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**Cohort profile: Biomarkers related to folate-dependent one-carbon metabolism in colorectal cancer recurrence and survival:
The FOCUS Consortium**

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Cohort profile: Biomarkers related to folate-dependent one-carbon metabolism in colorectal cancer recurrence and survival: The FOCUS Consortium

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44 Abstract

45 **Purpose:** The overarching goal of the FOCUS (Biomarkers related to folate-dependent one-
46 carbon metabolism in colorectal cancer recurrence and survival) Consortium is to unravel the
47 effect of folate and folate-mediated one-carbon metabolism (FOCM) biomarkers on colorectal
48 cancer (CRC) prognosis to provide clinically relevant advice on folate intake to cancer
49 patients and define future tertiary prevention strategies.

50 **Participants:** The FOCUS Consortium is an international, prospective cohort of 2,401
51 women and men above 18 years of age who were diagnosed with a primary invasive non-
52 metastatic (stage I-III) CRC. The Consortium comprises patients from Austria, two sites from
53 the Netherlands, Germany and two sites from the USA. Patients are recruited after CRC
54 diagnosis and followed at six and twelve months after enrolment. At each time point,
55 sociodemographic data, data on health behavior, and clinical data are collected, blood samples
56 are drawn.

57 **Findings to date:** An increased risk of cancer recurrences was observed among patients with
58 higher compared to lower circulating folic acid concentrations. Furthermore, specific folate
59 species within the FOCM pathway were associated with both inflammation and angiogenesis
60 pathways among CRC patients. In addition, higher vitamin B₆ status was associated with
61 better quality of life at six months post treatment.

62 **Future plans:** Better insights into the research on associations between folate and FOCM
63 biomarkers and clinical outcomes in CRC patients will facilitate the development of
64 guidelines regarding folate intake in order to provide clinically relevant advice to cancer
65 patients, health professionals involved in patient care, and ultimately further tertiary
66 prevention strategies in the future.

67 Strengths and limitations of this study

- 68 • FOCUS is the largest consortium to date addressing the research question of folate and
69 FOCM biomarkers in relation to survival, recurrence, treatment toxicity, and health-
70 related quality of life outcomes in CRC patients
- 71 • The cohorts included in the FOCUS Consortium are designed to enable future pooling
72 of data using harmonized and standardized methods to collect data and biospecimens
- 73 • The pooled sample size provides sufficient power to investigate subgroup analyses
74 across CRC patients
- 75 • Study time point definitions differ between some of the cohorts and have to be adapted
76 for specific projects
- 77 • A selection bias for follow-up can arise because it is possible that patients who
78 experience more severe toxicities, worse clinical outcome or health-related quality of
79 life are underrepresented among those completing follow-ups

81 **Why was the FOCUS Consortium set up?**

82 Among men and women world-wide, colorectal cancer (CRC) accounts for nearly 10% of all
83 incident cancer cases.¹ In Europe, 5-year survival for CRC patients is approximately 55%,
84 with substantial differences by stage.^{2,3} The number of CRC patients continues to increase due
85 to implementation of improved screening strategies and/or enhanced treatment modalities.⁴
86 Many cancer patients seek information on what they can do themselves to improve survival -
87 for instance by improving their dietary habits and other lifestyle factors.⁵ However, there may
88 be behavioural aspects among individuals diagnosed with CRC, which may be harmful in
89 some cases, such as the use of high-dose nutritional supplements containing synthetic folate.
90 In general, knowledge on short and long-term effects is insufficient to make sound
91 recommendations on use of dietary supplements, in particular folate, to cancer survivors, even
92 though dietary supplements are used by 20%-85% of patients with cancer⁶⁻⁸, highlighting the
93 importance of thorough evaluation of potential benefits and harms and to support
94 development of evidence-based recommendations on use of dietary supplements to cancer
95 patients.

96 Folic acid is the synthetic form of the B-vitamin folate and is often used in dietary
97 supplements or fortified foods. Folate and folic acid play an important role in one-carbon
98 metabolism, which is a complex series of biochemical reactions essential in nucleotide
99 synthesis, methylation reactions and amino acid homeostasis (REF PMID: 27641100). One-
100 carbon metabolism refers to a complex network of biochemical reactions linked to nucleotide
101 synthesis and provides methyl groups for DNA, RNA or protein methylation. Thus, one-
102 carbon metabolism is directly controlling processes determining DNA synthesis and integrity,
103 both processes known to be linked to tumor growth.⁹ To what extent folic acid supplement
104 use and biomarkers of folate-mediated one-carbon metabolism (FOCM) impact cancer
105 survival and treatment efficacy and toxicity still needs to be clarified.^{9 10} Folate and FOCM
106 biomarker deficiencies may increase cancer risk, but high levels, especially of synthetic folic
107 acid, may also be driving factors in carcinogenesis.^{6 11} An increasing body of evidence
108 suggests that folate plays a dual role in carcinogenesis, involving both the prevention of early
109 lesions and potential harm once pre-neoplastic or neoplastic lesions have developed.^{6 10 12-18}

110 The overarching goal of the FOCUS (biomarkers related to Folate-dependent one-
111 carbon metabolism in Colorectal cancer recUrrance and Survival) Consortium is to study
112 associations between folate and FOCM biomarkers and recurrence and survival in CRC
113 patients. Better insights into these associations will facilitate the development of guidelines

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3 114 regarding folate intake in order to provide clinically relevant advice to cancer patients, health
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5 115 professionals involved in patient care, and ultimately further tertiary prevention strategies in
6
7 116 the future. The FOCUS Consortium is a large-scale international consortium with CRC
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9 117 patients from six prospective cohort studies. The primary objectives of the FOCUS
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11 118 Consortium are: (i) to determine possible associations of folate and other FOCM biomarkers
12
13 119 at diagnosis with recurrence or survival in non-metastatic (stage I-III) CRC; (ii) to elucidate
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15 120 whether biomarkers related to FOCM are associated with dietary and supplemental intake of
16
17 121 these respective nutrients; (iii) to explore whether FOCM biomarkers are associated with
18
19 122 treatment toxicity in CRC patients undergoing chemotherapy; and (iv) to collect
20
21 123 comprehensive data of patient characteristics at baseline and follow-up including biomarkers
22
23 124 of FOCM to establish a unique resource for future scientific research. The FOCUS
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25 125 Consortium is funded by the European Research Area Network (ERA-NET) on Translational
26
27 126 Cancer Research (TRANSCAN).

28
29 127 This joint research may lead to a better understanding of the role of folate- and
30
31 128 FOCM-related mechanisms in the prognosis of CRC, which will be critical for the
32
33 129 development of guidelines regarding folate intake among CRC patients.

34 130 **Who is in the FOCUS Consortium?**

35
36 131 The FOCUS Consortium is an international, prospective consortium including six cohort
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38 132 studies recruiting women and men age 18 years and older diagnosed with a primary CRC. The
39
40 133 FOCUS Consortium is comprised of patients from the ColoCare Study at the University of
41
42 134 Heidelberg, Germany (n=298, 12.3%), the ColoCare Study at the Huntsman Cancer Institute
43
44 135 in Salt Lake City, Utah, USA (n=80, 3.3%), the ColoCare Study at the Fred Hutchinson
45
46 136 Cancer Research Center in Seattle, Washington, USA (n=157, 6.3%), the COLON Study
47
48 137 (Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that
49
50 138 may influence colorectal tumor recurrence, survival and quality of life) at Wageningen
51
52 139 University & Research in the Netherlands (n=1,365, 56.1%), the CORSA Study (Colorectal
53
54 140 Cancer Study of Austria) at the Medical University of Vienna in Austria (n=218, 9.0%), and
55
56 141 the EnCoRe Study (Energy for life after ColoRectal cancer) at Maastricht University Medical
57
58 142 Center+ in the Netherlands (n=317, 13.0%).

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60 143 In total, n=2,435 CRC patients were considered for the FOCUS Consortium, of which
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145 n=34 patients were excluded due to tumor staging being either 0 or IV leading to a total
number of n=2,401 stage I-III CRC patients included in further analyses.

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3 146 Patients were recruited after CRC diagnosis and repeated study measurements were
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5 147 conducted at time of recruitment, and at six and twelve months thereafter. At each study time
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7 148 point, sociodemographic data, data on lifestyle factors (e.g., diet, supplement use, physical
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9 149 activity), and clinical data (e.g., tumor site and stage, cancer treatment, treatment-induced
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11 150 toxicities, recurrence, survival) were collected, and blood samples were drawn (**Figure 1**). All
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13 151 individuals signed informed consent and the Institutional Review Board at each site approved
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15 152 the corresponding study. Below, a more specific description of each included study is
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17 153 provided.

18 154 The ColoCare Study

19
20 155 The ColoCare Study (ClinicalTrials.gov identifier: NCT02328677) is an ongoing
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22 156 international, multi-center prospective cohort study among women and men newly diagnosed
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24 157 with a primary invasive CRC, with the goal to investigate predictors of cancer recurrence,
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26 158 survival, treatment toxicities and health-related quality of life.^{4 19} Three ColoCare Consortium
27
28 159 sites participate in the FOCUS Consortium: the Fred Hutchinson Cancer Research Center in
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30 160 Seattle (Washington, USA), and the University Hospital Heidelberg, Heidelberg (Germany)
31
32 161 as well as the Huntsman Cancer Institute in Salt Lake City (Utah, USA). Patients are enrolled
33
34 162 prior to undergoing CRC surgery according to the following inclusion criteria: individuals
35
36 163 who are 18 years of age and older, newly diagnosed (i.e., non-recurrent) with invasive CRC.
37
38 164 Blood draws and other biospecimens are obtained prior to surgery and at regular intervals
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40 165 (e.g., six, twelve, twenty-four months). Questionnaires are administered to assess lifestyle
41
42 166 behavior, health-related quality of life, and clinical outcomes such as CRC recurrence,
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44 167 treatment, and treatment symptoms at each study time point. Clinical data are obtained
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46 168 through reviews of patient medical records, pathology and imaging reports. Vital status is
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48 169 obtained through medical records, routine follow-up mailings, periodic requests for outside
49
50 170 medical records, and state or national cancer and death registries. The Heidelberg ColoCare
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52 171 Study was approved by the Ethics Committee of the Medical Faculty of Heidelberg (approval
53
54 172 no. S-310/2001 and S-134/2016). The ColoCare Study at the Huntsman Cancer Institute and
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56 173 the Fred Hutchinson Cancer Research Center were approved by the respective IRBs (#77147
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58 174 and #6407).

55 175 The COLON Study

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58 176 The COLON Study (ClinicalTrials.gov identifier: NCT03191110) started in 2010 and is an
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60 177 ongoing, multicenter prospective cohort study specifically designed to assess associations

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3 178 between nutrition, lifestyle, and dietary supplement use with quality of life, CRC recurrence
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5 179 and survival among CRC patients (stages I-IV).²⁰ Persons with a history of CRC or (partial)
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7 180 bowel resection, chronic inflammatory bowel disease, or a known hereditary CRC syndrome
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9 181 are excluded from the study. Patients are recruited from eleven regional and academic
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11 182 hospitals prior to surgery. Individuals donate blood samples and provide information on diet,
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13 183 lifestyle, and dietary supplement use at CRC diagnosis, i.e. baseline, and at six, twelve
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15 184 (chemotherapy patients only), twenty-four and sixty months after diagnosis. Clinical data are
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17 185 collected through review of medical records (treatment-induced toxicity) or through linkage
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19 186 with the Dutch ColoRectal Audit (DCRA). Mortality data (i.e. death or alive and date of
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21 187 death) are retrieved from the Municipal Personal Records Database. Recurrence data have
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23 188 been retrieved in collaboration with the Netherlands Cancer Registry. Ethical approval for the
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25 189 study was granted by the Committee on Research involving Human Subjects, region Arnhem-
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27 190 Nijmegen under file number 2009/349.

26 191 The CORSA Study

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29 192 CORSA is an ongoing case-control study of women and men recruiting CRC patients,
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31 193 patients with high and low risk adenomas and population-based colonoscopy negative
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33 194 controls, with an age range between 30 and 90 years. Since 2003, more than 13,500
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35 195 participants have been recruited across nine sites in Austria. The multicentre recruitment
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37 196 within CORSA follows standardized protocols resulting in consistent data from all
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39 197 recruitment sites. These sites include the Medical University of Vienna (Department of
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41 198 Surgery), two hospitals in Vienna (Clinic Favoriten and Clinic Landstraße), and the Hospital
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43 199 Wiener Neustadt in Lower Austria. Furthermore, the recruitment for CORSA was performed
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45 200 in four hospitals in the federal state Burgenland within the population-based screening
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47 201 program "Burgenland PREvention Trial of colorectal cancer Disease with ImmunologiCal
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49 202 Testing" (B-PREDICT). B-PREDICT is a two-stage screening project where more than
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51 203 150,000 inhabitants of Burgenland aged between 40 and 80 are invited annually to participate
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53 204 in this program using a Fecal Immunochemical Test (FIT) as an initial screening. FIT-positive
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55 205 (≥ 10 μg hemoglobin / g feces) tested individuals are offered a complete colonoscopy and are
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57 206 asked to participate in CORSA. Biospecimen are collected at each site using harmonized
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59 207 protocols. CORSA participants provide a basic CORSA questionnaire assessing data on body
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208 mass index (BMI), smoking history, alcohol consumption, education level, family status,
209 profession, basic dietary habits, and diabetes. Clinical data are abstracted from medical
210 records and processed in a structured database following standardized documentation

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3 211 guidelines and according to the General Data Protection Regulation (GDPR). All subjects
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5 212 gave written informed consent and the study was approved by the institutional review boards:
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7 213 'Ethikkommission Burgenland' (33/2010), by the ethical review committee of the Medical
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9 214 University of Vienna (1160/2016) and by the "Ethikkommission der Stadt Wien" (EK 06-
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11 215 150VK).

12 13 216 The EnCoRe Study

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15 217 The EnCoRe Study is a prospective cohort study initiated in 2012 at the Maastricht University
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17 218 Medical Center+ in the Netherlands that focuses on the importance of lifestyle factors for
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19 219 quality of life, recurrence and survival of CRC patients.²¹ The EnCoRe Study is registered in
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21 220 the Netherlands Trial Registry for experimental and observational studies
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23 221 (www.trialregister.nl, registration number 7099). Stage I-III CRC patients are enrolled at
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25 222 diagnosis at three hospitals in the South-Eastern region of the Netherlands and followed up
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27 223 until five years after completion of treatment with repeated measurements at diagnosis (pre-
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29 224 treatment), and at six weeks and six, twelve, twenty-four, and sixty months after the end of
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31 225 the initial anti-cancer treatment (i.e., surgery, chemotherapy, radiotherapy). Patients with
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33 226 stage IV CRC, an inability to understand the Dutch language in speech or writing, with
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35 227 comorbidities obstructing successful participation (e.g., Alzheimer disease), or with severe
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37 228 visibility or hearing disorders are excluded from the study. Repeated home visits by trained
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39 229 dieticians are conducted and data are collected amongst others on sociodemographic factors,
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41 230 quality of life, functioning, physical activity, comorbidity, dietary intake, supplement use, and
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43 231 anthropometry. In addition, clinical data are collected from hospital records and blood
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45 232 samples are drawn at all time points. Recurrence data have been retrieved in collaboration
46
47 233 with the Netherlands Cancer Registry. Mortality data (i.e. death or alive and date of death) are
48
49 234 retrieved from the Municipal Personal Records Database. The EnCoRe Study was approved
50
51 235 by the Medical Ethics Committee of the Maastricht University Hospital and Maastricht
52
53 236 University (METC 11-3-075).

54 55 237 The FOCUS Consortium

56
57 238 Sociodemographic, clinical and lifestyle characteristics of the 2,401 participants in the
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59 239 FOCUS Consortium are presented in **Table 1**. The mean age at CRC diagnosis was 65.4 years
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240 (standard deviation (SD): 10.2; range: 22 to 93 years), and the majority of participants were
241
male (64.0%). The mean BMI was 27.0 kg/m² (SD: 4.6).

Table 1: Sociodemographic, clinical, and lifestyle factors of eligible FOCUS Consortium participants at baseline (n=2,401)

	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe
n (%)	2,401 (100)	298 (12.4)	150 (6.3)	80 (3.3)	1,338 (55.7)	218 (9.1)	317 (13.2)
Age							
Mean y ± SD	65.4 ± 10.2	63.7 ± 12.2	58.3 ± 12.8	61.3 ± 11.2	66.1 ± 8.7	67.3 ± 12.0	66.9 ± 9.3
Sex							
Female n (%)	864 (36.0)	100 (33.6)	67 (44.7)	32 (40.0)	486 (36.3)	76 (34.9)	103 (32.5)
Male n (%)	1,537 (64.0)	198 (66.4)	83 (55.3)	48 (60.0)	852 (63.7)	142 (65.1)	214 (67.5)
Education							
Low n (%)	861 (35.9)	123 (41.3)	18 (12.0)	17 (21.3)	543 (40.6)	73 (33.5)	87 (27.4)
Intermediate n (%)	694 (28.9)	57 (19.1)	71 (47.3)	40 (50.0)	332 (24.8)	73 (33.5)	121 (38.2)
High n (%)	658 (27.4)	88 (29.5)	28 (18.7)	8 (10.0)	417 (31.7)	14 (6.4)	103 (32.5)
Unknown n (%)	188 (7.8)	30 (10.1)	33 (22.0)	15 (18.7)	46 (3.4)	58 (26.6)	6 (1.9)
Marital status							
Unmarried n (%)	122 (5.1)	19 (6.4)	15 (10.0)	2 (2.5)	60 (4.5)	10 (4.6)	16 (5.1)
Married n (%)	1,738 (72.4)	193 (64.8)	71 (47.3)	50 (62.5)	1,071 (80.0)	113 (51.8)	240 (75.7)
Divorced/Separated n (%)	138 (5.7)	24 (8.0)	11 (7.3)	10 (12.5)	64 (4.8)	14 (6.4)	15 (4.7)
Widowed n (%)	194 (8.1)	31 (10.4)	7 (4.7)	1 (1.3)	90 (6.7)	26 (11.9)	39 (12.3)
Living community n (%)	20 (0.8)	0	13 (8.7)	2 (2.5)	4 (0.3)	1 (0.5)	0
Unknown n (%)	189 (8.9)	31 (10.4)	33 (22.0)	15 (18.7)	49 (3.7)	54 (24.8)	7 (2.2)
Smoking status							
Current n (%)	290 (12.1)	53 (17.8)	8 (5.3)	6 (7.5)	143 (10.7)	38 (17.4)	42 (13.3)
Former n (%)	1,219 (50.8)	132 (44.3)	53 (35.32)	21 (26.3)	767 (57.3)	76 (34.9)	170 (53.6)
Never n (%)	767 (31.9)	90 (30.2)	56 (37.3)	39 (48.7)	386 (28.9)	97 (44.5)	99 (31.2)
Unknown n (%)	125 (5.2)	23 (7.7)	33 (22.0)	14 (17.5)	42 (3.1)	7 (3.2)	6 (1.9)
Alcohol intake							
Mean g/day ± SD	13.6 ± 17.1	16.1 ± 19.2	8.8 ± 18.5	2.3 ± 10.2	14.0 ± 17.0		12.9 ± 16.5
Unknown n (%)	633 (26.4)	208 (69.8)	81 (54.0)	60 (75.0)	54 (4.1)	218 (100)	12 (3.8)
BMI at diagnosis							
Mean kg/m² ± SD	27.1 ± 4.6	26.6 ± 4.1	28.7 ± 7.3	29.4 ± 7.5	26.6 ± 4.0	27.7 ± 4.4	28.3 ± 4.6
Unknown n (%)	42 (1.7)	2 (0.7)	0	14 (17.5)	10 (0.8)	14 (6.4)	2 (0.6)
	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe

