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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)

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

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4 1 **CLINICAL STUDY PROTOCOL**
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9 3 **Randomized Phase II Trial of CAPTEM or FOLFIRI as**
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11 4 **SEcond-line Therapy in NEuroendocrine CARcinomas and**
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13 5 **Exploratory Analysis of Predictive Role of PET/CT Imaging**
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15 6 **and Biological Markers (SENECA Study)**
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17 7 **Running title: Second-line CAPTEM/FOLFIRI in NECs**
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45 Abstract

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

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

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

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4 69 **Ethics and dissemination:** Seneca trial , supported by IRST, was authorized by the
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6 70 locals Ethics Committee and the Italian Medicines Agency (AIFA). Results will be
7
8 71 widely disseminated via peer-reviewed manuscripts, conference presentations and
9
10 72 reports to relevant authorities.
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14 73

15 74 The study is currently open in Italy. Clinical trial registration: NCT03387592.
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20 76 **Keywords:** neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine,
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22 77 temozolomide, CAPTEM
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25 78 **Strengths and limitations of the study**

- 26
27 79 • the SENECA trial randomizes patients to receive two different treatments,
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29 80 FOLFIRI or CAPTEM, providing important information on the activity of both
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31 81 combinations in different NEC subtypes (NET G3 and NEC G3).
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34 82 • The SENECA trial analyzes the role of miRNAs and other biological markers
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36 83 as prognostic and predictive factors. A further aim is to assess ⁶⁸Ga-PET/CT as
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38 84 a tool to improve current histological classification.

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41 85 The major limitation of the study are:

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43 86 • The rarity of disease and patient's prognosis. However the involvement of
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45 87 several centers along the Italian Country try to overcome this problem.
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48 88 • Poor prognosis of NEC patients. Patients progressed to platinum chemotherapy
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50 89 usually have a rapidly worsening clinical conditions
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91 **Introduction**

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

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

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

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4 115 bevacizumab-FOLFIRI combination after progression on platinum-etoposide (7).

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6 116 Different second-line chemotherapy combinations have been evaluated but shown
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9 117 poor results (6, 8, 9). In a monocenter retrospective clinical trial, Hentic et al.
10
11 118 hypothesized the potential efficacy of FOLFIRI as second-line chemotherapy in
12
13 119 patients with G3 extra-pulmonary NECs (10). An objective response rate was obtained
14
15 120 in 31% of patients, with a disease control rate (DCR) of 62%. Median progression-free
16
17 121 survival (PFS) and overall survival (OS) were 4 and 18 months, respectively.

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19
20 122 In another retrospective study, a 71% DCR was obtained with temozolomide-based
21
22 123 chemotherapy. A PFS of 12 months (95% CI, 5.5- 24) and OS of 22 months (95% CI,
23
24 124 12-31) was reported in patients who responded to treatment or showed stable disease
25
26 125 (SD), whereas OS was only 8 months (95% CI, 0-8) in non-responders. The authors
27
28 126 observed a higher response rate in patients with Ki-67 \leq 60%. There were also more
29
30 127 responders in the group with high uptake in somatostatin receptor scintigraphy (SRS)
31
32 128 and in those with positive staining for chromogranin A (CgA). Both factors are often
33
34 129 associated with more differentiated tumors (11).

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38 130 Literature data on lung NECs in progression after first-line chemotherapy are based
39
40 131 on small patient series (12). Moreover, there is increasing evidence of some
41
42 132 discrepancies in the current grading of NECs, highlighting the need for more accurate
43
44 133 biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise 2
45
46 134 distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived
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48 135 from well differentiated neuroendocrine tumors (NETs) and characterized by mutations
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50 136 in MEN1, DAXX and ATRX, and a poorly differentiated group derived from
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52 137 neuroendocrine-differentiated adenocarcinomas and characterized by a mutation in
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54 138 RB1. Both subgroups display a distinct prognosis and different sensitivity to
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4 139 chemotherapy (13-15). Micro(mi)RNAs are a class of small, non-coding, highly
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6 140 conserved single-stranded RNAs involved in the post-transcriptional regulation of cell
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9 141 proliferation, differentiation, survival, and apoptosis (16). They are often associated
10
11 142 with resistance to therapy (17, 18). Whilst miRNAs are known to show a specific
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13 143 expression pattern in NETs (19), little is known about differential miRNA profiles in
14
15 144 NEC patients. At present, no data are available on the deregulation of specific miRNAs
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18 145 in this setting.

19
20 146 In a study recently published by our group on GEP-NEC patients undergoing
21
22 147 first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months
23
24 148 ($p < 0.01$) in patients with Ki-67% $< 50\%$ or $> 50\%$, respectively (19). Median (m)OS
25
26 149 was 8.1 months in the latter group but was not reached in the former group ($p = 0.039$).
27
28 150 Patients with a positive ^{68}Ga -PET/CT had a longer OS than those with a negative scan
29
30 151 (75% vs. 34.3%, respectively, at 18 months), but the difference was not significant ($p =$
31
32 152 0.06). Our data highlighted that ^{68}Ga -PET/CT positivity may be a discriminating factor
33
34 153 (20,15) in predicting prognosis, especially important in the metastatic setting where
35
36 154 histological material is not always available for evaluation. Also ^{18}F fluorodeoxyglucose
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38 155 (^{18}F FDG)-PET/CT could be useful to discriminate patients with different prognosis. (21)
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41 156 Given the above premises, we decided to investigate the efficacy and safety of
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43 157 second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression
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45 158 after first-line platinum-based treatment. We also aimed to study the serum miRNA
46
47 159 profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1,
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49 160 patient prognosis and response to therapy, and to assess the prognostic and predictive
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51 161 role of ^{18}F FDG-PET/CT, ^{68}Ga -PET/CT and Ki-67 score.
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67 164 **Methods and Analysis**
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1112 166
1314 167 **Study design**
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17 168 The SENECA study is a multicentre randomised non-comparative phase II study
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19 169 (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin
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21 170 (lung or gastroenteropancreatic) in progression after first-line treatment are randomized
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24 171 to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or
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26 172 unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until
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28 173 progression or unacceptable toxicity.

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30 174 The treatments arms are as follows:
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3435 176 **FOLFIRI regimen**
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38 177 • Irinotecan 180 mg/m², given as a 60-min. intravenous (i.v.) infusion on day 1 every
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40 178 2 weeks followed by
41
42 179 • Leucovorin 200 mg/m², given as a 2-h i.v. infusion on day 1 every 2 weeks followed
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44 180 by
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46 181 • 5-fluorouracil (5-FU) 400 mg/m² given as bolus, and then 5-FU 2400 mg/m² given
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48 182 as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a
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50 183 maximum of 12 cycles.
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56 185 **CAPTEM regimen**
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4 186 Capecitabine 750 mg/m² twice a day on days 1-14 in combination with temozolomide
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6 187 200 mg/m² daily on days 10-14, every 4 weeks, until progression or for a maximum of
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9 188 6 cycles.

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11 189 The study includes patients aged ≥ 18 years with a histological diagnosis of G3
12
13 190 neuroendocrine carcinoma (GEP-NEC and lung NEC), Ki-67 $>20\%$ and measurable
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15 191 disease according to Response evaluation criteria in solid tumors (RECIST) 1.1 criteria. All
16
17 192 patients must have an Eastern Cooperative Oncology Group (ECOG) performance
18
19 193 status ≤ 2 with a life expectancy > 3 months and must have already undergone first-line
20
21 194 treatment for metastatic disease with platinum -based chemotherapy
22
23 195 (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological,
24
25 196 liver and renal function is required and effective contraceptive methods must be used by
26
27 197 female patients of childbearing age. Written informed consent is obtained from all
28
29 198 patients to take part in the study. Exclusion criteria are as follows: metastatic NECs
30
31 199 previously treated with an irinotecan regimen, known hypersensitivity to 5-FU, calcium
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33 200 levofolinate, irinotecan or their recipients. All acute toxic effects of any prior therapy
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35 201 (including surgery, radiation therapy and chemotherapy) must have resolved to grade ≤ 1
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37 202 according to National Cancer Institute Common Terminology Criteria for Adverse
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39 203 Events Version 4.03 (CTCAE). Patients taking part in another clinical trial with any
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41 204 investigational agent < 30 days prior to study screening or with a history of allergic
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43 205 reactions attributable to compounds of similar chemical or biological composition are
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45 206 excluded Patients who have undergone chemotherapy or radiotherapy < 4 weeks (6
46
47 207 weeks for nitrosoureas or mitomycin C) prior to entering the study, have not recovered
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49 208 from adverse events caused by agents administered > 4 weeks earlier, or have known
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51 209 brain metastases are also not eligible for the study. Patients with other malignancies
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4 210 with a disease-free interval of < 5 years (with the exception of non melanoma skin
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6 211 cancer or low-grade superficial bladder cancer) are excluded, as are those with any
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8 212 severe and/or uncontrolled medical condition or other condition that could affect their
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10 213 participation in the study such as:
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13 214 • unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction
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15 215 < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or
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17 216 any other clinically significant cardiac disease;
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19 217 • severely impaired lung function (spirometry and DLCO 50% of the normal predicted
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21 218 value and/or oxygen saturation \leq 88% at rest, in room air);
22
23 219 • uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of
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25 220 normal (ULN);
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27 221 • any active (acute or chronic) or uncontrolled infections/disorders.

22 222 Tumor evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest,
23
24 223 abdomen, pelvis, and any additional known sites of disease. These tests are performed
25
26 224 at baseline, every three months during treatment and after therapy discontinuation in
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28 225 non-progressing patients until progression. When possible, ^{68}Ga -PET/CT and ^{18}F FDG-
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30 226 PET/CT is performed at baseline.
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228 Study endpoints

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48 229 The primary endpoint of the study is the DCR of each treatment, defined as the
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50 230 percentage of patients who have achieved complete or partial response or stable disease
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52 231 for at least 12 weeks from the start of therapy. DCR will be evaluated using the new
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54 232 international criteria proposed by the RECIST version 1.1. Acute and late toxicity will
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56 233 be evaluated by CTCAE Version 4.03, the latter defined as toxicity occurring at least 30
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4 234 days after the end of the last treatment cycle. Secondary endpoints are the evaluation of
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6 235 OS, calculated from the start of treatment to death from any cause and PFS, calculated
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9 236 from the start of treatment to the date of the first documented evidence of disease
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11 237 progression or of death from any cause. Patients without events at the time of analysis
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13 238 will be censored at their last-known-alive date for OS and at their last date of tumor
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15 239 evaluation for PFS. A further secondary endpoint is the evaluation of quality of life
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17 240 using the European Organization for the Research and Treatment of Cancer Quality of
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19 241 Life Questionnaire (EORTC QLQ-C30). When data are available, the impact of
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21 242 baseline ⁶⁸Ga-PET/CT and ¹⁸F-FDG-PET/CT on PFS will be analysed with exploratory
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23 243 intent.- After signing the informed consent for biomarker evaluation, patients will
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25 244 undergo evaluation of the mutation status of MEN1, DAXX, ATRX and RB-1 in
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27 245 primary tumor tissue and of miRNA in blood samples. Assessment of the miRNA
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29 246 profile will be performed on the first 20 patients who agree to participate in the
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31 247 biological part of the study.
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39 249 Ethical considerations

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41 250 The present clinical trial (EudraCT 2016-000767-17), supported by IRST, was
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43 251 authorized by the local Ethics Committee and the Italian Medicines Agency (AIFA); it
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45 252 is also registered on the ClinicalTrials.gov website (NCT03387592). The study
46
47 253 complies with the ethical standards laid down in the 1964 Declaration of Helsinki and
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49 254 the principles of Good Clinical Practice guidelines (including written informed
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51 255 consent).
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56 256 Patient and Public Involvement

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58 257 This research was done without patient involvement.
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259 Statistical methods

260 The Bryant and Day design is used to estimate a sample size that takes into account
261 both treatment activity and toxicity. Although randomisation is used to allocate patients
262 to the 2 arms, no formal statistical comparisons between treatment regimens is planned.

263 The purpose of randomisation is to reduce bias due to patient assignment to a specific
264 treatment arm. The hypothesis for the control arm is based on literature data (22, 23).

265 An α level of 0.10 (both for toxicity and DCR) and a power of 90% were adopted. A
266 DCR rate $\geq 60\%$ and a relevant toxicity rate $\leq 20\%$ are considered acceptable rates while
267 a DCR rate $\leq 40\%$ and a relevant toxicity rate $\geq 40\%$ are considered unacceptable rates.

268 Given these hypotheses, the first step of the study will require 25 patients. If ≥ 10
269 patients with a DCR are observed and ≥ 15 patients do not have relevant toxicity, the
270 study will enrol patients in the next step. A total of 53 patients will be enrolled. If ≥ 25
271 patients with DCR and ≥ 36 patients without any relevant toxicity are observed,
272 treatment will be considered active and not toxic. This design is used for each treatment
273 scheme and all analyses will be performed separately. If one of the schemes does not
274 obtain the expected proportions of the first step, the arm will be closed and patients will
275 be enrolled in the other arm until the target is reached; if the expected proportions are
276 not reached in any arm, the study will be prematurely closed. If no premature stop
277 occurs, a total of 106 evaluable patients are needed (53 patients in each arm). Taking
278 into account a 5% dropout rate, 56 patients must be enrolled in each arm (total 112
279 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopenia, prolonged G3-G4
280 neutropenia (> 7 days) and drug-related hospitalizations are considered relevant
281 toxicity. The stratification factors of this study are Ki-67 (21%-55 % vs. $>55\%$) and site

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4 282 of primary tumor (lung vs. GEP).
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6 283 Complete response, partial response or stable disease for at least 12 weeks will be
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9 284 considered as the DCR. The proportion of patients in this category will be determined
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11 285 and 95% confidence intervals (95% CIs) for the DCR will be calculated. OS and PFS
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13 286 will be estimated using the Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate
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15 287 statistical analyses will be performed on the basis of the data available to compare
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17 288 QLQ-C30 scores between baseline and subsequent follow-up visits.
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20 289 When data are available, the impact of ^{68}Ga -PET/CT result on PFS will be analyzed
21
22 290 with exploratory intent. The Shapiro-Wilk test will be used to determine the normality
23
24 291 distribution of each biomarker and, in the event of a non-normal distribution,
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26 292 nonparametric statistics will be used to analyze the relationship between the serum
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28 293 levels of each marker, considered as continuous variables, and response to treatment. In
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30 294 case of normality distribution of biomarkers parametric test will be used. All endpoints
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32 295 will be analysed separately for each treatment group.
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37 38 39 297 **Discussion**

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41 298 There is still no truly effective second-line chemotherapy for neuroendocrine
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43 299 carcinoma. The overall prognosis of patients is poor, with an OS of 5 months in the
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45 300 metastatic setting according to the SEER (Surveillance, Epidemiology, and End
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47 301 Results) data (22). Only 5% of all patients are long-term survivors. There is also a
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49 302 marked lack of prognostic and predictive factors (5).
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52 303 Three phase II studies registered at *ClinicalTrials.gov* are currently investigating the
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54 304 second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab
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56 305 (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab
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4 306 (NCT03147404). Some abstracts were presented at ESMO (European Society for
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6 307 Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019
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8 308 on the use of immunotherapy in GEP-NECs with inconclusive results. The SENECA
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10 309 study uses a promising approach to the treatment of patients with metastatic NECS.
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12 310 First, both the activity and safety of 2 regimens are assessed in the same setting with a
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14 311 sizeable patient population (56 patients/arm). In addition, patients are stratified
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16 312 according to Ki-67 index and morphology to investigate the role of each treatment
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18 313 combination in both poorly differentiated and well differentiated NECs. Another aim of
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20 314 this study is to integrate both biological and metabolic imaging data in an effort to
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22 315 improve the current GEP-NEC classification.
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27 316 In conclusion, there are still no FDA/EMA (Food and Drug Administration/European
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29 317 Medicines Agency)-approved second-line therapeutic options for patients with
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31 318 metastatic NECs. The SENECA trial could provide evidence of a novel therapeutic
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33 319 option for patients. Moreover, the integration of biological and imaging data could be
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35 320 lead to a better understanding of the natural history of the disease and help to identify
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37 321 potential responders.
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43 324 **Confidentiality**

