HJM, et al. Molecular subtypes of pulmonary large-cell neuroendocrine carcinoma predict chemotherapy treatment outcome. *Clin Cancer Res* (2018) 24(1):33-42. doi: 10.1158/1078-0432.CCR-17-1921

15. Tang LH, Untch BR, Reidy DL, O'Reilly E, Dhall D, Jih L, et al. Well-differentiated neuroendocrine tumors with a morphologically apparent high-grade component: a pathway distinct from poorly differentiated neuroendocrine carcinomas. *Clin Cancer Res* (2016) 22(4):1011-7. doi: 10.1158/1078-0432.CCR-15-0548

16. Liverani C, Bongiovanni A, Mercatali L, Foca F, Pieri F, De Vita A, Spadazzi C, Miserocchi G, Recine F, Riva N, Nicolini S, Severi S, Martinelli G, Ibrahim T. Grading of Neuroendocrine Carcinomas: Correlation of 68Ga-PET/CT Scan with Tissue Biomarkers. *Dis Markers.* 2018 Dec 2;2018:6878409.

17. Grolmusz VK, Kövesdi A, Borks K, Igaz P, Patócs A. Prognostic relevance of proliferation-related miRNAs in pancreatic neuroendocrine neoplasms Eur J Endocrinol (2018) 179(4):219-28. doi: 10.1530/EJE-18-0305

18. Gill P, Kim E, Chua TC, Clifton-Bligh RJ, Nahm CB, Mittal A, et al. MiRNA-3653 is a potential tissue biomarker for increased metastatic risk in pancreatic neuroendocrine tumours. *Endocr Pathol* (2019). doi: 10.1007/s12022-019-9570-y [Epub ahead of print]

19. Panarelli N, Tyryshkin K, Wong JJM, Majewski A, Yang X, Scognamiglio T, et al. Evaluating gastroenteropancreatic neuroendocrine tumors through microRNA sequencing. *Endocr Relat Cancer* (2019) 26(1):47-57. doi: 10.1530/ERC-18-0244

20. Malczewska A, Kidd M, Matar S, Kos-Kudla B, Modlin IM. A comprehensive

assessment of the role of mirnas as biomarkers in gastroenteropancreatic neuroendocrine tumors. *Neuroendocrinology* (2018)107(1):73-90. doi: 10.1159/000487326

21. Bongiovanni A, Riva N, Ricci M, Liverani C, La Manna F, De Vita A, et al. First-line chemotherapy in patients with metastatic gastroenteropancreatic neuroendocrinecarcinoma. *Onco Targets Ther* (2015) 8:3613-9. doi: 10.2147/OTT.S91971
22. Sansovini M, Severi S, Ianniello A, Nicolini S, Fantini L, Mezzenga E, Ferroni F, Scarpi E, Monti M, Bongiovanni A, Cingarlini S, Grana CM, Bodei L, Paganelli G. Long-term follow-up and role of FDG PET in advanced pancreatic neuroendocrine patients treated with 177Lu-D OTATATE. *Eur J Nucl Med Mol Imaging.* 2017 Mar;44(3):490-499.
23. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced Gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60. doi: 10.1093/annonc/mds276
24. Perren A, Couvelard A, Scoazec JY, Costa F, Borbath I, Delle Fave G, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: pathology: diagnosis and prognostic stratification. *Neuroendocrinology* (2017) 105(3):196-200. doi: 10.1159/000457956
25. Sorbye H, Köhne CH, Sargent DJ, Glimelius B. Patient characteristics and stratification in medical treatment studies for metastatic colorectal cancer: a proposal for standardization of patient characteristic reporting and stratification.

Ann Oncol (2007) 18(10):1666-72.

26. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J. Amer. *Statist Assoc* (1958) 53:457-81.

27. Strosberg JR, Fine RL, Choi J, Nasir A, Coppola D, Chen DT, et al. First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. *Cancer* (2011) 117 (2):268-75.

Figure legend

FIGURE 1 | SENECA study design.

Fig. 1

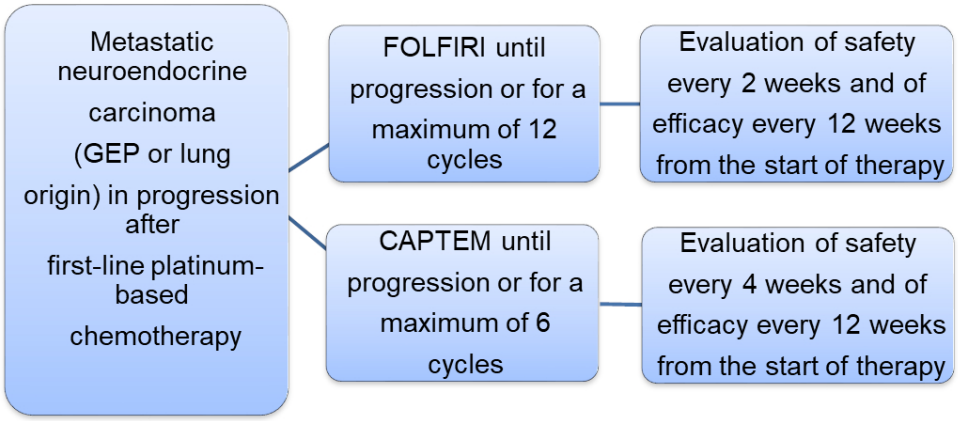


FIGURE 1 | SENECA study design.

90x91mm (300 x 300 DPI)

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5-7
	2b	Specific objectives or hypotheses	7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8-11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	not Applicable
Participants	4a	Eligibility criteria for participants	9
	4b	Settings and locations where the data were collected	14-15.
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8,9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10, 11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	12
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	12
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	12
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	12
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	12
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	N/A

		assessing outcomes) and how	N/A
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	12, 13
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	12
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	8, 12
	13b	For each group, losses and exclusions after randomisation, together with reasons	12
Recruitment	14a	Dates defining the periods of recruitment and follow-up	12
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	N/A
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	N/A
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	12
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	4
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13, 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13, 14
Other information			
Registration	23	Registration number and name of trial registry	13
Protocol	24	Where the full trial protocol can be accessed, if available	14, 16
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial
Methods: Data collection, management, and analysis		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
Methods: Monitoring		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

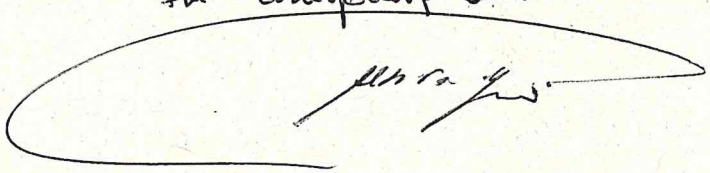
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

The above items were been addressed when applicable.
the corresponding author.



BMJ Open

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Trial): A Study Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-034393.R3
Article Type:	Protocol
Date Submitted by the Author:	08-May-2020
Complete List of Authors:	Bongiovanni, Alberto; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center Liverani, Chiara; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center Pusceddu, Sara; Istituto Nazionale per lo Studio e la Cura dei Tumori, Department of Medical Oncology Leo, Silvana; Ospedale Vito Fazzi, Oncology Unit Di Meglio, Giovanni; Ospedale di Bolzano, Oncology Unit Tamberi, Stefano; Ospedale degli Infermi di Faenza Santini, Daniele; Campus Bio-Medico University, Department of Medical Oncology Gelsomino, Fabio; Azienda Ospedaliero-Universitaria di Modena, Department of Oncology and Hematology Pucci, Francesca; Azienda Ospedaliero-Universitaria di Parma, Medical Oncology Unit Berardi, Rossana; University Hospital of Ancona Umberto I G M Lancisi G Salesi, Oncology Clinic Lolli, Ivan; Istituto Nazionale di Ricovero e Cura a Carattere Scientifico Saverio de Bellis, Department of Oncology Bergamo, Francesca; Istituto Oncologico Veneto Istituto di Ricovero e Cura a Carattere Scientifico, Department of Clinical and Experimental Oncology Ricci, Sergio; Santa Chiara Hospital, Internal Medicine and Medical Oncology Foca, Flavia; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Unit of Biostatistics and Clinical Trials Severi, Stefano; Nuclear Medicine Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS Ibrahim, Toni; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Oncology
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

CLINICAL STUDY PROTOCOL

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Trial): A Study Protocol

Running title: Second-line CAPTEM/FOLFIRI in NECs

Alberto Bongiovanni[1], Chiara Liverani[1], Sara Pusceddu[2], Silvana Leo[3], Giovanni Di Meglio[4], Stefano Tamberi[5], Daniele Santini[6] , Fabio Gelsomino[7], Francesca Pucci[8], Rossana Berardi[9], Ivan Lolli[10], Francesca Bergamo[11], Sergio Ricci[12] , Flavia Foca [13] Stefano Severi [14], Toni Ibrahim[1], and the SENECA Study Team Investigators**

[1]*Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [2] *Department of Medical Oncology, Istituto Nazionale Tumori Milano IRCCS, Milan, Italy,* [3]*Oncology Unit, Vito Fazzi Hospital, Lecce, Italy,* [4]*Onocology Unit, Ospedale di Bolzano, Bolzano, Italy,* [5]*Medical Oncology, Ospedale degli Infermi, Faenza, Italy,* [6] *Department of Medical Oncology, Università Campus Bio-Medico, Rome, Italy,* [7] *Department of Oncology and Hematology, Azienda Ospedaliera-Universitaria di Modena, Modena, Italy,* [8] *Medical Oncology Unit, Azienda Ospedaliera-Universitaria di Parma, Parma, Italy,* [9] *Oncology Clinic, AOU-Ospedali Riuniti Umberto I - G.M. Lancisi - G. Salesi, Ancona, Italy,* [10] *Department of Oncology, IRCCS "Saverio De Bellis", Castellana Grotte, Italy,* [11] *Department of Clinical and Experimental Oncology, Istituto Oncologico Veneto (IOV), Padua, Italy,* [12] *Internal Medicine and Medical Oncology, Ospedale S.Chiara -*

AOU Pisana, Pisa, Italy, [13]*Unit of Biostatistics and Clinical Trials, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [14]*Nuclear Medicine Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy*

***Correspondence:** Alberto Bongiovanni, MD

Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Via Piero Maroncelli 40, 47014 Meldola (FC), Italy

Tel.: +39-0543-739100; Fax: +39-0543-739123

E-mail: alberto.bongiovanni@irst.emr.it

Language style: This article is formatted in British English.