Tumor site								
Colon n (%)	1,450 (60.4)	140 (57.0)	75 (50.0)	45 (56.3)	867 (64.8)	131 (60.1)	192 (60.6)	
Rectum n (%)	862 (35.9)	143 (48.0)	60 (40.0)	22 (27.5)	434 (32.4)	78 (35.8)	125 (39.4)	
Rectosigmoid n (%)	42 (1.7)	15 (5.0)	15 (10.0)	7 (8.7)	0	5 (2.3)	0	
Unknown n (%)	47 (2.0)	0	0	6 (7.5)	37 (2.8)	4 (1.8)	0	
Tumor stage								
I n (%)	629 (26.2)	76 (25.5)	35 (23.3)	21 (26.3)	326 (24.4)	82 (37.6)	89 (28.1)	
II n (%)	694 (28.9)	114 (38.3)	49 (32.7)	23 (28.7)	386 (28.9)	52 (23.9)	70 (22.1)	
III n (%)	973 (40.5)	108 (36.2)	66 (44.0)	36 (45.0)	564 (42.1)	52 (23.9)	147 (46.4)	
Unspecified* n (%)	16 (0.7)	0	0	0	4 (0.3)	12 (5.5)	0	
Unknown n (%)	89 (3.7)	0	0	0	58 (4.3)	20 (9.2)	11 (3.5)	
Treatment								
Neoadjuvant chemotherapy n (%)	331 (13.8)	55 (18.5)	47 (31.3)	15 (18.8)	138 (10.3)	14 (6.4)	62 (19.6)	
Unknown n (%)	58 (2.4)	2 (0.7)	1 (0.7)	10 (12.5)	38 (2.8)	7 (3.2)	0	
Neoadjuvant radiotherapy n (%)	523 (21.8)	72 (24.2)	48 (32.0)	13 (16.3)	290 (21.7)	14 (6.4)	86 (27.1)	
Unknown n (%)	58 (2.4)	2 (0.7)	1 (0.7)	10 (12.5)	38 (2.8)	7 (3.2)	0	
Adjuvant chemotherapy n (%)	682 (28.4)	100 (33.6)	80 (53.3)	32 (40.0)	315 (23.8)	56 (25.7)	99 (31.2)	
Unknown n (%)	121 (5.0)	17 (5.7)	3 (2.0)	28 (35.0)	63 (4.7)	9 (4.1)	1 (0.3)	
Adjuvant radiotherapy n (%)	227 (9.5)	188 (63.1)	7 (4.7)	1 (1.3)	16 (1.2)	13 (6.0)	2 (0.6)	
Unknown n (%)	164 (6.8)	17 (5.7)	3 (2.0)	28 (35.0)	106 (7.9)	9 (4.1)	1 (0.3)	
Surgery n (%)	2,291 (95.4)	298 (100.0)	149 (99.3)	71 (88.7)	1,290 (96.4)	195 (89.5)	288 (90.9)	
Unknown n (%)	44 (1.8)	0	0	3 (3.8)	39 (2.9)	1 (0.5)	1 (0.3)	
Folic acid supplementation								
Yes n (%)	405 (16.9)	4 (1.3)	9 (5.9)	2 (2.5)	325 (24.3)		65 (20.5)	
Unknown n (%)	383 (15.9)	27 (9.1)	86 (56.6)	14 (17.5)	38 (2.8)	218 (100)	0	
Vitamin B₁₂ supplementation								
Yes n (%)	430 (17.9)	10 (3.4)	8 (5.3)	14 (17.5)	336 (25.1)		62 (19.6)	
Unknown n (%)	384 (16.0)	27 (9.1)	86 (56.6)	14 (17.5)	39 (2.9)	218 (100)	0	
Vitamin B₆ supplementation								
Yes n (%)	77 (3.2)	8 (2.7)	4 (2.6)	4 (5.0)			61 (19.2)	
Unknown n (%)	1,684 (70.1)	27 (9.1)	86 (56.6)	14 (17.5)	1,339 (100)	218 (100)	0	

	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe
Vitamin B₂ supplementation							
Yes n (%)	62 (2.6)	0	2 (1.3)	0			60 (18.9)
Unknown n (%)	1,684 (70.1)	27 (9.1)	86 (56.6)	14 (17.5)	1,339 (100)	218 (100)	0
Dietary intake of folate equivalents							
Mean µg/day ± SD	227.1 ± 94.3	115.1 ± 38.1	149.6 ± 97.5	116.2 ± 112.8	229.0 ± 88.7		276.3 ± 82.2
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₁₂							
Mean µg/day ± SD	4.6 ± 2.4	6.2 ± 2.9	7.1 ± 5.8	4.2 ± 2.2	4.3 ± 2.1		4.6 ± 2.0
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₆							
Mean mg/day ± SD	1.5 ± 0.5	1.7 ± 0.5	2.0 ± 0.7	1.5 ± 0.7	1.5 ± 0.5		1.8 ± 0.5
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₂							
Mean mg/day ± SD	1.4 ± 0.5	1.5 ± 0.5	2.2 ± 1.0	1.6 ± 0.8	1.3 ± 0.4		1.4 ± 0.5
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Total energy intake							
Mean kcal/day ± SD	1,941.8 ± 593.6	2,330.9 ± 714.0	1835.0 ± 775.7	1,398.5 ± 597.8	1,856.9 ± 517.2		2,243.7 ± 651.0
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Adherence to physical activity guidelines**							
Yes n (%)	1,310 (54.5)	84 (28.2)	35 (23.0)	20 (25.0)	941 (70.3)		230 (72.6)
Unknown n (%)	370 (15.4)	33 (11.1)	53 (34.9)	15 (18.7)	45 (3.4)	218 (100)	6 (1.9)

*Distinguishing between stage I and II or II and III not possible

**Self-reported engagement in at least 150 minutes per week of moderate-to-vigorous physical activity

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2
3
4 244 In terms of education status, 35.9% of the patients reported lower education, 28.8% reported
5
6 245 intermediate education and 27.3% high education. Most of the patients were married or part
7
8 246 of a living community (73.1%). About half of the overall cohort reported to be a former
9
10 247 smoker (50.8%), 31.7% were never smokers, and 12.1% were current smokers (5.4% were
11
12 248 unknown). Median alcohol intake was 7.9 g/day. In total, 16.9% of the patients reported
13
14 249 regular dietary supplementation (i.e., at least once per week during the last four weeks) of
15
16 250 folic acid. Over half of all participants (54.6%) reported adherence to the physical activity
17
18 251 guidelines of at least 150 minutes per week of moderate-to-vigorous physical activity.

19 252 Regarding clinical characteristics, 57.0% of participants were diagnosed with colon
20
21 253 cancer, 33.9% with rectal cancer, and 1.1% with rectosigmoid cancer (8.1% were of unknown
22
23 254 tumor subsite). In total, 26.0% were diagnosed with stage I, 28.7% with stage II, and 40.2%
24
25 255 with stage III CRC. Approximately 1% of participants were classified with an unspecified
26
27 256 cancer stage, as distinction between stage I and II or II and III was not possible, and for 4.4%
28
29 257 of the total population the cancer stage was unknown. In total, 89.3% of patients underwent
30
31 258 surgery, whereas neoadjuvant chemotherapy was administered to 10.6% of patients and
32
33 259 26.8% received adjuvant chemotherapy. Neoadjuvant radiotherapy was administered to
34
35 260 20.7% of patients and adjuvant radiotherapy in 9.4% of the population.

36 261 **Patient and public involvement**

37 262 Patients and the public were not involved in the design, conduct, reporting or dissemination
38
39 263 plans of this research.

41 264 **How often have study participants been followed up?**

42 265 At each study site, collection of biospecimen, clinical, demographic, questionnaire and
43
44 266 anthropometric data occurred at baseline, and at six and twelve months following recruitment.
45
46 267 Baseline measurements in CORSA, COLON and EnCoRe have been performed at diagnosis
47
48 268 (preferably prior to any cancer treatment) while in the ColoCare Study such measurements
49
50 269 have been done prior to surgery (i.e. neoadjuvant treatment might have been applied prior to
51
52 270 baseline blood and data collection).

53
54
55 271 Blood samples at baseline were collected from n=2,132 (88.8%) study participants.
56
57 272 N=1,537 (64.0%) participants donated blood at the 6 month and n=614 (25.6%) at the 12
58
59 273 month follow-up time point. Additionally, n=251 (10.5%) EnCoRe patients provided blood
60

274 samples at 6 weeks post-treatment, a subset specifically of interest at the Maastricht study
275 site.

276 **What has been measured/recorded within the FOCUS Consortium?**

277 Harmonized baseline and follow-up time points are summarized in **Table 2** and are briefly
278 described below.

Table 2: Variables available at baseline, and at 6 and 12 month follow-up within the FOCUS Consortium.

Category	Variables	Baseline	6m	12m
Demographics	Age	x	x	x
	Gender	x		
	Highest education	x		
	Social status	x		
	Height	x		
	Weight	x	x	x
	BMI	x	x	x
	Smoking status	x	x	x
	Smoking duration	x	x	x
	Smoking pack years	x	x	x
	Menopausal status	x		
	Postmenopausal hormone use	x		
	Race	x		
Cancer characteristics	Cancer site	x		
	Cancer stage	x		
	TNM classification	x		
Treatment	Preoperative chemotherapy	x		
	Preoperative radiotherapy	x		
	Surgery	x	x	
	Postoperative chemotherapy		x	x
	Postoperative radiotherapy		x	x
Supplement intake	Folic acid supplements	x	x	x
	Vitamin B ₂ supplements	x	x	x
	Vitamin B ₆ supplements	x	x	x
	Vitamin B ₁₂ supplements	x	x	x
	Vitamin A supplements	x	x	x
	Vitamin C supplements	x	x	x
	Vitamin D supplements	x	x	x

279

Supplement intake	Vitamin E supplements	x	x	x
	Calcium supplements	x	x	x
	Magnesium supplements	x	x	x
	Iron supplements	x	x	x
	Multivitamins	x	x	x
Dietary nutrients	Folate equivalents	x	x	x
	Vitamin B ₂	x	x	x
	Vitamin B ₆	x	x	x
	Vitamin B ₁₂	x	x	x
	Vitamin A	x	x	x
	Vitamin C	x	x	x
	Vitamin D	x	x	x
	Vitamin E	x	x	x
	Total protein	x	x	x
	Total fat	x	x	x
	Total carbohydrate	x	x	x
	Fibre	x	x	x
	Saturated fatty acids	x	x	x
	Monounsaturated fatty acids	x	x	x
	Polyunsaturated fatty acids	x	x	x
	Alcohol	x	x	x
	Total energy	x	x	x
Physical activity	Light physical activity	x	x	x
	Moderate physical activity	x	x	x
	Vigorous physical activity	x	x	x
	Adherence to physical activity guidelines	x	x	x
Medical history	Diabetes mellitus	x	x	x
	Asthma, Chronic Bronchitis, COPD, Emphysema	x		
	Heart attack, Heart Failure	x		
	Hypertension	x		
	Stroke	x		
	Ulcer of Stomach or Duodenum	x		
	Hypothyroidism/Hyperthyroidism	x		
	Systemic Lupus Erythematosus	x		
	Inflammatory Bowel Disease (Crohn's disease, ulcerative colitis)	x		
	Familial Adenomatous Polyposis	x		

280

Medical history	Lynch Syndrome (hereditary nonpolyposis colorectal cancer)	x		
Regular medication use	Aspirin	x	x	x
	NSAID	x	x	x
	Ibuprofen	x	x	x
	Naproxen	x	x	x
	Celecoxib/Etoricoxib	x	x	x
Health-related quality of life	EORTC QLQ-C30	x	x	x
	EORTC QLQ-CR29	x	x	x
	EORTC QLQ-CIPN20	x	x	X
General information	Date of questionnaire completion	x	x	x
	Date of blood collection	x	x	x
	Freeze-thaw cycles of blood samples	x	x	x
	Hemolysis	x	x	x
	Time between blood draw and processing/storage	x	x	x

NSAID=non-steroidal anti-inflammatory drug; EORTC=European Organization for Research and Treatment of Cancer; QLQ=quality of life questionnaire; C30=core 30-items; CR29=colon/rectum 29-items; CIPN20=chemotherapy-induced polyneuropathy 20-items.

281

282 Lifestyle and demographic data

283 ColoCare questionnaires and standardized Food Frequency Questionnaires (FFQ) are used to
 284 assess intake of dietary supplements and medication, smoking, dietary intake and other health
 285 behaviors at each study time point. In COLON, patients provide information on diet, lifestyle,
 286 and supplement use via COLON questionnaires and FFQs. Patients from the CORSA Study
 287 are requested to complete a questionnaire assessing anthropometric and demographic factors.
 288 Patients enrolled in EnCoRe receive repeated home visits by trained dietitians, where
 289 extensive measurements are performed that include assessment of demographic data, physical
 290 activity (both questionnaire- and accelerometry-based) and smoking behavior (questionnaire
 291 data), dietary intake (FFQs at diagnosis and 7-day food diaries at follow-up measurements),
 292 supplement use (registered by dietitians), and anthropometry measurements.

293 Clinical data and outcomes - Medical chart abstraction

294 All cancers and medical diagnoses are classified according to ICD-10 (International
 295 Classification of Diseases, Tenth Revision) codes. Details on CRC treatment, including type

1
2
3 296 of treatment regimens and treatment toxicity, are abstracted via medical record for all cohorts.
4
5 297 Detailed information on the primary outcomes of interest, CRC recurrence and survival, are
6
7 298 ascertained through reviews of medical records, pathology reports, and imaging reports
8
9 299 documenting the diagnosis of a recurrence. Data on recurrence and vital status is
10
11 300 supplemented from the clinical cancer registries and survival data is verified by the inhabitant
12
13 301 registries that exist at all study sites. For COLON and EnCoRe, all data on recurrence are
14
15 302 retrieved in collaboration with the Netherlands Cancer Registry. Moreover, survival data for
16
17 303 CORSA participants was obtained by the Main Association of Austrian Social Insurance
18
19 304 Carriers as well as from Statistics Austria.

20 305 Patient-reported outcomes

21
22 306 Health-related quality of life is assessed by the validated and widely used cancer-specific 30-
23
24 307 item core questionnaire QLQ-C30 and the 29-item CRC module QLQ-CR29, developed by
25
26 308 the European Organization for Research and Treatment of Cancer (EORTC).²²
27
28 309 Chemotherapy-induced peripheral neuropathy (CIPN), which is a common toxicity in CRC
29
30 310 patients, is measured by the EORTC QLQ-CIPN20.²³ Patient-reported outcomes are available
31
32 311 for ColoCare Heidelberg, COLON and EnCoRe.

33 312 Biomarkers of FOCM

34
35 313 Blood is processed in identical settings across all study centers. Plasma (CORSA, COLON
36
37 314 and EnCoRe) and serum (for the three ColoCare Study sites) samples were collected and
38
39 315 immediately centrifuged, aliquoted, and stored at -80°C.

40
41 316 All biological analyses were performed at BEVITAL AS (Bergen, Norway,
42
43 317 <http://www.bevital.no>), which carried out metabolic profiling of biomarkers allocated to seven
44
45 318 complementary analytical platforms. Apart from analyses of microbiological active folate²⁴
46
47 319 and vitamin B₁₂²⁵), all analyses were based on mass spectrometry. Circulating folate, separate
48
49 320 folate species, 5-methyltetrahydrofolate (the main form in plasma), folic acid (folate in
50
51 321 supplements), 4a-OH 5-methyltetrahydrofolate (the primary oxidation form of circulating
52
53 322 folate), folate catabolites (p-aminobenzoylglutamate and acetyl p-aminobenzoylglutamate)²⁶,
54
55 323 B₆, B₁ and B₃ vitamins, kynurenines, cotinine, trans-3'-hydroxycotinine, trigonelline²⁷,
56
57 324 choline and its metabolites, creatinine, methylhistidines, arginine and methylated arginines²⁸
58
59 325 were analyzed by liquid chromatography-tandem mass spectroscopy, whereas other amino
60
326 acids, in addition to total homocysteine, total cysteine and methylmalonic acid were analyzed

327 by gas chromatography-tandem mass spectroscopy.²⁹ C-reactive protein (hsCRP) and
 328 cystatin C and its variants were measured by MALDI-TOF mass spectroscopy.³⁰

329 A comprehensive overview of the panel of biomarkers measured in blood samples of
 330 patients enrolled in the FOCUS Consortium is provided in **Table 3**.

Table 3: Measured metabolites and biomarkers within the FOCUS Consortium

Folate and one-carbon metabolites	Abbreviation	Description
Anthranilic acid	AA	Tryptophan metabolite
Asymmetric dimethylarginine	ADMA	Inhibitor of nitric oxide synthase
α-Ketoglutaric acid	aKG	Keto acid of the Krebs cycle
Alanine	Ala	Amino acid
Acetamidobenzoylglutamate	apABG	Folate catabolite
Arginine	Arg	Amino acid
Asparagine	Asn	Amino acid
Aspartic acid	Asp	Amino acid
Betaine	Betaine	Methyl donor
Choline	Choline	Methyl donor
C-reactive protein	hsCRP	Inflammatory marker
Cystatin C-desS	CnCds	Cystatin C isoform
Cystatin C-desSSP	CnDcssp	Cystatin C isoform
Cystatin C	CnCn	Marker of kidney function
3Pro-OH cystatin C	CnCco	Cystatin C isoform
3Pro-OH cystatin C-desS	CnCods	Cystatin C isoform
Total concentration of detected cystatin C isoforms	CnCt	Marker of kidney function
Cobalamin	B ₁₂	Vitamin B ₁₂
Cotinine	Cot	Nicotine metabolite
Creatinine	Creat	Marker of kidney function
C-reactive protein	CRP	Inflammation
Cystathionine	Cysta	Thioether, transsulfuration intermediate
Dimethylglycine	DMG	Betaine metabolite
Folic acid	B ₉	Synthetic form of folate
Flavin mononucleotide	FMN	B ₂ vitamin
5-Formyl-tetrahydrofolate	fTHF	Folate species
Glutamine	Gln	Amino acid
Glutamic acid	Glu	Amino acid
Glycine	Gly	Amino acid
3-Hydroxyanthranilic acid	HAA	Tryptophan metabolite
Homoarginine	hArg	Amino acid
Histidine	Hist	Tryptophan metabolite
3-Hydroxykynurenine	HK	Tryptophan metabolite
4-Alfa-hydroxy-5-methyl-THF	hmTHF	Folate oxidation product
Isoleucine	Ile	Amino acid

Kynurenic acid	KA	Tryptophan metabolite
Kynurenine	Kyn	Tryptophan metabolite
Leucine	Leu	Amino acid
Lysine	Lys	Amino acid
3-Methylhistidine (3-MH)	m3His	Marker of muscle degradation and meat intake
1-Methylhistidine (1-MH)	m1His	Marker of muscle degradation and meat intake
Methionine	Met	Amino acid
Methionine sulfoxide	MetSo	Oxidation product of Met
Methylmalonic acid	MMA	Marker of B ₁₂ status
N1-methylnicotinamide	mNAM	B ₃ vitamin
5-Methyl-tetrahydrofolate	mTHF	Folate species
Nicotinic acid	NA	B ₃ vitamin
Nicotinamide	NAM	B ₃ vitamin
Neopterin	Neopt	Inflammatory marker
Trans-3'-hydroxycotinine	OHCot	Nicotine metabolite
Ornithine	Orn	Amino acid
4-Pyridoxic acid	PA	Vitamin B ₆ catabolite
Para-aminobenzoylglutamate	pABG	Folate catabolite
Phenylalanine	Phe	Amino acid
Picolinic acid	Pic	Tryptophan metabolite
Pyridoxal	PL	B ₆ vitamin
Pyridoxal 5'-phosphate	PLP	B ₆ vitamin
Pyridoxine	PN	Synthetic form of vitamin B ₆
Proline	Pro	Amino acid
Quinolinic acid	QA	Tryptophan metabolite
Riboflavin	Ribo	Main circulating B ₂ form
Symmetric dimethylarginine	SDMA	Marker of renal function
Serine	Ser	Amino acid
Folate	spFolate	Microbiologically active folate
Total cysteine	tCys	Amino acid
Homocysteine	tHcy	Marker of folate and B ₁₂ status
Thiamine	Thi	B ₁ vitamin
Threonine	Thr	Amino acid
Trimethylamineoxide	TMAO	Choline metabolite
Trimethyllysine	TML	Amino acid
Thiamine monophosphate	TMP	B ₁ vitamin
Trigonelline	Trig	Marker of coffee consumption
Tryptophan	Trp	Amino acid
Valine	Val	Amino acid
Leucine	Leu	Amino acid
Xanthurenic acid	XA	Tryptophan metabolite
Kyn/Trp ratio	KTR	Marker of immune activation
HK/XA ratio		Marker of B ₆ status

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What has the FOCUS Consortium found? Key findings and publications

The FOCUS Consortium provides a unique opportunity to conduct comprehensive research on folate and FOCM biomarkers status in the tertiary prevention of CRC using data collected both at diagnosis and during standardized follow-up time points. This well-characterized study design provides sufficient statistical power to discern prospective associations with relevant clinical outcomes, including CRC recurrence and survival within relevant subgroups.

Key findings and publications

We investigated associations of circulating concentrations of folate, folic acid, and folate catabolites pABG and apABG, measured around time of diagnosis, with recurrence and survival among 2024 patients diagnosed with stage I-III CRC within the international FOCUS Consortium. We did not observe any statistically significant associations for folate, pABG, and apABG concentrations. However, an increased risk of cancer recurrences was observed among patients with higher compared to lower circulating folic acid concentrations.³¹ Further, Kiblawi et al. measured associations between one-carbon metabolites, inflammation- and angiogenesis-related biomarkers in a cross-sectional analysis of 238 patients from the ColoCare Heidelberg cohort. The study showed that specific folate species within the one-carbon metabolism pathway are associated with both inflammation and angiogenesis pathways among CRC patients. Our findings reinforce the notion that B vitamins involved in the one-carbon metabolism may be correlated with carcinogenic processes.³² This and further research will support the evidence base needed for the development of dietary guidelines for CRC patients.

Further, we investigated circulating concentrations of nine biomarkers related to the B-vitamins folate, riboflavin, vitamin B₆, and cobalamin, measured at diagnosis and six months post-diagnosis, in association with health-related quality of life as assessed by the EORTC QLQ-C30 questionnaire six months post-treatment in three FOCUS cohorts (ColoCare Heidelberg, EnCoRe and COLON).³³ Higher pyridoxal 5'-phosphate (PLP) concentrations were cross-sectionally associated with better physical, role, and social functioning, and reduced fatigue six months post-diagnosis. Higher HKr (3-hydroxykynurenine:(kynurenic acid+xanthurenic acid+3-hydroxyanthranilic acid+anthranilic acid)), an inverse marker of vitamin B₆ status, was cross-sectionally associated with worse global quality of life, and lower physical and role functioning. Dose-response relations were observed for PLP with global

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3 363 quality of life, physical, role, and social functioning. No associations were observed for
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5 364 changes in biomarker concentrations between diagnosis and six months with quality of life
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7 365 outcomes. We therefore concluded that higher vitamin B₆ status was associated with better
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9 366 quality of life at six months post treatment and that further study is needed to clarify the role
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11 367 of vitamin B₆ in relation to quality of life.