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46 325 This study will be conducted in full conformity with ICH (The International Council for
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48 326 Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)
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50 327 Guidelines for Good Clinical Practice , Directive 2001/20/EEC of the European
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52 328 Parliament and other relevant current local legislation. Participants will be allocated a
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54 329 unique identification (ID) number at entry. The master list linking participant personal
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4 330 information and ID number will be maintained in a separate locked cabinet and
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6 331 password-protected hard-drive. Data will be analysed by ID number only. Patient files
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9 332 and other source data will for be kept a maximum of 15 years.
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13 334 **Dissemination**

15 335 After completing the study, all data, including beneficial and adverse events, of the trial
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17 336 will be communicated at scientific meetings and published in indexed peer-reviewed
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19 337 journals. If shown to be effective, the therapy program will be made available to the
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21 338 general public in an appropriate manner.
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23 339

27 340 **Abbreviations**

29 341 CAPTEM, capecitabine plus temozolomide; CgA, chromogranin A; DCR, disease
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31 342 control rate; GEP, gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET,
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33 343 neuroendocrine tumor; OS, overall survival; PFS, progression-free survival; SRS,
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35 344 somatostatin receptor scintigraphy.
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37 345

41 346 **Author contributions**

43 347 AB, CL and TI designed the study and drafted the article. AB was responsible for data
44
45 348 acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a
46
47 349 clinical information data extraction method for the protocol. FF performed the statistical
48
49 350 analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual
50
51 351 content. All authors read and approved the present version of the manuscript for
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53 352 submission.
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4 354 **Data availability statement**
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6 355 The datasets used and analysed in the present study are available from the
7
8
9 356 corresponding author on reasonable request.
10

11 357

12
13 358 **Funding**

14
15 359 The study was conducted in the absence of any commercial or financial relationships
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17
18 360 that could be construed as a potential conflict of interest.
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20 361

21
22 362 **Conflicts of interest**

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25 363 The authors declare no conflict of interest.
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27 364

28
29 365 **Acknowledgements**

30
31 366 *SENECA study Team investigators*: Davide Campana, Davide Pastorelli, Nicola
32

33 367 Silvestris, Francesco Silvestris, Angela Buonadonna, Giuseppe Badalamenti
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38 369 They also thank Gráinne Tierney and Cristiano Verna for editorial assistance.
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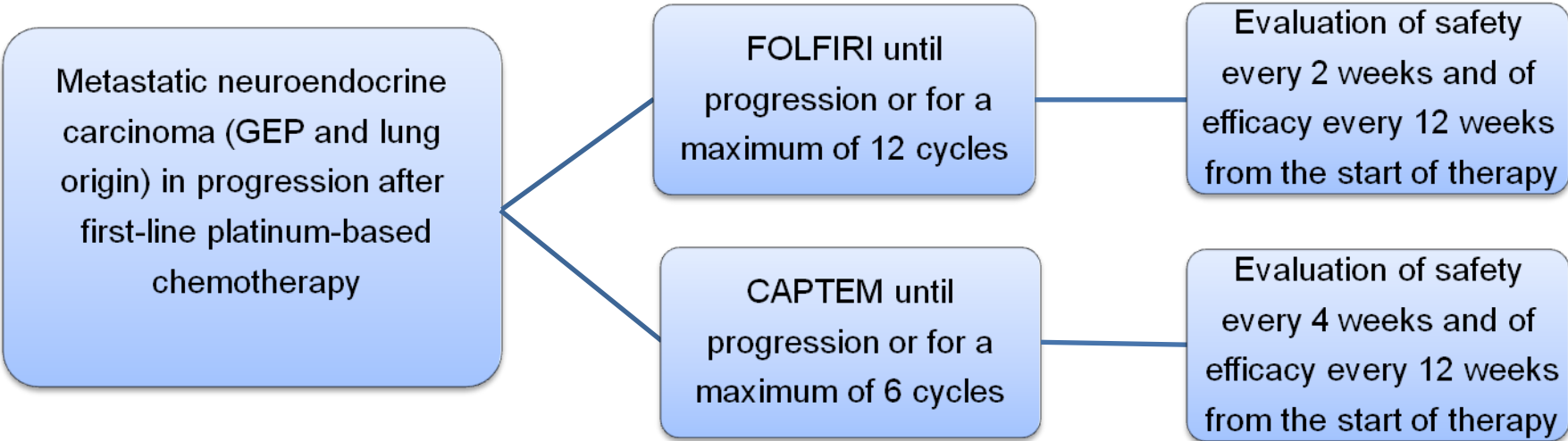
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27 **Figure legend**

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30 **FIGURE 1** | SENECA study design.

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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	2a	Scientific background and explanation of rationale	5-7
	2b	Specific objectives or hypotheses	7
Methods	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
	4a	Eligibility criteria for participants	9
	4b	Settings and locations where the data were collected	14-15
	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
	7a	How sample size was determined	12
Sample size	7a	How sample size was determined	N/A
	7b	When applicable, explanation of any interim analyses and stopping guidelines	12
	8a	Method used to generate the random allocation sequence	12
Randomisation:	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
	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	12
Blinding	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	N/A
	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	N/A

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_____ N/A
 _____ N/A
 11b If relevant, description of the similarity of interventions
 12a Statistical methods used to compare groups for primary and secondary outcomes
 12b Methods for additional analyses, such as subgroup analyses and adjusted analyses
 _____ 12, 13
 _____ 12

Results

13a For each group, the numbers of participants who were randomly assigned, received intended treatment, and diagram is strongly recommended) were analysed for the primary outcome
 13b For each group, losses and exclusions after randomisation, together with reasons
 14a Dates defining the periods of recruitment and follow-up
 14b Why the trial ended or was stopped
 _____ 8, 12
 _____ 12
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 _____ N/A

15 A table showing baseline demographic and clinical characteristics for each group
 16 For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups
 _____ N/A

17a For each primary and secondary outcome, results for each group, and the estimated effect size and its estimation precision (such as 95% confidence interval)
 17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended
 _____ 12
 _____ 12

18 Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory
 _____ 12

Discussion

19 All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)

20 Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses
 _____ 4

21 Generalisability (external validity, applicability) of the trial findings
 _____ 13, 14

22 Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence
 _____ 13, 14

Other information

23 Registration number and name of trial registry
 _____ 13

24 Where the full trial protocol can be accessed, if available
 _____ All, 15

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.

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1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1	Title
2	Trial identifier and registry name. If not yet registered, name of intended registry	2a	Trial registration
3	All items from the World Health Organization Trial Registration Data Set	2b	
4	Date and version identifier	3	Protocol version
5	Sources and types of financial, material, and other support	4	Funding
6	Names, affiliations, and roles of protocol contributors	5a	Roles and responsibilities
7	Name and contact information for the trial sponsor	5b	
8	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	5c	
9	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)	5d	
10	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	6a	Background and rationale
11	Explanation for choice of comparators	6b	
12	Specific objectives or hypotheses	7	Objectives
13	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	Trial design

Introduction

Administrative information

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

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS



Methods: Participants, interventions, and outcomes	
9	Study setting 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
10	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)
11a	Interventions 11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
11b	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	11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
11d	11d Relevant concomitant care and interventions that are permitted or prohibited during the trial
12	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
13	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)
14	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
15	Recruitment 15 Strategies for achieving adequate participant enrolment to reach target sample size
Methods: Assignment of interventions (for controlled trials)	
Allocation:	
16a	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

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5			level dataset, and statistical code
6	31c	Plans, if any, for granting public access to the full protocol, participant-	
7			writers
8			
9	31b	Authorship eligibility guidelines and any intended use of professional	
10			
11			data sharing arrangements), including any publication restrictions
12			groups (eg, via publication, reporting in results databases, or other
13			participants, healthcare professionals, the public, and other relevant
14	31a	Plans for investigators and sponsor to communicate trial results to	Dissemination policy
15			
16			compensation to those who suffer harm from trial participation
17	30	Provisions, if any, for ancillary and post-trial care, and for	Ancillary and post-trial care
18			
19			investigators
20			disclosure of contractual agreements that limit such access for
21			Statement of who will have access to the final trial dataset, and
22	29		Access to data
23			the overall trial and each study site
24			Financial and other competing interests for principal investigators for
25	28		Declaration of interests
26			before, during, and after the trial
27			be collected, shared, and maintained in order to protect confidentiality
28			How personal information about potential and enrolled participants will
29	27		Confidentiality
30			and biological specimens in ancillary studies, if applicable
31			Additional consent provisions for collection and use of participant data
32	26b		
33			participants or authorised surrogates, and how (see item 32)
34			Who will obtain informed consent or assent from potential trial
35	26a		Consent or assent
36			regulators)
37			(eg, investigators, REC/IRBs, trial participants, trial registries, journals,
38			changes to eligibility criteria, outcomes, analyses) to relevant parties
39			Plans for communicating important protocol modifications (eg,
40	25		Protocol amendments
41			(REC/IRB) approval
42			Plans for seeking research ethics committee/institutional review board
43	24		Research ethics approval
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50			sponsor
51			whether the process will be independent from investigators and the
52	23		Auditing
53			Frequency and procedures for auditing trial conduct, if any, and
54			of trial interventions or trial conduct
55			spontaneously reported adverse events and other unintended effects
56			Plans for collecting, assessing, reporting, and managing solicited and
57	22		Harms
58			decision to terminate the trial
59			who will have access to these interim results and make the final
60	21b	Description of any interim analyses and stopping guidelines, including	

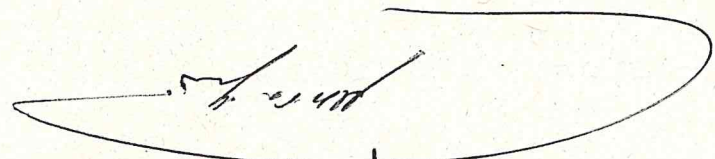
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Appendices

Model consent form and other related documentation given to participants and authorised surrogates	32	Informed consent
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	33	Biological specimens

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The above items have been advised when applicable.
The responsible author.



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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)

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Oncology
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

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4 1 **CLINICAL STUDY PROTOCOL**5
6 27
8 3 **Randomized Phase II Trial of CAPTEM or FOLFIRI as**
9 4 **SEcond-line Therapy in NEuroendocrine CARcinomas and**
10 5 **Exploratory Analysis of Predictive Role of PET/CT Imaging**
11 6 **and Biological Markers (SENECA Study)**12 7 **Running title: Second-line CAPTEM/FOLFIRI in NECs**

13 8

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20 33 **Language style:** This article is formatted in British English.
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60**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 ⁶⁸Ga-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

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4 69 participate in the biological sub-study.
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6 70 **Ethics and dissemination:** The SENECA trial, supported by IRST, was authorised by
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9 71 the locals Ethics Committee and the Italian Medicines Agency (AIFA). Results will be
10
11 72 widely disseminated via peer-reviewed manuscripts, conference presentations and
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13 73 reports to relevant authorities.
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18 75 The study is currently open in Italy. Clinical trial registration: NCT03387592.
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22 77 **Keywords:** neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine,
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24 78 temozolomide, CAPTEM
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27 79 **Strengths and limitations of the study**
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- 29 80
- 31 81 • the SENECA trial randomises patients to receive two different treatments,
32 FOLFIRI or CAPTEM, providing important information on the activity of both
33 82 combinations in different NEC subtypes (NET grade (G) 3 and NEC G3).
34
35 83 • The SENECA trial analyses the role of miRNAs and other biological markers as
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37 84 prognostic and predictive factors. A further aim is to assess ⁶⁸Ga-PET/CT as a
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39 85 tool to improve current histological classification.
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43 86 The major limitations of the study are:
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- 45 87
- 46 87 • The rarity of the disease and patient prognosis. However, the involvement of
47
48 88 several Italian centres will hopefully help to overcome this problem.
49
50 89 • Poor prognosis of NEC patients. Patients progressing on platinum chemotherapy
51
52 90 usually have rapid deterioration of clinical conditions
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93 **Introduction**

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

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

108 Given the rarity of this disease, prospective clinical data are lacking and treatment
109 recommendations are essentially expert-based opinions. Two phase II studies
110 investigating the second-line treatment of GEP-NECs are currently registered at
111 *ClinicalTrials.gov*, one evaluating the safety and tolerability of everolimus in
112 40 patients with G3 NEC/NET or G1/G2 NET switching to G3 NEN (National Clinical
113 Trial identifier NCT02113800) and the other investigating the efficacy of avelumab
114 (NCT03147404). A French study focusing on the identification of predictive molecular
115 markers of response to sunitinib in poorly differentiated digestive NETs

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4 116 (NCT01215578) has now closed recruitment and results are eagerly awaited. Another
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6 117 French multicentre prospective phase II trial is currently ongoing to investigate the
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8 118 efficacy of the bevacizumab-FOLFIRI combination after progression on a
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11 119 platinum/etoposide combination (7).

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13 120 Different second-line chemotherapy combinations have been evaluated but shown
14
15 121 poor results (8-10). In a monocentre retrospective clinical trial, Hentic et al.
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17 122 hypothesised the potential efficacy of FOLFIRI as second-line chemotherapy in
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19 123 19 patients with G3 extra-pulmonary NECs (11). An objective response rate was
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21 124 obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median PFS
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23 125 and OS were 4 and 18 months, respectively.

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27 126 In another retrospective study, a 71% DCR was obtained with temozolomide-based
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29 127 chemotherapy in 25 patients with metastatic poorly differentiated endocrine carcinoma
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31 128 of different sites and atypical bronchial carcinoid with Ki-67 > 20%. Small cell lung
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33 129 carcinoma and large cell lung carcinoma were excluded. A PFS of 12 months (95%
34
35 130 confidence interval [CI], 5.5- 24) and OS of 22 months (95% CI, 12-31) were reported
36
37 131 in patients who responded to treatment or showed stable disease (SD), whereas OS was
38
39 132 only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response
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41 133 rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with
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43 134 high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive
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45 135 staining for chromogranin A. Both factors are often associated with more highly
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47 136 differentiated tumours (12).

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52 137 Literature data on lung NECs in progression after first-line chemotherapy are based
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54 138 on small patient series (13). Moreover, there is increasing evidence of some
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57 139 discrepancies in the current grading of NECs, highlighting the need for more accurate
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4 140 biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise two
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6 141 distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived
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8 142 from well differentiated neuroendocrine tumours (NETs) and characterised by
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10 143 mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from
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12 144 neuroendocrine-differentiated adenocarcinomas and characterised by a mutation in
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14 145 RB1. Both subgroups display a distinct prognosis and different sensitivity to
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16 146 chemotherapy (14-16). Micro(mi)RNAs are a class of small, non-coding, highly
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18 147 conserved single-stranded RNAs involved in the post-transcriptional regulation of cell
19
20 148 proliferation, differentiation, survival, and apoptosis (17). They are often associated
21
22 149 with resistance to therapy (18, 19). Whilst miRNAs are known to show a specific
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24 150 expression pattern in NETs (20), little is known about differential miRNA profiles in
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26 151 NEC patients. At present, no data are available on the deregulation of specific miRNAs
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28 152 in this setting.