Abstract

Introduction: Patients with metastatic or locally advanced, non-resectable, grade 3 poorly-differentiated gastroenteropancreatic (GEP) and lung neuroendocrine carcinomas (NECs) are usually treated with in first-line platinum compounds. There is no standard second-line treatment upon progression. Accurate biomarkers are needed to facilitate diagnosis and prognostic assessment of NEC patients.

Methods and Analysis: The SENECA (SEcond-line therapy in NEuroendocrine CArcinomas) study is a randomised, non-comparative, multicentre phase II trial designed to evaluate the efficacy and safety of folinic acid, 5-fluorouracil and irinotecan (FOLFIRI) or capecitabine plus temozolomide (CAPTEM) regimens after failure of first-line-chemotherapy in lung NEC and GEP-NEC patients. Secondary aims are to correlate the serum miRNA profile and primary mutational status of MEN1, DAXX, ATRX and RB-1 with prognosis and outcome and to investigate the prognostic and predictive role of the Ki-67 score and FDG- or ^{68}Ga-PET/CT. The main eligibility criteria are age ≥18 years; metastatic or locally advanced, non-resectable, grade 3 lung or GEP-NECs; progression to first-line platinum-based chemotherapy. A Bryant and Day design taking into account treatment activity and toxicity was used to estimate the sample size. All analyses will be performed separately for each treatment group in the intention-to-treat population. A total of 112 patients (56/arm) will be randomly assigned (1:1) to receive FOLFIRI every 14 days or CAPTEM every 28 days until disease progression or unacceptable toxicity or for a maximum of six months. Patients undergo testing for specific biomarkers in primary tumour tissue and for miRNA in blood samples. MiRNA profiling will be performed in the first 20 patients who agree to participate in the biological sub-study.

Ethics and dissemination: The SENECA trial, supported by IRST, was authorised by the locals Ethics Committee and the Italian Medicines Agency (AIFA). Results will be widely disseminated via peer-reviewed manuscripts, conference presentations and reports to relevant authorities.

The study is currently open in Italy. ClinicalTrials.gov Identifier: NCT03387592.

EudraCT number: 2016-000767-17

Protocol version: Clinical Study Protocol Version 1, 07.11.2016.

Keywords: neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine, temozolomide, CAPTEM

Strengths and limitations of the study

- the SENECA trial randomises patients to receive two different treatments, FOLFIRI or CAPTEM, providing important information on the activity of both combinations in different NEC subtypes (NET grade (G) 3 and NEC G3).
- The SENECA trial analyses the role of miRNAs and other biological markers as prognostic and predictive factors. A further aim is to assess ^{68}Ga-PET/CT as a tool to improve current histological classification.

The major limitations of the study are:

- The rarity of the disease and patient prognosis. However, the involvement of several Italian centres will hopefully help to overcome this problem.
- Poor prognosis of NEC patients. Patients progressing on platinum chemotherapy usually have rapid deterioration of clinical conditions

Introduction

Poorly differentiated neuroendocrine carcinomas (NECs) are very rare malignancies, representing only 5%-10% of neuroendocrine neoplasias (NENs) (1-3). At the time of diagnosis, patients are generally in poor conditions due to aggressive and diffuse disease. These tumors are characterised by aggressive histological features (high Ki-67 index, extensive necrosis, and nuclear atypia) and are classified as grade (G) 3 NECs according to the 2010 World Health Organization (WHO) classification (4). The 2017 WHO classification recognized a further group called G3 NETs as having intermediate features between NETs and NECs (5).

An etoposide-platinum combination is the gold standard for the treatment of G3 NECs, several studies published in the 1990s reporting substantial anti-tumor activity and high response rates (41%-67%) (6). However, prognosis is generally poor with a median progression-free survival (PFS) of 9 months and a median overall survival (OS) of 15-19 months. When progression occurs after first-line chemotherapy, the disease is usually very aggressive and patients succumb rapidly (7).

Given the rarity of this disease, prospective clinical data are lacking and treatment recommendations are essentially expert-based opinions. Two phase II studies investigating the second-line treatment of GEP-NECs are currently registered at *ClinicalTrials.gov*, one evaluating the safety and tolerability of everolimus in 40 patients with G3 NEC/NET or G1/G2 NET switching to G3 NEN (National Clinical Trial identifier NCT02113800) and the other investigating the efficacy of avelumab (NCT03147404). A French study focusing on the identification of predictive molecular markers of response to sunitinib in poorly differentiated digestive NETs

(NCT01215578) has now closed recruitment and results are eagerly awaited. Another French multicentre prospective phase II trial is currently ongoing to investigate the efficacy of the bevacizumab-FOLFIRI combination after progression on a platinum/etoposide combination (7).

Different second-line chemotherapy combinations have been evaluated but shown poor results (8-10). In a monocentre retrospective clinical trial, Hentic et al. hypothesised the potential efficacy of FOLFIRI as second-line chemotherapy in 19 patients with G3 extra-pulmonary NECs (11). An objective response rate was obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median PFS and OS were 4 and 18 months, respectively.

In another retrospective study, a 71% DCR was obtained with temozolomide-based chemotherapy in 25 patients with metastatic poorly differentiated endocrine carcinoma of different sites and atypical bronchial carcinoid with Ki-67 > 20%. Small cell lung carcinoma and large cell lung carcinoma were excluded. A PFS of 12 months (95% confidence interval [CI], 5.5- 24) and OS of 22 months (95% CI, 12-31) were reported in patients who responded to treatment or showed stable disease (SD), whereas OS was only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive staining for chromogranin A. Both factors are often associated with more highly differentiated tumours (12).

Literature data on lung NECs in progression after first-line chemotherapy are based on small patient series (13). Moreover, there is increasing evidence of some discrepancies in the current grading of NECs, highlighting the need for more accurate

biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise two distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived from well differentiated neuroendocrine tumours (NETs) and characterised by mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from neuroendocrine-differentiated adenocarcinomas and characterised by a mutation in RB1. Both subgroups display a distinct prognosis and different sensitivity to chemotherapy (14-16). Micro(mi)RNAs are a class of small, non-coding, highly conserved single-stranded RNAs involved in the post-transcriptional regulation of cell proliferation, differentiation, survival, and apoptosis (17). They are often associated with resistance to therapy (18, 19). Whilst miRNAs are known to show a specific expression pattern in NETs (20), little is known about differential miRNA profiles in NEC patients. At present, no data are available on the deregulation of specific miRNAs in this setting.

In a study recently published by our group on GEP-NEC patients undergoing first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months (p <0.01) in patients with Ki-67 value between 20% and 50% or >50%, respectively (19). Median (m)OS was 8.1 months in the latter group but was not reached in the former group (p = 0.039). Patients with a positive ^{68}Ga-PET/CT had a higher 18-month OS rate than those with a negative scan (75% *vs*. 34.3%, respectively), but the difference was not statistically significant (p = 0.06). Our data highlighted that ^{68}Ga-PET/CT positivity may be a discriminating factor (16, 21) in predicting prognosis, especially in the metastatic setting where histological material is not always available for evaluation. Furthermore, ^{18}fludeoxyglucose (^{18}FDG)-PET/CT may be useful to discriminate between patients with different prognosis (22).

Given the above premises, we decided to investigate the efficacy and safety of second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression after first-line platinum-based treatment. We also aimed to study the serum miRNA profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1, patient prognosis and response to therapy, and to assess the prognostic and predictive role of ^{18}FDG-PET/CT, ^{68}Ga-PET/CT and Ki-67 score.

Methods and Analysis

Study design

The SENECA study is a multicentre randomised non-comparative phase II study (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin (lung or gastroenteropancreatic) in progression after first-line treatment are randomized to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until progression or unacceptable toxicity.

The treatment arms are as follows:

FOLFIRI regimen

- Irinotecan 180 mg/m^2, given as a 60-min. intravenous (i.v.) infusion on day 1 every 2 weeks followed by
- Leucovorin 200 mg/m^2, given as a 2-h i.v. infusion on day 1 every 2 weeks followed by

- 5-fluorouracil (5-FU) 400 mg/m^2 given as bolus, and then 5-FU 2400 mg/m^2 given as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a maximum of 12 cycles.