12 368 Previous relevant findings from individual cohorts within the consortium

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15 369 To date, individual cohorts from the FOCUS Consortium have initiated the examination of
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17 370 dietary supplement use and dietary habits over time. Among CRC patients enrolled in the
18
19 371 ColoCare Study the proportion of supplement users was found to be highest post-diagnosis
20
21 372 (35%).³⁴ Moreover, within an international investigation including ColoCare participants from
22
23 373 multiple sites, Ulrich et al. showed differences in plasma folate concentration between
24
25 374 Heidelberg and the US sites, probably reflecting variation in folic acid fortification and
26
27 375 supplement use.¹¹ Furthermore, ColoCare has published data on RECQ helicase expression³⁵,
28
29 376 NTRK3³⁶, RET³⁷, tumor-infiltrating lymphocytes and T cell receptor sequences³⁸, 25-25-
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31 377 hydroxyvitamin D₃^{11 39}, DNA methylation⁴⁰⁻⁴³, miRNAs^{44 45}, fecal microbiota⁴⁶⁻⁴⁸,
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33 378 metabolomics and transcriptomics⁴⁹⁻⁵¹, plasma proteins⁵², gene expression⁵³, branched-chain
34
35 379 amino acids⁵⁴, genetic variants⁵⁵, body composition^{51 56 57}, physical activity³⁹, and dietary
36
37 380 patterns⁴ in CRC patients. Within the COLON study, results have been published on body
38
39 381 weight trajectories⁵⁸, changes in lifestyle⁵⁹, 25-hydroxy vitamin D levels^{60 61}, and
40
41 382 inflammation markers⁶² over time, as well as vitamin D⁶², calcium or magnesium intake⁶³,
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43 383 physical activity⁶⁴, inflammation^{65 66}, skeletal muscle mass⁶⁷/ density⁶⁸ and other measures of
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45 384 body composition⁶⁹ in relation to cancer recurrence, survival, or physical functioning or
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47 385 fatigue. Moreover, in a subset of patients undergoing chemotherapy, dietary factors in relation
48
49 386 to chronic CIPN^{70 71} as well as chemosensory perception and food preferences⁷² were studied.
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51 387 The Austrian CORSA Study has published results on genomic data⁷³⁻⁷⁵, telomere length⁷⁶,
52
53 388 DNA repair processes⁷⁷, tumor autoantibodies⁷⁸ as well as metabolomics.^{31 79} To date,
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55 389 publications from the EnCoRe Study have reported on associations of physical activity and
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57 390 sedentary behaviour⁸⁰⁻⁸³, adherence to lifestyle guidelines⁸⁴, and parameters of body
58
59 391 composition^{85 86} measured through CT scans with quality of life, functioning and fatigue in
60
392 CRC survivors. Recently, longitudinal associations between supplement use and fatigue were
393 investigated from diagnosis to 2 years post-CRC treatment. No overall association between
394 supplement use and fatigue was found but results suggest that increased levels of fatigue may
395 be a reason for the use of supplements among CRC survivors.⁸⁷ Higher concentrations of

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3 396 25OHD3 were longitudinally associated with better global quality of life and less fatigue in
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5 397 colorectal cancer survivors within EnCoRe suggesting a potential beneficial role of vitamin D
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7 398 in colorectal cancer survivors.³³ In a mixed-method study using data of the EnCoRe study,
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9 399 colorectal cancer (treatment) related health and functioning problems negatively impacted the
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11 400 ability of nearly 1 in 5 long-term CRC survivors to participate in everyday life situations and
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13 401 their satisfaction with participation.⁸⁸ The validity of the food frequency questionnaire for
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15 402 measuring dietary intake among survivors of colorectal cancer within the EnCoRe study
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17 403 appeared to be moderate to good for most nutrients and food groups, relative to a 7-day
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19 404 dietary record.⁸⁹ Prediction models that we developed for estimating 1-year risk of low health-
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21 405 related quality of life in seven domains in colorectal cancer survivors performed well when
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23 406 externally validated among survivors within the EnCoRe and COLON studies.⁹⁰

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408 **What are the main strengths and weaknesses of the FOCUS Consortium?**

409 This is the largest consortium to date addressing the research question of folate and FOCM
410 biomarkers in relation to survival, recurrence, treatment toxicity, and health-related quality of
411 life outcomes in CRC patients. The cohorts included in the FOCUS Consortium are designed
412 to enable future pooling of data.²⁰ For that reason, methodologies, time points and
413 measurement instruments generally overlap, with each study presenting unique features such
414 as additional blood collection at the 6-week follow-up time point within the EnCoRe Study
415 and blood draws during chemotherapy within the COLON Study. The pooled sample size
416 provides sufficient power to investigate subgroup analyses across CRC patients, e.g., within
417 groups of patients who underwent 5-fluorouracil based chemotherapy or stratified by disease
418 stage. Furthermore, the assessment of biomarkers related to FOCM are conducted at a single,
419 state-of-the art laboratory and the biological materials are processed and stored according to
420 standardized operation protocols across all study sites, enabling precise and accurate
421 measurements of FOCM biomarkers. The study population is predominantly based on
422 European cohorts (90.4%) in countries that have not implemented mandatory folic acid
423 fortification. This enables us to study a population where dietary intake and dietary
424 supplement use determine differences in folate status, yielding information of direct relevance
425 to cancer patients. However, the generalizability of results to populations that have introduced
426 folic acid fortification, including United States, might therefore also be limited.⁹¹

427 Another limitation of the consortium includes differences in collection strategies of the
428 baseline study time point across included studies: CORSA, COLON and EnCoRe are
429 recruiting CRC patients at diagnosis, preferably prior to any cancer treatment while ColoCare
430 is recruiting patients prior to surgery (i.e. neoadjuvant treatment might have occurred prior to
431 baseline blood and data collection). Nevertheless, the ColoCare protocol requires that no
432 blood draw occurs within 2 weeks of neoadjuvant or adjuvant chemotherapy, limiting the
433 influence of ongoing treatments on blood biomarkers. In some cohorts, the timing with
434 respect to adjuvant chemotherapy is less clearly defined and this will be carefully considered
435 in sensitivity analyses. Furthermore, survivor bias – a type of selection bias – may be
436 introduced as some patients died or did not complete follow-up questionnaires or did not
437 provide blood samples. It is possible that patients who experience more severe toxicities,
438 worse clinical outcomes or worse health-related quality of life are underrepresented among
439 those patients who completed follow-up measurements.⁴

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3 441 **Can I get hold of the data? Where can I find out more?**
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5 442 A substantial amount of time has been spent in creating the harmonized dataset of baseline
6 443 variables from cohorts within the FOCUS Consortium, with follow-up data collection and
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8 444 FOCM biomarker analyses. Any person interested in learning about the FOCUS Consortium
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10 445 or in getting access to FOCUS data and in-depth analyses can contact the coordinating study
11
12 446 PIs Prof. Andrea Gsur (andrea.gsur@meduniwien.ac.at) and Prof. Cornelia Ulrich
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14 447 (neli.ulrich@hci.utah.edu). Requests for data will be discussed and decided by all PIs and will
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16 448 require a Data Transfer Agreement.
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449 **FOCUS Consortium profile in a nutshell**

- 450 • The FOCUS Consortium is an international prospective cohort with newly-diagnosed non-
451 metastatic (stage I-III) colorectal cancer (CRC) patients, designed to study the
452 associations between folate and folate-mediated one-carbon metabolism (FOCM)
453 biomarkers and CRC prognosis, to provide clinically relevant advice to cancer patients
454 and define future tertiary prevention strategies.
- 455 • 2,401 women and men age 18 years and older newly-diagnosed with a primary invasive
456 CRC, stage I-III, from the ColoCare Study at the University of Heidelberg in Heidelberg,
457 Germany; the ColoCare Study at the Huntsman Cancer Institute in Salt Lake City, Utah,
458 USA; the ColoCare Study at the Fred Hutchinson Cancer Research Center in Seattle,
459 Washington, USA; CORSA Study at the Medical University of Vienna in Austria; the
460 COLON Study at Wageningen University in the Netherlands; and the EnCoRe Study at
461 Maastricht University Medical Center in the Netherlands were eligible and recruited
462 between 2003 and 2016.
- 463 • Blood samples at baseline were collected from n=2,132 (88.8%) study participants.
464 N=1,537 (64.0%) participants donated blood at the 6 month and n=614 (25.6%) at the 12
465 month follow-up time points.
- 466 • The harmonized dataset comprises sociodemographic data and data on lifestyle behaviors
467 (e.g., dietary supplement use, dietary nutrient intake, and physical activity), clinical data
468 (e.g., cancer characteristics, treatment regimens), health-related quality of life, and
469 biomarkers of one-carbon metabolism. Follow-up data additionally includes CRC
470 recurrence, survival, treatment-induced toxicity, and health-related quality of life.
- 471 • We will welcome new collaborations, for which requests can be sent to the corresponding
472 authors, PIs Prof. Andrea Gsur (andrea.gsur@meduniwien.ac.at) and Prof. Cornelia Ulrich
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511 **Contributors**

512 BG, MW, EK, PMU, CMU, AG, ABU, NH: conceived of the project, developed the overall
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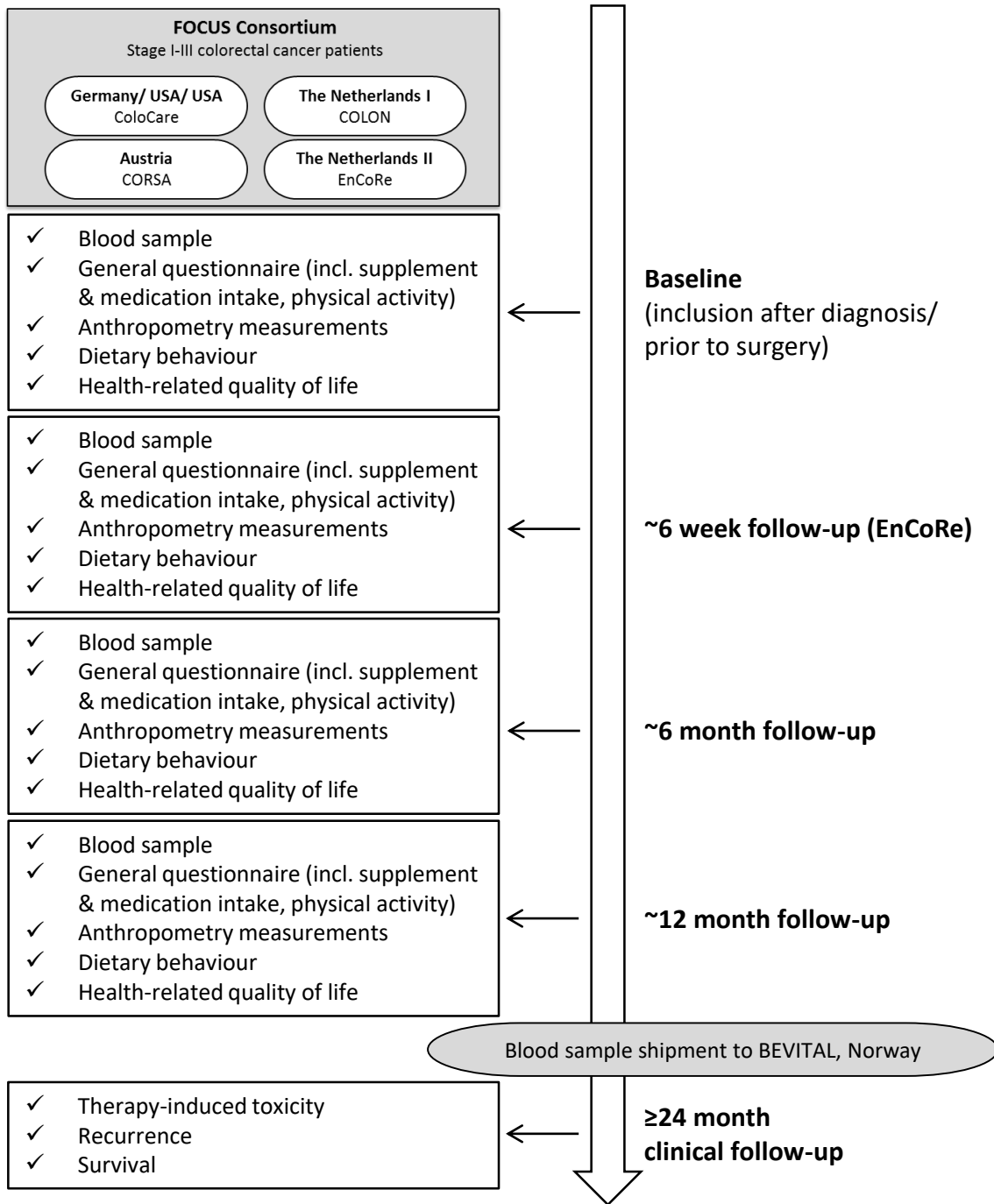
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3 893 **Figure legends**
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5 894 **Figure 1** FOCUS Consortium design
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**Cohort profile: Biomarkers related to folate-dependent one-carbon metabolism in colorectal cancer recurrence and survival:
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Cohort profile: Biomarkers related to folate-dependent one-carbon metabolism in colorectal cancer recurrence and survival: The FOCUS Consortium

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46 Abstract

47 **Purpose:** The overarching goal of the FOCUS (Biomarkers related to folate-dependent one-
48 carbon metabolism in colorectal cancer recurrence and survival) Consortium is to unravel the
49 effect of folate and folate-mediated one-carbon metabolism (FOCM) biomarkers on colorectal
50 cancer (CRC) prognosis to provide clinically relevant advice on folate intake to cancer
51 patients and define future tertiary prevention strategies.

52 **Participants:** The FOCUS Consortium is an international, prospective cohort of 2,401
53 women and men above 18 years of age who were diagnosed with a primary invasive non-
54 metastatic (stage I-III) CRC. The Consortium comprises patients from Austria, two sites from
55 the Netherlands, Germany and two sites from the USA. Patients are recruited after CRC
56 diagnosis and followed at six and twelve months after enrolment. At each time point,
57 sociodemographic data, data on health behavior, and clinical data are collected, blood samples
58 are drawn.

59 **Findings to date:** An increased risk of cancer recurrences was observed among patients with
60 higher compared to lower circulating folic acid concentrations. Furthermore, specific folate
61 species within the FOCM pathway were associated with both inflammation and angiogenesis
62 pathways among CRC patients. In addition, higher vitamin B₆ status was associated with
63 better quality of life at six months post treatment.

64 **Future plans:** Better insights into the research on associations between folate and FOCM
65 biomarkers and clinical outcomes in CRC patients will facilitate the development of
66 guidelines regarding folate intake in order to provide clinically relevant advice to cancer
67 patients, health professionals involved in patient care, and ultimately further tertiary
68 prevention strategies in the future. The FOCUS Consortium offers an excellent infrastructure
69 for short- and long-term research projects and for combining additional biomarkers and data
70 resulting from the individual cohorts within the next years, e.g. microbiome data, omics- and
71 multi-omics data or CT-quantified body composition data.

72 Strengths and limitations of this study

- 73 • FOCUS is the largest consortium to date addressing the research question of folate and
74 FOCM biomarkers in relation to survival, recurrence, treatment toxicity, and health-
75 related quality of life outcomes in CRC patients
- 76 • The cohorts included in the FOCUS Consortium are designed to enable future pooling
77 of data using harmonized and standardized methods to collect data and biospecimens
- 78 • The pooled sample size provides sufficient power to investigate subgroup analyses
79 across CRC patients
- 80 • Study time point definitions differ between some of the cohorts and have to be adapted
81 for specific projects
- 82 • A selection bias for follow-up can arise because it is possible that patients who
83 experience more severe toxicities, worse clinical outcome or health-related quality of
84 life are underrepresented among those completing follow-ups

86 Introduction

87 Among men and women world-wide, colorectal cancer (CRC) accounts for nearly 10% of all
88 incident cancer cases.¹ In Europe, 5-year survival for CRC patients is approximately 55%,
89 with substantial differences by stage.^{2,3} The number of CRC patients continues to increase due
90 to implementation of improved screening strategies and/or enhanced treatment modalities.⁴
91 Many cancer patients seek information on what they can do themselves to improve survival -
92 for instance by improving their dietary habits and other lifestyle factors.⁵ However, there may
93 be behavioural aspects among individuals diagnosed with CRC, which may be harmful in
94 some cases, such as the use of high-dose nutritional supplements containing synthetic folate.
95 In general, knowledge on short and long-term effects is insufficient to make sound
96 recommendations on use of dietary supplements, in particular folate, to cancer survivors, even
97 though dietary supplements are used by 20%-85% of patients with cancer⁶⁻⁸, highlighting the
98 importance of thorough evaluation of potential benefits and harms and to support
99 development of evidence-based recommendations on use of dietary supplements to cancer
100 patients.

101 Folic acid is the synthetic form of the B-vitamin folate and is often used in dietary
102 supplements or fortified foods. However, there is broad agreement that food folate is less
103 bioavailable than folic acid with a median relative bioavailability of 65% (range: 44–80%), an
104 estimate that approximates the 60% value derived from the Dietary Folate Equivalents
105 equation.⁹ Folate and folic acid play an important role in one-carbon metabolism, which is a
106 complex series of biochemical reactions essential in nucleotide synthesis, methylation
107 reactions and amino acid homeostasis.¹⁰ One-carbon metabolism refers to a complex network
108 of biochemical reactions linked to nucleotide synthesis and provides methyl groups for DNA,
109 RNA or protein methylation. Thus, one-carbon metabolism is directly controlling processes
110 determining DNA synthesis and integrity, both processes known to be linked to tumor
111 growth.¹¹ To what extent folic acid supplement use and biomarkers of folate-mediated one-
112 carbon metabolism (FOCM) impact cancer survival and treatment efficacy and toxicity still
113 needs to be clarified.^{11,12} Folate and FOCM biomarker deficiencies may increase cancer risk,
114 but high levels, especially of synthetic folic acid, may also be driving factors in
115 carcinogenesis.^{6,13} An increasing body of evidence suggests that folate plays a dual role in
116 carcinogenesis, involving both the prevention of early lesions and potential harm once pre-
117 neoplastic or neoplastic lesions have developed.^{6,12,14-20}

1
2
3 118 The overarching goal of the FOCUS (biomarkers related to Folate-dependent one-
4
5 119 carbon metabolism in Colorectal cancer recurrence and Survival) Consortium is to study
6
7 120 associations between folate and FOCM biomarkers and recurrence and survival in CRC
8
9 121 patients. Better insights into these associations will facilitate the development of guidelines
10
11 122 regarding folate intake in order to provide clinically relevant advice to cancer patients, health
12
13 123 professionals involved in patient care, and ultimately further tertiary prevention strategies in
14
15 124 the future. The FOCUS Consortium is a large-scale international consortium with CRC
16
17 125 patients from six prospective cohort studies. The primary objectives of the FOCUS
18
19 126 Consortium are: (i) to determine possible associations of folate and other FOCM biomarkers
20
21 127 at diagnosis with recurrence or survival in non-metastatic (stage I-III) CRC; (ii) to elucidate
22
23 128 whether biomarkers related to FOCM are associated with dietary and supplemental intake of
24
25 129 these respective nutrients; (iii) to explore whether FOCM biomarkers are associated with
26
27 130 treatment toxicity in CRC patients undergoing chemotherapy; and (iv) to collect
28
29 131 comprehensive data of patient characteristics at baseline and follow-up including biomarkers
30
31 132 of FOCM to establish a unique resource for future scientific research. The FOCUS
32
33 133 Consortium is funded by the European Research Area Network (ERA-NET) on Translational
34
35 134 Cancer Research (TRANSCAN).

36
37 135 The main purpose of the FOCUS Cohort profile is to (1) inform the scientific
38
39 136 community about the FOCUS Consortium, (2) describe the complex methodology of a large
40
41 137 consortium, (3) present ongoing studies using this infrastructure as well as (4) advise
42
43 138 interested researchers of opportunities for collaboration. This joint research may lead to a
44
45 139 better understanding of the role of folate- and FOCM-related mechanisms in the prognosis of
46
47 140 CRC and be a precursor for data for future randomized controlled trials, which will be critical
48
49 141 for the development of guidelines regarding folate intake among CRC patients.

142 **Cohort description**

143 The FOCUS Consortium is an international, prospective consortium including six cohort
144
145 144 studies recruiting women and men age 18 years and older diagnosed with a primary CRC. The
146
147 145 FOCUS Consortium is comprised of patients from the ColoCare Study at the University of
148
149 146 Heidelberg, Germany (n=298, 12.3%), the ColoCare Study at the Huntsman Cancer Institute
150
151 147 in Salt Lake City, Utah, USA (n=80, 3.3%), the ColoCare Study at the Fred Hutchinson
152
153 148 Cancer Research Center in Seattle, Washington, USA (n=157, 6.3%), the COLON Study
154
155 149 (Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that
156
157 150 may influence colorectal tumor recurrence, survival and quality of life) at Wageningen
158
159 151 University & Research in the Netherlands (n=1,365, 56.1%), the CORSA Study (Colorectal
160

1
2
3 152 Cancer Study of Austria) at the Medical University of Vienna in Austria (n=218, 9.0%), and
4
5 153 the EnCoRe Study (Energy for life after ColoRectal cancer) at Maastricht University Medical
6
7 154 Center+ in the Netherlands (n=317, 13.0%).