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34 153 In a study recently published by our group on GEP-NEC patients undergoing
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36 154 first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months
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38 155 ($p < 0.01$) in patients with Ki-67 value between 20% and 50% or $>50\%$, respectively
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40 156 (19). Median (m)OS was 8.1 months in the latter group but was not reached in the
41
42 157 former group ($p = 0.039$). Patients with a positive ^{68}Ga -PET/CT had a higher 18-month
43
44 158 OS rate than those with a negative scan (75% vs. 34.3%, respectively), but the
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46 159 difference was not statistically significant ($p = 0.06$). Our data highlighted that ^{68}Ga -
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48 160 PET/CT positivity may be a discriminating factor (16, 21) in predicting prognosis,
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50 161 especially ~~important~~ in the metastatic setting where histological material is not always
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52 162 available for evaluation. Furthermore, ^{18}F fludeoxyglucose (^{18}F FDG)-PET/CT may be
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54 163 useful to discriminate between patients with different prognosis (22).
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4 164 Given the above premises, we decided to investigate the efficacy and safety of
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6 165 second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression
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9 166 after first-line platinum-based treatment. We also aimed to study the serum miRNA
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11 167 profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1,
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13 168 patient prognosis and response to therapy, and to assess the prognostic and predictive
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15 169 role of ¹⁸F-FDG-PET/CT, ⁶⁸Ga-PET/CT and Ki-67 score.
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23 172 **Methods and Analysis**

24 173 **Study design**

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26 174 The SENECA study is a multicentre randomised non-comparative phase II study
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29 175 (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin
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31 176 (lung or gastroenteropancreatic) in progression after first-line treatment are randomized
32
33 177 to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or
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35 178 unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until
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37 179 progression or unacceptable toxicity.
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42 180 The treatment arms are as follows:
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47 182 **FOLFIRI regimen**

- 48 183 • Irinotecan 180 mg/m², given as a 60-min. intravenous (i.v.) infusion on day 1 every
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50 184 2 weeks followed by
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52 185 • Leucovorin 200 mg/m², given as a 2-h i.v. infusion on day 1 every 2 weeks followed
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4 187 • 5-fluorouracil (5-FU) 400 mg/m² given as bolus, and then 5-FU 2400 mg/m² given
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6 188 as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a
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8 189 maximum of 12 cycles.

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11 190

13 191 CAPTEM regimen

16 192 Capecitabine 750 mg/m² twice a day on days 1-14 in combination with temozolomide
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18 193 200 mg/m² daily on days 10-14, every 4 weeks, until progression or for a maximum of
19
20 194 6 cycles.

23 195 The study includes patients aged ≥ 18 years with a histological diagnosis of G3
24
25 196 neuroendocrine carcinoma (GEP-NEC and lung NEC) according to the 2010 and 2015
26
27 197 GEP and Lung NEN WHO classifications, respectively, Ki-67 $>20\%$ and measurable
28
29 198 disease according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria.
30
31 199 All patients must have an Eastern Cooperative Oncology Group (ECOG) performance
32
33 200 status ≤ 2 with a life expectancy > 3 months and must have already undergone first-line
34
35 201 treatment for metastatic disease with platinum -based chemotherapy
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37 202 (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological,
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39 203 liver and renal function is required and effective contraceptive methods must be used by
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41 204 female patients of childbearing age. Written informed consent is obtained from all
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43 205 patients to take part in the study. Exclusion criteria are as follows: metastatic NECs
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45 206 previously treated with an irinotecan or temozolomide regimen, known hypersensitivity
46
47 207 to 5-FU/capecitabine, calcium levofolinate, irinotecan or their recipients. All acute
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49 208 toxic effects of any prior therapy (including surgery, radiation therapy and
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51 209 chemotherapy) must have resolved to grade ≤ 1 according to National Cancer Institute
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53 210 Common Terminology Criteria for Adverse Events version 4.03 (CTCAE). Patients
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4 211 taking part in another clinical trial with any investigational agent < 30 days prior to
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6 212 study screening or with a history of allergic reactions attributable to compounds of
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8 213 similar chemical or biological composition are excluded. Patients who have undergone
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10 214 chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C)
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12 215 prior to entering the study, have not recovered from adverse events caused by agents
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14 216 administered > 4 weeks earlier, or have known brain metastases are also not eligible for
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16 217 the study. Patients with other malignancies with a disease-free interval of < 5 years
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18 218 (with the exception of non melanoma skin cancer or low-grade superficial bladder
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20 219 cancer) are excluded, as are those with any severe and/or uncontrolled medical
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22 220 condition or other condition that could affect their participation in the study such as:
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25 221 • unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction
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27 222 < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or
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29 223 any other clinically significant cardiac disease;
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31 224 • severely impaired lung function (spirometry and DLCO 50% of the normal predicted
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33 225 value and/or oxygen saturation \leq 88% at rest, in room air);
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35 226 • uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of
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37 227 normal;
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39 228 • any active (acute or chronic) or uncontrolled infections/disorders.
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43 229 Tumour evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest,
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45 230 abdomen, pelvis, and any additional known sites of disease. These tests are performed
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47 231 at baseline, every three months during treatment and after the end of treatment in
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49 232 non-progressing patients until progression. When possible, ^{68}Ga -PET/CT and ^{18}F FDG-
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51 233 PET/CT are performed at baseline. An EORTC quality of life questionnaire is
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53 234 administered at baseline and every three months thereafter during the treatment period.
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236 Study endpoints

237 The primary endpoint of the study is the DCR of each treatment, defined as the
238 percentage of patients who have achieved complete or partial response or stable disease
239 for ≥ 12 weeks from the start of therapy. DCR will be evaluated using the new
240 international criteria proposed by the RECIST version 1.1. Acute and late toxicity will
241 be assessed by CTCAE Version 4.03, the latter defined as toxicity occurring at least
242 30 days after the end of the last treatment cycle. Secondary endpoints are OS, calculated
243 from the start of treatment to death from any cause, and PFS, calculated from the start of
244 treatment to the date of the first documented evidence of disease progression or of death
245 from any cause. All the analyses will be performed in the intention-to-treat population.
246 Patients without events at the time of analysis will be censored at their last-known-alive
247 date for OS and at their last date of tumour evaluation for PFS. A further secondary
248 endpoint is the evaluation of quality of life using the European Organization for the
249 Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30).
250 When data are available, the impact of baseline ^{68}Ga -PET/CT and ^{18}F FDG-PET/CT on
251 PFS will be analysed with exploratory intent. After signing the informed consent for
252 biomarker assessment, patients will undergo evaluation of the mutation status of MEN1,
253 DAXX, ATRX and RB-1 in primary tumour tissue and of miRNA in blood samples.
254 Assessment of the miRNA profile will be performed on the first 20 patients who agree
255 to participate in the biological part of the study.

256

257 Ethical considerations

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4 258 The present clinical trial (EudraCT 2016-000767-17), supported by IRST, was
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6 259 authorised by the local Ethics Committee and by the Italian Medicines Agency (AIFA).
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9 260 The study is also registered on the *ClinicalTrials.gov* website (NCT03387592). The
10
11 261 study complies with the ethical standards laid down in the 1964 Declaration of Helsinki
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13 262 and the principles of Good Clinical Practice guidelines (including written informed
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15
16 263 consent).

19 264 Patient and public involvement

21 265 This research work was performed without patient involvement in the study design,
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23 266 execution or outcome measures.
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29 268 Statistical methods

31 269 The Bryant and Day design is used to estimate a sample size that takes into account
32
33 270 both treatment activity and toxicity. Although randomisation is used to allocate patients
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35 271 to the two arms, no formal statistical comparisons between treatment regimens are
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37 272 planned. The purpose of randomisation is to reduce bias due to patient assignment to a
38
39 273 specific treatment arm. The hypothesis for the control arm is based on literature data
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41 274 (23, 24).

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45 275 An α level of 0.10 (both for toxicity and DCR) and a power of 90% have been
46
47 276 adopted. A DCR rate $\geq 60\%$ and a relevant toxicity rate $\leq 20\%$ are considered acceptable
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49 277 rates, while a DCR rate $\leq 40\%$ and a relevant toxicity rate $\geq 40\%$ are considered
50
51 278 unacceptable rates. Given these hypotheses, the first step of the study will require
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53 279 25 patients. If ≥ 10 patients with a DCR are observed and ≥ 15 patients do not have
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55 280 significant toxicity, the study will enrol patients in the next step. A total of 53 patients
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4 281 will be enrolled. If ≥ 25 patients with DCR and ≥ 36 patients without any relevant
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6 282 toxicity are observed, treatment will be considered active and not toxic. This design is
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9 283 used for each treatment scheme and all analyses will be performed separately. If one of
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11 284 the schemes does not obtain the expected proportions of the first step, the arm will be
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13 285 closed and patients will be enrolled in the other arm until the target is reached; if the
14
15 286 expected proportions are not reached in any arm, the study will be prematurely closed.
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18 287 If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in
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20 288 each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each
21
22 289 arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopenia,
23
24 290 prolonged G3-G4 neutropenia (> 7 days) and drug-related hospitalisations are
25
26 291 considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55
27
28 292 % vs. $> 55\%$) and site of primary tumour (lung vs. GEP).
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32 293 Complete response, partial response or stable disease for at least 12 weeks will be
33
34 294 considered as the DCR. The proportion of patients in this category will be determined
35
36 295 and 95% CIs for the DCR will be calculated. OS and PFS will be estimated using the
37
38 296 Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate statistical analyses will be
39
40 297 performed on the basis of the data available to compare QLQ-C30 scores between
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42 298 baseline and subsequent follow-up visits.
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45 299 When data are available, the impact of ^{68}Ga -PET/CT result on PFS will be analysed
46
47 300 with exploratory intent. The Shapiro-Wilk test will be used to determine the normality
48
49 301 distribution of each clinical, demographic and biological biomarker (25). In the event
50
51 302 of a non-normal distribution, nonparametric statistics will be used to analyse the
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53 303 relationship between the serum levels of each marker, considered as continuous
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55 304 variables, and response to treatment. In the event of normal biomarker distribution,
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4 305 a parametric test will be used. All endpoints will be analysed separately for each
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6 306 treatment group.

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9 307 **Discussion**

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11 308 There is still no truly effective second-line chemotherapy for neuroendocrine
12
13 309 carcinoma. Overall prognosis of patients is poor, with an OS of 5 months in the
14
15 310 metastatic setting according to SEER (Surveillance, Epidemiology, and End Results)
16
17 311 data (26). Only 5% of all patients are long-term survivors. There is also a marked lack
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19 312 of prognostic and predictive factors (5).

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23 313 Three phase II studies registered at *ClinicalTrials.gov* are currently investigating
24
25 314 second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab
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27 315 (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab
28
29 316 (NCT03147404). Some abstracts were presented at ESMO (European Society for
30
31 317 Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019
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33 318 on the use of immunotherapy in GEP-NECs, all showing inconclusive results. The
34
35 319 SENECA study uses a promising approach to the treatment of patients with metastatic
36
37 320 NECs. First, both the activity and safety of 2 regimens are assessed in the same setting
38
39 321 with a sizeable patient population (56 patients/arm). In addition, patients are stratified
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41 322 according to Ki-67 index and morphology to investigate the role of each treatment
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43 323 combination in both poorly differentiated and well differentiated NECs. Another aim of
44
45 324 this study is to integrate both biological and metabolic imaging data in an effort to
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47 325 improve the current GEP-NEC classification.

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53 326 In conclusion, there are still no FDA/EMA (Food and Drug Administration/European
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55 327 Medicines Agency)-approved second-line therapeutic options for patients with
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57 328 metastatic NECs, and the SENECA trial could represent a step forward in finding novel
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4 329 therapies to prolong survival and maintain quality of life. Moreover, the integration of
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6 330 biological and imaging data could -lead to a better understanding of the natural history
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9 331 of the disease and help to identify potential responders.

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12 334 **Confidentiality**

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16 335 This study will be conducted in full conformity with ICH (The International Council for
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18 336 Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)
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20 337 Guidelines for Good Clinical Practice, Directive 2001/20/EEC of the European
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23 338 Parliament and other relevant current local legislation. Participants will be allocated a
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25 339 unique identification (ID) number at entry. The master list linking participant personal
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27 340 information and ID number will be maintained in a separate locked cabinet and
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29 341 password-protected hard-drive. Data will be analysed by ID number only. Patient files
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31
32 342 and other source data will for be kept a maximum of 15 years.
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37 344 **Dissemination**

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40 345 After completing the study, all data, including beneficial and adverse events, of the trial
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42 346 will be communicated at scientific meetings and published in indexed peer-reviewed
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44 347 journals. If shown to be effective, the therapy program will be made available to the
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46 348 general public in an appropriate manner.
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4 353 **Abbreviations**
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6 354 CAPTEM, capecitabine plus temozolomide; DCR, disease control rate; GEP,
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8 355 gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour;
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10 356 OS, overall survival; PFS, progression-free survival
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16 358 **Author contributions**
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18 359 AB, CL and TI designed the study and drafted the article. AB was responsible for data
19
20 360 acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a
21
22 361 clinical information data extraction method for the protocol. FF performed the statistical
23
24 362 analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual
25
26 363 content. All authors read and approved the present version of the manuscript for
27
28 364 submission.
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34 366 **Data availability statement**
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36 367 The datasets used and analysed in the present study are available from the
37
38 368 corresponding author on reasonable request.
39
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41 369

42
43 370 **Funding**
44

45 371 The study was conducted in the absence of any commercial or financial relationships
46
47 372 that could be construed as a potential conflict of interest.
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51
52 374 **Conflicts of interest**
53

54 375 The authors declare no conflict of interest.
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5
6 378 *SENECA study Team investigators*: Davide Campana, Davide Pastorelli, Nicola
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9 379 Silvestris, Francesco Silvestris, Angela Buonadonna and Giuseppe Badalamenti.
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11 380 The authors thank Gráinne Tierney and Cristiano Verna for editorial assistance.
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6 **Figure legend**
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8
9 **FIGURE 1** | SENECA study design.
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For peer review only

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Fig. 1

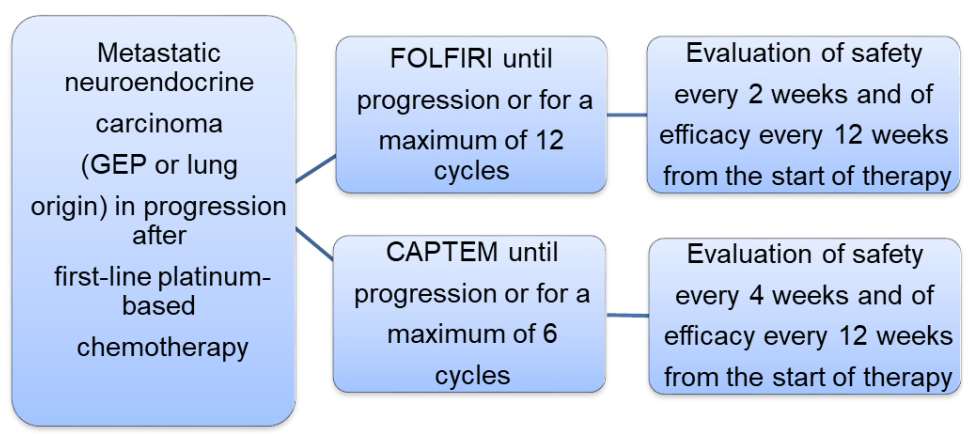


FIGURE 1 | SENECA study design.

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	2a	Scientific background and explanation of rationale	5-7
	2b	Specific objectives or hypotheses	7
Methods	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
	4a	Eligibility criteria for participants	9
	4b	Settings and locations where the data were collected	14-15
	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
	7a	How sample size was determined	12
Sample size	7a	How sample size was determined	N/A
	7b	When applicable, explanation of any interim analyses and stopping guidelines	12
	8a	Method used to generate the random allocation sequence	12
Randomisation:	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	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
	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	12
Blinding	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	12
	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	N/A

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_____ N/A
 _____ N/A
 11b If relevant, description of the similarity of interventions
 12a Statistical methods used to compare groups for primary and secondary outcomes
 12b Methods for additional analyses, such as subgroup analyses and adjusted analyses
 _____ 12, 13
 _____ 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
 _____ N/A
 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, 15
 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.