CAPTEM regimen

Capecitabine 750 mg/m^2 twice a day on days 1-14 in combination with temozolomide 200 mg/m^2 daily on days 10-14, every 4 weeks, until progression or for a maximum of 6 cycles.

The study includes patients aged ≥ 18 years with a histological diagnosis of G3 neuroendocrine carcinoma (GEP-NEC and lung NEC) according to the 2010 and 2015 GEP and Lung NEN WHO classifications, respectively, Ki-67 >20% and measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria. All patients must have an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 with a life expectancy > 3 months and must have already undergone first-line treatment for metastatic disease with platinum -based chemotherapy (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological, liver and renal function is required and effective contraceptive methods must be used by female patients of childbearing age. Written informed consent is obtained from all patients to take part in the study. Exclusion criteria are as follows: metastatic NECs previously treated with an irinotecan or temozolomide regimen, known hypersensitivity to 5-FU/capecitabine, calcium levofolinate, irinotecan or their recipients. All acute toxic effects of any prior therapy (including surgery, radiation therapy and chemotherapy) must have resolved to grade ≤1 according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (CTCAE). Patients

taking part in another clinical trial with any investigational agent < 30 days prior to study screening or with a history of allergic reactions attributable to compounds of similar chemical or biological composition are excluded. Patients who have undergone chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study, have not recovered from adverse events caused by agents administered > 4 weeks earlier, or have known brain metastases are not eligible for the study. Patients with other malignancies with a disease-free interval of < 5 years (with the exception of non melanoma skin cancer or low-grade superficial bladder cancer) are excluded, as are those with any severe and/or uncontrolled medical condition or other condition that could affect their participation in the study such as:

- unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or any other clinically significant cardiac disease;
- severely impaired lung function (spirometry and DLCO 50% of the normal predicted value and/or oxygen saturation ≤ 88% at rest, in room air);
- uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of normal;
- any active (acute or chronic) or uncontrolled infections/disorders.

Tumour evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest, abdomen, pelvis, and any additional known sites of disease. These tests are performed at baseline, every three months during treatment as per national regulatory agency indications, and after the end of treatment in non-progressing patients until progression. It is recommended that ^{68}Ga-PET/CT and ^{18}FDG-PET/CT scans be performed at baseline or a maximum of 90 days before study enrollment. An EORTC quality of life

questionnaire is administered at baseline and every three months thereafter during the treatment period.

Study endpoints

The primary endpoint of the study is the DCR of each treatment, defined as the percentage of patients who have achieved complete or partial response or stable disease for ≥12 weeks from the start of therapy. DCR will be evaluated using the new international criteria proposed by the RECIST version 1.1. Acute and late toxicity will be assessed by CTCAE Version 4.03, the latter defined as toxicity occurring at least 30 days after the end of the last treatment cycle. Secondary endpoints are OS, calculated from the start of treatment to death from any cause, and PFS, calculated from the start of treatment to the date of the first documented evidence of disease progression or of death from any cause. All the analyses will be performed in the intention-to-treat population. Patients without events at the time of analysis will be censored at their last-known-alive date for OS and at their last date of tumour evaluation for PFS. A further secondary endpoint is the evaluation of quality of life using the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). When data are available, the impact of baseline ^{68}Ga-PET/CT and ^{18}FDG-PET/CT on PFS will be analysed with exploratory intent. After signing the informed consent for biomarker assessment, patients will undergo evaluation of the mutation status of MEN1, DAXX, ATRX and RB-1 in primary tumour tissue and of miRNA in blood samples. Assessment of the miRNA profile will be performed on the first 20 patients who agree to participate in the biological part of the study.

Ethical considerations

The present clinical trial, supported by IRST, was authorised by the local Ethics Committee and by the Italian Medicines Agency (AIFA). The request for EudraCT registration (mandatory for studies in Europe) was send to AIFA in December 2016 and we received a EudraCT number (EudraCT 2016-000767-17). However, technical problems at AIFA resulted in some clinical trials, including ours, being uploaded onto the EudraCT website after enrolment of the first patients.

The study is also registered on the *ClinicalTrials.gov* website (NCT03387592). The study complies with the ethical standards laid down in the 1964 Declaration of Helsinki and the principles of Good Clinical Practice guidelines (including written informed consent).

Patient and public involvement

This research work was performed without patient involvement in the study design, execution or outcome measures.

Statistical methods

The Bryant and Day design is used to estimate a sample size that takes into account both treatment activity and toxicity. Although randomisation is used to allocate patients to the two arms, no formal statistical comparisons between treatment regimens are planned. The purpose of randomisation is to reduce bias due to patient assignment to a specific treatment arm. The hypothesis for the control arm is based on literature data (23, 24).

An α level of 0.10 (both for toxicity and DCR) and a power of 90% have been

adopted. A DCR rate ≥60% and a relevant toxicity rate ≤20% are considered acceptable rates, while a DCR rate ≤40% and a relevant toxicity rate ≥40% are considered inacceptable rates. Given these hypotheses, the first step of the study will require 25 patients. If ≥10 patients with a DCR are observed and ≥15 patients do not have significant toxicity, the study will enrol patients in the next step. A total of 53 patients will be enrolled. If ≥25 patients with DCR and ≥36 patients without any relevant toxicity are observed, treatment will be considered active and not toxic. This design is used for each treatment scheme and all analyses will be performed separately. If one of the schemes does not obtain the expected proportions of the first step, the arm will be closed and patients will be enrolled in the other arm until the target is reached; if the expected proportions are not reached in any arm, the study will be prematurely closed. If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopoenia, prolonged G3-G4 neutropoenia (> 7 days) and drug-related hospitalisations are considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55 % *vs*. >55%) and site of primary tumour (lung *vs*. GEP). A subgroup analysis of the efficacy of both treatments according to these stratification factors has been planned.

Complete response, partial response or stable disease for at least 12 weeks will be considered as the DCR. The proportion of patients in this category will be determined and 95% CIs for the DCR will be calculated. OS and PFS will be estimated using the Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate statistical analyses will be performed on the basis of the data available to compare QLQ-C30 scores between baseline and subsequent follow-up visits.

When data are available, the impact of ^{68}Ga-PET/CT result on PFS will be analysed with exploratory intent. The Shapiro-Wilk test will be used to determine the normality distribution of each clinical, demographic and biological biomarker (25). In the event of a non-normal distribution, nonparametric statistics will be used to analyse the relationship between the serum levels of each marker, considered as continuous variables, and response to treatment. In the event of normal biomarker distribution, a parametric test will be used. All endpoints will be analysed separately for each treatment group.

Discussion

There is still no truly effective second-line chemotherapy for neuroendocrine carcinoma. Overall prognosis of patients is poor, with an OS of 5 months in the metastatic setting according to SEER (Surveillance, Epidemiology, and End Results) data (26). Only 5% of all patients are long-term survivors. There is also a marked lack of prognostic and predictive factors (5).

Three phase II studies registered at *ClinicalTrials.gov* are currently investigating second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab (NCT03147404). Some abstracts were presented at ESMO (European Society for Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019 on the use of immunotherapy in GEP-NECs, all showing inconclusive results. The SENECA study uses a promising approach to the treatment of patients with metastatic NECs. First, both the activity and safety of 2 regimens are assessed in the same setting with a sizeable patient population (56 patients/arm). In addition, patients are stratified according to Ki-67 index and morphology to investigate the role of each treatment

combination in both poorly differentiated and well differentiated NECs. Another aim of this study is to integrate both biological and metabolic imaging data in an effort to improve the current GEP-NEC classification.

The duration of treatments in the metastatic setting is a dilemma in NENs and especially in neuroendocrine carcinomas. Given the lack of evidence-based recommendations on treatment duration of second-line chemotherapy in NECs, we decided to opt for a fixed duration of treatments to avoid unnecessary exposure to cytotoxic agents and consequent bone marrow reserve depletion (27).

In conclusion, there are still no FDA/EMA (Food and Drug Administration/European Medicines Agency)-approved second-line therapeutic options for patients with metastatic NECs, and the SENECA trial could represent a step forward in finding novel therapies to prolong survival and maintain quality of life. Moreover, the integration of biological and imaging data could -lead to a better understanding of the natural history of the disease and help to identify potential responders.

Confidentiality

This study will be conducted in full conformity with ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) Guidelines for Good Clinical Practice, Directive 2001/20/EEC of the European Parliament and other relevant current local legislation. Participants will be allocated a unique identification (ID) number at entry. The master list linking participant personal information and ID number will be maintained in a separate locked cabinet and

password-protected hard-drive. Data will be analysed by ID number only. Patient files and other source data will for be kept a maximum of 15 years.

Ethics and Dissemination

The SENECA trial, supported by IRST, involves several Italian centres and was authorised by the local Ethics Committees of the centres taking part and by the Italian Medicines Agency (AIFA) (see list of all centers in the supplementary table 1). After completing the study, all data, including beneficial and adverse events, of the trial will be communicated at scientific meetings and published in indexed peer-reviewed journals. If shown to be effective, the therapy program will be made available to the general public in an appropriate manner.

Abbreviations

CAPTEM, capecitabine plus temozolomide; DCR, disease control rate; GEP, gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour; OS, overall survival; PFS, progression-free survival

Author contributions

AB, CL and TI designed the study and drafted the article. AB was responsible for data acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a clinical information data extraction method for the protocol. FF performed the statistical analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual

content. All authors read and approved the present version of the manuscript for submission.

