***Cell lines***

**UWSEA:** The CRC cell lines RKO, LoVo, SW480, LS174T, AAC1/SB10, HCT116, SW48, FET, VACO400, VACO411, VACO5 and the adenoma AAC1 cell line were cultured as described previously ([1](#_ENREF_1)). All cell lines were authenticated prior to use by DNA fingerprinting (IDEXX/Radil Bioresearch). CIMP and MSI status on these cell lines was assessed as described previously ([1](#_ENREF_1), [2](#_ENREF_2)).

**VUmc:** CRC cell lines Colo205, Colo320, HCT116, HCT15, HT29, LIM1863, LS174T, LS513, RKO, SW480, and SW1398, were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Lonza Biowhittaker, Verviers, Belgium) containing 10% fetal bovine serum (Hyclone, Perbio, UK). SW48 and Caco2 were cultured in RPMI 1640 (Lonza Biowhittaker) containing 10% (SW48) or 20% (Caco2) fetal bovine serum. All cell culture media were supplemented with 2 mM L-glutamine, 100 IU/ml sodium penicillin (Astellas Pharma B.V., Leiderdorp, the Netherlands) and 100 mg/ml streptomycin (Fisiopharma, Palomonta (SA), Italy). All cell lines were authenticated by array comparative genomic hybridization (aCGH, 244 k Agilent oligonucleotide platform) prior to use at the VU University Medical Center (VUmc), Amsterdam, the Netherlands, most recently in October 2008. The patterns of chromosomal changes observed were in concordance to the previously described chromosomal changes in these cell lines ([3](#_ENREF_3), [4](#_ENREF_4)).

 In order to investigate re-expression of *WRN* after inhibition of DNA methyltransfer-ases, HCT116 and Colo205 cells were treated with 5-aza-2’-deoxycytidine (DAC, 200 or 5000 nM) for 3 days (Sigma Chemical Co., St. Louis, MO, USA). To investigate the re-expression after inhibition of DNA methyltransferases and inhibition of histone deacetylation, cells were treated with 200 nM DAC for 96 hours, of which the last 24 hours were in combination with 300 nM Trichostatin A (TSA) (Sigma Chemical).

***Tissues***

**UWSEA:** Twenty-six primary CRC cases with matched normal colon tissue were obtained from the Cooperative Human Tissue Network (CHTN) as fresh frozen tissue. All cases contained > 60% tumor cells upon histologic examination, and were studied following IRB approved protocols and in accordance with the ethical regulations of the institution.

**VUmc:** Formalin-fixed and paraffin-embedded (FFPE) tissues from 21 normal colons from cancer-free patients were collected from the tissue archive of the Department of Pathology at the VU University Medical Center, Amsterdam, the Netherlands, in accordance with the Code for Proper Secondary Use of Human Tissue in the Netherlands([5](#_ENREF_5)).

FFPE tumor samples from a subgroup of patients that participated in a previously conducted randomized phase III trial (CAIRO study (NCT00312000) of the Dutch Colorectal Cancer Group (DCCG)([6](#_ENREF_6))) were retrieved through the Dutch National Pathology Registry PALGA ([7](#_ENREF_7)). In the CAIRO study, patients with metastatic CRC were randomized between either a sequential (Arm A) or combination treatment arm (Arm B). Patients in Arm A received first-line capecitabine (CAP), followed upon disease progression by second-line irinotecan and third-line capecitabine plus oxaliplatin (CAPOX). Patients in Arm B received first-line capecitabine plus irinotecan (CAPIRI), followed upon disease progression by second-line CAPOX. Primary tumors were resected before chemotherapy. Tumor response was assessed over 3 cycles (9 weeks) of treatment according to RECIST criteria ([8](#_ENREF_8)). Follow-up after completion of treatment was done every 3 months until death. Written informed consent was required from all patients before study entry, and included consent for collecting paraffin-embedded blocks of patient material. The study was registered at ClinicalTrials.gov with the number NCT00312000, and by the local ethics committees of all participating centers. Further details of the study have been previously reported ([6](#_ENREF_6)).

A total of 183 patients were selected for analysis, of which 90 were treated in sequential treatment Arm A, and 93 were treated in combination Arm B. These patients were matched for stratification data according to the stratification factors in the original study for the subgroup of patients that underwent resection of the primary tumor, i.e. resection status, WHO performance status, predominant localization of metastases, previous adjuvant therapy and serum lactate dehydrogenase levels. Samples were also selected based on a high proportion of tumor cells in sections (at least 70%). A large proportion of these sample overlap with samples described in ([9](#_ENREF_9)). For progression-free survival (PFS) analysis, only patients who had received at least 3 cycles of 1st line therapy, or 2 cycles when death followed due to progressive disease were analyzed. For overall survival (OS) analysis, all patients were analyzed.