8
9 155 In total, n=2,435 CRC patients were considered for the FOCUS Consortium, of which
10
11 156 n=34 patients were excluded due to tumor staging being either 0 or IV leading to a total
12
13 157 number of n=2,401 stage I-III CRC patients included in further analyses.

14
15 158 Patients were recruited after CRC diagnosis and repeated study measurements were
16
17 159 conducted at time of recruitment, and at six and twelve months thereafter. At each study time
18
19 160 point, sociodemographic data, data on lifestyle factors (e.g., diet, supplement use, physical
20
21 161 activity), and clinical data (e.g., tumor site and stage, cancer treatment, treatment-induced
22
23 162 toxicities, recurrence, survival) were collected, and blood samples were drawn (**Figure 1**). All
24
25 163 individuals signed informed consent and the Institutional Review Board at each site approved
26
27 164 the corresponding study. Below, a more specific description of each included study is
28
29 165 provided.

30 166 The ColoCare Study

31 167 The ColoCare Study (ClinicalTrials.gov identifier: NCT02328677) is an ongoing
32
33 168 international, multi-center prospective cohort study among women and men newly diagnosed
34
35 169 with a primary invasive CRC, with the goal to investigate predictors of cancer recurrence,
36
37 170 survival, treatment toxicities and health-related quality of life.^{4 21} Three ColoCare Consortium
38
39 171 sites participate in the FOCUS Consortium: the Fred Hutchinson Cancer Research Center in
40
41 172 Seattle (Washington, USA), and the University Hospital Heidelberg, Heidelberg (Germany)
42
43 173 as well as the Huntsman Cancer Institute in Salt Lake City (Utah, USA). Patients are enrolled
44
45 174 prior to undergoing CRC surgery according to the following inclusion criteria: individuals
46
47 175 who are 18 years of age and older, newly diagnosed (i.e., non-recurrent) with invasive CRC.
48
49 176 Blood draws and other biospecimens are obtained prior to surgery and at regular intervals
50
51 177 (e.g., six, twelve, twenty-four months). Questionnaires are administered to assess lifestyle
52
53 178 behavior, health-related quality of life, and clinical outcomes such as CRC recurrence,
54
55 179 treatment, and treatment symptoms at each study time point. Clinical data are obtained
56
57 180 through reviews of patient medical records, pathology and imaging reports. Vital status is
58
59 181 obtained through medical records, routine follow-up mailings, periodic requests for outside
60
182 medical records, and state or national cancer and death registries. The Heidelberg ColoCare
183 Study was approved by the Ethics Committee of the Medical Faculty of Heidelberg (approval
184 no. S-310/2001 and S-134/2016). The ColoCare Study at the Huntsman Cancer Institute and

1
2
3 185 the Fred Hutchinson Cancer Research Center were approved by the respective IRBs (#77147
4
5 186 and #6407).

6
7
8 187 The COLON Study

9
10 188 The COLON Study (ClinicalTrials.gov identifier: NCT03191110) started in 2010 and is an
11
12 189 ongoing, multicenter prospective cohort study specifically designed to assess associations
13
14 190 between nutrition, lifestyle, and dietary supplement use with quality of life, CRC recurrence
15
16 191 and survival among CRC patients (stages I-IV).²² Persons with a history of CRC or (partial)
17
18 192 bowel resection, chronic inflammatory bowel disease, or a known hereditary CRC syndrome
19
20 193 are excluded from the study. Patients are recruited from eleven regional and academic
21
22 194 hospitals prior to surgery. Individuals donate blood samples and provide information on diet,
23
24 195 lifestyle, and dietary supplement use at CRC diagnosis, i.e. baseline, and at six, twelve
25
26 196 (chemotherapy patients only), twenty-four and sixty months after diagnosis. Clinical data are
27
28 197 collected through review of medical records (treatment-induced toxicity) or through linkage
29
30 198 with the Dutch ColoRectal Audit (DCRA). Mortality data (i.e. death or alive and date of
31
32 199 death) are retrieved from the Municipal Personal Records Database. Recurrence data have
33
34 200 been retrieved in collaboration with the Netherlands Cancer Registry. Ethical approval for the
35
36 201 study was granted by the Committee on Research involving Human Subjects, region Arnhem-
37
38 202 Nijmegen under file number 2009/349.

39
40 203 The CORSA Study

41
42 204 CORSA is an ongoing case-control study of women and men recruiting CRC patients,
43
44 205 patients with high and low risk adenomas and population-based colonoscopy negative
45
46 206 controls, with an age range between 30 and 90 years. Since 2003, more than 13,500
47
48 207 participants have been recruited across nine sites in Austria. The multicentre recruitment
49
50 208 within CORSA follows standardized protocols resulting in consistent data from all
51
52 209 recruitment sites. These sites include the Medical University of Vienna (Department of
53
54 210 Surgery), two hospitals in Vienna (Clinic Favoriten and Clinic Landstraße), and the Hospital
55
56 211 Wiener Neustadt in Lower Austria. Furthermore, the recruitment for CORSA was performed
57
58 212 in four hospitals in the federal state Burgenland within the population-based screening
59
60 213 program "Burgenland PREvention Trial of colorectal cancer DIsease with ImmunologiCal
214
215 214 Testing" (B-PREDICT). B-PREDICT is a two-stage screening project where more than
216
215 215 150,000 inhabitants of Burgenland aged between 40 and 80 are invited annually to participate
216
216 216 in this program using a Fecal Immunochemical Test (FIT) as an initial screening. FIT-positive

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2
3 217 (≥ 10 μg hemoglobin / g feces) tested individuals are offered a complete colonoscopy and are
4
5 218 asked to participate in CORSA. Biospecimen are collected at each site using harmonized
6
7 219 protocols. CORSA participants provide a basic CORSA questionnaire assessing data on body
8
9 220 mass index (BMI), smoking history, alcohol consumption, education level, family status,
10
11 221 profession, basic dietary habits, and diabetes. Clinical data are abstracted from medical
12
13 222 records and processed in a structured database following standardized documentation
14
15 223 guidelines and according to the General Data Protection Regulation (GDPR). All subjects
16
17 224 gave written informed consent and the study was approved by the institutional review boards:
18
19 225 ‘Ethikkommission Burgenland’ (33/2010), by the ethical review committee of the Medical
20
21 226 University of Vienna (1160/2016) and by the “Ethikkommission der Stadt Wien” (EK 06-
22
23 227 150VK).

23 228 The EnCoRe Study

24
25
26 229 The EnCoRe Study is a prospective cohort study initiated in 2012 at the Maastricht University
27
28 230 Medical Center+ in the Netherlands that focuses on the importance of lifestyle factors for
29
30 231 quality of life, recurrence and survival of CRC patients.²³ The EnCoRe Study is registered in
31
32 232 the Netherlands Trial Registry for experimental and observational studies
33
34 233 (www.trialregister.nl, registration number 7099). Stage I-III CRC patients are enrolled at
35
36 234 diagnosis at three hospitals in the South-Eastern region of the Netherlands and followed up
37
38 235 until five years after completion of treatment with repeated measurements at diagnosis (pre-
39
40 236 treatment), and at six weeks and six, twelve, twenty-four, and sixty months after the end of
41
42 237 the initial anti-cancer treatment (i.e., surgery, chemotherapy, radiotherapy). Patients with
43
44 238 stage IV CRC, an inability to understand the Dutch language in speech or writing, with
45
46 239 comorbidities obstructing successful participation (e.g., Alzheimer disease), or with severe
47
48 240 visibility or hearing disorders are excluded from the study. Repeated home visits by trained
49
50 241 dieticians are conducted and data are collected amongst others on sociodemographic factors,
51
52 242 quality of life, functioning, physical activity, comorbidity, dietary intake, supplement use, and
53
54 243 anthropometry. In addition, clinical data are collected from hospital records and blood
55
56 244 samples are drawn at all time points. Recurrence data have been retrieved in collaboration
57
58 245 with the Netherlands Cancer Registry. Mortality data (i.e. death or alive and date of death) are
59
60 246 retrieved from the Municipal Personal Records Database. The EnCoRe Study was approved
247
248 by the Medical Ethics Committee of the Maastricht University Hospital and Maastricht
University (METC 11-3-075).

249 The FOCUS Consortium

250 Sociodemographic and clinical characteristics of the 2,401 participants in the FOCUS
251 Consortium are presented in **Table 1**. The mean age at CRC diagnosis was 65.4 years
252 (standard deviation (SD): 10.2; range: 22 to 93 years), and the majority of participants were
253 male (64.0%). The mean BMI was 27.0 kg/m² (SD: 4.6).

254 In terms of education status, 35.9% of the patients reported lower education, 28.8% reported
255 intermediate education and 27.3% high education. Most of the patients were married or part
256 of a living community (73.1%). About half of the overall cohort reported to be a former
257 smoker (50.8%), 31.7% were never smokers, and 12.1% were current smokers (5.4% were
258 unknown). Median alcohol intake was 7.9 g/day. In total, 16.9% of the patients reported
259 regular dietary supplementation (i.e., at least once per week during the last four weeks) of
260 folic acid. Over half of all participants (54.6%) reported adherence to the physical activity
261 guidelines of at least 150 minutes per week of moderate-to-vigorous physical activity.
262 Lifestyle characteristics of the FOCUS cohort are presented in **Table 2**.

263 Regarding clinical characteristics, 57.0% of participants were diagnosed with colon cancer,
264 33.9% with rectal cancer, and 1.1% with rectosigmoid cancer (8.1% were of unknown tumor
265 subsite). In total, 26.0% were diagnosed with stage I, 28.7% with stage II, and 40.2% with
266 stage III CRC. Approximately 1% of participants were classified with an unspecified cancer
267 stage, as distinction between stage I and II or II and III was not possible, and for 4.4% of the
268 total population the cancer stage was unknown. In total, 89.3% of patients underwent surgery,
269 whereas neoadjuvant chemotherapy was administered to 10.6% of patients and 26.8%
270 received adjuvant chemotherapy. Neoadjuvant radiotherapy was administered to 20.7% of
271 patients and adjuvant radiotherapy in 9.4% of the population.

272 **Patient and public involvement**

273 Patients and the public were not involved in the design, conduct, reporting or dissemination
274 plans of this research.

Table 1: Sociodemographic and clinical factors of eligible FOCUS Consortium participants at baseline (n=2,401)

	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe
n (%)	2,401 (100)	298 (12.4)	150 (6.3)	80 (3.3)	1,338 (55.7)	218 (9.1)	317 (13.2)
Age							
Mean y ± SD	65.4 ± 10.2	63.7 ± 12.2	58.3 ± 12.8	61.3 ± 11.2	66.1 ± 8.7	67.3 ± 12.0	66.9 ± 9.3
Sex							
Female n (%)	864 (36.0)	100 (33.6)	67 (44.7)	32 (40.0)	486 (36.3)	76 (34.9)	103 (32.5)
Male n (%)	1,537 (64.0)	198 (66.4)	83 (55.3)	48 (60.0)	852 (63.7)	142 (65.1)	214 (67.5)
Education							
Low n (%)	861 (35.9)	123 (41.3)	18 (12.0)	17 (21.3)	543 (40.6)	73 (33.5)	87 (27.4)
Intermediate n (%)	694 (28.9)	57 (19.1)	71 (47.3)	40 (50.0)	332 (24.8)	73 (33.5)	121 (38.2)
High n (%)	658 (27.4)	88 (29.5)	28 (18.7)	8 (10.0)	417 (31.7)	14 (6.4)	103 (32.5)
Unknown n (%)	188 (7.8)	30 (10.1)	33 (22.0)	15 (18.7)	46 (3.4)	58 (26.6)	6 (1.9)
Marital status							
Unmarried n (%)	122 (5.1)	19 (6.4)	15 (10.0)	2 (2.5)	60 (4.5)	10 (4.6)	16 (5.1)
Married n (%)	1,738 (72.4)	193 (64.8)	71 (47.3)	50 (62.5)	1,071 (80.0)	113 (51.8)	240 (75.7)
Divorced/Separated n (%)	138 (5.7)	24 (8.0)	11 (7.3)	10 (12.5)	64 (4.8)	14 (6.4)	15 (4.7)
Widowed n (%)	194 (8.1)	31 (10.4)	7 (4.7)	1 (1.3)	90 (6.7)	26 (11.9)	39 (12.3)
Living community n (%)	20 (0.8)	0	13 (8.7)	2 (2.5)	4 (0.3)	1 (0.5)	0
Unknown n (%)	189 (8.9)	31 (10.4)	33 (22.0)	15 (18.7)	49 (3.7)	54 (24.8)	7 (2.2)
Smoking status							
Current n (%)	290 (12.1)	53 (17.8)	8 (5.3)	6 (7.5)	143 (10.7)	38 (17.4)	42 (13.3)
Former n (%)	1,219 (50.8)	132 (44.3)	53 (35.32)	21 (26.3)	767 (57.3)	76 (34.9)	170 (53.6)
Never n (%)	767 (31.9)	90 (30.2)	56 (37.3)	39 (48.7)	386 (28.9)	97 (44.5)	99 (31.2)
Unknown n (%)	125 (5.2)	23 (7.7)	33 (22.0)	14 (17.5)	42 (3.1)	7 (3.2)	6 (1.9)
Alcohol intake							
Mean g/day ± SD	13.6 ± 17.1	16.1 ± 19.2	8.8 ± 18.5	2.3 ± 10.2	14.0 ± 17.0		12.9 ± 16.5
Unknown n (%)	633 (26.4)	208 (69.8)	81 (54.0)	60 (75.0)	54 (4.1)	218 (100)	12 (3.8)
BMI at diagnosis							
Mean kg/m² ± SD	27.1 ± 4.6	26.6 ± 4.1	28.7 ± 7.3	29.4 ± 7.5	26.6 ± 4.0	27.7 ± 4.4	28.3 ± 4.6
Unknown n (%)	42 (1.7)	2 (0.7)	0	14 (17.5)	10 (0.8)	14 (6.4)	2 (0.6)

	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe
Tumor site							
Colon n (%)	1,450 (60.4)	140 (57.0)	75 (50.0)	45 (56.3)	867 (64.8)	131 (60.1)	192 (60.6)
Rectum n (%)	862 (35.9)	143 (48.0)	60 (40.0)	22 (27.5)	434 (32.4)	78 (35.8)	125 (39.4)
Rectosigmoid n (%)	42 (1.7)	15 (5.0)	15 (10.0)	7 (8.7)	0	5 (2.3)	0
Unknown n (%)	47 (2.0)	0	0	6 (7.5)	37 (2.8)	4 (1.8)	0
Tumor stage							
I n (%)	629 (26.2)	76 (25.5)	35 (23.3)	21 (26.3)	326 (24.4)	82 (37.6)	89 (28.1)
II n (%)	694 (28.9)	114 (38.3)	49 (32.7)	23 (28.7)	386 (28.9)	52 (23.9)	70 (22.1)
III n (%)	973 (40.5)	108 (36.2)	66 (44.0)	36 (45.0)	564 (42.1)	52 (23.9)	147 (46.4)
Unspecified* n (%)	16 (0.7)	0	0	0	4 (0.3)	12 (5.5)	0
Unknown n (%)	89 (3.7)	0	0	0	58 (4.3)	20 (9.2)	11 (3.5)
Treatment							
Neoadjuvant chemotherapy n (%)	331 (13.8)	55 (18.5)	47 (31.3)	15 (18.8)	138 (10.3)	14 (6.4)	62 (19.6)
Unknown n (%)	58 (2.4)	2 (0.7)	1 (0.7)	10 (12.5)	38 (2.8)	7 (3.2)	0
Neoadjuvant radiotherapy n (%)	523 (21.8)	72 (24.2)	48 (32.0)	13 (16.3)	290 (21.7)	14 (6.4)	86 (27.1)
Unknown n (%)	58 (2.4)	2 (0.7)	1 (0.7)	10 (12.5)	38 (2.8)	7 (3.2)	0
Adjuvant chemotherapy n (%)	682 (28.4)	100 (33.6)	80 (53.3)	32 (40.0)	315 (23.8)	56 (25.7)	99 (31.2)
Unknown n (%)	121 (5.0)	17 (5.7)	3 (2.0)	28 (35.0)	63 (4.7)	9 (4.1)	1 (0.3)
Adjuvant radiotherapy n (%)	227 (9.5)	188 (63.1)	7 (4.7)	1 (1.3)	16 (1.2)	13 (6.0)	2 (0.6)
Unknown n (%)	164 (6.8)	17 (5.7)	3 (2.0)	28 (35.0)	106 (7.9)	9 (4.1)	1 (0.3)
Surgery n (%)	2,291 (95.4)	298 (100.0)	149 (99.3)	71 (88.7)	1,290 (96.4)	195 (89.5)	288 (90.9)
Unknown n (%)	44 (1.8)	0	0	3 (3.8)	39 (2.9)	1 (0.5)	1 (0.3)

*Distinguishing between stage I and II or II and III not possible

Table 2: Lifestyle characteristics of eligible FOCUS Consortium participants at baseline (n=2,401)

	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe
n (%)	2,401 (100)	298 (12.4)	150 (6.3)	80 (3.3)	1,338 (55.7)	218 (9.1)	317 (13.2)
Folic acid supplementation							
Yes n (%)	405 (16.9)	4 (1.3)	9 (5.9)	2 (2.5)	325 (24.3)		65 (20.5)
Unknown n (%)	383 (15.9)	27 (9.1)	86 (56.6)	14 (17.5)	38 (2.8)	218 (100)	0
Vitamin B₁₂ supplementation							
Yes n (%)	430 (17.9)	10 (3.4)	8 (5.3)	14 (17.5)	336 (25.1)		62 (19.6)
Unknown n (%)	384 (16.0)	27 (9.1)	86 (56.6)	14 (17.5)	39 (2.9)	218 (100)	0
Vitamin B₆ supplementation							
Yes n (%)	77 (3.2)	8 (2.7)	4 (2.6)	4 (5.0)			61 (19.2)
Unknown n (%)	1,684 (70.1)	27 (9.1)	86 (56.6)	14 (17.5)	1,339 (100)	218 (100)	0
Vitamin B₂ supplementation							
Yes n (%)	62 (2.6)	0	2 (1.3)	0			60 (18.9)
Unknown n (%)	1,684 (70.1)	27 (9.1)	86 (56.6)	14 (17.5)	1,339 (100)	218 (100)	0
Dietary intake of folate equivalents							
Mean µg/day ± SD	227.1 ± 94.3	115.1 ± 38.1	149.6 ± 97.5	116.2 ± 112.8	229.0 ± 88.7		276.3 ± 82.2
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₁₂							
Mean µg/day ± SD	4.6 ± 2.4	6.2 ± 2.9	7.1 ± 5.8	4.2 ± 2.2	4.3 ± 2.1		4.6 ± 2.0
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₆							
Mean mg/day ± SD	1.5 ± 0.5	1.7 ± 0.5	2.0 ± 0.7	1.5 ± 0.7	1.5 ± 0.5		1.8 ± 0.5
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₂							
Mean mg/day ± SD	1.4 ± 0.5	1.5 ± 0.5	2.2 ± 1.0	1.6 ± 0.8	1.3 ± 0.4		1.4 ± 0.5
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Total energy intake							
Mean kcal/day ± SD	1,941.8 ± 593.6	2,330.9 ± 714.0	1835.0 ± 775.7	1,398.5 ± 597.8	1,856.9 ± 517.2		2,243.7 ± 651.0
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Adherence to physical activity guidelines*							

Yes n (%)	1,310 (54.5)	84 (28.2)	35 (23.0)	20 (25.0)	941 (70.3)		230 (72.6)
Unknown n (%)	370 (15.4)	33 (11.1)	53 (34.9)	15 (18.7)	45 (3.4)	218 (100)	6 (1.9)

*Self-reported engagement in at least 150 minutes per week of moderate-to-vigorous physical activity

For peer review only

280 **Cohort follow-up**

281 At each study site, collection of biospecimen, clinical, demographic, questionnaire and
282 anthropometric data occurred at baseline, and at six and twelve months following recruitment.
283 Baseline measurements in CORSA, COLON and EnCoRe have been performed at diagnosis
284 (preferably prior to any cancer treatment) while in the ColoCare Study such measurements
285 have been done prior to surgery (i.e. neoadjuvant treatment might have been applied prior to
286 baseline blood and data collection).

287 Blood samples at baseline were collected from n=2,132 (88.8%) study participants.
288 N=1,537 (64.0%) participants donated blood at the 6 month and n=614 (25.6%) at the 12
289 month follow-up time point. Additionally, n=251 (10.5%) EnCoRe patients provided blood
290 samples at 6 weeks post-treatment, a subset specifically of interest at the Maastricht study
291 site.

292 **Data collection**

293 Harmonized baseline and follow-up time points are summarized in **Table 3** and are briefly
294 described below.

295 Lifestyle and demographic data

296 ColoCare questionnaires and standardized Food Frequency Questionnaires (FFQ) are used to
297 assess intake of dietary supplements and medication, smoking, dietary intake and other health
298 behaviors at each study time point. In COLON, patients provide information on diet, lifestyle,
299 and supplement use via COLON questionnaires and FFQs. Patients from the CORSA Study
300 are requested to complete a questionnaire assessing anthropometric and demographic factors.
301 Patients enrolled in EnCoRe receive repeated home visits by trained dietitians, where
302 extensive measurements are performed that include assessment of demographic data, physical
303 activity (both questionnaire- and accelerometry-based) and smoking behavior (questionnaire
304 data), dietary intake (FFQs at diagnosis and 7-day food diaries at follow-up measurements),
305 supplement use (registered by dietitians), and anthropometry measurements.