Data availability statement

The datasets used and analysed in the present study are available from the corresponding author on reasonable request.

Funding

The study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

SENECA study Team investigators: Davide Campana, Davide Pastorelli, Nicola Silvestris, Francesco Silvestris, Angela Buonadonna and Giuseppe Badalamenti.

The authors thank Gráinne Tierney and Cristiano Verna for editorial assistance.

References

1. Kulke MH, Shah MH, Benson AB 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, et al. Neuroendocrine tumors, version 1.2015. *J Natl Compr Canc Netw* (2015) 13(1):78-108
2. Modlin IM, Moss SF, Chung DC, Jensen RT, Snyderwine E. Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors. *J Natl Cancer Inst* (2008)100(18):1282-9. doi: 10.1093/jnci/djn275
3. Yao YC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* (2008) 26(18):3063-72
4. Fraenkel M, Kim M, Faggiano A, de Herder WW, Valk GD; Knowledge NETwork. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocr Relat Cancer* (2014) 21(3):R153-63. doi: 10.1530/ERC-13-0125
5. Lloyd RV, Osamura RY, Klöppel G, Rosai J. WHO classification of tumours of endocrine organs. 4th ed (2017). Lyon: International Agency for Research on Cancer (IARC)
6. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60.
7. Heetfeld M, Chougnet CN, Olsen IH, Rinke A, Borbath I, Crespo G, et al. Characteristics and treatment of patients with G3 gastroenteropancreatic neuroendocrine neoplasms. *Endocr Relat Cancer* (2015) 22(4):657-64. doi:

10.1530/ERC-15-0119

8. Walter T, Malka D, Hentic O, Lombard-Bohas C, Le Malicot K, Smith D, et al. Evaluating bevacizumab in combination with FOLFIRI after the failure of platinum-etoposide regimen in patients with advanced poorly differentiated neuroendocrine carcinoma: The PRODIGE 41-BEVANEC randomized phase II study. *Dig Liver Dis* (2018) 50(2):195-8. doi: 10.1016/j.dld.2017.11.020
9. Hadoux J, Malka D, Planchard D, Scoazec JY, Caramella C, Guigay J, et al. Post-first-line FOLFOX chemotherapy for grade 3 neuroendocrine carcinoma. *Endocr Relat Cancer* (2015) 22(3)289-98. doi: 10.1530/ERC-15-0075
10. Olsen IH, Sørensen JB, Federspiel B, Kjaer A, Hansen CP, Knigge U, et al. Temozolomide as second or third line treatment of patients with neuroendocrine carcinomas. *Sci World J* (2012) 2012;2012:170496. doi: 10.1100/2012/170496
11. Hentic O, Hammel P, Couvelard A, Rebours V, Zappa M, Palazzo M, et al. FOLFIRI regimen: an effective second-line chemotherapy after failure of etoposide-platinum combination in patients with neuroendocrine carcinomas grade 3. *Endocr Relat Cancer* (2012) 9(6):751-7. doi: 10.1530/ERC-12-0002
12. Welin S, Sorbye H, Sebjornsen S, Knappskog S, Busch C, Oberg K. Clinical effect of temozolomide-based chemotherapy in poorly differentiated endocrine carcinoma after progression on first-line chemotherapy. *Cancer* (2011) 117(20):4617-22. doi: 10.1002/cncr.26124
13. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* (2002) 346(2):85-91. doi: 10.1056/NEJMoa003034

14. Derks JL, Leblay N, Thunnissen E, van Suylen RJ, den Bakker M, Groen HJM, et al. Molecular subtypes of pulmonary large-cell neuroendocrine carcinoma predict chemotherapy treatment outcome. *Clin Cancer Res* (2018) 24(1):33-42. doi: 10.1158/1078-0432.CCR-17-1921

15. Tang LH, Untch BR, Reidy DL, O'Reilly E, Dhall D, Jih L, et al. Well-differentiated neuroendocrine tumors with a morphologically apparent high-grade component: a pathway distinct from poorly differentiated neuroendocrine carcinomas. *Clin Cancer Res* (2016) 22(4):1011-7. doi: 10.1158/1078-0432.CCR-15-0548

16. Liverani C, Bongiovanni A, Mercatali L, Foca F, Pieri F, De Vita A, Spadazzi C, Miserocchi G, Recine F, Riva N, Nicolini S, Severi S, Martinelli G, Ibrahim T. Grading of Neuroendocrine Carcinomas: Correlation of 68Ga-PET/CT Scan with Tissue Biomarkers. *Dis Markers.* 2018 Dec 2;2018:6878409.

17. Grolmusz VK, Kövesdi A, Borks K, Igaz P, Patócs A. Prognostic relevance of proliferation-related miRNAs in pancreatic neuroendocrine neoplasms Eur J Endocrinol (2018) 179(4):219-28. doi: 10.1530/EJE-18-0305

18. Gill P, Kim E, Chua TC, Clifton-Bligh RJ, Nahm CB, Mittal A, et al. MiRNA-3653 is a potential tissue biomarker for increased metastatic risk in pancreatic neuroendocrine tumours. *Endocr Pathol* (2019). doi: 10.1007/s12022-019-9570-y [Epub ahead of print]

19. Panarelli N, Tyryshkin K, Wong JJM, Majewski A, Yang X, Scognamiglio T, et al. Evaluating gastroenteropancreatic neuroendocrine tumors through microRNA sequencing. *Endocr Relat Cancer* (2019) 26(1):47-57. doi: 10.1530/ERC-18-0244

20. Malczewska A, Kidd M, Matar S, Kos-Kudla B, Modlin IM. A comprehensive

assessment of the role of mirnas as biomarkers in gastroenteropancreatic neuroendocrine tumors. *Neuroendocrinology* (2018)107(1):73-90. doi: 10.1159/000487326

21. Bongiovanni A, Riva N, Ricci M, Liverani C, La Manna F, De Vita A, et al. First-line chemotherapy in patients with metastatic gastroenteropancreatic neuroendocrinecarcinoma. *Onco Targets Ther* (2015) 8:3613-9. doi: 10.2147/OTT.S91971

22. Sansovini M, Severi S, Ianniello A, Nicolini S, Fantini L, Mezzenga E, Ferroni F, Scarpi E, Monti M, Bongiovanni A, Cingarlini S, Grana CM, Bodei L, Paganelli G. Long-term follow-up and role of FDG PET in advanced pancreatic neuroendocrine patients treated with 177Lu-D OTATATE. *Eur J Nucl Med Mol Imaging.* 2017 Mar;44(3):490-499.

23. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced Gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60. doi: 10.1093/annonc/mds276

24. Perren A, Couvelard A, Scoazec JY, Costa F, Borbath I, Delle Fave G, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: pathology: diagnosis and prognostic stratification. *Neuroendocrinology* (2017) 105(3):196-200. doi: 10.1159/000457956

25. Sorbye H, Köhne CH, Sargent DJ, Glimelius B. Patient characteristics and stratification in medical treatment studies for metastatic colorectal cancer: a proposal for standardization of patient characteristic reporting and stratification.

Ann Oncol (2007) 18(10):1666-72.

26. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J. Amer. *Statist Assoc* (1958) 53:457-81.

27. Strosberg JR, Fine RL, Choi J, Nasir A, Coppola D, Chen DT, et al. First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. *Cancer* (2011) 117 (2):268-75.

Figure legend

FIGURE 1 | SENECA study design.

Fig. 1

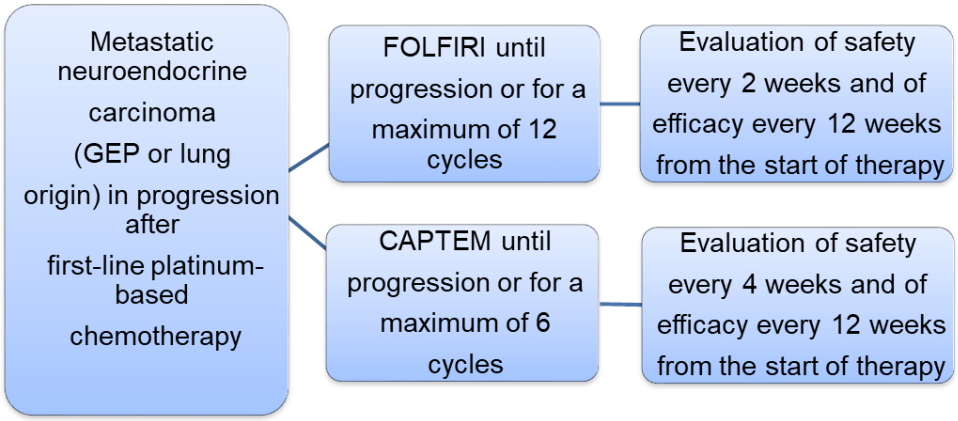


FIGURE 1 | SENECA study design.