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1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1	Title
2	Trial identifier and registry name. If not yet registered, name of intended registry	2a	Trial registration
3	All items from the World Health Organization Trial Registration Data Set	2b	
4	Date and version identifier	3	Protocol version
5	Sources and types of financial, material, and other support	4	Funding
6	Names, affiliations, and roles of protocol contributors	5a	Roles and responsibilities
7	Name and contact information for the trial sponsor	5b	
8	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	5c	
9	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)	5d	
10	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	6a	Background and rationale
11	Explanation for choice of comparators	6b	
12	Specific objectives or hypotheses	7	Objectives
13	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	Trial design

Introduction

Administrative information

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

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS



1			
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3			
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5			level dataset, and statistical code
6	31c	Plans, if any, for granting public access to the full protocol, participant-	
7			writers
8			
9	31b	Authorship eligibility guidelines and any intended use of professional	
10			
11			data sharing arrangements), including any publication restrictions
12			groups (eg, via publication, reporting in results databases, or other
13			participants, healthcare professionals, the public, and other relevant
14	31a	Plans for investigators and sponsor to communicate trial results to	Dissemination policy
15			
16			compensation to those who suffer harm from trial participation
17	30	Provisions, if any, for ancillary and post-trial care, and for	Ancillary and post-trial care
18			
19			investigators
20			
21			disclosure of contractual agreements that limit such access for
22	29	Statement of who will have access to the final trial dataset, and	Access to data
23			
24			the overall trial and each study site
25	28	Financial and other competing interests for principal investigators for	Declaration of interests
26			
27			before, during, and after the trial
28			be collected, shared, and maintained in order to protect confidentiality
29	27	How personal information about potential and enrolled participants will	Confidentiality
30			
31			and biological specimens in ancillary studies, if applicable
32	26b	Additional consent provisions for collection and use of participant data	
33			
34			participants or authorised surrogates, and how (see item 32)
35	26a	Who will obtain informed consent or assent from potential trial	Consent or assent
36			
37			regulators)
38			(eg, investigators, REC/IRBs, trial participants, trial registries, journals,
39			changes to eligibility criteria, outcomes, analyses) to relevant parties
40	25	Plans for communicating important protocol modifications (eg,	Protocol amendments
41			
42	24	Plans for seeking research ethics committee/institutional review board	Research ethics approval
43			(REC/IRB) approval
44			
45			
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47			
48			Ethics and dissemination
49			
50			sponsor
51			whether the process will be independent from investigators and the
52	23	Frequency and procedures for auditing trial conduct, if any, and	Auditing
53			
54			of trial interventions or trial conduct
55			spontaneously reported adverse events and other unintended effects
56	22	Plans for collecting, assessing, reporting, and managing solicited and	Harms
57			
58			decision to terminate the trial
59	21b	Description of any interim analyses and stopping guidelines, including	
60			who will have access to these interim results and make the final

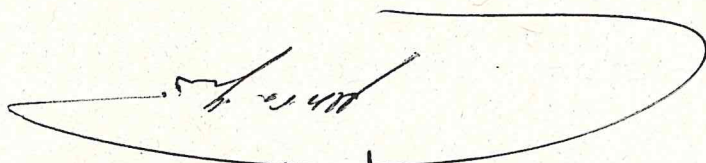
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Appendices

Model consent form and other related documentation given to participants and authorised surrogates	32	Informed consent
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	33	Biological specimens

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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

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Oncology
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

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4 1 **CLINICAL STUDY PROTOCOL**5
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11 4 **SEcond-line Therapy in NEuroendocrine CARcinomas and**
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13 5 **Exploratory Analysis of Predictive Role of PET/CT Imaging**
14
15 6 **and Biological Markers (SENECA Trial): A Study Protocol**16
17 7 **Running title: Second-line CAPTEM/FOLFIRI in NECs**18
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30 37 **Language style:** This article is formatted in British English.
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49 Abstract

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

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

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4 73 **Ethics and dissemination:** The SENECA trial, supported by IRST, was authorised by
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6 74 the local Ethics Committee and the Italian Medicines Agency (AIFA). Results will be
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8 75 widely disseminated via peer-reviewed manuscripts, conference presentations and
9
10 76 reports to relevant authorities.
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14 77

15 78 The study is currently open in Italy. Clinical trial registration: NCT03387592.
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18 79

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20 80 **Keywords:** neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine,
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22 81 temozolomide, CAPTEM
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25 82 **Strengths and limitations of the study**

- 26
27 83 • the SENECA trial randomises patients to receive two different treatments,
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29 84 FOLFIRI or CAPTEM, providing important information on the activity of both
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31 85 combinations in different NEC subtypes (NET grade (G) 3 and NEC G3).
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34 86 • The SENECA trial analyses the role of miRNAs and other biological markers as
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36 87 prognostic and predictive factors. A further aim is to assess ⁶⁸Ga-PET/CT as a
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38 88 tool to improve current histological classification.

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41 89 The major limitations of the study are:

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43 90 • The rarity of the disease and patient prognosis. However, the involvement of
44
45 91 several Italian centres will hopefully help to overcome this problem.
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48 92 • Poor prognosis of NEC patients. Patients progressing on platinum chemotherapy
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50 93 usually have rapid deterioration of clinical conditions
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96 **Introduction**

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

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

111 Given the rarity of this disease, prospective clinical data are lacking and treatment
112 recommendations are essentially expert-based opinions. Two phase II studies
113 investigating the second-line treatment of GEP-NECs are currently registered at
114 *ClinicalTrials.gov*, one evaluating the safety and tolerability of everolimus in
115 40 patients with G3 NEC/NET or G1/G2 NET switching to G3 NEN (National Clinical
116 Trial identifier NCT02113800) and the other investigating the efficacy of avelumab
117 (NCT03147404). A French study focusing on the identification of predictive molecular
118 markers of response to sunitinib in poorly differentiated digestive NETs

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4 119 (NCT01215578) has now closed recruitment and results are eagerly awaited. Another
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6 120 French multicentre prospective phase II trial is currently ongoing to investigate the
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8 121 efficacy of the bevacizumab-FOLFIRI combination after progression on a
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10 122 platinum/etoposide combination (7).

13 123 Different second-line chemotherapy combinations have been evaluated but shown
14
15 124 poor results (8-10). In a monocentre retrospective clinical trial, Hentic et al.
16
17 125 hypothesised the potential efficacy of FOLFIRI as second-line chemotherapy in
18
19 126 19 patients with G3 extra-pulmonary NECs (11). An objective response rate was
20
21 127 obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median PFS
22
23 128 and OS were 4 and 18 months, respectively.

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27 129 In another retrospective study, a 71% DCR was obtained with temozolomide-based
28
29 130 chemotherapy in 25 patients with metastatic poorly differentiated endocrine carcinoma
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31 131 of different sites and atypical bronchial carcinoid with Ki-67 > 20%. Small cell lung
32
33 132 carcinoma and large cell lung carcinoma were excluded. A PFS of 12 months (95%
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35 133 confidence interval [CI], 5.5- 24) and OS of 22 months (95% CI, 12-31) were reported
36
37 134 in patients who responded to treatment or showed stable disease (SD), whereas OS was
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39 135 only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response
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41 136 rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with
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43 137 high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive
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45 138 staining for chromogranin A. Both factors are often associated with more highly
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47 139 differentiated tumours (12).

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50 140 Literature data on lung NECs in progression after first-line chemotherapy are based
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52 141 on small patient series (13). Moreover, there is increasing evidence of some
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54 142 discrepancies in the current grading of NECs, highlighting the need for more accurate
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4 143 biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise two
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6 144 distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived
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9 145 from well differentiated neuroendocrine tumours (NETs) and characterised by
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11 146 mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from
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13 147 neuroendocrine-differentiated adenocarcinomas and characterised by a mutation in
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16 148 RB1. Both subgroups display a distinct prognosis and different sensitivity to
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18 149 chemotherapy (14-16). Micro(mi)RNAs are a class of small, non-coding, highly
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20 150 conserved single-stranded RNAs involved in the post-transcriptional regulation of cell
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22 151 proliferation, differentiation, survival, and apoptosis (17). They are often associated
23
24 152 with resistance to therapy (18, 19). Whilst miRNAs are known to show a specific
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27 153 expression pattern in NETs (20), little is known about differential miRNA profiles in
28
29 154 NEC patients. At present, no data are available on the deregulation of specific miRNAs
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32 155 in this setting.

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34 156 In a study recently published by our group on GEP-NEC patients undergoing
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36 157 first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months
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38 158 ($p < 0.01$) in patients with Ki-67 value between 20% and 50% or $>50\%$, respectively
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40 159 (19). Median (m)OS was 8.1 months in the latter group but was not reached in the
41
42 160 former group ($p = 0.039$). Patients with a positive ^{68}Ga -PET/CT had a higher 18-month
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44 161 OS rate than those with a negative scan (75% vs. 34.3%, respectively), but the
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46 162 difference was not statistically significant ($p = 0.06$). Our data highlighted that ^{68}Ga -
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48 163 PET/CT positivity may be a discriminating factor (16, 21) in predicting prognosis,
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50 164 especially in the metastatic setting where histological material is not always available
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52 165 for evaluation. Furthermore, ^{18}F fluorodeoxyglucose (^{18}F FDG)-PET/CT may be useful to
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55 166 discriminate between patients with different prognosis (22).
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4 167 Given the above premises, we decided to investigate the efficacy and safety of
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6 168 second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression
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9 169 after first-line platinum-based treatment. We also aimed to study the serum miRNA
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11 170 profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1,
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14 171 patient prognosis and response to therapy, and to assess the prognostic and predictive
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16 172 role of ¹⁸FDG-PET/CT, ⁶⁸Ga-PET/CT and Ki-67 score.
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22 23 175 **Methods and Analysis**

24 25 26 176 Study design

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28
29 177 The SENECA study is a multicentre randomised non-comparative phase II study
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31 178 (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin
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34 179 (lung or gastroenteropancreatic) in progression after first-line treatment are randomized
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36 180 to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or
37
38 181 unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until
39
40 182 progression or unacceptable toxicity.

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43 183 The treatment arms are as follows:
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46 47 185 FOLFIRI regimen

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49
50 186 • Irinotecan 180 mg/m², given as a 60-min. intravenous (i.v.) infusion on day 1 every
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52 187 2 weeks followed by
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54 188 • Leucovorin 200 mg/m², given as a 2-h i.v. infusion on day 1 every 2 weeks followed
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4 190 • 5-fluorouracil (5-FU) 400 mg/m² given as bolus, and then 5-FU 2400 mg/m² given
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6 191 as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a
7
8 192 maximum of 12 cycles.

193

194 CAPTEM regimen

195 Capecitabine 750 mg/m² twice a day on days 1-14 in combination with temozolomide
196 200 mg/m² daily on days 10-14, every 4 weeks, until progression or for a maximum of
197 6 cycles.

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

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4 214 taking part in another clinical trial with any investigational agent < 30 days prior to
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6 215 study screening or with a history of allergic reactions attributable to compounds of
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8 216 similar chemical or biological composition are excluded. Patients who have undergone
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10 217 chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C)
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12 218 prior to entering the study, have not recovered from adverse events caused by agents
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14 219 administered > 4 weeks earlier, or have known brain metastases are not eligible for the
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16 220 study. Patients with other malignancies with a disease-free interval of < 5 years (with
17
18 221 the exception of non-melanoma skin cancer or low-grade superficial bladder cancer) are
19
20 222 excluded, as are those with any severe and/or uncontrolled medical condition or other
21
22 223 condition that could affect their participation in the study such as:

- 23 224 • unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction
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25 225 < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or
26
27 226 any other clinically significant cardiac disease;
- 28 227 • severely impaired lung function (spirometry and DLCO 50% of the normal predicted
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30 228 value and/or oxygen saturation \leq 88% at rest, in room air);
- 31 229 • uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of
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33 230 normal;
- 34 231 • any active (acute or chronic) or uncontrolled infections/disorders.

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36 232 Tumour evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest,
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38 233 abdomen, pelvis, and any additional known sites of disease. These tests are performed
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40 234 at baseline, every three months during treatment as per national regulatory agency
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42 235 indications, and after the end of treatment in non-progressing patients until progression.
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44 236 It is recommended that ^{68}Ga -PET/CT and ^{18}F FDG-PET/CT scans be performed at
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46 237 baseline or a maximum of 90 days before study enrollment. An EORTC quality of life
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4 238 questionnaire is administered at baseline and every three months thereafter during the
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6 239 treatment period.
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10 241 Study endpoints

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14 242 The primary endpoint of the study is the DCR of each treatment, defined as the
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16 243 percentage of patients who have achieved complete or partial response or stable disease
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18 244 for ≥ 12 weeks from the start of therapy. DCR will be evaluated using the new
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20 245 international criteria proposed by the RECIST version 1.1. Acute and late toxicity will
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22 246 be assessed by CTCAE Version 4.03, the latter defined as toxicity occurring at least
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24 247 30 days after the end of the last treatment cycle. Secondary endpoints are OS, calculated
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26 248 from the start of treatment to death from any cause, and PFS, calculated from the start of
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28 249 treatment to the date of the first documented evidence of disease progression or of death
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30 250 from any cause. All the analyses will be performed in the intention-to-treat population.
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32 251 Patients without events at the time of analysis will be censored at their last-known-alive
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34 252 date for OS and at their last date of tumour evaluation for PFS. A further secondary
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36 253 endpoint is the evaluation of quality of life using the European Organization for the
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38 254 Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30).
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40 255 When data are available, the impact of baseline ^{68}Ga -PET/CT and ^{18}F FDG-PET/CT on
41
42 256 PFS will be analysed with exploratory intent. After signing the informed consent for
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44 257 biomarker assessment, patients will undergo evaluation of the mutation status of MEN1,
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46 258 DAXX, ATRX and RB-1 in primary tumour tissue and of miRNA in blood samples.
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48 259 Assessment of the miRNA profile will be performed on the first 20 patients who agree
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50 260 to participate in the biological part of the study.
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262 Ethical considerations

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

269 Patient and public involvement

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

273 Statistical methods

274 The Bryant and Day design is used to estimate a sample size that takes into account
275 both treatment activity and toxicity. Although randomisation is used to allocate patients
276 to the two arms, no formal statistical comparisons between treatment regimens are
277 planned. The purpose of randomisation is to reduce bias due to patient assignment to a
278 specific treatment arm. The hypothesis for the control arm is based on literature data
279 (23, 24).

280 An α level of 0.10 (both for toxicity and DCR) and a power of 90% have been
281 adopted. A DCR rate $\geq 60\%$ and a relevant toxicity rate $\leq 20\%$ are considered acceptable
282 rates, while a DCR rate $\leq 40\%$ and a relevant toxicity rate $\geq 40\%$ are considered
283 unacceptable rates. Given these hypotheses, the first step of the study will require
284 25 patients. If ≥ 10 patients with a DCR are observed and ≥ 15 patients do not have

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4 285 significant toxicity, the study will enrol patients in the next step. A total of 53 patients
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6 286 will be enrolled. If ≥ 25 patients with DCR and ≥ 36 patients without any relevant
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8 287 toxicity are observed, treatment will be considered active and not toxic. This design is
9
10 288 used for each treatment scheme and all analyses will be performed separately. If one of
11
12 289 the schemes does not obtain the expected proportions of the first step, the arm will be
13
14 290 closed and patients will be enrolled in the other arm until the target is reached; if the
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16 291 expected proportions are not reached in any arm, the study will be prematurely closed.
17
18 292 If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in
19
20 293 each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each
21
22 294 arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopenia,
23
24 295 prolonged G3-G4 neutropenia (> 7 days) and drug-related hospitalisations are
25
26 296 considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55
27
28 297 % vs. $>55\%$) and site of primary tumour (lung vs. GEP). A subgroup analysis of the
29
30 298 efficacy of both treatments according to these stratification factors has been planned.
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36 299 Complete response, partial response or stable disease for at least 12 weeks will be
37
38 300 considered as the DCR. The proportion of patients in this category will be determined
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40 301 and 95% CIs for the DCR will be calculated. OS and PFS will be estimated using the
41
42 302 Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate statistical analyses will be
43
44 303 performed on the basis of the data available to compare QLQ-C30 scores between
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46 304 baseline and subsequent follow-up visits.
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50 305 When data are available, the impact of ^{68}Ga -PET/CT result on PFS will be analysed
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52 306 with exploratory intent. The Shapiro-Wilk test will be used to determine the normality
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54 307 distribution of each clinical, demographic and biological biomarker (25). In the event
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56 308 of a non-normal distribution, nonparametric statistics will be used to analyse the
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4 309 relationship between the serum levels of each marker, considered as continuous
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6 310 variables, and response to treatment. In the event of normal biomarker distribution,
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9 311 a parametric test will be used. All endpoints will be analysed separately for each
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11 312 treatment group.