Table 3: Variables available at baseline, and at 6 and 12 month follow-up within the FOCUS Consortium.

Category	Variables	Baseline	6m	12m
Demographics	Age	x	x	x
	Gender	x		
	Highest education	x		
	Social status	x		
	Height	x		
	Weight	x	x	x
	BMI	x	x	x
	Smoking status	x	x	x
	Smoking duration	x	x	x
	Smoking pack years	x	x	x
	Menopausal status	x		
	Postmenopausal hormone use	x		
	Race	x		
	Cancer characteristics	Cancer site	x	
Cancer stage		x		
TNM classification		x		
Treatment	Preoperative chemotherapy	x		
	Preoperative radiotherapy	x		
	Surgery	x	x	
	Postoperative chemotherapy		x	x
	Postoperative radiotherapy		x	x
Supplement intake	Folic acid supplements	x	x	x
	Vitamin B ₂ supplements	x	x	x
	Vitamin B ₆ supplements	x	x	x
	Vitamin B ₁₂ supplements	x	x	x
	Vitamin A supplements	x	x	x
	Vitamin C supplements	x	x	x
	Vitamin D supplements	x	x	x
Supplement intake	Vitamin E supplements	x	x	x
	Calcium supplements	x	x	x
	Magnesium supplements	x	x	x
	Iron supplements	x	x	x
	Multivitamins	x	x	x
	Dietary nutrients	Folate equivalents	x	x
Vitamin B ₂		x	x	x
Vitamin B ₆		x	x	x
Vitamin B ₁₂		x	x	x
Vitamin A		x	x	x
Vitamin C		x	x	x
Vitamin D		x	x	x
Vitamin E		x	x	x
Total protein		x	x	x
Total fat	x	x	x	
Total carbohydrate	x	x	x	

Dietary nutrients	Fibre	x	x	x
	Saturated fatty acids	x	x	x
	Monounsaturated fatty acids	x	x	x
	Polyunsaturated fatty acids	x	x	x
	Alcohol	x	x	x
	Total energy	x	x	x
Physical activity	Light physical activity	x	x	x
	Moderate physical activity	x	x	x
	Vigorous physical activity	x	x	x
	Adherence to physical activity guidelines	x	x	x
Medical history	Diabetes mellitus	x	x	x
	Asthma, Chronical Bronchitis, COPD, Emphysema	x		
	Heart attack, Heart Failure	x		
	Hypertension	x		
	Stroke	x		
	Ulcer of Stomach or Duodenum	x		
	Hypothyroidism/Hyperthyroidism	x		
	Systemic Lupus Erythematosus	x		
	Inflammatory Bowel Disease (Crohn's disease, ulcerative colitis)	x		
	Familial Adenomatous Polyposis	x		
Medical history	Lynch Syndrome (hereditary nonpolyposis colorectal cancer)	x		
Regular medication use	Aspirin	x	x	x
	NSAID	x	x	x
	Ibuprofen	x	x	x
	Naproxen	x	x	x
	Celecoxib/Etoricoxib	x	x	x
Health-related quality of life	EORTC QLQ-C30	x	x	x
	EORTC QLQ-CR29	x	x	x
	EORTC QLQ-CIPN20	x	x	x
General information	Date of questionnaire completion	x	x	x
	Date of blood collection	x	x	x
	Freeze-thaw cycles of blood samples	x	x	x
	Hemolysis	x	x	x
	Time between blood draw and processing/storage	x	x	x

NSAID=non-steroidal anti-inflammatory drug; EORTC=European Organization for Research and Treatment of Cancer; QLQ=quality of life questionnaire; C30=core 30-items; CR29=colon/rectum 29-items; CIPN20=chemotherapy-induced polyneuropathy 20-items.

306

307 Clinical data and outcomes - Medical chart abstraction

308 All cancers and medical diagnoses are classified according to ICD-10 (International
309 Classification of Diseases, Tenth Revision) codes. Details on CRC treatment, including type
310 of treatment regimens and treatment toxicity, are abstracted via medical record for all cohorts.
311 Detailed information on the primary outcomes of interest, CRC recurrence and survival, are
312 ascertained through reviews of medical records, pathology reports, and imaging reports
313 documenting the diagnosis of a recurrence. Data on recurrence and vital status is
314 supplemented from the clinical cancer registries and survival data is verified by the inhabitant
315 registries that exist at all study sites. For COLON and EnCoRe, all data on recurrence are
316 retrieved in collaboration with the Netherlands Cancer Registry. Moreover, survival data for
317 CORSA participants was obtained by the Main Association of Austrian Social Insurance
318 Carriers as well as from Statistics Austria.

319 Patient-reported outcomes

320 Health-related quality of life is assessed by the validated and widely used cancer-specific 30-
321 item core questionnaire QLQ-C30 and the 29-item CRC module QLQ-CR29, developed by
322 the European Organization for Research and Treatment of Cancer (EORTC).²⁴
323 Chemotherapy-induced peripheral neuropathy (CIPN), which is a common toxicity in CRC
324 patients, is measured by the EORTC QLQ-CIPN20.²⁵ Patient-reported outcomes are available
325 for ColoCare Heidelberg, COLON and EnCoRe.

326 Biomarkers of FOCM

327 Blood is processed in identical settings across all study centers. Plasma (CORSA, COLON
328 and EnCoRe) and serum (for the three ColoCare Study sites) samples were collected and
329 immediately centrifuged, aliquoted, and stored at -80°C.

330 All biological analyses were performed at BEVITAL AS (Bergen, Norway,
331 <http://www.bevital.no>), which carried out metabolic profiling of biomarkers allocated to seven
332 complementary analytical platforms. Apart from analyses of microbiological active folate²⁶
333 and vitamin B₁₂²⁷, all analyses were based on mass spectrometry. Circulating folate, separate
334 folate species, 5-methyltetrahydrofolate (the main form in plasma), folic acid (folate in
335 supplements), 4a-OH 5-methyltetrahydrofolate (the primary oxidation form of circulating
336 folate), folate catabolites (p-aminobenzoylglutamate and acetyl p-aminobenzoylglutamate)²⁸,
337 B₆, B₁ and B₃ vitamins, kynurenines, cotinine, trans-3'-hydroxycotinine, trigonelline²⁹,
338 choline and its metabolites, creatinine, methylhistidines, arginine and methylated arginines³⁰

1
2
3 339 were analyzed by liquid chromatography-tandem mass spectroscopy, whereas other amino
4
5 340 acids, in addition to total homocysteine, total cysteine and methylmalonic acid were analyzed
6
7 341 by gass chromatography-tandem mass spectroscopy.³¹ C-reactive protein (hsCRP) and
8
9 342 cystatin C and its variants were measured by MALDI-TOF mass spectroscopy.³²

10
11 343 A comprehensive overview of the panel of biomarkers measured in blood samples of
12
13 344 patients enrolled in the FOCUS Consortium is provided in **Table 4**.

14 15 345 **Findings to date**

16
17 346 The FOCUS Consortium provides a unique opportunity to conduct comprehensive research
18
19 347 on folate and FOCM biomarkers status in the tertiary prevention of CRC using data collected
20
21 348 both at diagnosis and during standardized follow-up time points. This well-characterized
22
23 349 study design provides sufficient statistical power to discern prospective associations with
24
25 350 relevant clinical outcomes, including CRC recurrence and survival within relevant subgroups.

26 27 351 Key findings and publications

28
29 352 We investigated associations of circulating concentrations of folate, folic acid, and folate
30
31 353 catabolites pABG and apABG, measured around time of diagnosis, with recurrence and survival
32
33 354 among 2024 patients diagnosed with stage I-III CRC within the international FOCUS
34
35 355 Consortium. We did not observe any statistically significant associations for folate, pABG, and
36
37 356 apABG concentrations. However, an increased risk of cancer recurrences was observed among
38
39 357 patients with higher compared to lower circulating folic acid concentrations.³³ Further, Kiblawi
40
41 358 et al. measured associations between one-carbon metabolites, inflammation- and angiogenesis-
42
43 359 related biomarkers in a cross-sectional analysis of 238 patients from the ColoCare Heidelberg
44
45 360 cohort. The study showed that specific folate species within the one-carbon metabolism
46
47 361 pathway are associated with both inflammation and angiogenesis pathways among CRC
48
49 362 patients. In particular, vitamin B6 species, pyridoxal 5'-phosphate (PLP), pyridoxal (PL) and
50
51 363 pyridoxic acid (PA), were inversely associated with inflammatory biomarkers C-reactive
52
53 364 protein (CRP), serum amyloid A (SAA), IL-6 and IL-8. Thiamine and thiamine monophosphate
54
55 365 were inversely correlated with the CRP and IL-6. In addition, positive correlations of PA, PL
56
57 366 and PLP with angiogenesis biomarker VEGF-D were observed. Our findings reinforce the
58
59 367 notion that B vitamins involved in the one-carbon metabolism may be correlated with
60
370 368 carcinogenic processes.³⁴ This and further research will support the evidence base needed for
the development of dietary guidelines for CRC patients.

Table 4: Measured metabolites and biomarkers within the FOCUS Consortium

Folate and one-carbon metabolites	Abbreviation	Description
Anthranilic acid	AA	Tryptophan metabolite
Asymmetric dimethylarginine	ADMA	Inhibitor of nitric oxide synthase
α-Ketoglutaric acid	aKG	Keto acid of the Krebs cycle
Alanine	Ala	Amino acid
Acetamidobenzoylglutamate	apABG	Folate catabolite
Arginine	Arg	Amino acid
Asparagine	Asn	Amino acid
Aspartic acid	Asp	Amino acid
Betaine	Betaine	Methyl donor
Choline	Choline	Methyl donor
C-reactive protein	hsCRP	Inflammatory marker
Cystatin C-desS	CnCds	Cystatin C isoform
Cystatin C-desSSP	CnDcssp	Cystatin C isoform
Cystatin C	CnCn	Marker of kidney function
3Pro-OH cystatin C	CnCo	Cystatin C isoform
3Pro-OH cystatin C-desS	CnCods	Cystatin C isoform
Total concentration of detected cystatin C isoforms	CnCt	Marker of kidney function
Cobalamin	B ₁₂	Vitamin B ₁₂
Cotinine	Cot	Nicotine metabolite
Creatinine	Creat	Marker of kidney function
C-reactive protein	CRP	Inflammation
Cystathionine	Cysta	Thioether, transsulfuration intermediate
Dimethylglycine	DMG	Betaine metabolite
Folic acid	B ₉	Synthetic form of folate
Flavin mononucleotide	FMN	B ₂ vitamin
5-Formyl-tetrahydrofolate	fTHF	Folate species
Glutamine	Gln	Amino acid
Glutamic acid	Glu	Amino acid
Glycine	Gly	Amino acid
3-Hydroxyanthranilic acid	HAA	Tryptophan metabolite
Homoarginine	hArg	Amino acid
Histidine	Hist	Tryptophan metabolite
3-Hydroxykynurenine	HK	Tryptophan metabolite
4-Alfa-hydroxy-5-methyl-THF	hmTHF	Folate oxidation product
Isoleucine	Ile	Amino acid
Kynurenic acid	KA	Tryptophan metabolite
Kynurenine	Kyn	Tryptophan metabolite
Leucine	Leu	Amino acid
Lysine	Lys	Amino acid
3-Methylhistidine (3-MH)	m3His	Marker of muscle degradation and meat intake
1-Methylhistidine (1-MH)	m1His	Marker of muscle degradation and meat intake
Methionine	Met	Amino acid
Methionine sulfoxide	MetSo	Oxidation product of Met
Methylmalonic acid	MMA	Marker of B ₁₂ status
N1-methylnicotinamide	mNAM	B ₃ vitamin
5-Methyl-tetrahydrofolate	mTHF	Folate species

Nicotinic acid	NA	B ₃ vitamin
Nicotinamide	NAM	B ₃ vitamin
Neopterin	Neopt	Inflammatory marker
Trans-3'-hydroxycotinine	OHCot	Nicotine metabolite
Ornithine	Orn	Amino acid
4-Pyridoxic acid	PA	Vitamin B ₆ catabolite
Para-aminobenzoylglutamate	pABG	Folate catabolite
Phenylalanine	Phe	Amino acid
Picolinic acid	Pic	Tryptophan metabolite
Pyridoxal	PL	B ₆ vitamin
Pyridoxal 5'-phosphate	PLP	B ₆ vitamin
Pyridoxine	PN	Synthetic form of vitamin B ₆
Proline	Pro	Amino acid
Quinolinic acid	QA	Tryptophan metabolite
Riboflavin	Ribo	Main circulating B ₂ form
Symmetric dimethylarginine	SDMA	Marker of renal function
Serine	Ser	Amino acid
Folate	spFolate	Microbiologically active folate
Total cysteine	tCys	Amino acid
Homocysteine	tHcy	Marker of folate and B ₁₂ status
Thiamine	Thi	B ₁ vitamin
Threonine	Thr	Amino acid
Trimethylamineoxide	TMAO	Choline metabolite
Trimethyllysine	TML	Amino acid
Thiamine monophosphate	TMP	B ₁ vitamin
Trigonelline	Trig	Marker of coffee consumption
Tryptophan	Trp	Amino acid
Valine	Val	Amino acid
Leucine	Leu	Amino acid
Xanthurenic acid	XA	Tryptophan metabolite
Kyn/Trp ratio	KTR	Marker of immune activation
HK/XA ratio		Marker of B ₆ status

371

372 Further, we investigated circulating concentrations of nine biomarkers related to the B-

373 vitamins folate, riboflavin, vitamin B₆, and cobalamin, measured at diagnosis and six months

374 post-diagnosis, in association with health-related quality of life as assessed by the EORTC

375 QLQ-C30 questionnaire six months post-treatment in three FOCUS cohorts (ColoCare

376 Heidelberg, EnCoRe and COLON).³⁵ Higher PLP concentrations were cross-sectionally

377 associated with better physical, role, and social functioning, and reduced fatigue six months

378 post-diagnosis. Higher HKr (3-hydroxykynurenine:(kynurenic acid+xanthurenic acid+3-

379 hydroxyanthranilic acid+anthranilic acid)), an inverse marker of vitamin B₆ status, was cross-

380 sectionally associated with worse global quality of life, and lower physical and role

381 functioning. Dose-response relations were observed for PLP with global quality of life,

382 physical, role, and social functioning. No associations were observed for changes in

383 biomarker concentrations between diagnosis and six months with quality of life outcomes. We

1
2
3 384 therefore concluded that higher vitamin B₆ status was associated with better quality of life at
4
5 385 six months post treatment and that further study is needed to clarify the role of vitamin B₆ in
6
7 386 relation to quality of life.

8
9 387 Previous relevant findings from individual cohorts within the consortium

10
11 388 To date, individual cohorts from the FOCUS Consortium have initiated the examination of
12
13 389 dietary supplement use and dietary habits over time. Among CRC patients enrolled in the
14
15 390 ColoCare Study the proportion of supplement users was found to be highest post-diagnosis
16
17 391 (35%).³⁶ Moreover, within an international investigation including ColoCare participants from
18
19 392 multiple sites, Ulrich et al. showed differences in plasma folate concentration between
20
21 393 Heidelberg and the US sites, probably reflecting variation in folic acid fortification and
22
23 394 supplement use.¹³ Furthermore, ColoCare has published data on RECQ helicase expression³⁷,
24
25 395 NTRK3³⁸, RET³⁹, tumor-infiltrating lymphocytes and T cell receptor sequences⁴⁰, 25-25-
26
27 396 hydroxyvitamin D₃^{13 41}, DNA methylation⁴²⁻⁴⁵, miRNAs^{46 47}, fecal microbiota⁴⁸⁻⁵⁰,
28
29 397 metabolomics and transcriptomics⁵¹⁻⁵³, plasma proteins⁵⁴, gene expression⁵⁵, branched-chain
30
31 398 amino acids⁵⁶, genetic variants⁵⁷, body composition^{53 58 59}, physical activity⁴¹, and dietary
32
33 399 patterns⁴ in CRC patients. Within the COLON study, results have been published on body
34
35 400 weight trajectories⁶⁰, changes in lifestyle⁶¹, 25-hydroxy vitamin D levels^{62 63}, and
36
37 401 inflammation markers⁶⁴ over time, as well as vitamin D⁶⁴, calcium or magnesium intake⁶⁵,
38
39 402 physical activity⁶⁶, inflammation^{67 68}, skeletal muscle mass⁶⁹/ density⁷⁰ and other measures of
40
41 403 body composition⁷¹ in relation to cancer recurrence, survival, or physical functioning or
42
43 404 fatigue. Moreover, in a subset of patients undergoing chemotherapy, dietary factors in relation
44
45 405 to chronic CIPN^{72 73} as well as chemosensory perception and food preferences⁷⁴ were studied.
46
47 406 The Austrian CORSA Study has published results on genomic data⁷⁵⁻⁷⁷, telomere length⁷⁸,
48
49 407 DNA repair processes⁷⁹, tumor autoantibodies⁸⁰ as well as metabolomics.^{33 81} To date,
50
51 408 publications from the EnCoRe Study have reported on associations of physical activity and
52
53 409 sedentary behaviour⁸²⁻⁸⁵, adherence to lifestyle guidelines⁸⁶, and parameters of body
54
55 410 composition^{87 88} measured through CT scans with quality of life, functioning and fatigue in
56
57 411 CRC survivors. Recently, longitudinal associations between supplement use and fatigue were
58
59 412 investigated from diagnosis to 2 years post-CRC treatment. No overall association between
60
413 supplement use and fatigue was found but results suggest that increased levels of fatigue may
414
415 be a reason for the use of supplements among CRC survivors.⁸⁹ Higher concentrations of
416
25OHD₃ were longitudinally associated with better global quality of life and less fatigue in
colorectal cancer survivors within EnCoRe suggesting a potential beneficial role of vitamin D

1
2
3 417 in colorectal cancer survivors.³⁵ In a mixed-method study using data of the EnCoRe study,
4 418 colorectal cancer (treatment) related health and functioning problems negatively impacted the
5 419 ability of nearly 1 in 5 long-term CRC survivors to participate in everyday life situations and
6 420 their satisfaction with participation.⁹⁰ The validity of the food frequency questionnaire for
7 421 measuring dietary intake among survivors of colorectal cancer within the EnCoRe study
8 422 appeared to be moderate to good for most nutrients and food groups, relative to a 7-day
9 423 dietary record.⁹¹ Prediction models that we developed for estimating 1-year risk of low health-
10 424 related quality of life in seven domains in colorectal cancer survivors performed well when
11 425 externally validated among survivors within the EnCoRe and COLON studies.⁹²

426 **Future plans**

427 The consortium specified a comprehensive manuscript list of future projects using data from
428 the FOCUS Consortium. Some selected projects are described below:

429 a) Recently, the investigation of longitudinal associations of adherence to the dietary World
430 Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) and Dutch
431 Healthy Diet (DHD) recommendations with plasma kynurenine levels in colorectal cancer
432 survivors after treatment has been finalized and the corresponding manuscript is under review
433 at an international journal.

434 b) Further, near-term future plans include the investigation of (1) biomarkers related to
435 FOCM and associations with folate intake (from diet and supplements); (2) associations
436 between FOCM biomarkers such as vitamin B12 and tryptophan and recurrence, survival, and
437 patient-reported outcomes in CRC; (3) the impact of folate status (FOCM biomarkers and
438 diet/supplements) on treatment toxicity in patients treated with 5-FU modifiers; (4) the
439 interaction between biomarkers related to FOCM and polymorphisms in FOCM-related genes
440 in relation to CRC prognosis (recurrence & survival); (5) prognosis (disease-free and overall
441 survival) in stage I-III CRC and associations with dietary and supplement use at diagnosis and
442 changes during and after treatment; (6) FOCM-related biomarkers and their association with
443 body composition in stage I to III CRC patients; (7) associations between folate status
444 (FOCM biomarkers and diet/supplement use) and recurrence, survival and patient-reported
445 outcomes in young-onset CRC.

446 c) Long-term plans include the combination of additional biomarkers measured by the
447 individual cohorts within the next years (e.g. microbiome data, omics- and multi-omics data
448 etc.).

449 **Strengths and limitations**

450 This is the largest consortium to date addressing the research question of folate and FOCM
451 biomarkers in relation to survival, recurrence, treatment toxicity, and health-related quality of
452 life outcomes in CRC patients. The cohorts included in the FOCUS Consortium are designed
453 to enable future pooling of data.²² For that reason, methodologies, time points and
454 measurement instruments generally overlap, with each study presenting unique features such
455 as additional blood collection at the 6-week follow-up time point within the EnCoRe Study
456 and blood draws during chemotherapy within the COLON Study. The pooled sample size
457 provides sufficient power to investigate subgroup analyses across CRC patients, e.g., within
458 groups of patients who underwent 5-fluorouracil based chemotherapy or stratified by disease
459 stage. Furthermore, the assessment of biomarkers related to FOCM are conducted at a single,
460 state-of-the art laboratory and the biological materials are processed and stored according to
461 standardized operation protocols across all study sites, enabling precise and accurate
462 measurements of FOCM biomarkers. Another advantage of this cohort is the ability to include
463 a time-varying exposure on dietary supplement intake for future studies to consider. The study
464 population is predominantly based on European cohorts (90.4%) in countries that have not
465 implemented mandatory folic acid fortification. This enables us to study a population where
466 dietary intake and dietary supplement use determine differences in folate status, yielding
467 information of direct relevance to cancer patients. However, the generalizability of results to
468 populations that have introduced folic acid fortification, including the United States, might
469 therefore also be limited.⁹³ Performing sensitivity analyses by excluding countries without
470 folic acid fortification (e.g. Germany) or investigating analyses separately for Germany and
471 the US might help to address differences in fortification status. Moreover, patients were
472 predominantly White, thus, it is not possible to address racial and ethnic minorities.
473 Ethnicity/race is an important determinant of folate status and metabolism may be different
474 between African Americans and Hispanics⁹⁴, thus, recommendations should be limited to this
475 current population. Future studies are warranted in diverse populations and compared with the
476 FOCUS cohort.