90x91mm (300 x 300 DPI)

Center Ethical Committee	Approval ID number
Comitato Etico della Romagna CEROM	1660
Comitato Etico di INT - Milano	146/16
Comitato etico per la sperimentazione clinica - Comprensorio di Bolzano	8-2017
Comitato Etico dell'Area Vasta Emilia Nord	220/16
Comitato Etico Area Vasta Nord Ovest- Pisa	1220/2016
Comitato Etico IRCCS "De Bellis" - Castellana Grotte	34/CE De Bellis
Comitato Etico dell'Area Vasta Emilia Nord	24-2017
Istituto Oncologico Veneto - Padova	2016/63
Comitato Etico ASL - Lecce	140686
Comitato Etico di Area Vasta Emilia Centro (CE-AVEC)	32/2017/O/Sper
Comitato Etico Regionale Marche di AOU - Ospedali Riuniti di Ancona	2017 0068 OR
Comitato Etico dell'Università Campus Bio-Medico di Roma	02.17
Comitato Etico per la Pratica Clinica dell'ULSS 1 Dolomiti,	399/CE Dolomiti
Comitato Etico Istituto Tumori - Bari	621/CE
Comitato Etico Interregionale - Bari	5165
Comitato Etico Palermo 1	02/2018
Comitato Etico Unico Regionale (C.E.U.R.)	Ceur-2018-Sper-076-CRO
Comitato etico per la Sperimentazione Clinica (CESC) delle Province di Verona e Rovigo	2350CESC
Comitato Etico degli IRCCS Istituto Europeo di Oncologia e Centro Cardiologico Monzino.	IEO 1133 - RE2189/NC
Comitato Etico Interaziendale A.O.U. San Luigi Gonzaga	192/2019
Comitato Etico di Brescia	3729
Comitato Etico Area Vasta Centro - AOU Careggi	12447_spe

Supplementary Table 1. List of Ethical committee of Italian Centers with the ID approval number involved in SENECA trial

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5-7
	2b	Specific objectives or hypotheses	7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8-11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	not Applicable
Participants	4a	Eligibility criteria for participants	9
	4b	Settings and locations where the data were collected	14-15.
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8,9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10, 11
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	12
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	12
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	12
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	12
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	12
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	N/A

		assessing outcomes) and how	N/A
	11b	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	12, 13
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	12
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	8, 12
	13b	For each group, losses and exclusions after randomisation, together with reasons	12
Recruitment	14a	Dates defining the periods of recruitment and follow-up	12
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	N/A
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	N/A
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	12
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	12
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	12
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	4
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13, 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13, 14
Other information			
Registration	23	Registration number and name of trial registry	13
Protocol	24	Where the full trial protocol can be accessed, if available	14, 16
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size

Methods: Assignment of interventions (for controlled trials)

Allocation:

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed

	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor

Ethics and dissemination

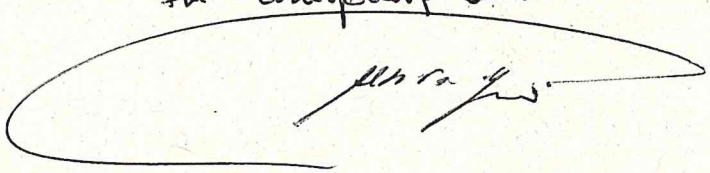
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code

Appendices

Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

The above items were been addressed when applicable.
the corresponding author.



BMJ Open

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Trial): A Study Protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2019-034393.R4
Article Type:	Protocol
Date Submitted by the Author:	14-May-2020
Complete List of Authors:	Bongiovanni, Alberto; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center Liverani, Chiara; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center Pusceddu, Sara; Istituto Nazionale per lo Studio e la Cura dei Tumori, Department of Medical Oncology Leo, Silvana; Ospedale Vito Fazzi, Oncology Unit Di Meglio, Giovanni; Ospedale di Bolzano, Oncology Unit Tamberi, Stefano; Ospedale degli Infermi di Faenza Santini, Daniele; Campus Bio-Medico University, Department of Medical Oncology Gelsomino, Fabio; Azienda Ospedaliero-Universitaria di Modena, Department of Oncology and Hematology Pucci, Francesca; Azienda Ospedaliero-Universitaria di Parma, Medical Oncology Unit Berardi, Rossana; University Hospital of Ancona Umberto I G M Lancisi G Salesi, Oncology Clinic Lolli, Ivan; Istituto Nazionale di Ricovero e Cura a Carattere Scientifico Saverio de Bellis, Department of Oncology Bergamo, Francesca; Istituto Oncologico Veneto Istituto di Ricovero e Cura a Carattere Scientifico, Department of Clinical and Experimental Oncology Ricci, Sergio; Santa Chiara Hospital, Internal Medicine and Medical Oncology Foca, Flavia; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Unit of Biostatistics and Clinical Trials Severi, Stefano; Nuclear Medicine Unit Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS Ibrahim, Toni; Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Osteoncology and Rare Tumors Center
Primary Subject Heading:	Oncology
Secondary Subject Heading:	Oncology
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

CLINICAL STUDY PROTOCOL

Randomized Phase II Trial of CAPTEM or FOLFIRI as SEcond-line Therapy in NEuroendocrine CArcinomas and Exploratory Analysis of Predictive Role of PET/CT Imaging and Biological Markers (SENECA Trial): A Study Protocol

Running title: Second-line CAPTEM/FOLFIRI in NECs

Alberto Bongiovanni[1], Chiara Liverani[1], Sara Pusceddu[2], Silvana Leo[3], Giovanni Di Meglio[4], Stefano Tamberi[5], Daniele Santini[6] , Fabio Gelsomino[7], Francesca Pucci[8], Rossana Berardi[9], Ivan Lolli[10], Francesca Bergamo[11], Sergio Ricci[12] , Flavia Foca [13] Stefano Severi [14], Toni Ibrahim[1], and the SENECA Study Team Investigators**

[1]*Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [2] *Department of Medical Oncology, Istituto Nazionale Tumori Milano IRCCS, Milan, Italy,* [3]*Oncology Unit, Vito Fazzi Hospital, Lecce, Italy,* [4]*Onocology Unit, Ospedale di Bolzano, Bolzano, Italy,* [5]*Medical Oncology, Ospedale degli Infermi, Faenza, Italy,* [6] *Department of Medical Oncology, Università Campus Bio-Medico, Rome, Italy,* [7] *Department of Oncology and Hematology, Azienda Ospedaliera-Universitaria di Modena, Modena, Italy,* [8] *Medical Oncology Unit, Azienda Ospedaliera-Universitaria di Parma, Parma, Italy,* [9] *Oncology Clinic, AOU-Ospedali Riuniti Umberto I - G.M. Lancisi - G. Salesi, Ancona, Italy,* [10] *Department of Oncology, IRCCS "Saverio De Bellis", Castellana Grotte, Italy,* [11] *Department of Clinical and Experimental Oncology, Istituto Oncologico Veneto (IOV), Padua, Italy,* [12] *Internal Medicine and Medical Oncology, Ospedale S.Chiara -*

AOU Pisana, Pisa, Italy, [13]*Unit of Biostatistics and Clinical Trials, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy,* [14]*Nuclear Medicine Unit, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Meldola, Italy*

***Correspondence:** Alberto Bongiovanni, MD

Osteoncology and Rare Tumors Center, Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) IRCCS, Via Piero Maroncelli 40, 47014 Meldola (FC), Italy

Tel.: +39-0543-739100; Fax: +39-0543-739123

E-mail: alberto.bongiovanni@irst.emr.it

Language style: This article is formatted in British English.

Abstract

Introduction: Patients with metastatic or locally advanced, non-resectable, grade 3 poorly-differentiated gastroenteropancreatic (GEP) and lung neuroendocrine carcinomas (NECs) are usually treated with in first-line platinum compounds. There is no standard second-line treatment upon progression. Accurate biomarkers are needed to facilitate diagnosis and prognostic assessment of NEC patients.

Methods and Analysis: The SENECA (SEcond-line therapy in NEuroendocrine CArcinomas) study is a randomised, non-comparative, multicentre phase II trial designed to evaluate the efficacy and safety of folinic acid, 5-fluorouracil and irinotecan (FOLFIRI) or capecitabine plus temozolomide (CAPTEM) regimens after failure of first-line-chemotherapy in lung NEC and GEP-NEC patients. Secondary aims are to correlate the serum miRNA profile and primary mutational status of MEN1, DAXX, ATRX and RB-1 with prognosis and outcome and to investigate the prognostic and predictive role of the Ki-67 score and FDG- or ^{68}Ga-PET/CT. The main eligibility criteria are age ≥18 years; metastatic or locally advanced, non-resectable, grade 3 lung or GEP-NECs; progression to first-line platinum-based chemotherapy. A Bryant and Day design taking into account treatment activity and toxicity was used to estimate the sample size. All analyses will be performed separately for each treatment group in the intention-to-treat population. A total of 112 patients (56/arm) will be randomly assigned (1:1) to receive FOLFIRI every 14 days or CAPTEM every 28 days until disease progression or unacceptable toxicity or for a maximum of six months. Patients undergo testing for specific biomarkers in primary tumour tissue and for miRNA in blood samples. MiRNA profiling will be performed in the first 20 patients who agree to participate in the biological sub-study.

Ethics and dissemination: The SENECA trial, supported by IRST, was authorised by the locals Ethics Committee and the Italian Medicines Agency (AIFA). Results will be widely disseminated via peer-reviewed manuscripts, conference presentations and reports to relevant authorities.

The study is currently open in Italy. ClinicalTrials.gov Identifier: NCT03387592.