13 313 **Discussion**

16 314 There is still no truly effective second-line chemotherapy for neuroendocrine
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18 315 carcinoma. Overall prognosis of patients is poor, with an OS of 5 months in the
19
20 316 metastatic setting according to SEER (Surveillance, Epidemiology, and End Results)
21
22 317 data (26). Only 5% of all patients are long-term survivors. There is also a marked lack
23
24 318 of prognostic and predictive factors (5).

27 319 Three phase II studies registered at *ClinicalTrials.gov* are currently investigating
28
29 320 second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab
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31 321 (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab
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33 322 (NCT03147404). Some abstracts were presented at ESMO (European Society for
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35 323 Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019
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37 324 on the use of immunotherapy in GEP-NECs, all showing inconclusive results. The
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39 325 SENECA study uses a promising approach to the treatment of patients with metastatic
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41 326 NECs. First, both the activity and safety of 2 regimens are assessed in the same setting
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43 327 with a sizeable patient population (56 patients/arm). In addition, patients are stratified
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45 328 according to Ki-67 index and morphology to investigate the role of each treatment
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47 329 combination in both poorly differentiated and well differentiated NECs. Another aim of
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49 330 this study is to integrate both biological and metabolic imaging data in an effort to
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51 331 improve the current GEP-NEC classification.
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4 332 The duration of treatments in the metastatic setting is a dilemma in NENs and
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6 333 especially in neuroendocrine carcinomas. Given the lack of evidence-based
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9 334 recommendations on treatment duration of second-line chemotherapy in NECs, we
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11 335 decided to opt for a fixed duration of treatments to avoid unnecessary exposure to
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13 336 cytotoxic agents and consequent bone marrow reserve depletion (27).

15 337 In conclusion, there are still no FDA/EMA (Food and Drug Administration/European
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18 338 Medicines Agency)-approved second-line therapeutic options for patients with
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20 339 metastatic NECs, and the SENECA trial could represent a step forward in finding novel
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22 340 therapies to prolong survival and maintain quality of life. Moreover, the integration of
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24 341 biological and imaging data could -lead to a better understanding of the natural history
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26 342 of the disease and help to identify potential responders.

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34 345 **Confidentiality**

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36 346 This study will be conducted in full conformity with ICH (The International Council for
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38 347 Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)
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40 348 Guidelines for Good Clinical Practice, Directive 2001/20/EEC of the European
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42 349 Parliament and other relevant current local legislation. Participants will be allocated a
43
44 350 unique identification (ID) number at entry. The master list linking participant personal
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46 351 information and ID number will be maintained in a separate locked cabinet and
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48 352 password-protected hard-drive. Data will be analysed by ID number only. Patient files
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50 353 and other source data will for be kept a maximum of 15 years.

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4 **356 Ethics and Dissemination**
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6 357 The SENECA trial, supported by IRST, involves several Italian centres and was
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8 358 authorised by the local Ethics Committees of the centres taking part and by the Italian
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10 359 Medicines Agency (AIFA). After completing the study, all data, including beneficial
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12 360 and adverse events, of the trial will be communicated at scientific meetings and
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14 361 published in indexed peer-reviewed journals. If shown to be effective, the therapy
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16 362 program will be made available to the general public in an appropriate manner.
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25 **365 Abbreviations**
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27 366 CAPTEM, capecitabine plus temozolomide; DCR, disease control rate; GEP,
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29 367 gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour;
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31 368 OS, overall survival; PFS, progression-free survival
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36 **370 Author contributions**
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38 371 AB, CL and TI designed the study and drafted the article. AB was responsible for data
39
40 372 acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a
41
42 373 clinical information data extraction method for the protocol. FF performed the statistical
43
44 374 analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual
45
46 375 content. All authors read and approved the present version of the manuscript for
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48 376 submission.
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4 380 **Data availability statement**
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6 381 The datasets used and analysed in the present study are available from the
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8
9 382 corresponding author on reasonable request.
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11 383

12
13 384 **Funding**
14

15 385 The study was conducted in the absence of any commercial or financial relationships
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17
18 386 that could be construed as a potential conflict of interest.
19

20 387

21
22 388 **Conflicts of interest**
23

24
25 389 The authors declare no conflict of interest.
26

27 390

28
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30

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35
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25 501 **Figure legend**

26
27 502 **FIGURE 1** | SENECA study design.

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Fig. 1

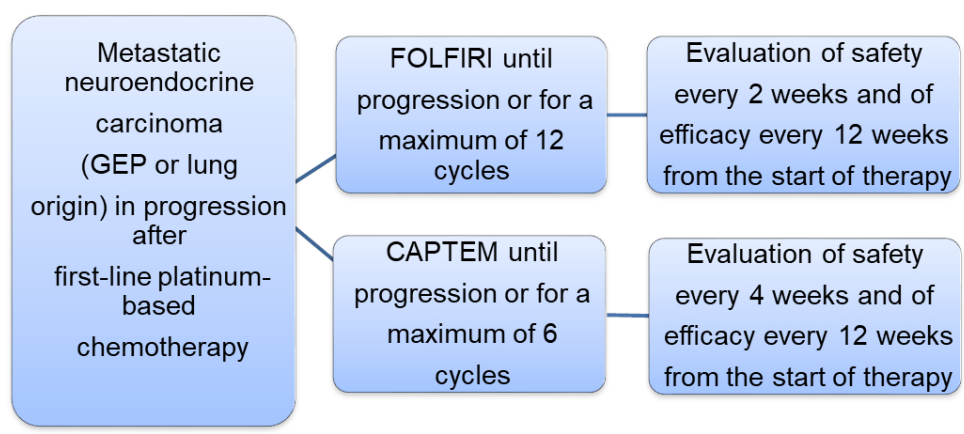


FIGURE 1 | SENECA study design.

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	2a	Scientific background and explanation of rationale	5-7
	2b	Specific objectives or hypotheses	7
Methods	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
	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10, 11
Outcomes	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
	7a	How sample size was determined	12
Sample size	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
	8a	Method used to generate the random allocation sequence	12
Randomisation:	8b	Type of randomisation; details of any restriction (such as blocking and block size)	12
	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
	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	N/A

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assessing outcomes) and how

11b If relevant, description of the similarity of interventions N/A

12a Statistical methods used to compare groups for primary and secondary outcomes N/A

12b Methods for additional analyses, such as subgroup analyses and adjusted analyses 12, 13

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 N/A

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, 15

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.

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1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1	Title
2	Trial identifier and registry name. If not yet registered, name of intended registry	2a	Trial registration
3	All items from the World Health Organization Trial Registration Data Set	2b	
4	Date and version identifier	3	Protocol version
5	Sources and types of financial, material, and other support	4	Funding
6	Names, affiliations, and roles of protocol contributors	5a	Roles and responsibilities
7	Name and contact information for the trial sponsor	5b	
8	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	5c	
9	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)	5d	
10	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	6a	Background and rationale
11	Explanation for choice of comparators	6b	
12	Specific objectives or hypotheses	7	Objectives
13	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	Trial design

Introduction

Administrative information

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



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Methods: Participants, interventions, and outcomes		
9	Study setting	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
10	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)
11a	Interventions	11a Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
11b		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		11c Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
11d		11d Relevant concomitant care and interventions that are permitted or prohibited during the trial
12	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
13	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)
14	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
15	Recruitment	15 Strategies for achieving adequate participant enrolment to reach target sample size
Methods: Assignment of interventions (for controlled trials)		
Allocation:		
16a	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

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5			level dataset, and statistical code
6	31c	Plans, if any, for granting public access to the full protocol, participant-	
7			writers
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9	31b	Authorship eligibility guidelines and any intended use of professional	
10			
11			data sharing arrangements), including any publication restrictions
12			groups (eg, via publication, reporting in results databases, or other
13			participants, healthcare professionals, the public, and other relevant
14	31a	Plans for investigators and sponsor to communicate trial results to	Dissemination policy
15			
16			compensation to those who suffer harm from trial participation
17	30	Provisions, if any, for ancillary and post-trial care, and for	Ancillary and post-trial care
18			
19			investigators
20			disclosure of contractual agreements that limit such access for
21			Statement of who will have access to the final trial dataset, and
22	29		Access to data
23			the overall trial and each study site
24			Financial and other competing interests for principal investigators for
25	28		Declaration of interests
26			before, during, and after the trial
27			be collected, shared, and maintained in order to protect confidentiality
28			How personal information about potential and enrolled participants will
29	27		Confidentiality
30			and biological specimens in ancillary studies, if applicable
31			Additional consent provisions for collection and use of participant data
32	26b		
33			participants or authorised surrogates, and how (see item 32)
34			Who will obtain informed consent or assent from potential trial
35	26a		Consent or assent
36			regulators)
37			(eg, investigators, REC/IRBs, trial participants, trial registries, journals,
38			changes to eligibility criteria, outcomes, analyses) to relevant parties
39			Plans for communicating important protocol modifications (eg,
40	25		Protocol amendments
41			(REC/IRB) approval
42			Plans for seeking research ethics committee/institutional review board
43	24		Research ethics approval
44			
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48			Ethics and dissemination
49			
50			sponsor
51			whether the process will be independent from investigators and the
52	23		Auditing
53			Frequency and procedures for auditing trial conduct, if any, and
54			of trial interventions or trial conduct
55			spontaneously reported adverse events and other unintended effects
56			Plans for collecting, assessing, reporting, and managing solicited and
57	22		Harms
58			decision to terminate the trial
59			who will have access to these interim results and make the final
60	21b	Description of any interim analyses and stopping guidelines, including	

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Appendices

Model consent form and other related documentation given to participants and authorised surrogates	32	Informed consent
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	33	Biological specimens

*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 have been advised when applicable.
The responsible author.

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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:	<i>BMJ Open</i>
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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Oncology
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

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4 1 **CLINICAL STUDY PROTOCOL**5
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9 3 **Randomized Phase II Trial of CAPTEM or FOLFIRI as**
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11 4 **SEcond-line Therapy in NEuroendocrine CARcinomas and**
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13 5 **Exploratory Analysis of Predictive Role of PET/CT Imaging**
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15 6 **and Biological Markers (SENECA Trial): A Study Protocol**16
17 7 **Running title: Second-line CAPTEM/FOLFIRI in NECs**18
19 820
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30 37 **Language style:** This article is formatted in British English.
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4 73 **Ethics and dissemination:** The SENECA trial, supported by IRST, was authorised by
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6 74 the local Ethics Committee and the Italian Medicines Agency (AIFA). Results will be
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9 75 widely disseminated via peer-reviewed manuscripts, conference presentations and
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11 76 reports to relevant authorities.
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14 77
15 78 The study is currently open in Italy. ClinicalTrials.gov Identifier: NCT03387592.

16 79 EudraCT number: 2016-000767-17

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18 80 Protocol version: Clinical Study Protocol Version 1, 07.11.2016.
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23 82 **Keywords:** neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine,
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26 83 temozolomide, CAPTEM
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28 29 84 **Strengths and limitations of the study**

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31 85
- 32 • the SENECA trial randomises patients to receive two different treatments,
33 FOLFIRI or CAPTEM, providing important information on the activity of both
34 86 combinations in different NEC subtypes (NET grade (G) 3 and NEC G3).
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36 87
 - 37 • The SENECA trial analyses the role of miRNAs and other biological markers as
38 88 prognostic and predictive factors. A further aim is to assess ⁶⁸Ga-PET/CT as a
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40 89 tool to improve current histological classification.
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44 91 The major limitations of the study are:

- 45
46 92 • The rarity of the disease and patient prognosis. However, the involvement of
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48 93 several Italian centres will hopefully help to overcome this problem.
- 49
50 94 • Poor prognosis of NEC patients. Patients progressing on platinum chemotherapy
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52 95 usually have rapid deterioration of clinical conditions
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98 Introduction

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

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

113 Given the rarity of this disease, prospective clinical data are lacking and treatment
114 recommendations are essentially expert-based opinions. Two phase II studies
115 investigating the second-line treatment of GEP-NECs are currently registered at
116 *ClinicalTrials.gov*, one evaluating the safety and tolerability of everolimus in
117 40 patients with G3 NEC/NET or G1/G2 NET switching to G3 NEN (National Clinical
118 Trial identifier NCT02113800) and the other investigating the efficacy of avelumab
119 (NCT03147404). A French study focusing on the identification of predictive molecular
120 markers of response to sunitinib in poorly differentiated digestive NETs

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4 121 (NCT01215578) has now closed recruitment and results are eagerly awaited. Another
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6 122 French multicentre prospective phase II trial is currently ongoing to investigate the
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8 123 efficacy of the bevacizumab-FOLFIRI combination after progression on a
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10 124 platinum/etoposide combination (7).

13 125 Different second-line chemotherapy combinations have been evaluated but shown
14
15 126 poor results (8-10). In a monocentre retrospective clinical trial, Hentic et al.
16
17 127 hypothesised the potential efficacy of FOLFIRI as second-line chemotherapy in
18
19 128 19 patients with G3 extra-pulmonary NECs (11). An objective response rate was
20
21 129 obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median PFS
22
23 130 and OS were 4 and 18 months, respectively.

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27 131 In another retrospective study, a 71% DCR was obtained with temozolomide-based
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29 132 chemotherapy in 25 patients with metastatic poorly differentiated endocrine carcinoma
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31 133 of different sites and atypical bronchial carcinoid with Ki-67 > 20%. Small cell lung
32
33 134 carcinoma and large cell lung carcinoma were excluded. A PFS of 12 months (95%
34
35 135 confidence interval [CI], 5.5- 24) and OS of 22 months (95% CI, 12-31) were reported
36
37 136 in patients who responded to treatment or showed stable disease (SD), whereas OS was
38
39 137 only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response
40
41 138 rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with
42
43 139 high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive
44
45 140 staining for chromogranin A. Both factors are often associated with more highly
46
47 141 differentiated tumours (12).

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50 142 Literature data on lung NECs in progression after first-line chemotherapy are based
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52 143 on small patient series (13). Moreover, there is increasing evidence of some
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54 144 discrepancies in the current grading of NECs, highlighting the need for more accurate
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4 145 biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise two
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6 146 distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived
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8 147 from well differentiated neuroendocrine tumours (NETs) and characterised by
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10 148 mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from
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12 149 neuroendocrine-differentiated adenocarcinomas and characterised by a mutation in
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14 150 RB1. Both subgroups display a distinct prognosis and different sensitivity to
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16 151 chemotherapy (14-16). Micro(mi)RNAs are a class of small, non-coding, highly
17
18 152 conserved single-stranded RNAs involved in the post-transcriptional regulation of cell
19
20 153 proliferation, differentiation, survival, and apoptosis (17). They are often associated
21
22 154 with resistance to therapy (18, 19). Whilst miRNAs are known to show a specific
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24 155 expression pattern in NETs (20), little is known about differential miRNA profiles in
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26 156 NEC patients. At present, no data are available on the deregulation of specific miRNAs
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28 157 in this setting.