477 Another limitation of the consortium includes differences in collection strategies of the
478 baseline study time point across included studies: CORSA, COLON and EnCoRe are
479 recruiting CRC patients at diagnosis, preferably prior to any cancer treatment while ColoCare
480 is recruiting patients prior to surgery (i.e. neoadjuvant treatment might have occurred prior to
481 baseline blood and data collection). Nevertheless, the ColoCare protocol requires that no
482 blood draw occurs within 2 weeks of neoadjuvant or adjuvant chemotherapy, limiting the

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3 483 influence of ongoing treatments on blood biomarkers. In some cohorts, the timing with
4
5 484 respect to adjuvant chemotherapy is less clearly defined and this will be carefully considered
6
7 485 in sensitivity analyses. Furthermore, survivor bias – a type of selection bias – may be
8
9 486 introduced as some patients died or did not complete follow-up questionnaires or did not
10
11 487 provide blood samples. It is possible that patients who experience more severe toxicities,
12
13 488 worse clinical outcomes, or worse health-related quality of life, are underrepresented among
14
15 489 those patients who completed follow-up measurements.⁴ Cohort studies such as the one
16
17 490 presented here generate critical knowledge about preventable causes of disease. However,
18
19 491 selection bias may affect estimates. This is particularly true for non-participation at follow-up
20
21 492 that may depend on both the exposure and outcome. Within a review, Nohr et al. showed a
22
23 493 range of methods to quantify and adjust for selection bias. Even with limited data on
24
25 494 nonparticipants and those lost to follow up, it is possible to examine how effect estimates in a
26
27 495 specific study may be biased by selection.⁹⁵ The likelihood for reverse causation is small in
28
29 496 this prospective cohort, as the exposure measurements (blood folate levels and intake through
30
31 497 diet/supplements) were collected before the outcome (survival, recurrence, and quality of life)
32
33 498 occurred. Therefore, these outcomes are unlikely to have influenced the exposure
34
35 499 measurements. Given the robust follow-up in these cohorts for outcomes and data availability,
36
37 500 future studies will be able to consider key confounders as well as predictors of recurrence and
38
39 501 survival.

502 **Collaboration**

503 A substantial amount of time has been spent in creating the harmonized dataset of baseline
504 variables from cohorts within the FOCUS Consortium, with follow-up data collection and
505 FOCM biomarker analyses. Any person interested in collaborating, learning about the
506 FOCUS Consortium or in getting access to FOCUS data and in-depth analyses can contact the
507 coordinating study PIs Prof. Andrea Gsur (andrea.gsur@meduniwien.ac.at) and Prof. Cornelia
508 Ulrich (neli.ulrich@hci.utah.edu). Requests for data will be discussed and decided by all PIs
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10
11 546 BG, MW, EK, PMU, CMU, AG, ABU, NH: conceived of the project, developed the overall
12 547 research plan, and provided study oversight; BG, EHvR, ANH, SB, AJMRG, AU, JO, JLK,
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600 **Competing interests**

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602 **Patient consent for publication**

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3 605 **Patient and public involvement**

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9 608 **Ethics approval**

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11 609 The Heidelberg ColoCare Study was approved by the Ethics Committee of the Medical
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14
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28 618 **Data availability**

29
30 619 Data described in the manuscript, code book, and analytic code have been generated from
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32 620 European-based consortia and as such are subject to regulations from multiple European
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34 621 countries, which limit our availability to share data. The consortium’s funding has ended, and
35
36 622 no centralized staff is available to support
37
38 623 data requests. However, the FOCUS PIs have agreed to answer any queries or discuss
39
40 624 potential projects with anyone interested in future collaborative research. For further
41
42 625 questions please contact colocarestudy_admin@hci.utah.edu.

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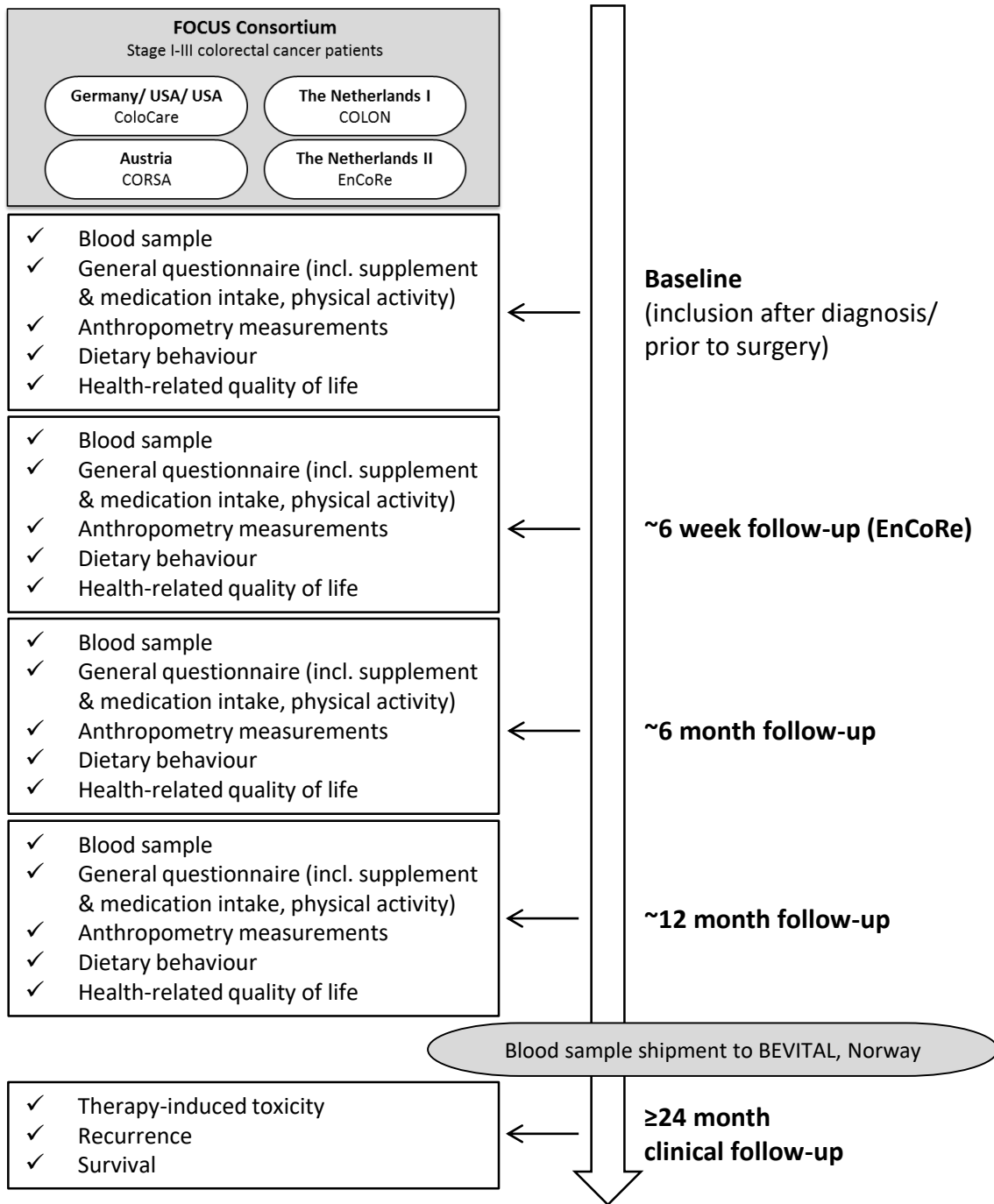
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3 945 **Figure legends**
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5 946 **Figure 1** FOCUS Consortium design
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**Cohort profile: Biomarkers related to folate-dependent one-carbon metabolism in colorectal cancer recurrence and survival:
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Cohort profile: Biomarkers related to folate-dependent one-carbon metabolism in colorectal cancer recurrence and survival: The FOCUS Consortium

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46 **Abstract**

47 **Purpose:** The overarching goal of the FOCUS (Biomarkers related to folate-dependent one-
48 carbon metabolism in colorectal cancer recurrence and survival) Consortium is to unravel the
49 effect of folate and folate-mediated one-carbon metabolism (FOCM) biomarkers on colorectal
50 cancer (CRC) prognosis to provide clinically relevant advice on folate intake to cancer
51 patients and define future tertiary prevention strategies.

52 **Participants:** The FOCUS Consortium is an international, prospective cohort of 2,401
53 women and men above 18 years of age who were diagnosed with a primary invasive non-
54 metastatic (stage I-III) CRC. The Consortium comprises patients from Austria, two sites from
55 the Netherlands, Germany and two sites from the USA. Patients are recruited after CRC
56 diagnosis and followed at six and twelve months after enrolment. At each time point,
57 sociodemographic data, data on health behavior, and clinical data are collected, blood samples
58 are drawn.

59 **Findings to date:** An increased risk of cancer recurrences was observed among patients with
60 higher compared to lower circulating folic acid concentrations. Furthermore, specific folate
61 species within the FOCM pathway were associated with both inflammation and angiogenesis
62 pathways among CRC patients. In addition, higher vitamin B₆ status was associated with
63 better quality of life at six months post treatment.

64 **Future plans:** Better insights into the research on associations between folate and FOCM
65 biomarkers and clinical outcomes in CRC patients will facilitate the development of
66 guidelines regarding folate intake in order to provide clinically relevant advice to cancer
67 patients, health professionals involved in patient care, and ultimately further tertiary
68 prevention strategies in the future. The FOCUS Consortium offers an excellent infrastructure
69 for short- and long-term research projects and for combining additional biomarkers and data
70 resulting from the individual cohorts within the next years, e.g. microbiome data, omics- and
71 multi-omics data or CT-quantified body composition data.

72 **Strengths and limitations of this study**

- 73 • FOCUS is the largest consortium to date addressing the research question of folate and
74 FOCM biomarkers in relation to survival, recurrence, treatment toxicity, and health-
75 related quality of life outcomes in CRC patients
- 76 • The cohorts included in the FOCUS Consortium are designed to enable future pooling
77 of data using harmonized and standardized methods to collect data and biospecimens
- 78 • The pooled sample size provides sufficient power to investigate subgroup analyses
79 across CRC patients
- 80 • Study time point definitions differ between some of the cohorts and have to be adapted
81 for specific projects
- 82 • A selection bias for follow-up can arise because it is possible that patients who
83 experience more severe toxicities, worse clinical outcome or health-related quality of
84 life are underrepresented among those completing follow-ups

85

86 Introduction

87 Among men and women world-wide, colorectal cancer (CRC) accounts for nearly 10% of all
88 incident cancer cases.¹ In Europe, 5-year survival for CRC patients is approximately 55%,
89 with substantial differences by stage.^{2,3} The number of CRC patients continues to increase due
90 to implementation of improved screening strategies and/or enhanced treatment modalities.⁴
91 Many cancer patients seek information on what they can do themselves to improve survival -
92 for instance by improving their dietary habits and other lifestyle factors.⁵ However, there may
93 be behavioural aspects among individuals diagnosed with CRC, which may be harmful in
94 some cases, such as the use of high-dose nutritional supplements containing synthetic folate.
95 In general, knowledge on short and long-term effects is insufficient to make sound
96 recommendations on use of dietary supplements, in particular folate, to cancer survivors, even
97 though dietary supplements are used by 20%-85% of patients with cancer⁶⁻⁸, highlighting the
98 importance of thorough evaluation of potential benefits and harms and to support
99 development of evidence-based recommendations on use of dietary supplements to cancer
100 patients.

101 Folic acid is the synthetic form of the B-vitamin folate and is often used in dietary
102 supplements or fortified foods. However, there is broad agreement that food folate is less
103 bioavailable than folic acid with a median relative bioavailability of 65% (range: 44–80%), an
104 estimate that approximates the 60% value derived from the Dietary Folate Equivalents
105 equation.⁹ Folate and folic acid play an important role in one-carbon metabolism, which is a
106 complex series of biochemical reactions essential in nucleotide synthesis, methylation
107 reactions and amino acid homeostasis.¹⁰ One-carbon metabolism refers to a complex network
108 of biochemical reactions linked to nucleotide synthesis and provides methyl groups for DNA,
109 RNA or protein methylation. Thus, one-carbon metabolism is directly controlling processes
110 determining DNA synthesis and integrity, both processes known to be linked to tumor
111 growth.¹¹ To what extent folic acid supplement use and biomarkers of folate-mediated one-
112 carbon metabolism (FOCM) impact cancer survival and treatment efficacy and toxicity still
113 needs to be clarified.^{11,12} Folate and FOCM biomarker deficiencies may increase cancer risk,
114 but high levels, especially of synthetic folic acid, may also be driving factors in
115 carcinogenesis.^{6,13} An increasing body of evidence suggests that folate plays a dual role in
116 carcinogenesis, involving both the prevention of early lesions and potential harm once pre-
117 neoplastic or neoplastic lesions have developed.^{6,12,14-20}

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3 118 The overarching goal of the FOCUS (biomarkers related to Folate-dependent one-
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5 119 carbon metabolism in Colorectal cancer recurrence and Survival) Consortium is to study
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7 120 associations between folate and FOCM biomarkers and recurrence and survival in CRC
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9 121 patients. Better insights into these associations will facilitate the development of guidelines
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11 122 regarding folate intake in order to provide clinically relevant advice to cancer patients, health
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13 123 professionals involved in patient care, and ultimately further tertiary prevention strategies in
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15 124 the future. The FOCUS Consortium is a large-scale international consortium with CRC
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17 125 patients from six prospective cohort studies. The primary objectives of the FOCUS
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19 126 Consortium are: (i) to determine possible associations of folate and other FOCM biomarkers
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21 127 at diagnosis with recurrence or survival in non-metastatic (stage I-III) CRC; (ii) to elucidate
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23 128 whether biomarkers related to FOCM are associated with dietary and supplemental intake of
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25 129 these respective nutrients; (iii) to explore whether FOCM biomarkers are associated with
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27 130 treatment toxicity in CRC patients undergoing chemotherapy; and (iv) to collect
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29 131 comprehensive data of patient characteristics at baseline and follow-up including biomarkers
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31 132 of FOCM to establish a unique resource for future scientific research. The FOCUS
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33 133 Consortium is funded by the European Research Area Network (ERA-NET) on Translational
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35 134 Cancer Research (TRANSCAN).

36
37 135 The main purpose of the FOCUS Cohort profile is to (1) inform the scientific
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39 136 community about the FOCUS Consortium, (2) describe the complex methodology of a large
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41 137 consortium, (3) present ongoing studies using this infrastructure as well as (4) advise
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43 138 interested researchers of opportunities for collaboration. This joint research may lead to a
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45 139 better understanding of the role of folate- and FOCM-related mechanisms in the prognosis of
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47 140 CRC and be a precursor for data for future randomized controlled trials (RCTs), which will be
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49 141 critical for the development of guidelines regarding folate intake among CRC patients.

142 **Cohort description**

143 The FOCUS Consortium is an international, prospective consortium including six cohort
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145 studies recruiting women and men age 18 years and older diagnosed with a primary CRC. The
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147 FOCUS Consortium is comprised of patients from the ColoCare Study at the University of
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149 Heidelberg, Germany (n=298, 12.3%), the ColoCare Study at the Huntsman Cancer Institute
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151 in Salt Lake City, Utah, USA (n=80, 3.3%), the ColoCare Study at the Fred Hutchinson
Cancer Research Center in Seattle, Washington, USA (n=157, 6.3%), the COLON Study
(Colorectal cancer: Longitudinal, Observational study on Nutritional and lifestyle factors that
may influence colorectal tumor recurrence, survival and quality of life) at Wageningen
University & Research in the Netherlands (n=1,365, 56.1%), the CORSA Study (Colorectal

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3 152 Cancer Study of Austria) at the Medical University of Vienna in Austria (n=218, 9.0%), and
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5 153 the EnCoRe Study (Energy for life after ColoRectal cancer) at Maastricht University Medical
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7 154 Center+ in the Netherlands (n=317, 13.0%).
8

9 155 In total, n=2,435 CRC patients were considered for the FOCUS Consortium, of which
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11 156 n=34 patients were excluded due to tumor staging being either 0 or IV leading to a total
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13 157 number of n=2,401 stage I-III CRC patients included in further analyses.
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15 158 Patients were recruited after CRC diagnosis and repeated study measurements were
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17 159 conducted at time of recruitment, and at six and twelve months thereafter. At each study time
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19 160 point, sociodemographic data, data on lifestyle factors (e.g., diet, supplement use, physical
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21 161 activity), and clinical data (e.g., tumor site and stage, cancer treatment, treatment-induced
22
23 162 toxicities, recurrence, survival) were collected, and blood samples were drawn (**Figure 1**). All
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25 163 individuals signed informed consent and the Institutional Review Board at each site approved
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27 164 the corresponding study. Below, a more specific description of each included study is
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29 165 provided.

30 166 The ColoCare Study

31 167 The ColoCare Study (ClinicalTrials.gov identifier: NCT02328677) is an ongoing
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33 168 international, multi-center prospective cohort study among women and men newly diagnosed
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35 169 with a primary invasive CRC, with the goal to investigate predictors of cancer recurrence,
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37 170 survival, treatment toxicities and health-related quality of life.^{4 21} Three ColoCare Consortium
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39 171 sites participate in the FOCUS Consortium: the Fred Hutchinson Cancer Research Center in
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41 172 Seattle (Washington, USA), and the University Hospital Heidelberg, Heidelberg (Germany)
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43 173 as well as the Huntsman Cancer Institute in Salt Lake City (Utah, USA). Patients are enrolled
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45 174 prior to undergoing CRC surgery according to the following inclusion criteria: individuals
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47 175 who are 18 years of age and older, newly diagnosed (i.e., non-recurrent) with invasive CRC.
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49 176 Blood draws and other biospecimens are obtained prior to surgery and at regular intervals
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51 177 (e.g., six, twelve, twenty-four months). Questionnaires are administered to assess lifestyle
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53 178 behavior, health-related quality of life, and clinical outcomes such as CRC recurrence,
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55 179 treatment, and treatment symptoms at each study time point. Clinical data are obtained
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57 180 through reviews of patient medical records, pathology and imaging reports. Vital status is
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59 181 obtained through medical records, routine follow-up mailings, periodic requests for outside
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182 medical records, and state or national cancer and death registries. The Heidelberg ColoCare
183 Study was approved by the Ethics Committee of the Medical Faculty of Heidelberg (approval
184 no. S-310/2001 and S-134/2016). The ColoCare Study at the Huntsman Cancer Institute and

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3 185 the Fred Hutchinson Cancer Research Center were approved by the respective IRBs (#77147
4
5 186 and #6407).

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8 187 The COLON Study

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10 188 The COLON Study (ClinicalTrials.gov identifier: NCT03191110) started in 2010 and is an
11
12 189 ongoing, multicenter prospective cohort study specifically designed to assess associations
13
14 190 between nutrition, lifestyle, and dietary supplement use with quality of life, CRC recurrence
15
16 191 and survival among CRC patients (stages I-IV).²² Persons with a history of CRC or (partial)
17
18 192 bowel resection, chronic inflammatory bowel disease, or a known hereditary CRC syndrome
19
20 193 are excluded from the study. Patients are recruited from eleven regional and academic
21
22 194 hospitals prior to surgery. Individuals donate blood samples and provide information on diet,
23
24 195 lifestyle, and dietary supplement use at CRC diagnosis, i.e. baseline, and at six, twelve
25
26 196 (chemotherapy patients only), twenty-four and sixty months after diagnosis. Clinical data are
27
28 197 collected through review of medical records (treatment-induced toxicity) or through linkage
29
30 198 with the Dutch ColoRectal Audit (DCRA). Mortality data (i.e. death or alive and date of
31
32 199 death) are retrieved from the Municipal Personal Records Database. Recurrence data have
33
34 200 been retrieved in collaboration with the Netherlands Cancer Registry. Ethical approval for the
35
36 201 study was granted by the Committee on Research involving Human Subjects, region Arnhem-
37
38 202 Nijmegen under file number 2009/349.