EudraCT number: 2016-000767-17

Protocol version: Clinical Study Protocol Version 1, 07.11.2016.

Keywords: neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine, temozolomide, CAPTEM

Strengths and limitations of the study

- the SENECA trial randomises patients to receive two different treatments, FOLFIRI or CAPTEM, providing important information on the activity of both combinations in different NEC subtypes (NET grade (G) 3 and NEC G3).
- The SENECA trial analyses the role of miRNAs and other biological markers as prognostic and predictive factors. A further aim is to assess ^{68}Ga-PET/CT as a tool to improve current histological classification.

The major limitations of the study are:

- The rarity of the disease and patient prognosis. However, the involvement of several Italian centres will hopefully help to overcome this problem.
- Poor prognosis of NEC patients. Patients progressing on platinum chemotherapy usually have rapid deterioration of clinical conditions

Introduction

Poorly differentiated neuroendocrine carcinomas (NECs) are very rare malignancies, representing only 5%-10% of neuroendocrine neoplasias (NENs) (1-3). At the time of diagnosis, patients are generally in poor conditions due to aggressive and diffuse disease. These tumors are characterised by aggressive histological features (high Ki-67 index, extensive necrosis, and nuclear atypia) and are classified as grade (G) 3 NECs according to the 2010 World Health Organization (WHO) classification (4). The 2017 WHO classification recognized a further group called G3 NETs as having intermediate features between NETs and NECs (5).

An etoposide-platinum combination is the gold standard for the treatment of G3 NECs, several studies published in the 1990s reporting substantial anti-tumor activity and high response rates (41%-67%) (6). However, prognosis is generally poor with a median progression-free survival (PFS) of 9 months and a median overall survival (OS) of 15-19 months. When progression occurs after first-line chemotherapy, the disease is usually very aggressive and patients succumb rapidly (7).

Given the rarity of this disease, prospective clinical data are lacking and treatment recommendations are essentially expert-based opinions. Two phase II studies investigating the second-line treatment of GEP-NECs are currently registered at *ClinicalTrials.gov*, one evaluating the safety and tolerability of everolimus in 40 patients with G3 NEC/NET or G1/G2 NET switching to G3 NEN (National Clinical Trial identifier NCT02113800) and the other investigating the efficacy of avelumab (NCT03147404). A French study focusing on the identification of predictive molecular markers of response to sunitinib in poorly differentiated digestive NETs

(NCT01215578) has now closed recruitment and results are eagerly awaited. Another French multicentre prospective phase II trial is currently ongoing to investigate the efficacy of the bevacizumab-FOLFIRI combination after progression on a platinum/etoposide combination (7).

Different second-line chemotherapy combinations have been evaluated but shown poor results (8-10). In a monocentre retrospective clinical trial, Hentic et al. hypothesised the potential efficacy of FOLFIRI as second-line chemotherapy in 19 patients with G3 extra-pulmonary NECs (11). An objective response rate was obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median PFS and OS were 4 and 18 months, respectively.

In another retrospective study, a 71% DCR was obtained with temozolomide-based chemotherapy in 25 patients with metastatic poorly differentiated endocrine carcinoma of different sites and atypical bronchial carcinoid with Ki-67 > 20%. Small cell lung carcinoma and large cell lung carcinoma were excluded. A PFS of 12 months (95% confidence interval [CI], 5.5- 24) and OS of 22 months (95% CI, 12-31) were reported in patients who responded to treatment or showed stable disease (SD), whereas OS was only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive staining for chromogranin A. Both factors are often associated with more highly differentiated tumours (12).

Literature data on lung NECs in progression after first-line chemotherapy are based on small patient series (13). Moreover, there is increasing evidence of some discrepancies in the current grading of NECs, highlighting the need for more accurate

biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise two distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived from well differentiated neuroendocrine tumours (NETs) and characterised by mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from neuroendocrine-differentiated adenocarcinomas and characterised by a mutation in RB1. Both subgroups display a distinct prognosis and different sensitivity to chemotherapy (14-16). Micro(mi)RNAs are a class of small, non-coding, highly conserved single-stranded RNAs involved in the post-transcriptional regulation of cell proliferation, differentiation, survival, and apoptosis (17). They are often associated with resistance to therapy (18, 19). Whilst miRNAs are known to show a specific expression pattern in NETs (20), little is known about differential miRNA profiles in NEC patients. At present, no data are available on the deregulation of specific miRNAs in this setting.

In a study recently published by our group on GEP-NEC patients undergoing first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months (p <0.01) in patients with Ki-67 value between 20% and 50% or >50%, respectively (19). Median (m)OS was 8.1 months in the latter group but was not reached in the former group (p = 0.039). Patients with a positive ^{68}Ga-PET/CT had a higher 18-month OS rate than those with a negative scan (75% *vs*. 34.3%, respectively), but the difference was not statistically significant (p = 0.06). Our data highlighted that ^{68}Ga-PET/CT positivity may be a discriminating factor (16, 21) in predicting prognosis, especially in the metastatic setting where histological material is not always available for evaluation. Furthermore, ^{18}fludeoxyglucose (^{18}FDG)-PET/CT may be useful to discriminate between patients with different prognosis (22).

Given the above premises, we decided to investigate the efficacy and safety of second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression after first-line platinum-based treatment. We also aimed to study the serum miRNA profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1, patient prognosis and response to therapy, and to assess the prognostic and predictive role of ^{18}FDG-PET/CT, ^{68}Ga-PET/CT and Ki-67 score.

Methods and Analysis

Study design

The SENECA study is a multicentre randomised non-comparative phase II study (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin (lung or gastroenteropancreatic) in progression after first-line treatment are randomized to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until progression or unacceptable toxicity.

The treatment arms are as follows:

FOLFIRI regimen

- Irinotecan 180 mg/m^2, given as a 60-min. intravenous (i.v.) infusion on day 1 every 2 weeks followed by
- Leucovorin 200 mg/m^2, given as a 2-h i.v. infusion on day 1 every 2 weeks followed by

- 5-fluorouracil (5-FU) 400 mg/m^2 given as bolus, and then 5-FU 2400 mg/m^2 given as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a maximum of 12 cycles.

CAPTEM regimen

Capecitabine 750 mg/m^2 twice a day on days 1-14 in combination with temozolomide 200 mg/m^2 daily on days 10-14, every 4 weeks, until progression or for a maximum of 6 cycles.

The study includes patients aged ≥ 18 years with a histological diagnosis of G3 neuroendocrine carcinoma (GEP-NEC and lung NEC) according to the 2010 and 2015 GEP and Lung NEN WHO classifications, respectively, Ki-67 >20% and measurable disease according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria. All patients must have an Eastern Cooperative Oncology Group (ECOG) performance status ≤2 with a life expectancy > 3 months and must have already undergone first-line treatment for metastatic disease with platinum -based chemotherapy (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological, liver and renal function is required and effective contraceptive methods must be used by female patients of childbearing age. Written informed consent is obtained from all patients to take part in the study. Exclusion criteria are as follows: metastatic NECs previously treated with an irinotecan or temozolomide regimen, known hypersensitivity to 5-FU/capecitabine, calcium levofolinate, irinotecan or their recipients. All acute toxic effects of any prior therapy (including surgery, radiation therapy and chemotherapy) must have resolved to grade ≤1 according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (CTCAE). Patients

taking part in another clinical trial with any investigational agent < 30 days prior to study screening or with a history of allergic reactions attributable to compounds of similar chemical or biological composition are excluded. Patients who have undergone chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C) prior to entering the study, have not recovered from adverse events caused by agents administered > 4 weeks earlier, or have known brain metastases are not eligible for the study. Patients with other malignancies with a disease-free interval of < 5 years (with the exception of non melanoma skin cancer or low-grade superficial bladder cancer) are excluded, as are those with any severe and/or uncontrolled medical condition or other condition that could affect their participation in the study such as:

- unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or any other clinically significant cardiac disease;
- severely impaired lung function (spirometry and DLCO 50% of the normal predicted value and/or oxygen saturation ≤ 88% at rest, in room air);
- uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of normal;
- any active (acute or chronic) or uncontrolled infections/disorders.

Tumour evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest, abdomen, pelvis, and any additional known sites of disease. These tests are performed at baseline, every three months during treatment as per national regulatory agency indications, and after the end of treatment in non-progressing patients until progression. It is recommended that ^{68}Ga-PET/CT and ^{18}FDG-PET/CT scans be performed at baseline or a maximum of 90 days before study enrollment. An EORTC quality of life

questionnaire is administered at baseline and every three months thereafter during the treatment period.

Study endpoints

The primary endpoint of the study is the DCR of each treatment, defined as the percentage of patients who have achieved complete or partial response or stable disease for ≥12 weeks from the start of therapy. DCR will be evaluated using the new international criteria proposed by the RECIST version 1.1. Acute and late toxicity will be assessed by CTCAE Version 4.03, the latter defined as toxicity occurring at least 30 days after the end of the last treatment cycle. Secondary endpoints are OS, calculated from the start of treatment to death from any cause, and PFS, calculated from the start of treatment to the date of the first documented evidence of disease progression or of death from any cause. All the analyses will be performed in the intention-to-treat population. Patients without events at the time of analysis will be censored at their last-known-alive date for OS and at their last date of tumour evaluation for PFS. A further secondary endpoint is the evaluation of quality of life using the European Organization for the Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). When data are available, the impact of baseline ^{68}Ga-PET/CT and ^{18}FDG-PET/CT on PFS will be analysed with exploratory intent. After signing the informed consent for biomarker assessment, patients will undergo evaluation of the mutation status of MEN1, DAXX, ATRX and RB-1 in primary tumour tissue and of miRNA in blood samples. Assessment of the miRNA profile will be performed on the first 20 patients who agree to participate in the biological part of the study.