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34 158 In a study recently published by our group on GEP-NEC patients undergoing
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36 159 first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months
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38 160 ($p < 0.01$) in patients with Ki-67 value between 20% and 50% or $>50\%$, respectively
39
40 161 (19). Median (m)OS was 8.1 months in the latter group but was not reached in the
41
42 162 former group ($p = 0.039$). Patients with a positive ^{68}Ga -PET/CT had a higher 18-month
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44 163 OS rate than those with a negative scan (75% vs. 34.3%, respectively), but the
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46 164 difference was not statistically significant ($p = 0.06$). Our data highlighted that ^{68}Ga -
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48 165 PET/CT positivity may be a discriminating factor (16, 21) in predicting prognosis,
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50 166 especially in the metastatic setting where histological material is not always available
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52 167 for evaluation. Furthermore, ^{18}F fluorodeoxyglucose (^{18}F FDG)-PET/CT may be useful to
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54 168 discriminate between patients with different prognosis (22).
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4 169 Given the above premises, we decided to investigate the efficacy and safety of
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6 170 second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression
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9 171 after first-line platinum-based treatment. We also aimed to study the serum miRNA
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11 172 profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1,
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13 173 patient prognosis and response to therapy, and to assess the prognostic and predictive
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15 174 role of ¹⁸F-FDG-PET/CT, ⁶⁸Ga-PET/CT and Ki-67 score.
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22 23 177 **Methods and Analysis**

24 25 26 178 Study design

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29 179 The SENECA study is a multicentre randomised non-comparative phase II study
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31 180 (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin
32
33 181 (lung or gastroenteropancreatic) in progression after first-line treatment are randomized
34
35 182 to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or
36
37 183 unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until
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39 184 progression or unacceptable toxicity.
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41

42 185 The treatment arms are as follows:
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46 47 187 FOLFIRI regimen

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49 188 • Irinotecan 180 mg/m², given as a 60-min. intravenous (i.v.) infusion on day 1 every
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51 189 2 weeks followed by
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53 190 • Leucovorin 200 mg/m², given as a 2-h i.v. infusion on day 1 every 2 weeks followed
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4 192 • 5-fluorouracil (5-FU) 400 mg/m² given as bolus, and then 5-FU 2400 mg/m² given
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6 193 as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a
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8 194 maximum of 12 cycles.

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12 13 196 CAPTEM regimen

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16 197 Capecitabine 750 mg/m² twice a day on days 1-14 in combination with temozolomide
17
18 198 200 mg/m² daily on days 10-14, every 4 weeks, until progression or for a maximum of
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20
21 199 6 cycles.

22
23 200 The study includes patients aged ≥ 18 years with a histological diagnosis of G3
24
25 201 neuroendocrine carcinoma (GEP-NEC and lung NEC) according to the 2010 and 2015
26
27 202 GEP and Lung NEN WHO classifications, respectively, Ki-67 $>20\%$ and measurable
28
29 203 disease according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria.
30
31 204 All patients must have an Eastern Cooperative Oncology Group (ECOG) performance
32
33 205 status ≤ 2 with a life expectancy > 3 months and must have already undergone first-line
34
35 206 treatment for metastatic disease with platinum -based chemotherapy
36
37 207 (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological,
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39 208 liver and renal function is required and effective contraceptive methods must be used by
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41 209 female patients of childbearing age. Written informed consent is obtained from all
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43 210 patients to take part in the study. Exclusion criteria are as follows: metastatic NECs
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45 211 previously treated with an irinotecan or temozolomide regimen, known hypersensitivity
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47 212 to 5-FU/capecitabine, calcium levofolinate, irinotecan or their recipients. All acute
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49 213 toxic effects of any prior therapy (including surgery, radiation therapy and
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51 214 chemotherapy) must have resolved to grade ≤ 1 according to National Cancer Institute
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53 215 Common Terminology Criteria for Adverse Events version 4.03 (CTCAE). Patients
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4 216 taking part in another clinical trial with any investigational agent < 30 days prior to
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6 217 study screening or with a history of allergic reactions attributable to compounds of
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8 218 similar chemical or biological composition are excluded. Patients who have undergone
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10 219 chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C)
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12 220 prior to entering the study, have not recovered from adverse events caused by agents
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14 221 administered > 4 weeks earlier, or have known brain metastases are not eligible for the
15
16 222 study. Patients with other malignancies with a disease-free interval of < 5 years (with
17
18 223 the exception of non melanoma skin cancer or low-grade superficial bladder cancer) are
19
20 224 excluded, as are those with any severe and/or uncontrolled medical condition or other
21
22 225 condition that could affect their participation in the study such as:

- 23 226 • unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction
24 227 < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or
25 228 any other clinically significant cardiac disease;
- 26 229 • severely impaired lung function (spirometry and DLCO 50% of the normal predicted
27 230 value and/or oxygen saturation \leq 88% at rest, in room air);
- 28 231 • uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of
29 232 normal;
- 30 233 • any active (acute or chronic) or uncontrolled infections/disorders.

31 234 Tumour evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest,
32 235 abdomen, pelvis, and any additional known sites of disease. These tests are performed
33 236 at baseline, every three months during treatment as per national regulatory agency
34 237 indications, and after the end of treatment in non-progressing patients until progression.
35 238 It is recommended that ^{68}Ga -PET/CT and ^{18}F FDG-PET/CT scans be performed at
36 239 baseline or a maximum of 90 days before study enrollment. An EORTC quality of life
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4 240 questionnaire is administered at baseline and every three months thereafter during the
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6 241 treatment period.

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10 243 Study endpoints

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14 244 The primary endpoint of the study is the DCR of each treatment, defined as the
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16 245 percentage of patients who have achieved complete or partial response or stable disease
17
18 246 for ≥ 12 weeks from the start of therapy. DCR will be evaluated using the new
19
20 247 international criteria proposed by the RECIST version 1.1. Acute and late toxicity will
21
22 248 be assessed by CTCAE Version 4.03, the latter defined as toxicity occurring at least
23
24 249 30 days after the end of the last treatment cycle. Secondary endpoints are OS, calculated
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26 250 from the start of treatment to death from any cause, and PFS, calculated from the start of
27
28 251 treatment to the date of the first documented evidence of disease progression or of death
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30 252 from any cause. All the analyses will be performed in the intention-to-treat population.
31
32 253 Patients without events at the time of analysis will be censored at their last-known-alive
33
34 254 date for OS and at their last date of tumour evaluation for PFS. A further secondary
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36 255 endpoint is the evaluation of quality of life using the European Organization for the
37
38 256 Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30).
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40 257 When data are available, the impact of baseline ^{68}Ga -PET/CT and ^{18}F FDG-PET/CT on
41
42 258 PFS will be analysed with exploratory intent. After signing the informed consent for
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44 259 biomarker assessment, patients will undergo evaluation of the mutation status of MEN1,
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46 260 DAXX, ATRX and RB-1 in primary tumour tissue and of miRNA in blood samples.
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48 261 Assessment of the miRNA profile will be performed on the first 20 patients who agree
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50 262 to participate in the biological part of the study.
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264 Ethical considerations

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

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

275 Patient and public involvement

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

279 Statistical methods

280 The Bryant and Day design is used to estimate a sample size that takes into account
281 both treatment activity and toxicity. Although randomisation is used to allocate patients
282 to the two arms, no formal statistical comparisons between treatment regimens are
283 planned. The purpose of randomisation is to reduce bias due to patient assignment to a
284 specific treatment arm. The hypothesis for the control arm is based on literature data
285 (23, 24).

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

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4 287 adopted. A DCR rate $\geq 60\%$ and a relevant toxicity rate $\leq 20\%$ are considered acceptable
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6 288 rates, while a DCR rate $\leq 40\%$ and a relevant toxicity rate $\geq 40\%$ are considered
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8 289 unacceptable rates. Given these hypotheses, the first step of the study will require
9
10 290 25 patients. If ≥ 10 patients with a DCR are observed and ≥ 15 patients do not have
11
12 291 significant toxicity, the study will enrol patients in the next step. A total of 53 patients
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14 292 will be enrolled. If ≥ 25 patients with DCR and ≥ 36 patients without any relevant
15
16 293 toxicity are observed, treatment will be considered active and not toxic. This design is
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18 294 used for each treatment scheme and all analyses will be performed separately. If one of
19
20 295 the schemes does not obtain the expected proportions of the first step, the arm will be
21
22 296 closed and patients will be enrolled in the other arm until the target is reached; if the
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24 297 expected proportions are not reached in any arm, the study will be prematurely closed.
25
26 298 If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in
27
28 299 each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each
29
30 300 arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopenia,
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32 301 prolonged G3-G4 neutropenia (> 7 days) and drug-related hospitalisations are
33
34 302 considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55
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36 303 % vs. $> 55\%$) and site of primary tumour (lung vs. GEP). A subgroup analysis of the
37
38 304 efficacy of both treatments according to these stratification factors has been planned.
39
40 305 Complete response, partial response or stable disease for at least 12 weeks will be
41
42 306 considered as the DCR. The proportion of patients in this category will be determined
43
44 307 and 95% CIs for the DCR will be calculated. OS and PFS will be estimated using the
45
46 308 Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate statistical analyses will be
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48 309 performed on the basis of the data available to compare QLQ-C30 scores between
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50 310 baseline and subsequent follow-up visits.
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4 311 When data are available, the impact of ⁶⁸Ga-PET/CT result on PFS will be analysed
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6 312 with exploratory intent. The Shapiro-Wilk test will be used to determine the normality
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8 313 distribution of each clinical, demographic and biological biomarker (25). In the event
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10 314 of a non-normal distribution, nonparametric statistics will be used to analyse the
11
12 315 relationship between the serum levels of each marker, considered as continuous
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14 316 variables, and response to treatment. In the event of normal biomarker distribution,
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16 317 a parametric test will be used. All endpoints will be analysed separately for each
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18 318 treatment group.
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23 **Discussion**

24
25 320 There is still no truly effective second-line chemotherapy for neuroendocrine
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27 321 carcinoma. Overall prognosis of patients is poor, with an OS of 5 months in the
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29 322 metastatic setting according to SEER (Surveillance, Epidemiology, and End Results)
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31 323 data (26). Only 5% of all patients are long-term survivors. There is also a marked lack
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33 324 of prognostic and predictive factors (5).
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37 325 Three phase II studies registered at *ClinicalTrials.gov* are currently investigating
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39 326 second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab
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41 327 (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab
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43 328 (NCT03147404). Some abstracts were presented at ESMO (European Society for
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45 329 Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019
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47 330 on the use of immunotherapy in GEP-NECs, all showing inconclusive results. The
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49 331 SENECA study uses a promising approach to the treatment of patients with metastatic
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51 332 NECs. First, both the activity and safety of 2 regimens are assessed in the same setting
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53 333 with a sizeable patient population (56 patients/arm). In addition, patients are stratified
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55 334 according to Ki-67 index and morphology to investigate the role of each treatment
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4 335 combination in both poorly differentiated and well differentiated NECs. Another aim of
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6 336 this study is to integrate both biological and metabolic imaging data in an effort to
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9 337 improve the current GEP-NEC classification.

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11 338 The duration of treatments in the metastatic setting is a dilemma in NENs and
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13 339 especially in neuroendocrine carcinomas. Given the lack of evidence-based
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15 340 recommendations on treatment duration of second-line chemotherapy in NECs, we
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17 341 decided to opt for a fixed duration of treatments to avoid unnecessary exposure to
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20 342 cytotoxic agents and consequent bone marrow reserve depletion (27).

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23 343 In conclusion, there are still no FDA/EMA (Food and Drug Administration/European
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25 344 Medicines Agency)-approved second-line therapeutic options for patients with
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27 345 metastatic NECs, and the SENECA trial could represent a step forward in finding novel
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29 346 therapies to prolong survival and maintain quality of life. Moreover, the integration of
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31 347 biological and imaging data could -lead to a better understanding of the natural history
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33 348 of the disease and help to identify potential responders.

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40 41 351 **Confidentiality**

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43 352 This study will be conducted in full conformity with ICH (The International Council for
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45 353 Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)
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47 354 Guidelines for Good Clinical Practice, Directive 2001/20/EEC of the European
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49 355 Parliament and other relevant current local legislation. Participants will be allocated a
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51 356 unique identification (ID) number at entry. The master list linking participant personal
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53 357 information and ID number will be maintained in a separate locked cabinet and
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4 358 password-protected hard-drive. Data will be analysed by ID number only. Patient files
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6 359 and other source data will for be kept a maximum of 15 years.
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12 13 362 **Ethics and Dissemination**

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15 363 The SENECA trial, supported by IRST, involves several Italian centres and was
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17 364 authorised by the local Ethics Committees of the centres taking part and by the Italian
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19 365 Medicines Agency (AIFA) (see list of all centers in the supplementary table 1). After
20
21 366 completing the study, all data, including beneficial and adverse events, of the trial will
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23 367 be communicated at scientific meetings and published in indexed peer-reviewed
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25 368 journals. If shown to be effective, the therapy program will be made available to the
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27 369 general public in an appropriate manner.
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35 36 372 **Abbreviations**

37
38 373 CAPTEM, capecitabine plus temozolomide; DCR, disease control rate; GEP,
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40 374 gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour;
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42 375 OS, overall survival; PFS, progression-free survival
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49 377 **Author contributions**

50 378 AB, CL and TI designed the study and drafted the article. AB was responsible for data
51
52 379 acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a
53
54 380 clinical information data extraction method for the protocol. FF performed the statistical
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56 381 analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual
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4 382 content. All authors read and approved the present version of the manuscript for
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6 383 submission.
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16 387 **Data availability statement**

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18 388 The datasets used and analysed in the present study are available from the
19
20 389 corresponding author on reasonable request.
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24
25 391 **Funding**

26
27 392 The study was conducted in the absence of any commercial or financial relationships
28
29 393 that could be construed as a potential conflict of interest.
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34 395 **Conflicts of interest**

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36 396 The authors declare no conflict of interest.
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41 398 **Acknowledgements**

42
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44
45 400 Silvestris, Francesco Silvestris, Angela Buonadonna and Giuseppe Badalamenti.

46
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25 508 **Figure legend**

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27 509 **FIGURE 1** | SENECA study design.
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Fig. 1

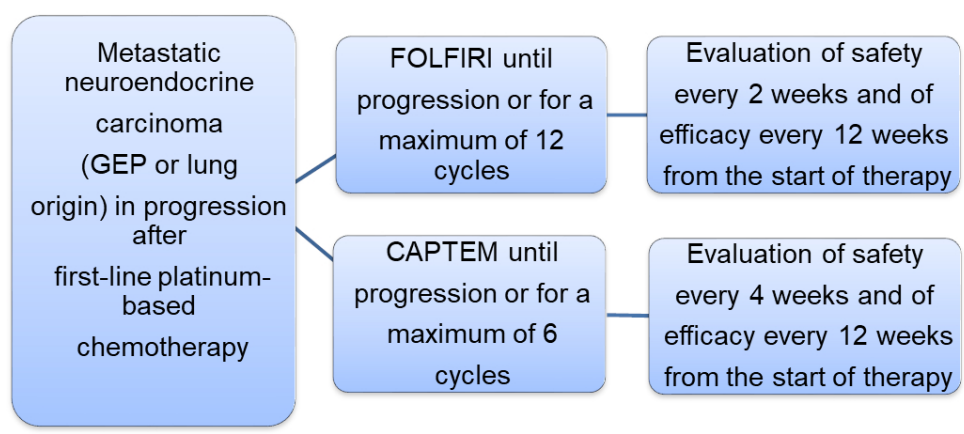


FIGURE 1 | SENECA study design.