39
40 203 The CORSA Study

41
42 204 CORSA is an ongoing case-control study of women and men recruiting CRC patients,
43
44 205 patients with high and low risk adenomas and population-based colonoscopy negative
45
46 206 controls, with an age range between 30 and 90 years. Since 2003, more than 13,500
47
48 207 participants have been recruited across nine sites in Austria. The multicentre recruitment
49
50 208 within CORSA follows standardized protocols resulting in consistent data from all
51
52 209 recruitment sites. These sites include the Medical University of Vienna (Department of
53
54 210 Surgery), two hospitals in Vienna (Clinic Favoriten and Clinic Landstraße), and the Hospital
55
56 211 Wiener Neustadt in Lower Austria. Furthermore, the recruitment for CORSA was performed
57
58 212 in four hospitals in the federal state Burgenland within the population-based screening
59
60 213 program "Burgenland PREvention Trial of colorectal cancer DIsease with ImmunologiCal
214
215 214 Testing" (B-PREDICT). B-PREDICT is a two-stage screening project where more than
216
215 215 150,000 inhabitants of Burgenland aged between 40 and 80 are invited annually to participate
216
216 216 in this program using a Fecal Immunochemical Test (FIT) as an initial screening. FIT-positive

1
2
3 217 (≥ 10 μg hemoglobin / g feces) tested individuals are offered a complete colonoscopy and are
4
5 218 asked to participate in CORSA. Biospecimen are collected at each site using harmonized
6
7 219 protocols. CORSA participants provide a basic CORSA questionnaire assessing data on body
8
9 220 mass index (BMI), smoking history, alcohol consumption, education level, family status,
10
11 221 profession, basic dietary habits, and diabetes. Clinical data are abstracted from medical
12
13 222 records and processed in a structured database following standardized documentation
14
15 223 guidelines and according to the General Data Protection Regulation (GDPR). All subjects
16
17 224 gave written informed consent and the study was approved by the institutional review boards:
18
19 225 ‘Ethikkommission Burgenland’ (33/2010), by the ethical review committee of the Medical
20
21 226 University of Vienna (1160/2016) and by the “Ethikkommission der Stadt Wien” (EK 06-
22
23 227 150VK).

23 228 The EnCoRe Study

24
25
26 229 The EnCoRe Study is a prospective cohort study initiated in 2012 at the Maastricht University
27
28 230 Medical Center+ in the Netherlands that focuses on the importance of lifestyle factors for
29
30 231 quality of life, recurrence and survival of CRC patients.²³ The EnCoRe Study is registered in
31
32 232 the Netherlands Trial Registry for experimental and observational studies
33
34 233 (www.trialregister.nl, registration number 7099). Stage I-III CRC patients are enrolled at
35
36 234 diagnosis at three hospitals in the South-Eastern region of the Netherlands and followed up
37
38 235 until five years after completion of treatment with repeated measurements at diagnosis (pre-
39
40 236 treatment), and at six weeks and six, twelve, twenty-four, and sixty months after the end of
41
42 237 the initial anti-cancer treatment (i.e., surgery, chemotherapy, radiotherapy). Patients with
43
44 238 stage IV CRC, an inability to understand the Dutch language in speech or writing, with
45
46 239 comorbidities obstructing successful participation (e.g., Alzheimer disease), or with severe
47
48 240 visibility or hearing disorders are excluded from the study. Repeated home visits by trained
49
50 241 dieticians are conducted and data are collected amongst others on sociodemographic factors,
51
52 242 quality of life, functioning, physical activity, comorbidity, dietary intake, supplement use, and
53
54 243 anthropometry. In addition, clinical data are collected from hospital records and blood
55
56 244 samples are drawn at all time points. Recurrence data have been retrieved in collaboration
57
58 245 with the Netherlands Cancer Registry. Mortality data (i.e. death or alive and date of death) are
59
60 246 retrieved from the Municipal Personal Records Database. The EnCoRe Study was approved
247
248 by the Medical Ethics Committee of the Maastricht University Hospital and Maastricht
University (METC 11-3-075).

249 The FOCUS Consortium

250 Sociodemographic and clinical characteristics of the 2,401 participants in the FOCUS
251 Consortium are presented in **Table 1**. The mean age at CRC diagnosis was 65.4 years
252 (standard deviation (SD): 10.2; range: 22 to 93 years), and the majority of participants were
253 male (64.0%). The mean BMI was 27.0 kg/m² (SD: 4.6).

254 In terms of education status, 35.9% of the patients reported lower education, 28.8% reported
255 intermediate education and 27.3% high education. Most of the patients were married or part
256 of a living community (73.1%). About half of the overall cohort reported to be a former
257 smoker (50.8%), 31.7% were never smokers, and 12.1% were current smokers (5.4% were
258 unknown). Median alcohol intake was 7.9 g/day. In total, 16.9% of the patients reported
259 regular dietary supplementation (i.e., at least once per week during the last four weeks) of
260 folic acid. Over half of all participants (54.6%) reported adherence to the physical activity
261 guidelines of at least 150 minutes per week of moderate-to-vigorous physical activity.

262 Lifestyle characteristics of the FOCUS cohort are presented in **Table 2**.

263 Regarding clinical characteristics, 57.0% of participants were diagnosed with colon cancer,
264 33.9% with rectal cancer, and 1.1% with rectosigmoid cancer (8.1% were of unknown tumor
265 subsite). In total, 26.0% were diagnosed with stage I, 28.7% with stage II, and 40.2% with
266 stage III CRC. Approximately 1% of participants were classified with an unspecified cancer
267 stage, as distinction between stage I and II or II and III was not possible, and for 4.4% of the
268 total population the cancer stage was unknown. In total, 89.3% of patients underwent surgery,
269 whereas neoadjuvant chemotherapy was administered to 10.6% of patients and 26.8%
270 received adjuvant chemotherapy. Neoadjuvant radiotherapy was administered to 20.7% of
271 patients and adjuvant radiotherapy in 9.4% of the population.

272 **Patient and public involvement**

273 Patients and the public were not involved in the design, conduct, reporting or dissemination
274 plans of this research.

Table 1: Sociodemographic and clinical factors of eligible FOCUS Consortium participants at baseline (n=2,401)

	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe
n (%)	2,401 (100)	298 (12.4)	150 (6.3)	80 (3.3)	1,338 (55.7)	218 (9.1)	317 (13.2)
Age							
Mean y ± SD	65.4 ± 10.2	63.7 ± 12.2	58.3 ± 12.8	61.3 ± 11.2	66.1 ± 8.7	67.3 ± 12.0	66.9 ± 9.3
Sex							
Female n (%)	864 (36.0)	100 (33.6)	67 (44.7)	32 (40.0)	486 (36.3)	76 (34.9)	103 (32.5)
Male n (%)	1,537 (64.0)	198 (66.4)	83 (55.3)	48 (60.0)	852 (63.7)	142 (65.1)	214 (67.5)
Education							
Low n (%)	861 (35.9)	123 (41.3)	18 (12.0)	17 (21.3)	543 (40.6)	73 (33.5)	87 (27.4)
Intermediate n (%)	694 (28.9)	57 (19.1)	71 (47.3)	40 (50.0)	332 (24.8)	73 (33.5)	121 (38.2)
High n (%)	658 (27.4)	88 (29.5)	28 (18.7)	8 (10.0)	417 (31.7)	14 (6.4)	103 (32.5)
Unknown n (%)	188 (7.8)	30 (10.1)	33 (22.0)	15 (18.7)	46 (3.4)	58 (26.6)	6 (1.9)
Marital status							
Unmarried n (%)	122 (5.1)	19 (6.4)	15 (10.0)	2 (2.5)	60 (4.5)	10 (4.6)	16 (5.1)
Married n (%)	1,738 (72.4)	193 (64.8)	71 (47.3)	50 (62.5)	1,071 (80.0)	113 (51.8)	240 (75.7)
Divorced/Separated n (%)	138 (5.7)	24 (8.0)	11 (7.3)	10 (12.5)	64 (4.8)	14 (6.4)	15 (4.7)
Widowed n (%)	194 (8.1)	31 (10.4)	7 (4.7)	1 (1.3)	90 (6.7)	26 (11.9)	39 (12.3)
Living community n (%)	20 (0.8)	0	13 (8.7)	2 (2.5)	4 (0.3)	1 (0.5)	0
Unknown n (%)	189 (8.9)	31 (10.4)	33 (22.0)	15 (18.7)	49 (3.7)	54 (24.8)	7 (2.2)
Smoking status							
Current n (%)	290 (12.1)	53 (17.8)	8 (5.3)	6 (7.5)	143 (10.7)	38 (17.4)	42 (13.3)
Former n (%)	1,219 (50.8)	132 (44.3)	53 (35.32)	21 (26.3)	767 (57.3)	76 (34.9)	170 (53.6)
Never n (%)	767 (31.9)	90 (30.2)	56 (37.3)	39 (48.7)	386 (28.9)	97 (44.5)	99 (31.2)
Unknown n (%)	125 (5.2)	23 (7.7)	33 (22.0)	14 (17.5)	42 (3.1)	7 (3.2)	6 (1.9)
Alcohol intake							
Mean g/day ± SD	13.6 ± 17.1	16.1 ± 19.2	8.8 ± 18.5	2.3 ± 10.2	14.0 ± 17.0		12.9 ± 16.5
Unknown n (%)	633 (26.4)	208 (69.8)	81 (54.0)	60 (75.0)	54 (4.1)	218 (100)	12 (3.8)
BMI at diagnosis							
Mean kg/m² ± SD	27.1 ± 4.6	26.6 ± 4.1	28.7 ± 7.3	29.4 ± 7.5	26.6 ± 4.0	27.7 ± 4.4	28.3 ± 4.6
Unknown n (%)	42 (1.7)	2 (0.7)	0	14 (17.5)	10 (0.8)	14 (6.4)	2 (0.6)

	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe
Tumor site							
Colon n (%)	1,450 (60.4)	140 (57.0)	75 (50.0)	45 (56.3)	867 (64.8)	131 (60.1)	192 (60.6)
Rectum n (%)	862 (35.9)	143 (48.0)	60 (40.0)	22 (27.5)	434 (32.4)	78 (35.8)	125 (39.4)
Rectosigmoid n (%)	42 (1.7)	15 (5.0)	15 (10.0)	7 (8.7)	0	5 (2.3)	0
Unknown n (%)	47 (2.0)	0	0	6 (7.5)	37 (2.8)	4 (1.8)	0
Tumor stage							
I n (%)	629 (26.2)	76 (25.5)	35 (23.3)	21 (26.3)	326 (24.4)	82 (37.6)	89 (28.1)
II n (%)	694 (28.9)	114 (38.3)	49 (32.7)	23 (28.7)	386 (28.9)	52 (23.9)	70 (22.1)
III n (%)	973 (40.5)	108 (36.2)	66 (44.0)	36 (45.0)	564 (42.1)	52 (23.9)	147 (46.4)
Unspecified* n (%)	16 (0.7)	0	0	0	4 (0.3)	12 (5.5)	0
Unknown n (%)	89 (3.7)	0	0	0	58 (4.3)	20 (9.2)	11 (3.5)
Treatment							
Neoadjuvant chemotherapy n (%)	331 (13.8)	55 (18.5)	47 (31.3)	15 (18.8)	138 (10.3)	14 (6.4)	62 (19.6)
Unknown n (%)	58 (2.4)	2 (0.7)	1 (0.7)	10 (12.5)	38 (2.8)	7 (3.2)	0
Neoadjuvant radiotherapy n (%)	523 (21.8)	72 (24.2)	48 (32.0)	13 (16.3)	290 (21.7)	14 (6.4)	86 (27.1)
Unknown n (%)	58 (2.4)	2 (0.7)	1 (0.7)	10 (12.5)	38 (2.8)	7 (3.2)	0
Adjuvant chemotherapy n (%)	682 (28.4)	100 (33.6)	80 (53.3)	32 (40.0)	315 (23.8)	56 (25.7)	99 (31.2)
Unknown n (%)	121 (5.0)	17 (5.7)	3 (2.0)	28 (35.0)	63 (4.7)	9 (4.1)	1 (0.3)
Adjuvant radiotherapy n (%)	227 (9.5)	188 (63.1)	7 (4.7)	1 (1.3)	16 (1.2)	13 (6.0)	2 (0.6)
Unknown n (%)	164 (6.8)	17 (5.7)	3 (2.0)	28 (35.0)	106 (7.9)	9 (4.1)	1 (0.3)
Surgery n (%)	2,291 (95.4)	298 (100.0)	149 (99.3)	71 (88.7)	1,290 (96.4)	195 (89.5)	288 (90.9)
Unknown n (%)	44 (1.8)	0	0	3 (3.8)	39 (2.9)	1 (0.5)	1 (0.3)

*Distinguishing between stage I and II or II and III not possible

Table 2: Lifestyle characteristics of eligible FOCUS Consortium participants at baseline (n=2,401)

	Overall	ColoCare HD	ColoCare FHCRC	ColoCare HCI	COLON	CORSA	EnCoRe
n (%)	2,401 (100)	298 (12.4)	150 (6.3)	80 (3.3)	1,338 (55.7)	218 (9.1)	317 (13.2)
Folic acid supplementation							
Yes n (%)	405 (16.9)	4 (1.3)	9 (5.9)	2 (2.5)	325 (24.3)		65 (20.5)
Unknown n (%)	383 (15.9)	27 (9.1)	86 (56.6)	14 (17.5)	38 (2.8)	218 (100)	0
Vitamin B₁₂ supplementation							
Yes n (%)	430 (17.9)	10 (3.4)	8 (5.3)	14 (17.5)	336 (25.1)		62 (19.6)
Unknown n (%)	384 (16.0)	27 (9.1)	86 (56.6)	14 (17.5)	39 (2.9)	218 (100)	0
Vitamin B₆ supplementation							
Yes n (%)	77 (3.2)	8 (2.7)	4 (2.6)	4 (5.0)			61 (19.2)
Unknown n (%)	1,684 (70.1)	27 (9.1)	86 (56.6)	14 (17.5)	1,339 (100)	218 (100)	0
Vitamin B₂ supplementation							
Yes n (%)	62 (2.6)	0	2 (1.3)	0			60 (18.9)
Unknown n (%)	1,684 (70.1)	27 (9.1)	86 (56.6)	14 (17.5)	1,339 (100)	218 (100)	0
Dietary intake of folate equivalents							
Mean µg/day ± SD	227.1 ± 94.3	115.1 ± 38.1	149.6 ± 97.5	116.2 ± 112.8	229.0 ± 88.7		276.3 ± 82.2
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₁₂							
Mean µg/day ± SD	4.6 ± 2.4	6.2 ± 2.9	7.1 ± 5.8	4.2 ± 2.2	4.3 ± 2.1		4.6 ± 2.0
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₆							
Mean mg/day ± SD	1.5 ± 0.5	1.7 ± 0.5	2.0 ± 0.7	1.5 ± 0.7	1.5 ± 0.5		1.8 ± 0.5
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Dietary intake of vitamin B₂							
Mean mg/day ± SD	1.4 ± 0.5	1.5 ± 0.5	2.2 ± 1.0	1.6 ± 0.8	1.3 ± 0.4		1.4 ± 0.5
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Total energy intake							
Mean kcal/day ± SD	1,941.8 ± 593.6	2,330.9 ± 714.0	1835.0 ± 775.7	1,398.5 ± 597.8	1,856.9 ± 517.2		2,243.7 ± 651.0
Unknown n (%)	638 (26.5)	208 (69.8)	83 (54.6)	60 (75.0)	57 (4.3)	218 (100)	12 (3.8)
Adherence to physical activity guidelines*							

Yes n (%)	1,310 (54.5)	84 (28.2)	35 (23.0)	20 (25.0)	941 (70.3)		230 (72.6)
Unknown n (%)	370 (15.4)	33 (11.1)	53 (34.9)	15 (18.7)	45 (3.4)	218 (100)	6 (1.9)

*Self-reported engagement in at least 150 minutes per week of moderate-to-vigorous physical activity

For peer review only

280 **Cohort follow-up**

281 At each study site, collection of biospecimen, clinical, demographic, questionnaire and
282 anthropometric data occurred at baseline, and at six and twelve months following recruitment.
283 Baseline measurements in CORSA, COLON and EnCoRe have been performed at diagnosis
284 (preferably prior to any cancer treatment) while in the ColoCare Study such measurements
285 have been done prior to surgery (i.e. neoadjuvant treatment might have been applied prior to
286 baseline blood and data collection).

287 Blood samples at baseline were collected from n=2,132 (88.8%) study participants.
288 N=1,537 (64.0%) participants donated blood at the 6 month and n=614 (25.6%) at the 12
289 month follow-up time point. Additionally, n=251 (10.5%) EnCoRe patients provided blood
290 samples at 6 weeks post-treatment, a subset specifically of interest at the Maastricht study
291 site.

292 **Data collection**

293 Harmonized baseline and follow-up time points are summarized in **Table 3** and are briefly
294 described below.

295 Lifestyle and demographic data

296 ColoCare questionnaires and standardized Food Frequency Questionnaires (FFQ) are used to
297 assess intake of dietary supplements and medication, smoking, dietary intake and other health
298 behaviors at each study time point. In COLON, patients provide information on diet, lifestyle,
299 and supplement use via COLON questionnaires and FFQs. Patients from the CORSA Study
300 are requested to complete a questionnaire assessing anthropometric and demographic factors.
301 Patients enrolled in EnCoRe receive repeated home visits by trained dietitians, where
302 extensive measurements are performed that include assessment of demographic data, physical
303 activity (both questionnaire- and accelerometry-based) and smoking behavior (questionnaire
304 data), dietary intake (FFQs at diagnosis and 7-day food diaries at follow-up measurements),
305 supplement use (registered by dietitians), and anthropometry measurements.

Table 3: Variables available at baseline, and at 6 and 12 month follow-up within the FOCUS Consortium.

Category	Variables	Baseline	6m	12m
Demographics	Age	x	x	x
	Gender	x		
	Highest education	x		
	Social status	x		
	Height	x		
	Weight	x	x	x
	BMI	x	x	x
	Smoking status	x	x	x
	Smoking duration	x	x	x
	Smoking pack years	x	x	x
	Menopausal status	x		
	Postmenopausal hormone use	x		
	Race	x		
	Cancer characteristics	Cancer site	x	
Cancer stage		x		
TNM classification		x		
Treatment	Preoperative chemotherapy	x		
	Preoperative radiotherapy	x		
	Surgery	x	x	
	Postoperative chemotherapy		x	x
	Postoperative radiotherapy		x	x
Supplement intake	Folic acid supplements	x	x	x
	Vitamin B ₂ supplements	x	x	x
	Vitamin B ₆ supplements	x	x	x
	Vitamin B ₁₂ supplements	x	x	x
	Vitamin A supplements	x	x	x
	Vitamin C supplements	x	x	x
	Vitamin D supplements	x	x	x
Supplement intake	Vitamin E supplements	x	x	x
	Calcium supplements	x	x	x
	Magnesium supplements	x	x	x
	Iron supplements	x	x	x
	Multivitamins	x	x	x
Dietary nutrients	Folate equivalents	x	x	x
	Vitamin B ₂	x	x	x
	Vitamin B ₆	x	x	x
	Vitamin B ₁₂	x	x	x
	Vitamin A	x	x	x
	Vitamin C	x	x	x
	Vitamin D	x	x	x
	Vitamin E	x	x	x
	Total protein	x	x	x
Total fat	x	x	x	
Total carbohydrate	x	x	x	

Dietary nutrients	Fibre	x	x	x
	Saturated fatty acids	x	x	x
	Monounsaturated fatty acids	x	x	x
	Polyunsaturated fatty acids	x	x	x
	Alcohol	x	x	x
	Total energy	x	x	x
Physical activity	Light physical activity	x	x	x
	Moderate physical activity	x	x	x
	Vigorous physical activity	x	x	x
	Adherence to physical activity guidelines	x	x	x
Medical history	Diabetes mellitus	x	x	x
	Asthma, Chronical Bronchitis, COPD, Emphysema	x		
	Heart attack, Heart Failure	x		
	Hypertension	x		
	Stroke	x		
	Ulcer of Stomach or Duodenum	x		
	Hypothyroidism/Hyperthyroidism	x		
	Systemic Lupus Erythematosus	x		
	Inflammatory Bowel Disease (Crohn's disease, ulcerative colitis)	x		
	Familial Adenomatous Polyposis	x		
Medical history	Lynch Syndrome (hereditary nonpolyposis colorectal cancer)	x		
Regular medication use	Aspirin	x	x	x
	NSAID	x	x	x
	Ibuprofen	x	x	x
	Naproxen	x	x	x
	Celecoxib/Etoricoxib	x	x	x
Health-related quality of life	EORTC QLQ-C30	x	x	x
	EORTC QLQ-CR29	x	x	x
	EORTC QLQ-CIPN20	x	x	x
General information	Date of questionnaire completion	x	x	x
	Date of blood collection	x	x	x
	Freeze-thaw cycles of blood samples	x	x	x
	Hemolysis	x	x	x
	Time between blood draw and processing/storage	x	x	x

NSAID=non-steroidal anti-inflammatory drug; EORTC=European Organization for Research and Treatment of Cancer; QLQ=quality of life questionnaire; C30=core 30-items; CR29=colon/rectum 29-items; CIPN20=chemotherapy-induced polyneuropathy 20-items.

306

307 Clinical data and outcomes - Medical chart abstraction

308 All cancers and medical diagnoses are classified according to ICD-10 (International
309 Classification of Diseases, Tenth Revision) codes. Details on CRC treatment, including type
310 of treatment regimens and treatment toxicity, are abstracted via medical record for all cohorts.
311 Detailed information on the primary outcomes of interest, CRC recurrence and survival, are
312 ascertained through reviews of medical records, pathology reports, and imaging reports
313 documenting the diagnosis of a recurrence. Data on recurrence and vital status is
314 supplemented from the clinical cancer registries and survival data is verified by the inhabitant
315 registries that exist at all study sites. For COLON and EnCoRe, all data on recurrence are
316 retrieved in collaboration with the Netherlands Cancer Registry. Moreover, survival data for
317 CORSA participants was obtained by the Main Association of Austrian Social Insurance
318 Carriers as well as from Statistics Austria.