Ethical considerations

The present clinical trial, supported by IRST, was authorised by the local Ethics Committee and by the Italian Medicines Agency (AIFA). The request for EudraCT registration (mandatory for studies in Europe) was send to AIFA in December 2016 and we received a EudraCT number (EudraCT 2016-000767-17). However, technical problems at AIFA resulted in some clinical trials, including ours, being uploaded onto the EudraCT website after enrolment of the first patients.

The study is also registered on the *ClinicalTrials.gov* website (NCT03387592). The study complies with the ethical standards laid down in the 1964 Declaration of Helsinki and the principles of Good Clinical Practice guidelines (including written informed consent).

Patient and public involvement

This research work was performed without patient involvement in the study design, execution or outcome measures.

Statistical methods

The Bryant and Day design is used to estimate a sample size that takes into account both treatment activity and toxicity. Although randomisation is used to allocate patients to the two arms, no formal statistical comparisons between treatment regimens are planned. The purpose of randomisation is to reduce bias due to patient assignment to a specific treatment arm. The hypothesis for the control arm is based on literature data (23, 24).

An α level of 0.10 (both for toxicity and DCR) and a power of 90% have been

adopted. A DCR rate ≥60% and a relevant toxicity rate ≤20% are considered acceptable rates, while a DCR rate ≤40% and a relevant toxicity rate ≥40% are considered inacceptable rates. Given these hypotheses, the first step of the study will require 25 patients. If ≥10 patients with a DCR are observed and ≥15 patients do not have significant toxicity, the study will enrol patients in the next step. A total of 53 patients will be enrolled. If ≥25 patients with DCR and ≥36 patients without any relevant toxicity are observed, treatment will be considered active and not toxic. This design is used for each treatment scheme and all analyses will be performed separately. If one of the schemes does not obtain the expected proportions of the first step, the arm will be closed and patients will be enrolled in the other arm until the target is reached; if the expected proportions are not reached in any arm, the study will be prematurely closed. If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopoenia, prolonged G3-G4 neutropoenia (> 7 days) and drug-related hospitalisations are considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55 % *vs*. >55%) and site of primary tumour (lung *vs*. GEP). A subgroup analysis of the efficacy of both treatments according to these stratification factors has been planned.

Complete response, partial response or stable disease for at least 12 weeks will be considered as the DCR. The proportion of patients in this category will be determined and 95% CIs for the DCR will be calculated. OS and PFS will be estimated using the Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate statistical analyses will be performed on the basis of the data available to compare QLQ-C30 scores between baseline and subsequent follow-up visits.

When data are available, the impact of ^{68}Ga-PET/CT result on PFS will be analysed with exploratory intent. The Shapiro-Wilk test will be used to determine the normality distribution of each clinical, demographic and biological biomarker (25). In the event of a non-normal distribution, nonparametric statistics will be used to analyse the relationship between the serum levels of each marker, considered as continuous variables, and response to treatment. In the event of normal biomarker distribution, a parametric test will be used. All endpoints will be analysed separately for each treatment group.

Discussion

There is still no truly effective second-line chemotherapy for neuroendocrine carcinoma. Overall prognosis of patients is poor, with an OS of 5 months in the metastatic setting according to SEER (Surveillance, Epidemiology, and End Results) data (26). Only 5% of all patients are long-term survivors. There is also a marked lack of prognostic and predictive factors (5).

Three phase II studies registered at *ClinicalTrials.gov* are currently investigating second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab (NCT03147404). Some abstracts were presented at ESMO (European Society for Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019 on the use of immunotherapy in GEP-NECs, all showing inconclusive results. The SENECA study uses a promising approach to the treatment of patients with metastatic NECs. First, both the activity and safety of 2 regimens are assessed in the same setting with a sizeable patient population (56 patients/arm). In addition, patients are stratified according to Ki-67 index and morphology to investigate the role of each treatment

combination in both poorly differentiated and well differentiated NECs. Another aim of this study is to integrate both biological and metabolic imaging data in an effort to improve the current GEP-NEC classification.

The duration of treatments in the metastatic setting is a dilemma in NENs and especially in neuroendocrine carcinomas. Given the lack of evidence-based recommendations on treatment duration of second-line chemotherapy in NECs, we decided to opt for a fixed duration of treatments to avoid unnecessary exposure to cytotoxic agents and consequent bone marrow reserve depletion (27).

In conclusion, there are still no FDA/EMA (Food and Drug Administration/European Medicines Agency)-approved second-line therapeutic options for patients with metastatic NECs, and the SENECA trial could represent a step forward in finding novel therapies to prolong survival and maintain quality of life. Moreover, the integration of biological and imaging data could -lead to a better understanding of the natural history of the disease and help to identify potential responders.

Confidentiality

This study will be conducted in full conformity with ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) Guidelines for Good Clinical Practice, Directive 2001/20/EEC of the European Parliament and other relevant current local legislation. Participants will be allocated a unique identification (ID) number at entry. The master list linking participant personal information and ID number will be maintained in a separate locked cabinet and

password-protected hard-drive. Data will be analysed by ID number only. Patient files and other source data will for be kept a maximum of 15 years.

Ethics and Dissemination

The SENECA trial, supported by IRST, involves several Italian centres and was authorised by the local Ethics Committees of the centres taking part and by the Italian Medicines Agency (AIFA) (see list of all centers in the supplementary table 1). After completing the study, all data, including beneficial and adverse events, of the trial will be communicated at scientific meetings and published in indexed peer-reviewed journals. If shown to be effective, the therapy program will be made available to the general public in an appropriate manner.

Abbreviations

CAPTEM, capecitabine plus temozolomide; DCR, disease control rate; GEP, gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour; OS, overall survival; PFS, progression-free survival

Author contributions

AB, CL and TI designed the study and drafted the article. AB was responsible for data acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a clinical information data extraction method for the protocol. FF performed the statistical analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual

content. All authors read and approved the present version of the manuscript for submission.

Data availability statement

The datasets used and analysed in the present study are available from the corresponding author on reasonable request.

Funding

The study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

SENECA study Team investigators: Davide Campana, Davide Pastorelli, Nicola Silvestris, Francesco Silvestris, Angela Buonadonna and Giuseppe Badalamenti.

The authors thank Gráinne Tierney and Cristiano Verna for editorial assistance.

References

1. Kulke MH, Shah MH, Benson AB 3rd, Bergsland E, Berlin JD, Blaszkowsky LS, et al. Neuroendocrine tumors, version 1.2015. *J Natl Compr Canc Netw* (2015) 13(1):78-108
2. Modlin IM, Moss SF, Chung DC, Jensen RT, Snyderwine E. Priorities for improving the management of gastroenteropancreatic neuroendocrine tumors. *J Natl Cancer Inst* (2008)100(18):1282-9. doi: 10.1093/jnci/djn275
3. Yao YC, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, et al. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* (2008) 26(18):3063-72
4. Fraenkel M, Kim M, Faggiano A, de Herder WW, Valk GD; Knowledge NETwork. Incidence of gastroenteropancreatic neuroendocrine tumours: a systematic review of the literature. *Endocr Relat Cancer* (2014) 21(3):R153-63. doi: 10.1530/ERC-13-0125
5. Lloyd RV, Osamura RY, Klöppel G, Rosai J. WHO classification of tumours of endocrine organs. 4th ed (2017). Lyon: International Agency for Research on Cancer (IARC)
6. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60.
7. Heetfeld M, Chougnet CN, Olsen IH, Rinke A, Borbath I, Crespo G, et al. Characteristics and treatment of patients with G3 gastroenteropancreatic neuroendocrine neoplasms. *Endocr Relat Cancer* (2015) 22(4):657-64. doi:

10.1530/ERC-15-0119

8. Walter T, Malka D, Hentic O, Lombard-Bohas C, Le Malicot K, Smith D, et al. Evaluating bevacizumab in combination with FOLFIRI after the failure of platinum-etoposide regimen in patients with advanced poorly differentiated neuroendocrine carcinoma: The PRODIGE 41-BEVANEC randomized phase II study. *Dig Liver Dis* (2018) 50(2):195-8. doi: 10.1016/j.dld.2017.11.020
9. Hadoux J, Malka D, Planchard D, Scoazec JY, Caramella C, Guigay J, et al. Post-first-line FOLFOX chemotherapy for grade 3 neuroendocrine carcinoma. *Endocr Relat Cancer* (2015) 22(3)289-98. doi: 10.1530/ERC-15-0075
10. Olsen IH, Sørensen JB, Federspiel B, Kjaer A, Hansen CP, Knigge U, et al. Temozolomide as second or third line treatment of patients with neuroendocrine carcinomas. *Sci World J* (2012) 2012;2012:170496. doi: 10.1100/2012/170496
11. Hentic O, Hammel P, Couvelard A, Rebours V, Zappa M, Palazzo M, et al. FOLFIRI regimen: an effective second-line chemotherapy after failure of etoposide-platinum combination in patients with neuroendocrine carcinomas grade 3. *Endocr Relat Cancer* (2012) 9(6):751-7. doi: 10.1530/ERC-12-0002
12. Welin S, Sorbye H, Sebjornsen S, Knappskog S, Busch C, Oberg K. Clinical effect of temozolomide-based chemotherapy in poorly differentiated endocrine carcinoma after progression on first-line chemotherapy. *Cancer* (2011) 117(20):4617-22. doi: 10.1002/cncr.26124
13. Noda K, Nishiwaki Y, Kawahara M, Negoro S, Sugiura T, Yokoyama A, et al. Irinotecan plus cisplatin compared with etoposide plus cisplatin for extensive small-cell lung cancer. *N Engl J Med* (2002) 346(2):85-91. doi: 10.1056/NEJMoa003034