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
- 1b Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)

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Introduction

- 2a Scientific background and explanation of rationale
- 2b Specific objectives or hypotheses

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Methods

Trial design

- 3a Description of trial design (such as parallel, factorial) including allocation ratio
- 3b Important changes to methods after trial commencement (such as eligibility criteria), with reasons

8-11

Participants

- 4a Eligibility criteria for participants
- 4b Settings and locations where the data were collected

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9

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
- 6b Any changes to trial outcomes after the trial commenced, with reasons

10, 11
N/A

Sample size

- 7a How sample size was determined
- 7b When applicable, explanation of any interim analyses and stopping guidelines

12
N/A

Randomisation:

- 8a Method used to generate the random allocation sequence
- 8b Type of randomisation; details of any restriction (such as blocking and block size)

12
12

- 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

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- 11a If done, who was blinded after assignment to interventions (for example, participants, care providers, those

N/A

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Statistical methods
 11b assessing outcomes) and how
 12a If relevant, description of the similarity of interventions
 12b Statistical methods used to compare groups for primary and secondary outcomes
 Methods for additional analyses, such as subgroup analyses and adjusted analyses
 N/A
 12, 13
 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
 13b For each group, losses and exclusions after randomisation, together with reasons
 14a Dates defining the periods of recruitment and follow-up
 14b Why the trial ended or was stopped
 8, 12
 12
 N/A
 N/A

Baseline data
 15 A table showing baseline demographic and clinical characteristics for each group
 16 For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups
 N/A
 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)
 17b For binary outcomes, presentation of both absolute and relative effect sizes is recommended
 12
 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)
 12

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, 15

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.

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1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1	Title
2	Trial identifier and registry name. If not yet registered, name of intended registry	2a	Trial registration
3	All items from the World Health Organization Trial Registration Data Set	2b	
4	Date and version identifier	3	Protocol version
5	Sources and types of financial, material, and other support	4	Funding
6	Names, affiliations, and roles of protocol contributors	5a	Roles and responsibilities
7	Name and contact information for the trial sponsor	5b	
8	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	5c	
9	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)	5d	
10	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	6a	Background and rationale
11	Explanation for choice of comparators	6b	
12	Specific objectives or hypotheses	7	Objectives
13	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	Trial design

Introduction

Administrative information

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

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS



1			
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5			interventions
6			that is unavailable to those who enrol participants or assign
7			restriction (eg, blocking) should be provided in a separate document
8			To reduce predictability of a random sequence, details of any planned
9			generated random numbers), and list of any factors for stratification.
10		generation	
11	16a	Sequence	Method of generating the allocation sequence (eg, computer-
12			
13		Allocation:	
14			
15		Methods: Assignment of interventions (for controlled trials)	
16			
17			target sample size
18	15	Recruitment	Strategies for achieving adequate participant enrolment to reach
19			
20			assumptions supporting any sample size calculations
21			and how it was determined, including clinical and statistical
22	14	Sample size	Estimated number of participants needed to achieve study objectives
23			
24			diagram is highly recommended (see Figure)
25			washouts), assessments, and visits for participants. A schematic
26		timeline	
27	13	Participant	Time schedule of enrolment, interventions (including any run-ins and
28			
29			harm outcomes is strongly recommended
30			outcome. Explanation of the clinical relevance of chosen efficacy and
31			aggregation (eg, median, proportion), and time point for each
32			(eg, change from baseline, final value, time to event), method of
33			measurement variable (eg, systolic blood pressure), analysis metric
34			Primary, secondary, and other outcomes, including the specific
35	12	Outcomes	
36			
37			prohibited during the trial
38	11d		Relevant concomitant care and interventions that are permitted or
39			
40			laboratory tests)
41			procedures for monitoring adherence (eg, drug tablet return,
42			
43	11c		Strategies to improve adherence to intervention protocols, and any
44			
45			participant request, or improving/worsening disease)
46			given trial participant (eg, drug dose change in response to harms,
47	11b		Criteria for discontinuing or modifying allocated interventions for a
48			
49			including how and when they will be administered
50	11a	Interventions	Interventions for each group with sufficient detail to allow replication,
51			
52			interventions (eg, surgeons, psychotherapists)
53			criteria for study centres and individuals who will perform the
54	10	Eligibility criteria	Inclusion and exclusion criteria for participants. If applicable, eligibility
55			
56			
57			list of study sites can be obtained
58			and list of countries where data will be collected. Reference to where
59	9	Study setting	Description of study settings (eg, community clinic, academic hospital)
60			

Methods: Participants, interventions, and outcomes

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5			level dataset, and statistical code
6	31c	Plans, if any, for granting public access to the full protocol, participant-	
7			writers
8			
9	31b	Authorship eligibility guidelines and any intended use of professional	
10			
11			data sharing arrangements), including any publication restrictions
12			groups (eg, via publication, reporting in results databases, or other
13			participants, healthcare professionals, the public, and other relevant
14	31a	Plans for investigators and sponsor to communicate trial results to	Dissemination policy
15			
16			compensation to those who suffer harm from trial participation
17	30	Provisions, if any, for ancillary and post-trial care, and for	Ancillary and post-trial care
18			
19			investigators
20			
21			disclosure of contractual agreements that limit such access for
22	29	Statement of who will have access to the final trial dataset, and	Access to data
23			
24			the overall trial and each study site
25	28	Financial and other competing interests for principal investigators for	Declaration of interests
26			
27			before, during, and after the trial
28			be collected, shared, and maintained in order to protect confidentiality
29	27	How personal information about potential and enrolled participants will	Confidentiality
30			
31			and biological specimens in ancillary studies, if applicable
32	26b	Additional consent provisions for collection and use of participant data	
33			
34			participants or authorised surrogates, and how (see item 32)
35	26a	Who will obtain informed consent or assent from potential trial	Consent or assent
36			
37			regulators)
38			(eg, investigators, REC/IRBs, trial participants, trial registries, journals,
39			changes to eligibility criteria, outcomes, analyses) to relevant parties
40	25	Plans for communicating important protocol modifications (eg,	Protocol amendments
41			
42	24	Plans for seeking research ethics committee/institutional review board	Research ethics approval
43			(REC/IRB) approval
44			
45			
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49			
50			sponsor
51			whether the process will be independent from investigators and the
52	23	Frequency and procedures for auditing trial conduct, if any, and	Auditing
53			
54			of trial interventions or trial conduct
55			spontaneously reported adverse events and other unintended effects
56	22	Plans for collecting, assessing, reporting, and managing solicited and	Harms
57			
58			decision to terminate the trial
59	21b	who will have access to these interim results and make the final	
60		Description of any interim analyses and stopping guidelines, including	

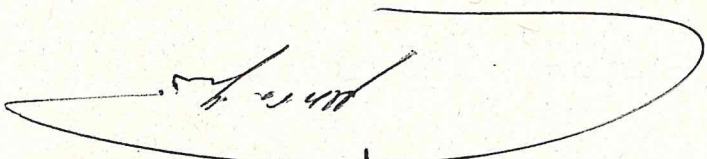
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Appendices

32	Informed consent	Model consent form and other related documentation given to participants and authorised surrogates
33	Biological specimens	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.

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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

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Primary Subject Heading:	Oncology
Secondary Subject Heading:	Oncology
Keywords:	CAPTEM, neuroendocrine carcinoma, FOLFIRI, capecitabine, temozolomide, second-line

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4 1 **CLINICAL STUDY PROTOCOL**5
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9 3 **Randomized Phase II Trial of CAPTEM or FOLFIRI as**
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11 4 **SEcond-line Therapy in NEuroendocrine CARcinomas and**
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13 5 **Exploratory Analysis of Predictive Role of PET/CT Imaging**
14
15 6 **and Biological Markers (SENECA Trial): A Study Protocol**16
17 7 **Running title: Second-line CAPTEM/FOLFIRI in NECs**18
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21
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29 37 **Language style:** This article is formatted in British English.
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4 73 **Ethics and dissemination:** The SENECA trial, supported by IRST, was authorised by
5
6 74 the local Ethics Committee and the Italian Medicines Agency (AIFA). Results will be
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8
9 75 widely disseminated via peer-reviewed manuscripts, conference presentations and
10
11 76 reports to relevant authorities.
12

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14 77
15 78 The study is currently open in Italy. ClinicalTrials.gov Identifier: NCT03387592.

16 79 EudraCT number: 2016-000767-17

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18 80 Protocol version: Clinical Study Protocol Version 1, 07.11.2016.
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24 82 **Keywords:** neuroendocrine carcinoma, second-line, FOLFIRI, capecitabine,
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26 83 temozolomide, CAPTEM
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29 84 **Strengths and limitations of the study**

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31 85
- 32 • the SENECA trial randomises patients to receive two different treatments,
33 FOLFIRI or CAPTEM, providing important information on the activity of both
34 86 combinations in different NEC subtypes (NET grade (G) 3 and NEC G3).
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36 87
 - 37 • The SENECA trial analyses the role of miRNAs and other biological markers as
38 88 prognostic and predictive factors. A further aim is to assess ⁶⁸Ga-PET/CT as a
39
40 89 tool to improve current histological classification.
41
42 90

43
44 91 The major limitations of the study are:

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46 92 • The rarity of the disease and patient prognosis. However, the involvement of
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48 93 several Italian centres will hopefully help to overcome this problem.
- 49
50 94 • Poor prognosis of NEC patients. Patients progressing on platinum chemotherapy
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52 95 usually have rapid deterioration of clinical conditions
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98 Introduction

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

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

113 Given the rarity of this disease, prospective clinical data are lacking and treatment
114 recommendations are essentially expert-based opinions. Two phase II studies
115 investigating the second-line treatment of GEP-NECs are currently registered at
116 *ClinicalTrials.gov*, one evaluating the safety and tolerability of everolimus in
117 40 patients with G3 NEC/NET or G1/G2 NET switching to G3 NEN (National Clinical
118 Trial identifier NCT02113800) and the other investigating the efficacy of avelumab
119 (NCT03147404). A French study focusing on the identification of predictive molecular
120 markers of response to sunitinib in poorly differentiated digestive NETs

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4 121 (NCT01215578) has now closed recruitment and results are eagerly awaited. Another
5
6 122 French multicentre prospective phase II trial is currently ongoing to investigate the
7
8 123 efficacy of the bevacizumab-FOLFIRI combination after progression on a
9
10 124 platinum/etoposide combination (7).

11
12
13 125 Different second-line chemotherapy combinations have been evaluated but shown
14
15 126 poor results (8-10). In a monocentre retrospective clinical trial, Hentic et al.
16
17 127 hypothesised the potential efficacy of FOLFIRI as second-line chemotherapy in
18
19 128 19 patients with G3 extra-pulmonary NECs (11). An objective response rate was
20
21 129 obtained in 31% of patients, with a disease control rate (DCR) of 62%. Median PFS
22
23 130 and OS were 4 and 18 months, respectively.

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27 131 In another retrospective study, a 71% DCR was obtained with temozolomide-based
28
29 132 chemotherapy in 25 patients with metastatic poorly differentiated endocrine carcinoma
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31 133 of different sites and atypical bronchial carcinoid with Ki-67 > 20%. Small cell lung
32
33 134 carcinoma and large cell lung carcinoma were excluded. A PFS of 12 months (95%
34
35 135 confidence interval [CI], 5.5- 24) and OS of 22 months (95% CI, 12-31) were reported
36
37 136 in patients who responded to treatment or showed stable disease (SD), whereas OS was
38
39 137 only 8 months (95% CI, 0-8) in non-responders. The authors observed a higher response
40
41 138 rate in patients with Ki-67 ≤ 60%. There were also more responders in the group with
42
43 139 high uptake in somatostatin receptor scintigraphy (SRS) and in those with positive
44
45 140 staining for chromogranin A. Both factors are often associated with more highly
46
47 141 differentiated tumours (12).

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50 142 Literature data on lung NECs in progression after first-line chemotherapy are based
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52 143 on small patient series (13). Moreover, there is increasing evidence of some
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54 144 discrepancies in the current grading of NECs, highlighting the need for more accurate
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4 145 biomarkers (4, 5). Recent research has shown that NECs may, in fact, comprise two
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6 146 distinct subgroups with different pathogenesis, *i.e.* a highly proliferative group derived
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8 147 from well differentiated neuroendocrine tumours (NETs) and characterised by
9
10 148 mutations in MEN1, DAXX and ATRX, and a poorly differentiated group derived from
11
12 149 neuroendocrine-differentiated adenocarcinomas and characterised by a mutation in
13
14 150 RB1. Both subgroups display a distinct prognosis and different sensitivity to
15
16 151 chemotherapy (14-16). Micro(mi)RNAs are a class of small, non-coding, highly
17
18 152 conserved single-stranded RNAs involved in the post-transcriptional regulation of cell
19
20 153 proliferation, differentiation, survival, and apoptosis (17). They are often associated
21
22 154 with resistance to therapy (18, 19). Whilst miRNAs are known to show a specific
23
24 155 expression pattern in NETs (20), little is known about differential miRNA profiles in
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26 156 NEC patients. At present, no data are available on the deregulation of specific miRNAs
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28 157 in this setting.

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34 158 In a study recently published by our group on GEP-NEC patients undergoing
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36 159 first-line platinum-based chemotherapy, median PFS was 19.3 months and 6.3 months
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38 160 ($p < 0.01$) in patients with Ki-67 value between 20% and 50% or $>50\%$, respectively
39
40 161 (19). Median (m)OS was 8.1 months in the latter group but was not reached in the
41
42 162 former group ($p = 0.039$). Patients with a positive ^{68}Ga -PET/CT had a higher 18-month
43
44 163 OS rate than those with a negative scan (75% vs. 34.3%, respectively), but the
45
46 164 difference was not statistically significant ($p = 0.06$). Our data highlighted that ^{68}Ga -
47
48 165 PET/CT positivity may be a discriminating factor (16, 21) in predicting prognosis,
49
50 166 especially in the metastatic setting where histological material is not always available
51
52 167 for evaluation. Furthermore, ^{18}F fluorodeoxyglucose (^{18}F FDG)-PET/CT may be useful to
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54 168 discriminate between patients with different prognosis (22).
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4 169 Given the above premises, we decided to investigate the efficacy and safety of
5
6 170 second-line FOLFIRI or CAPTEM in patients with GEP and lung NECs in progression
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9 171 after first-line platinum-based treatment. We also aimed to study the serum miRNA
10
11 172 profile in relation to the primary mutational status of MEN1, DAXX, ATRX and RB-1,
12
13 173 patient prognosis and response to therapy, and to assess the prognostic and predictive
14
15 174 role of ¹⁸FDG-PET/CT, ⁶⁸Ga-PET/CT and Ki-67 score.
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23 177 **Methods and Analysis**

26 178 Study design

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29 179 The SENECA study is a multicentre randomised non-comparative phase II study
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31 180 (Figure 1). Patients with metastatic neuroendocrine carcinomas of different origin
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33 181 (lung or gastroenteropancreatic) in progression after first-line treatment are randomized
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35 182 to receive FOLFIRI every 14 days for a maximum of 12 cycles or until progression or
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37 183 unacceptable toxicity, or CAPTEM every 28 days for a maximum of 6 cycles or until
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39 184 progression or unacceptable toxicity.
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42 185 The treatment arms are as follows:
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47 187 FOLFIRI regimen

- 50 188 • Irinotecan 180 mg/m², given as a 60-min. intravenous (i.v.) infusion on day 1 every
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52 189 2 weeks followed by
- 54 190 • Leucovorin 200 mg/m², given as a 2-h i.v. infusion on day 1 every 2 weeks followed
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4 192 • 5-fluorouracil (5-FU) 400 mg/m² given as bolus, and then 5-FU 2400 mg/m² given
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6 193 as a 48-h continuous infusion on day 1, every 2 weeks, until progression or for a
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8 194 maximum of 12 cycles.

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12 13 196 CAPTEM regimen

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16 197 Capecitabine 750 mg/m² twice a day on days 1-14 in combination with temozolomide
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18 198 200 mg/m² daily on days 10-14, every 4 weeks, until progression or for a maximum of
19
20
21 199 6 cycles.