***DNA isolation and bisulfite treatment procedures***

**UWSEA:** DNA from cell lines and fresh frozen tissues were extracted and bisulfite converted as previously described ([1](#_ENREF_1)).

**VUmc:** DNA from cell lines was isolated using TRIzol (Invitrogen, Breda, NL) following the supplier’s instructions. DNA from FFPE tissues was isolated by a column-based method (QIamp DNA microkit, Qiagen, Hilden, Germany) as described before ([10](#_ENREF_10)). DNA from FFPE tissue samples from primary tumors was manually macrodissected from areas containing >70% tumor cell content. DNA was subjected to sodium bisulfite conversion using the EZ DNA Methylation Kit (Zymo Research, Orange, CA, USA) according to the manufacturer’s protocol.

***Methylation Specific PCR***

**UWSEA**: Prior to methylation-specific PCR (MSP), isolated genomic DNA was first subjected to sodium bisulfite treatment as previously described ([1](#_ENREF_1)). The thermocycler conditions were: 94 ºC x 15 min, 45 x (94 ºC x 30 sec, 66 ºC x 45 sec, 72 ºC x 30 sec), 72 ºC x 10 min. All the samples were subjected to at least two independent rounds of sodium bisulfite treatment and MSP assays. The MSP assay targeted a region from -31 bp to +128 bp relative to the TSS at bp +1 (referred to as Region 1 in the manuscript). The methylation status was assigned only if consistent results were obtained. Control samples consisting of DNA from Peripheral Blood Lymphocytes (PBL) and DNA from SssI methylase-treated DNA from the colorectal cancer cell line SW48 were used, respectively, as ‘unmethylated template’ and ‘methylated template’ controls and were included in each MSP assay as technical/assay controls. MSP products were subjected to horizontal gel electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and visualized with UV transillumination using an Eagle Eye Imaging system (Stratagene, La Jolla, CA).

**VUmc**:

CRC cell lines and normal colon tissues were subjected to a quantitative MSP (qMSP) assay for *WRN*. The qMSP assay targeted the region located -410bp to -331 bp relative to the TSS (referred to as Region 2 in the manuscript). Quality control was performed with *in vitro* Methylated DNA as a positive control and *in vitro* Unmethylated DNA as a negative control (Chemicon, Temecula, CA). qMSP reactions were carried out using a 7500 Fast Real-Time PCR System (Applied Biosystem, Foster City, CA) in duplicate in 25 µl reaction volumes, where each reaction contained 40 ng of bisulfite-treated DNA, 10 pmol of each primer and 12.5 μl SYBR Green PCR Master Mix (Applied Biosystems). Each plate included no template controls and a standard curve with a serial dilution of bisulfite-modified DNA prepared from *in vitro* Methylated DNA (Chemicon). PCR conditions were 95°C for 15 min, 40 cycles at 95°C for 15 sec, 60°C for 60 sec, followed by Melt Curve Stage (95°C for 15 sec, 60°C for 60 sec, 95°C for 30 sec and 60°C for 15 sec). Melting curve analysis was performed to check the specificity of the amplification reaction. Amplification of beta-actin (ACTB) was used as an unmethylated reference gene, using the same PCR conditions. The Ct ratio per sample was calculated according to the formula 2e-[mean CtWRN – mean CtACTB]. A sample was called methylated when the Ct ratio was higher than observed in a panel of 21 normal colon mucosal samples (i.e. 0.03).

 Tumor samples from the CAIRO study were subjected to a high-throughput LightCycler MSP assay (LightCycler 480 SYBR Green I Master kit (Roche, Vilvoorde, Belgium)), using the same primers and controls for *WRN* as described above for the qMSP, where each reaction contained 20 ng bisulfite-modified DNA amplified with the following PCR conditions: 95°C for 10 min followed by 45 cycles of 95°C for 10 sec, 60°C for 30 sec and 72°C for 1 sec. Amplification of beta-actin was used as an unmethylated reference gene. The amplicons were checked for size and quantified by capillary electrophoresis (LC90 Labchip; Caliper Lifesciences).

See Supplementary Table 3 for all primer sequences.

***Bisulfite Sequencing***

**UWSEA**: Sodium bisulfite sequencing (BS) was carried out as previously described ([1](#_ENREF_1)) using newly designed PCR primers for the *WRN* promoter region beginning 193 bp upstream of the transcription start site (see Figure 1 and Supplementary Table 3). The sequencing thermocycler reaction conditions were as follow: 94 ºC x 15 min, 40 x (94 ºC x 30 sec, 54 ºC x 30 sec, 72 ºC x 30 sec), 72 ºC x 10 min. Sequencing of the amplicons was performed by the Genomics Core at the Fred Hutchinson Cancer Research Center. BS was done on the colon cancer cell lines HCT116 and SW480 and on 10 colon tissue (8 CRCs and 2 normal mucosa samples) using the same protocol.