14. Derks JL, Leblay N, Thunnissen E, van Suylen RJ, den Bakker M, Groen HJM, et al. Molecular subtypes of pulmonary large-cell neuroendocrine carcinoma predict chemotherapy treatment outcome. *Clin Cancer Res* (2018) 24(1):33-42. doi: 10.1158/1078-0432.CCR-17-1921

15. Tang LH, Untch BR, Reidy DL, O'Reilly E, Dhall D, Jih L, et al. Well-differentiated neuroendocrine tumors with a morphologically apparent high-grade component: a pathway distinct from poorly differentiated neuroendocrine carcinomas. *Clin Cancer Res* (2016) 22(4):1011-7. doi: 10.1158/1078-0432.CCR-15-0548

16. Liverani C, Bongiovanni A, Mercatali L, Foca F, Pieri F, De Vita A, Spadazzi C, Miserocchi G, Recine F, Riva N, Nicolini S, Severi S, Martinelli G, Ibrahim T. Grading of Neuroendocrine Carcinomas: Correlation of 68Ga-PET/CT Scan with Tissue Biomarkers. *Dis Markers.* 2018 Dec 2;2018:6878409.

17. Grolmusz VK, Kövesdi A, Borks K, Igaz P, Patócs A. Prognostic relevance of proliferation-related miRNAs in pancreatic neuroendocrine neoplasms Eur J Endocrinol (2018) 179(4):219-28. doi: 10.1530/EJE-18-0305

18. Gill P, Kim E, Chua TC, Clifton-Bligh RJ, Nahm CB, Mittal A, et al. MiRNA-3653 is a potential tissue biomarker for increased metastatic risk in pancreatic neuroendocrine tumours. *Endocr Pathol* (2019). doi: 10.1007/s12022-019-9570-y [Epub ahead of print]

19. Panarelli N, Tyryshkin K, Wong JJM, Majewski A, Yang X, Scognamiglio T, et al. Evaluating gastroenteropancreatic neuroendocrine tumors through microRNA sequencing. *Endocr Relat Cancer* (2019) 26(1):47-57. doi: 10.1530/ERC-18-0244

20. Malczewska A, Kidd M, Matar S, Kos-Kudla B, Modlin IM. A comprehensive

assessment of the role of mirnas as biomarkers in gastroenteropancreatic neuroendocrine tumors. *Neuroendocrinology* (2018)107(1):73-90. doi: 10.1159/000487326

21. Bongiovanni A, Riva N, Ricci M, Liverani C, La Manna F, De Vita A, et al. First-line chemotherapy in patients with metastatic gastroenteropancreatic neuroendocrinecarcinoma. *Onco Targets Ther* (2015) 8:3613-9. doi: 10.2147/OTT.S91971

22. Sansovini M, Severi S, Ianniello A, Nicolini S, Fantini L, Mezzenga E, Ferroni F, Scarpi E, Monti M, Bongiovanni A, Cingarlini S, Grana CM, Bodei L, Paganelli G. Long-term follow-up and role of FDG PET in advanced pancreatic neuroendocrine patients treated with 177Lu-D OTATATE. *Eur J Nucl Med Mol Imaging.* 2017 Mar;44(3):490-499.

23. Sorbye H, Welin S, Langer SW, Vestermark LW, Holt N, Osterlund P, et al. Predictive and prognostic factors for treatment and survival in 305 patients with advanced Gastrointestinal neuroendocrine carcinoma (WHO G3): the NORDIC NEC study. *Ann Oncol* (2013) 24(1):152-60. doi: 10.1093/annonc/mds276

24. Perren A, Couvelard A, Scoazec JY, Costa F, Borbath I, Delle Fave G, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: pathology: diagnosis and prognostic stratification. *Neuroendocrinology* (2017) 105(3):196-200. doi: 10.1159/000457956

25. Sorbye H, Köhne CH, Sargent DJ, Glimelius B. Patient characteristics and stratification in medical treatment studies for metastatic colorectal cancer: a proposal for standardization of patient characteristic reporting and stratification.

Ann Oncol (2007) 18(10):1666-72.

26. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J. Amer. *Statist Assoc* (1958) 53:457-81.

27. Strosberg JR, Fine RL, Choi J, Nasir A, Coppola D, Chen DT, et al. First-line chemotherapy with capecitabine and temozolomide in patients with metastatic pancreatic endocrine carcinomas. *Cancer* (2011) 117 (2):268-75.

Figure legend

FIGURE 1 | SENECA study design.

Fig. 1

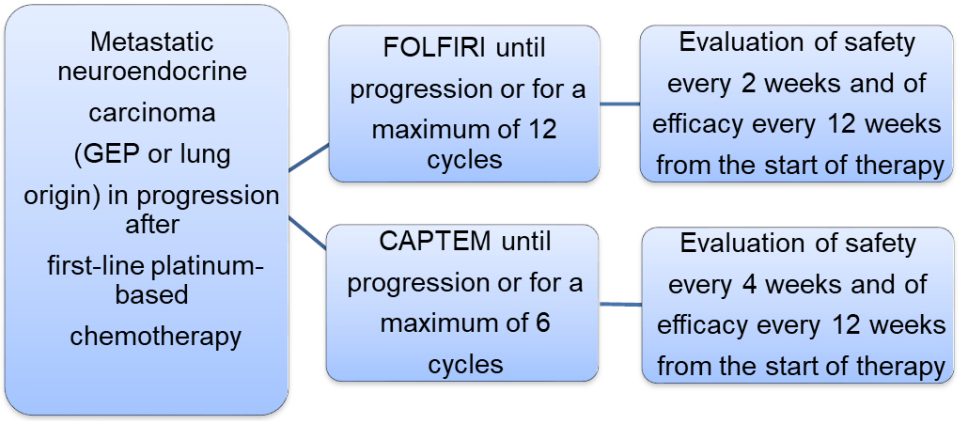


FIGURE 1 | SENECA study design.

90x91mm (300 x 300 DPI)

Supplementary Table 1. List of Ethical committee of Italian Centers with the ID approval number involved in SENECA trial

Center Ethical Committee	Approval ID number
Comitato Etico della Romagna CEROM	1660
Comitato Etico di INT - Milano	146/16
Comitato etico per la sperimentazione clinica - Comprensorio di Bolzano	8-2017
Comitato Etico dell'Area Vasta Emilia Nord	220/16
Comitato Etico Area Vasta Nord Ovest- Pisa	1220/2016
Comitato Etico IRCCS "De Bellis" - Castellana Grotte	34/CE De Bellis
Comitato Etico dell'Area Vasta Emilia Nord	24-2017
Istituto Oncologico Veneto - Padova	2016/63
Comitato Etico ASL - Lecce	140686
Comitato Etico di Area Vasta Emilia Centro (CE-AVEC)	32/2017/O/Sper
Comitato Etico Regionale Marche di AOU - Ospedali Riuniti di Ancona	2017 0068 OR
Comitato Etico dell'Università Campus Bio-Medico di Roma	02.17
Comitato Etico per la Pratica Clinica dell'ULSS 1 Dolomiti,	399/CE Dolomiti
Comitato Etico Istituto Tumori - Bari	621/CE
Comitato Etico Interregionale - Bari	5165
Comitato Etico Palermo 1	02/2018
Comitato Etico Unico Regionale (C.E.U.R.)	Ceur-2018-Sper-076-CRO
Comitato etico per la Sperimentazione Clinica (CESC) delle Province di Verona e Rovigo	2350CESC
Comitato Etico degli IRCCS Istituto Europeo di Oncologia e Centro Cardiologico Monzino.	IEO 1133 - RE2189/NC
Comitato Etico Interaziendale A.O.U. San Luigi Gonzaga	192/2019
Comitato Etico di Brescia	3729
Comitato Etico Area Vasta Centro - AOU Careggi	12447_spe

SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	4
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	4
Funding	4	Sources and types of financial, material, and other support	17
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 2 ; 16, 17
	5b	Name and contact information for the trial sponsor	N/A
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	16
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5, 6, 7
	6b	Explanation for choice of comparators	6, 7, 8
Objectives	7	Specific objectives or hypotheses	8
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	8

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	8
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	9, 10
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8, 9
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	8-10
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	10
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	11
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	13

2

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12, 13
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	12, 13
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	12
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	15
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	15
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	10, 11
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	10, 11

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15.
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	12, 13
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	12, 13
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	12, 13
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	13
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	9
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	11
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	17
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/a
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	See protocol
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	See protocol

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.