22
23 200 The study includes patients aged ≥ 18 years with a histological diagnosis of G3
24
25 201 neuroendocrine carcinoma (GEP-NEC and lung NEC) according to the 2010 and 2015
26
27 202 GEP and Lung NEN WHO classifications, respectively, Ki-67 $>20\%$ and measurable
28
29 203 disease according to Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 criteria.
30
31 204 All patients must have an Eastern Cooperative Oncology Group (ECOG) performance
32
33 205 status ≤ 2 with a life expectancy > 3 months and must have already undergone first-line
34
35 206 treatment for metastatic disease with platinum -based chemotherapy
36
37 207 (cisplatin/carboplatin and etoposide, FOLFOX4 or CAPOX). Adequate haematological,
38
39 208 liver and renal function is required and effective contraceptive methods must be used by
40
41 209 female patients of childbearing age. Written informed consent is obtained from all
42
43 210 patients to take part in the study. Exclusion criteria are as follows: metastatic NECs
44
45 211 previously treated with an irinotecan or temozolomide regimen, known hypersensitivity
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47 212 to 5-FU/capecitabine, calcium levofolinate, irinotecan or their recipients. All acute
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49 213 toxic effects of any prior therapy (including surgery, radiation therapy and
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51 214 chemotherapy) must have resolved to grade ≤ 1 according to National Cancer Institute
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57 215 Common Terminology Criteria for Adverse Events version 4.03 (CTCAE). Patients
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4 216 taking part in another clinical trial with any investigational agent < 30 days prior to
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6 217 study screening or with a history of allergic reactions attributable to compounds of
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8 218 similar chemical or biological composition are excluded. Patients who have undergone
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10 219 chemotherapy or radiotherapy < 4 weeks (6 weeks for nitrosoureas or mitomycin C)
11
12 220 prior to entering the study, have not recovered from adverse events caused by agents
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14 221 administered > 4 weeks earlier, or have known brain metastases are not eligible for the
15
16 222 study. Patients with other malignancies with a disease-free interval of < 5 years (with
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18 223 the exception of non melanoma skin cancer or low-grade superficial bladder cancer) are
19
20 224 excluded, as are those with any severe and/or uncontrolled medical condition or other
21
22 225 condition that could affect their participation in the study such as:

- 23 226 • unstable angina pectoris, symptomatic congestive heart failure, myocardial infarction
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25 227 < 6 months before the start of the study, serious uncontrolled cardiac arrhythmia or
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27 228 any other clinically significant cardiac disease;
- 28 229 • severely impaired lung function (spirometry and DLCO 50% of the normal predicted
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30 230 value and/or oxygen saturation \leq 88% at rest, in room air);
- 31 231 • uncontrolled diabetes as defined by fasting serum glucose >1.5 x upper limit of
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33 232 normal;
- 34 233 • any active (acute or chronic) or uncontrolled infections/disorders.

35 234 Tumour evaluation by anatomic imaging (multiphase CT and/or MRI) includes chest,
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37 235 abdomen, pelvis, and any additional known sites of disease. These tests are performed
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39 236 at baseline, every three months during treatment as per national regulatory agency
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41 237 indications, and after the end of treatment in non-progressing patients until progression.
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43 238 It is recommended that ^{68}Ga -PET/CT and ^{18}F FDG-PET/CT scans be performed at
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45 239 baseline or a maximum of 90 days before study enrollment. An EORTC quality of life
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4 240 questionnaire is administered at baseline and every three months thereafter during the
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6 241 treatment period.
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10 243 Study endpoints

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14 244 The primary endpoint of the study is the DCR of each treatment, defined as the
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16 245 percentage of patients who have achieved complete or partial response or stable disease
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18 246 for ≥ 12 weeks from the start of therapy. DCR will be evaluated using the new
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20 247 international criteria proposed by the RECIST version 1.1. Acute and late toxicity will
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22 248 be assessed by CTCAE Version 4.03, the latter defined as toxicity occurring at least
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24 249 30 days after the end of the last treatment cycle. Secondary endpoints are OS, calculated
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26 250 from the start of treatment to death from any cause, and PFS, calculated from the start of
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28 251 treatment to the date of the first documented evidence of disease progression or of death
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30 252 from any cause. All the analyses will be performed in the intention-to-treat population.
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32 253 Patients without events at the time of analysis will be censored at their last-known-alive
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34 254 date for OS and at their last date of tumour evaluation for PFS. A further secondary
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36 255 endpoint is the evaluation of quality of life using the European Organization for the
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38 256 Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30).
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40 257 When data are available, the impact of baseline ^{68}Ga -PET/CT and ^{18}F FDG-PET/CT on
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42 258 PFS will be analysed with exploratory intent. After signing the informed consent for
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44 259 biomarker assessment, patients will undergo evaluation of the mutation status of MEN1,
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46 260 DAXX, ATRX and RB-1 in primary tumour tissue and of miRNA in blood samples.
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48 261 Assessment of the miRNA profile will be performed on the first 20 patients who agree
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50 262 to participate in the biological part of the study.
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264 Ethical considerations

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

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

275 Patient and public involvement

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

279 Statistical methods

280 The Bryant and Day design is used to estimate a sample size that takes into account
281 both treatment activity and toxicity. Although randomisation is used to allocate patients
282 to the two arms, no formal statistical comparisons between treatment regimens are
283 planned. The purpose of randomisation is to reduce bias due to patient assignment to a
284 specific treatment arm. The hypothesis for the control arm is based on literature data
285 (23, 24).

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

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4 287 adopted. A DCR rate $\geq 60\%$ and a relevant toxicity rate $\leq 20\%$ are considered acceptable
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6 288 rates, while a DCR rate $\leq 40\%$ and a relevant toxicity rate $\geq 40\%$ are considered
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8 289 unacceptable rates. Given these hypotheses, the first step of the study will require
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10 290 25 patients. If ≥ 10 patients with a DCR are observed and ≥ 15 patients do not have
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12 291 significant toxicity, the study will enrol patients in the next step. A total of 53 patients
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14 292 will be enrolled. If ≥ 25 patients with DCR and ≥ 36 patients without any relevant
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16 293 toxicity are observed, treatment will be considered active and not toxic. This design is
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18 294 used for each treatment scheme and all analyses will be performed separately. If one of
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20 295 the schemes does not obtain the expected proportions of the first step, the arm will be
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22 296 closed and patients will be enrolled in the other arm until the target is reached; if the
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24 297 expected proportions are not reached in any arm, the study will be prematurely closed.
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26 298 If no premature stop occurs, a total of 106 evaluable patients are needed (53 patients in
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28 299 each arm). Taking into account a 5% dropout rate, 56 patients must be enrolled in each
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30 300 arm (total 112 patients). G3-4 gastrointestinal toxicity, G4 thrombocytopenia,
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32 301 prolonged G3-G4 neutropenia (> 7 days) and drug-related hospitalisations are
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34 302 considered relevant toxicity. The stratification factors of this study are Ki-67 (21%-55
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36 303 % vs. $> 55\%$) and site of primary tumour (lung vs. GEP). A subgroup analysis of the
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38 304 efficacy of both treatments according to these stratification factors has been planned.
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40 305 Complete response, partial response or stable disease for at least 12 weeks will be
41
42 306 considered as the DCR. The proportion of patients in this category will be determined
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44 307 and 95% CIs for the DCR will be calculated. OS and PFS will be estimated using the
45
46 308 Kaplan-Meier method (two-sided 95% CIs) (24). Appropriate statistical analyses will be
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48 309 performed on the basis of the data available to compare QLQ-C30 scores between
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50 310 baseline and subsequent follow-up visits.
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4 311 When data are available, the impact of ⁶⁸Ga-PET/CT result on PFS will be analysed
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6 312 with exploratory intent. The Shapiro-Wilk test will be used to determine the normality
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8 313 distribution of each clinical, demographic and biological biomarker (25). In the event
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10 314 of a non-normal distribution, nonparametric statistics will be used to analyse the
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12 315 relationship between the serum levels of each marker, considered as continuous
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14 316 variables, and response to treatment. In the event of normal biomarker distribution,
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16 317 a parametric test will be used. All endpoints will be analysed separately for each
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18 318 treatment group.
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23 **Discussion**

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25 320 There is still no truly effective second-line chemotherapy for neuroendocrine
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27 321 carcinoma. Overall prognosis of patients is poor, with an OS of 5 months in the
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29 322 metastatic setting according to SEER (Surveillance, Epidemiology, and End Results)
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31 323 data (26). Only 5% of all patients are long-term survivors. There is also a marked lack
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33 324 of prognostic and predictive factors (5).
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37 325 Three phase II studies registered at *ClinicalTrials.gov* are currently investigating
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39 326 second-line treatment of GEP-NECs, the first focusing on FOLFIRI and bevacizumab
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41 327 (NCT02820857), the second on everolimus (NCT02113800) and the third on avelumab
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43 328 (NCT03147404). Some abstracts were presented at ESMO (European Society for
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45 329 Medical Oncology) 2018 and ASCO (American Society of Clinical Oncology) 2019
46
47 330 on the use of immunotherapy in GEP-NECs, all showing inconclusive results. The
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49 331 SENECA study uses a promising approach to the treatment of patients with metastatic
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51 332 NECs. First, both the activity and safety of 2 regimens are assessed in the same setting
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53 333 with a sizeable patient population (56 patients/arm). In addition, patients are stratified
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55 334 according to Ki-67 index and morphology to investigate the role of each treatment
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4 335 combination in both poorly differentiated and well differentiated NECs. Another aim of
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6 336 this study is to integrate both biological and metabolic imaging data in an effort to
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9 337 improve the current GEP-NEC classification.

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11 338 The duration of treatments in the metastatic setting is a dilemma in NENs and
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13 339 especially in neuroendocrine carcinomas. Given the lack of evidence-based
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15 340 recommendations on treatment duration of second-line chemotherapy in NECs, we
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17 341 decided to opt for a fixed duration of treatments to avoid unnecessary exposure to
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20 342 cytotoxic agents and consequent bone marrow reserve depletion (27).

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23 343 In conclusion, there are still no FDA/EMA (Food and Drug Administration/European
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25 344 Medicines Agency)-approved second-line therapeutic options for patients with
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27 345 metastatic NECs, and the SENECA trial could represent a step forward in finding novel
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29 346 therapies to prolong survival and maintain quality of life. Moreover, the integration of
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31 347 biological and imaging data could -lead to a better understanding of the natural history
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34 348 of the disease and help to identify potential responders.

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40 41 351 **Confidentiality**

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43 352 This study will be conducted in full conformity with ICH (The International Council for
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45 353 Harmonisation of Technical Requirements for Pharmaceuticals for Human Use)
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47 354 Guidelines for Good Clinical Practice, Directive 2001/20/EEC of the European
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50 355 Parliament and other relevant current local legislation. Participants will be allocated a
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52 356 unique identification (ID) number at entry. The master list linking participant personal
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55 357 information and ID number will be maintained in a separate locked cabinet and
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4 358 password-protected hard-drive. Data will be analysed by ID number only. Patient files
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6 359 and other source data will for be kept a maximum of 15 years.
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12 13 362 **Ethics and Dissemination**

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15 363 The SENECA trial, supported by IRST, involves several Italian centres and was
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17 364 authorised by the local Ethics Committees of the centres taking part and by the Italian
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19 365 Medicines Agency (AIFA) (see list of all centers in the supplementary table 1). After
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21 366 completing the study, all data, including beneficial and adverse events, of the trial will
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23 367 be communicated at scientific meetings and published in indexed peer-reviewed
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25 368 journals. If shown to be effective, the therapy program will be made available to the
26
27 369 general public in an appropriate manner.
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35 36 372 **Abbreviations**

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38 373 CAPTEM, capecitabine plus temozolomide; DCR, disease control rate; GEP,
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40 374 gastroenteropancreatic; NEC, neuroendocrine carcinoma; NET, neuroendocrine tumour;
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42 375 OS, overall survival; PFS, progression-free survival
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47 48 377 **Author contributions**

49
50 378 AB, CL and TI designed the study and drafted the article. AB was responsible for data
51
52 379 acquisition. SP, SL, GDM, SS and ST provided methodological support and designed a
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54 380 clinical information data extraction method for the protocol. FF performed the statistical
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56 381 analysis. DS, FG, FP, RB, IL, FB and SR revised the paper for important intellectual
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4 382 content. All authors read and approved the present version of the manuscript for
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6 383 submission.

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16 387 **Data availability statement**

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18 388 The datasets used and analysed in the present study are available from the
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20 389 corresponding author on reasonable request.

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24
25 391 **Funding**

26
27 392 The study was conducted in the absence of any commercial or financial relationships
28
29 393 that could be construed as a potential conflict of interest.

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34 395 **Conflicts of interest**

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36 396 The authors declare no conflict of interest.

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40
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42
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44
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25 508 **Figure legend**

26
27 509 **FIGURE 1** | SENECA study design.
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Fig. 1

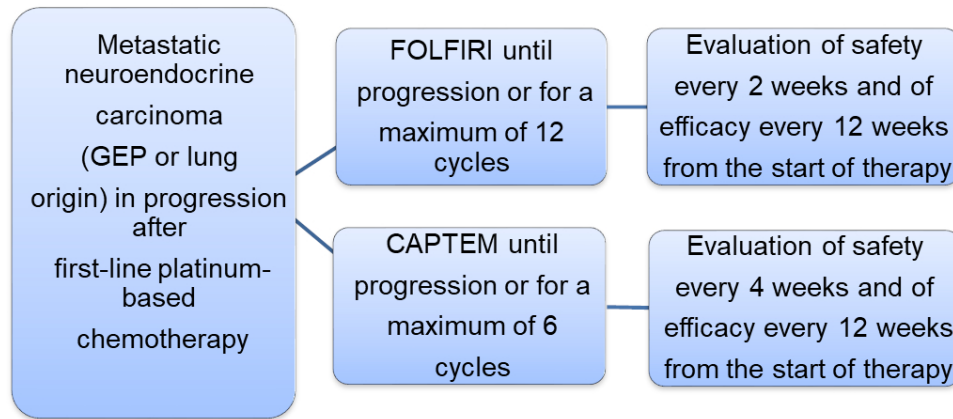
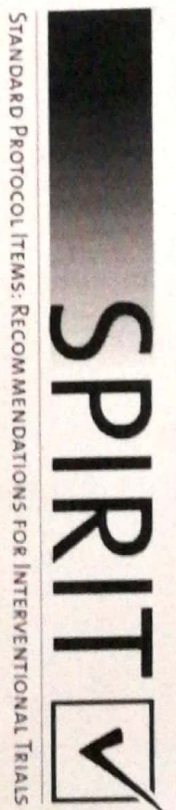


FIGURE 1 | SENECA study design.

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

Section/Item	Item No	Description	Addressed on page number
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Administrative information

Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	<u>4</u>
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	<u>4</u>
	2b	All items from the World Health Organization Trial Registration Data Set	<u>N/A</u>
Protocol version	3	Date and version identifier	<u>4</u>
Funding	4	Sources and types of financial, material, and other support	<u>12</u>
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	<u>1, 2, 16, 17</u>
	5b	Name and contact information for the trial sponsor	<u>N/A</u>
	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	<u>16</u>
	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)	<u>N/A</u>

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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	<u>5, 6, 7</u>
	6b	Explanation for choice of comparators	<u>6, 7, 8</u>
Objectives	7	Specific objectives or hypotheses	<u>8</u>
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)	<u>8</u>

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	<u>8</u>
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)	<u>9, 10</u>
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	<u>8, 9</u>
	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)	<u>8-10</u>
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	<u>10</u>
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	<u>10</u>
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	<u>11</u>
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)	<u>13</u>

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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	<u>12, 13</u>
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	<u>12, 13</u>
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	<u>12</u>
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	<u>15</u>
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	<u>15</u>
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	<u>N/A</u>
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	<u>N/A</u>
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	<u>10, 11</u>
	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	<u>10, 11</u>

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	<u>15</u>
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	<u>12, 13</u>
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	<u>12, 13</u>
	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)	<u>10, 13</u>
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	<u>N/A</u>
	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	<u>13</u>
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	<u>N/A</u>
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	<u>N/A</u>
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	<u>N6</u>
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)	<u>N/A</u>

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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.

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