***RT-PCR***

**UWSEA**: RNA was isolated from cell lines and fresh frozen tissue using Trizol as recommended by the manufacturer (Life Technologies), followed by purification using the RNeasy Kit (Qiagen). cDNA was made using Superscript II reverse transcriptase (Invitrogen), 1 x PCR buffer, MgCl2 (8mM), RNase Out and 1-4μg of total RNA. Real-time quantitative PCR assays were conducted using Taqman probe and primers (Applied Biosystems) for *WRN* (Hs00172155\_m1) and *GusB* (Hs99999908\_m1) along with Taqman Universal PCR Mix (Applied Biosystems) as recommended by the manufacturer. *GusB* expression was used as a loading control. The relative standard curve method was used to determine *WRN* expression levels. Control RNA was obtained from the following CRC cell lines: LS174T for *WRN*, and SW480 for *GUSB*.

**VUmc**: Total RNA was isolated from cell lines using TriZol reagent (Invitrogen, Breda, the Netherlands), and subjected to purification using RNeasy Mini Kit (Qiagen). After DNAse treatment (RQ1 DNAse, Promega, Leiden, the Netherlands), cDNA was made with the Iscript cDNA Synthesis Kit (BioRad, Veenendaal, the Netherlands). Quantitative RT-PCR was done using TaqMan® Gene Expression Assays from Applied Biosystems directed to *WRN* (Hs00172155\_m1) and β-2-microglobulin (*B2M;* Hs00984230\_m1). Relative expression levels were determined by calculating the Ct-ratio (Ct ratio = 2e-(CtWRN–CtB2M))x1000.

***Western Blotting***

**UWSEA**: Western blotting studies were conducted on 10 CRC cell lines (RKO, LoVo, SW480, LS174T, AAC1/SB10, HCT116, SW48, FET, VACO400, VACO411) as described previously ([11](#_ENREF_11)). Briefly, the cells were lysed using RIPA buffer on ice (0°C). The protein extracts (100 μg/sample) were subjected to electrophoresis through 12% Bis-Trispolyacrylamide gels (BioRad, USA) and transferred to PVDF membranes. Mouse anti-WRN (monoclonal W0393; Sigma) antibody was used to detect the endogenous WRN protein expression, and anti-β-actin (monoclonal 13E5, Cell Signaling Technologies) was used to normalize the protein loading. Immunoreactive proteins were then visualized by incubating PVDF membranes with ECL plus detection reagents, followed by imaging of chemiluminescence on a Geldoc Imaging system (BioRad).

***TCGA data***

*WRN* DNA methylation (Illumina Infinium HM27 bead array; HM27) and mRNA expression (Agilent  microarray) data were obtained via cBioPortal for Cancer Genomics (<http://www.cbioportal.org>; ([12](#_ENREF_12))) on 223 CRC tumors included in The Cancer Genome Atlas (TCGA) Colorectal Cancer project ([13](#_ENREF_13)). This data set was downloaded on 2 March 2014 from all available tumors with methylation data (HM27) data from the *Colorectal Adenocarcinoma (TCGA, Nature 2012)* dataset.

***Statistical analysis***

Student’s T-test was used for compare *WRN* mRNA expression levels in HCT116 and Colo205 before and after DAC and/or TSA treatment. Pearson correlation analysis was used to determine the correlation between *WRN* methylation and mRNA expression levels. Progression-free survival (PFS) for first-line treatment was considered the time lapse between the date of randomization and the date of first observation of disease progression or death reported after first-line treatment. The predictive value of *WRN* methylation was assessed by survival analysis including Kaplan-Meier curves. Overall survival (OS) was measured from the date of randomization to date of death due to cancer. Other causes of death were censored. Cox Proportional Hazard models were used to estimate Hazard Ratios (HR) and 95% confidence intervals (95% CI). A multivariate Cox regression model using Wald test was used to calculate the interaction effect between treatment arm and *WRN* methylation status after adjusting for potentially confounding factors including age, gender, serum LDH, WHO performance status, previous adjuvant therapy and predominant location of metastasis. Multivariate Cox regression analysis was also used to assess and adjust for possible prognostic variables Microsatellite Instability (MSI) status, *BRAF* mutational status and mucinous differentiation, for which information was available on a sub-set of the samples (136 out of 183)([14](#_ENREF_14), [15](#_ENREF_15)). Results were considered significant when *p*-values were ≤ 0.05.

Statistical analyses were performed using the computing environment R version 3.1.2([16](#_ENREF_16)), including the package '*survival*'([17](#_ENREF_17), [18](#_ENREF_18)).

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