319 Patient-reported outcomes

320 Health-related quality of life is assessed by the validated and widely used cancer-specific 30-
321 item core questionnaire QLQ-C30 and the 29-item CRC module QLQ-CR29, developed by
322 the European Organization for Research and Treatment of Cancer (EORTC).²⁴
323 Chemotherapy-induced peripheral neuropathy (CIPN), which is a common toxicity in CRC
324 patients, is measured by the EORTC QLQ-CIPN20.²⁵ Patient-reported outcomes are available
325 for ColoCare Heidelberg, COLON and EnCoRe.

326 Biomarkers of FOCM

327 Blood is processed in identical settings across all study centers. Plasma (CORSA, COLON
328 and EnCoRe) and serum (for the three ColoCare Study sites) samples were collected and
329 immediately centrifuged, aliquoted, and stored at -80°C.

330 All biological analyses were performed at BEVITAL AS (Bergen, Norway,
331 <http://www.bevital.no>), which carried out metabolic profiling of biomarkers allocated to seven
332 complementary analytical platforms. Apart from analyses of microbiological active folate²⁶
333 and vitamin B₁₂²⁷, all analyses were based on mass spectrometry. Circulating folate, separate
334 folate species, 5-methyltetrahydrofolate (the main form in plasma), folic acid (folate in
335 supplements), 4a-OH 5-methyltetrahydrofolate (the primary oxidation form of circulating
336 folate), folate catabolites (p-aminobenzoylglutamate and acetyl p-aminobenzoylglutamate)²⁸,
337 B₆, B₁ and B₃ vitamins, kynurenines, cotinine, trans-3'-hydroxycotinine, trigonelline²⁹,
338 choline and its metabolites, creatinine, methylhistidines, arginine and methylated arginines³⁰

1
2
3 339 were analyzed by liquid chromatography-tandem mass spectroscopy, whereas other amino
4
5 340 acids, in addition to total homocysteine, total cysteine and methylmalonic acid were analyzed
6
7 341 by gass chromatography-tandem mass spectroscopy.³¹ C-reactive protein (hsCRP) and
8
9 342 cystatin C and its variants were measured by MALDI-TOF mass spectroscopy.³²

10
11 343 A comprehensive overview of the panel of biomarkers measured in blood samples of
12
13 344 patients enrolled in the FOCUS Consortium is provided in **Table 4**.

14 15 345 **Findings to date**

16
17 346 The FOCUS Consortium provides a unique opportunity to conduct comprehensive research
18
19 347 on folate and FOCM biomarkers status in the tertiary prevention of CRC using data collected
20
21 348 both at diagnosis and during standardized follow-up time points. This well-characterized
22
23 349 study design provides sufficient statistical power to discern prospective associations with
24
25 350 relevant clinical outcomes, including CRC recurrence and survival within relevant subgroups.

26 27 351 Key findings and publications

28
29 352 We investigated associations of circulating concentrations of folate, folic acid, and folate
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31 353 catabolites pABG and apABG, measured around time of diagnosis, with recurrence and survival
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33 354 among 2024 patients diagnosed with stage I-III CRC within the international FOCUS
34
35 355 Consortium. We did not observe any statistically significant associations for folate, pABG, and
36
37 356 apABG concentrations. However, an increased risk of cancer recurrences was observed among
38
39 357 patients with higher compared to lower circulating folic acid concentrations.³³ Further, Kiblawi
40
41 358 et al. measured associations between one-carbon metabolites, inflammation- and angiogenesis-
42
43 359 related biomarkers in a cross-sectional analysis of 238 patients from the ColoCare Heidelberg
44
45 360 cohort. The study showed that specific folate species within the one-carbon metabolism
46
47 361 pathway are associated with both inflammation and angiogenesis pathways among CRC
48
49 362 patients. In particular, vitamin B6 species, pyridoxal 5'-phosphate (PLP), pyridoxal (PL) and
50
51 363 pyridoxic acid (PA), were inversely associated with inflammatory biomarkers C-reactive
52
53 364 protein (CRP), serum amyloid A (SAA), IL-6 and IL-8. Thiamine and thiamine monophosphate
54
55 365 were inversely correlated with the CRP and IL-6. In addition, positive correlations of PA, PL
56
57 366 and PLP with angiogenesis biomarker VEGF-D were observed. Our findings reinforce the
58
59 367 notion that B vitamins involved in the one-carbon metabolism may be correlated with
60
370 368 carcinogenic processes.³⁴ This and further research will support the evidence base needed for
the development of dietary guidelines for CRC patients.

Table 4: Measured metabolites and biomarkers within the FOCUS Consortium

Folate and one-carbon metabolites	Abbreviation	Description
Anthranilic acid	AA	Tryptophan metabolite
Asymmetric dimethylarginine	ADMA	Inhibitor of nitric oxide synthase
α-Ketoglutaric acid	aKG	Keto acid of the Krebs cycle
Alanine	Ala	Amino acid
Acetamidobenzoylglutamate	apABG	Folate catabolite
Arginine	Arg	Amino acid
Asparagine	Asn	Amino acid
Aspartic acid	Asp	Amino acid
Betaine	Betaine	Methyl donor
Choline	Choline	Methyl donor
C-reactive protein	hsCRP	Inflammatory marker
Cystatin C-desS	CnCds	Cystatin C isoform
Cystatin C-desSSP	CnDcssp	Cystatin C isoform
Cystatin C	CnCn	Marker of kidney function
3Pro-OH cystatin C	CnCo	Cystatin C isoform
3Pro-OH cystatin C-desS	CnCods	Cystatin C isoform
Total concentration of detected cystatin C isoforms	CnCt	Marker of kidney function
Cobalamin	B ₁₂	Vitamin B ₁₂
Cotinine	Cot	Nicotine metabolite
Creatinine	Creat	Marker of kidney function
C-reactive protein	CRP	Inflammation
Cystathionine	Cysta	Thioether, transsulfuration intermediate
Dimethylglycine	DMG	Betaine metabolite
Folic acid	B ₉	Synthetic form of folate
Flavin mononucleotide	FMN	B ₂ vitamin
5-Formyl-tetrahydrofolate	fTHF	Folate species
Glutamine	Gln	Amino acid
Glutamic acid	Glu	Amino acid
Glycine	Gly	Amino acid
3-Hydroxyanthranilic acid	HAA	Tryptophan metabolite
Homoarginine	hArg	Amino acid
Histidine	Hist	Tryptophan metabolite
3-Hydroxykynurenine	HK	Tryptophan metabolite
4-Alfa-hydroxy-5-methyl-THF	hmTHF	Folate oxidation product
Isoleucine	Ile	Amino acid
Kynurenic acid	KA	Tryptophan metabolite
Kynurenine	Kyn	Tryptophan metabolite
Leucine	Leu	Amino acid
Lysine	Lys	Amino acid
3-Methylhistidine (3-MH)	m3His	Marker of muscle degradation and meat intake
1-Methylhistidine (1-MH)	m1His	Marker of muscle degradation and meat intake
Methionine	Met	Amino acid
Methionine sulfoxide	MetSo	Oxidation product of Met
Methylmalonic acid	MMA	Marker of B ₁₂ status
N1-methylnicotinamide	mNAM	B ₃ vitamin
5-Methyl-tetrahydrofolate	mTHF	Folate species

Nicotinic acid	NA	B ₃ vitamin
Nicotinamide	NAM	B ₃ vitamin
Neopterin	Neopt	Inflammatory marker
Trans-3'-hydroxycotinine	OHCot	Nicotine metabolite
Ornithine	Orn	Amino acid
4-Pyridoxic acid	PA	Vitamin B ₆ catabolite
Para-aminobenzoylglutamate	pABG	Folate catabolite
Phenylalanine	Phe	Amino acid
Picolinic acid	Pic	Tryptophan metabolite
Pyridoxal	PL	B ₆ vitamin
Pyridoxal 5'-phosphate	PLP	B ₆ vitamin
Pyridoxine	PN	Synthetic form of vitamin B ₆
Proline	Pro	Amino acid
Quinolinic acid	QA	Tryptophan metabolite
Riboflavin	Ribo	Main circulating B ₂ form
Symmetric dimethylarginine	SDMA	Marker of renal function
Serine	Ser	Amino acid
Folate	spFolate	Microbiologically active folate
Total cysteine	tCys	Amino acid
Homocysteine	tHcy	Marker of folate and B ₁₂ status
Thiamine	Thi	B ₁ vitamin
Threonine	Thr	Amino acid
Trimethylamineoxide	TMAO	Choline metabolite
Trimethyllysine	TML	Amino acid
Thiamine monophosphate	TMP	B ₁ vitamin
Trigonelline	Trig	Marker of coffee consumption
Tryptophan	Trp	Amino acid
Valine	Val	Amino acid
Leucine	Leu	Amino acid
Xanthurenic acid	XA	Tryptophan metabolite
Kyn/Trp ratio	KTR	Marker of immune activation
HK/XA ratio		Marker of B ₆ status

371
372 Further, we investigated circulating concentrations of nine biomarkers related to the B-
373 vitamins folate, riboflavin, vitamin B₆, and cobalamin, measured at diagnosis and six months
374 post-diagnosis, in association with health-related quality of life as assessed by the EORTC
375 QLQ-C30 questionnaire six months post-treatment in three FOCUS cohorts (ColoCare
376 Heidelberg, EnCoRe and COLON).³⁵ Higher PLP concentrations were cross-sectionally
377 associated with better physical, role, and social functioning, and reduced fatigue six months
378 post-diagnosis. Higher HKr (3-hydroxykynurenine:(kynurenic acid+xanthurenic acid+3-
379 hydroxyanthranilic acid+anthranilic acid)), an inverse marker of vitamin B₆ status, was cross-
380 sectionally associated with worse global quality of life, and lower physical and role
381 functioning. Dose-response relations were observed for PLP with global quality of life,
382 physical, role, and social functioning. No associations were observed for changes in
383 biomarker concentrations between diagnosis and six months with quality of life outcomes. We

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2
3 384 therefore concluded that higher vitamin B₆ status was associated with better quality of life at
4
5 385 six months post treatment and that further study is needed to clarify the role of vitamin B₆ in
6
7 386 relation to quality of life.

8
9 387 Previous relevant findings from individual cohorts within the consortium

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11 388 To date, individual cohorts from the FOCUS Consortium have initiated the examination of
12
13 389 dietary supplement use and dietary habits over time. Among CRC patients enrolled in the
14
15 390 ColoCare Study the proportion of supplement users was found to be highest post-diagnosis
16
17 391 (35%).³⁶ Moreover, within an international investigation including ColoCare participants from
18
19 392 multiple sites, Ulrich et al. showed differences in plasma folate concentration between
20
21 393 Heidelberg and the US sites, probably reflecting variation in folic acid fortification and
22
23 394 supplement use.¹³ Furthermore, ColoCare has published data on RECQ helicase expression³⁷,
24
25 395 NTRK3³⁸, RET³⁹, tumor-infiltrating lymphocytes and T cell receptor sequences⁴⁰, 25-25-
26
27 396 hydroxyvitamin D₃^{13 41}, DNA methylation⁴²⁻⁴⁵, miRNAs^{46 47}, fecal microbiota⁴⁸⁻⁵⁰,
28
29 397 metabolomics and transcriptomics⁵¹⁻⁵³, plasma proteins⁵⁴, gene expression⁵⁵, branched-chain
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31 398 amino acids⁵⁶, genetic variants⁵⁷, body composition^{53 58 59}, physical activity⁴¹, and dietary
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33 399 patterns⁴ in CRC patients. Within the COLON study, results have been published on body
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35 400 weight trajectories⁶⁰, changes in lifestyle⁶¹, 25-hydroxy vitamin D levels^{62 63}, and
36
37 401 inflammation markers⁶⁴ over time, as well as vitamin D⁶⁴, calcium or magnesium intake⁶⁵,
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39 402 physical activity⁶⁶, inflammation^{67 68}, skeletal muscle mass⁶⁹/ density⁷⁰ and other measures of
40
41 403 body composition⁷¹ in relation to cancer recurrence, survival, or physical functioning or
42
43 404 fatigue. Moreover, in a subset of patients undergoing chemotherapy, dietary factors in relation
44
45 405 to chronic CIPN^{72 73} as well as chemosensory perception and food preferences⁷⁴ were studied.
46
47 406 The Austrian CORSA Study has published results on genomic data⁷⁵⁻⁷⁷, telomere length⁷⁸,
48
49 407 DNA repair processes⁷⁹, tumor autoantibodies⁸⁰ as well as metabolomics.^{33 81} To date,
50
51 408 publications from the EnCoRe Study have reported on associations of physical activity and
52
53 409 sedentary behaviour⁸²⁻⁸⁵, adherence to lifestyle guidelines⁸⁶, and parameters of body
54
55 410 composition^{87 88} measured through CT scans with quality of life, functioning and fatigue in
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57 411 CRC survivors. Recently, longitudinal associations between supplement use and fatigue were
58
59 412 investigated from diagnosis to 2 years post-CRC treatment. No overall association between
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413 supplement use and fatigue was found but results suggest that increased levels of fatigue may
414
415 be a reason for the use of supplements among CRC survivors.⁸⁹ Higher concentrations of
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25OHD₃ were longitudinally associated with better global quality of life and less fatigue in
colorectal cancer survivors within EnCoRe suggesting a potential beneficial role of vitamin D

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2
3 417 in colorectal cancer survivors.³⁵ In a mixed-method study using data of the EnCoRe study,
4 418 colorectal cancer (treatment) related health and functioning problems negatively impacted the
5 419 ability of nearly 1 in 5 long-term CRC survivors to participate in everyday life situations and
6 420 their satisfaction with participation.⁹⁰ The validity of the food frequency questionnaire for
7 421 measuring dietary intake among survivors of colorectal cancer within the EnCoRe study
8 422 appeared to be moderate to good for most nutrients and food groups, relative to a 7-day
9 423 dietary record.⁹¹ Prediction models that we developed for estimating 1-year risk of low health-
10 424 related quality of life in seven domains in colorectal cancer survivors performed well when
11 425 externally validated among survivors within the EnCoRe and COLON studies.⁹²

426 **Future plans**

427 The consortium specified a comprehensive manuscript list of future projects using data from
428 the FOCUS Consortium. Some selected projects are described below:

429 a) Recently, the investigation of longitudinal associations of adherence to the dietary World
430 Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) and Dutch
431 Healthy Diet (DHD) recommendations with plasma kynurenine levels in colorectal cancer
432 survivors after treatment has been finalized and the corresponding manuscript is under review
433 at an international journal.

434 b) Further, near-term future plans include the investigation of (1) biomarkers related to
435 FOCM and associations with folate intake (from diet and supplements); (2) associations
436 between FOCM biomarkers such as vitamin B12 and tryptophan and recurrence, survival, and
437 patient-reported outcomes in CRC; (3) the impact of folate status (FOCM biomarkers and
438 diet/supplements) on treatment toxicity in patients treated with 5-FU modifiers; (4) the
439 interaction between biomarkers related to FOCM and polymorphisms in FOCM-related genes
440 in relation to CRC prognosis (recurrence & survival); (5) prognosis (disease-free and overall
441 survival) in stage I-III CRC and associations with dietary and supplement use at diagnosis and
442 changes during and after treatment; (6) FOCM-related biomarkers and their association with
443 body composition in stage I to III CRC patients; (7) associations between folate status
444 (FOCM biomarkers and diet/supplement use) and recurrence, survival and patient-reported
445 outcomes in young-onset CRC.

446 c) Long-term plans include the combination of additional biomarkers measured by the
447 individual cohorts within the next years (e.g. microbiome data, omics- and multi-omics data
448 etc.).

449 **Strengths and limitations**

450 This is the largest consortium to date addressing the research question of folate and FOCM
451 biomarkers in relation to survival, recurrence, treatment toxicity, and health-related quality of
452 life outcomes in CRC patients. The cohorts included in the FOCUS Consortium are designed
453 to enable future pooling of data.²² For that reason, methodologies, time points and
454 measurement instruments generally overlap, with each study presenting unique features such
455 as additional blood collection at the 6-week follow-up time point within the EnCoRe Study
456 and blood draws during chemotherapy within the COLON Study. The pooled sample size
457 provides sufficient power to investigate subgroup analyses across CRC patients, e.g., within
458 groups of patients who underwent 5-fluorouracil based chemotherapy or stratified by disease
459 stage. Furthermore, the assessment of biomarkers related to FOCM are conducted at a single,
460 state-of-the art laboratory and the biological materials are processed and stored according to
461 standardized operation protocols across all study sites, enabling precise and accurate
462 measurements of FOCM biomarkers. While RCTs are the gold standard for establishing
463 causality, the FOCUS cohort with its longitudinal design can contribute to establish causal
464 relationships, with appropriate statistical analyses. Further, the FOCUS data includes a time-
465 varying exposure on dietary supplement intake for future studies to consider. The collection
466 of the longitudinal data on dietary supplement intake, a key-exposure, is essential to obtain
467 meaningful estimates and thus required for developing recommendations and guidelines
468 regarding dietary intakes among CRC patients. The study population is predominantly based
469 on European cohorts (90.4%) in countries that have not implemented mandatory folic acid
470 fortification. This enables us to study a population where dietary intake and dietary
471 supplement use determine differences in folate status, yielding information of direct relevance
472 to cancer patients. However, the generalizability of results to populations that have introduced
473 folic acid fortification, including the United States, might therefore also be limited.⁹³
474 Performing sensitivity analyses by excluding countries without folic acid fortification (e.g.
475 Germany) or investigating analyses separately for Germany and the US might help to address
476 differences in fortification status. Moreover, patients were predominantly White, thus, it is not
477 possible to address racial and ethnic minorities. Ethnicity/race is an important determinant of
478 folate status and metabolism may be different between African Americans and Hispanics⁹⁴,
479 thus, recommendations should be limited to this current population. Future studies are
480 warranted in diverse populations and compared with the FOCUS cohort.

481 Another limitation of the consortium includes differences in collection strategies of the
482 baseline study time point across included studies: CORSA, COLON and EnCoRe are

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2
3 483 recruiting CRC patients at diagnosis, preferably prior to any cancer treatment while ColoCare
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5 484 is recruiting patients prior to surgery (i.e. neoadjuvant treatment might have occurred prior to
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7 485 baseline blood and data collection). Nevertheless, the ColoCare protocol requires that no
8
9 486 blood draw occurs within 2 weeks of neoadjuvant or adjuvant chemotherapy, limiting the
10
11 487 influence of ongoing treatments on blood biomarkers. In some cohorts, the timing with
12
13 488 respect to adjuvant chemotherapy is less clearly defined and this will be carefully considered
14
15 489 in sensitivity analyses. Furthermore, survivor bias – a type of selection bias – may be
16
17 490 introduced as some patients died or did not complete follow-up questionnaires or did not
18
19 491 provide blood samples. It is possible that patients who experience more severe toxicities,
20
21 492 worse clinical outcomes, or worse health-related quality of life, are underrepresented among
22
23 493 those patients who completed follow-up measurements.⁴ Cohort studies such as the one
24
25 494 presented here generate critical knowledge about preventable causes of disease. However,
26
27 495 selection bias may affect estimates. This is particularly true for non-participation at follow-up
28
29 496 that may depend on both the exposure and outcome. Within a review, Nohr et al. showed a
30
31 497 range of methods to quantify and adjust for selection bias. Even with limited data on
32
33 498 nonparticipants and those lost to follow up, it is possible to examine how effect estimates in a
34
35 499 specific study may be biased by selection.⁹⁵ The likelihood for reverse causation is small in
36
37 500 this prospective cohort, as the exposure measurements (blood folate levels and intake through
38
39 501 diet/supplements) were collected before the outcome (survival, recurrence, and quality of life)
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41 502 occurred. Therefore, these outcomes are unlikely to have influenced the exposure
42
43 503 measurements. Given the robust follow-up in these cohorts for outcomes and data availability,
44
45 504 future studies will be able to consider key confounders as well as predictors of recurrence and
46
47 505 survival.

506 **Collaboration**

507 A substantial amount of time has been spent in creating the harmonized dataset of baseline
508 variables from cohorts within the FOCUS Consortium, with follow-up data collection and
509 FOCM biomarker analyses. Any person interested in collaborating, learning about the
510 FOCUS Consortium or in getting access to FOCUS data and in-depth analyses can contact the
511 coordinating study PIs Prof. Andrea Gsur (andrea.gsur@meduniwien.ac.at) and Prof. Cornelia
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10
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604 **Competing interests**

605 None declared.

606 **Patient consent for publication**

607 Not applicable.

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3 609 **Ethics approval**
4

5 610 The Heidelberg ColoCare Study was approved by the Ethics Committee of the Medical
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8
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11
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15
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21
22 619 **Data availability**
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24 620 Data described in the manuscript, code book, and analytic code have been generated from
25 621 European-based consortia and as such are subject to regulations from multiple European
26 622 countries, which limit our availability to share data. The consortium’s funding has ended, and
27
28 623 no centralized staff is available to support
29
30 624 data requests. However, the FOCUS PIs have agreed to answer any queries or discuss
31
32 625 potential projects with anyone interested in future collaborative research. For further
33
34 626 questions please contact colocarestudy_admin@hci.utah.edu.
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3 946 **Figure legends**
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5 947 **Figure 1** FOCUS Consortium design
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