1. PROTOCOL SYNOPSIS

TITLE	*Mesalamine for Colorectal Cancer Prevention Program in Lynch syndrome*
OBJECTIVES	**Primary Objective** • *The primary objective of the study is to test whether mesalamine (5-ASA) reduces the occurrence of any colorectal neoplasia (both benign and malignant tumors) compared to placebo in Lynch syndrome (LS) patients as detected by any colonoscopy until the end of treatment (24 months +/- 1 month) and end of study.* **Secondary Objectives** • *To test whether 5-ASA reduces the number of any colorectal neoplasia, both benign and malignant tumors, (tumor multiplicity) and tumor progression to placebo in LS patients at defined time points. Advanced adenomas are defined by a diameter above 1 cm villous or tubulovillous histology or high grade dysplasia.* • *To investigate if differences between 5-ASA effects and placebo effects on the occurrence of colorectal neoplasia, tumor multiplicity or tumor progression depend on the history of colorectal cancer, sex and patients age (LS patients below 45 years of age or 45 years of age and older).* • *To investigate differences between low and high dose 5-ASA with respect to the occurrence of colorectal neoplasia, to tumor multiplicity and tumor progression.* • *To determine the safety concerning 5-ASA in LS patients* **Descriptive Objectives** • *Tumor occurrence in the right and left colon and rectum and the occurrence and location of serrated benign or malignant colorectal neoplasia are described for the three treatment groups.* • *The occurrence of colorectal neoplasia, tumor multiplicity or tumor progression are described for each sublocation.* • *To investigate if 5-ASA effects differs between major genotypes specific for LS.* • *To investigate if differences between low and high dose 5-ASA on the occurrence of colorectal neoplasia, on tumor multiplicity and on tumor progression depend on the history of cancer, sex and patients age (LS patients below 45 years of age or 45 years of age and older).* • *To determine compliance concerning 5-ASA in LS patients*
DESIGN / PHASE	*Multicenter, multinational, randomized, 3-arm, double-blind, phase II clinical study with 2400mg 5-ASA, 1200mg 5-ASA or placebo in LS patients for 2 years*

<table>
<tr><td>STUDY TREATMENT PLANNED DURATION</td><td rowspan="2">First patient
First visit</td><td rowspan="2">4Q
2017</td><td rowspan="2">Last patient
First visit</td><td rowspan="2">2Q
2019</td><td>Last patient
Last visit</td><td>2Q
2021</td></tr>
<tr><td>STUDY END PLANNED DURATION</td><td>Last patient last phone FU visit</td><td>2Q
2025</td></tr>
<tr><td>COUNTRIES</td><td colspan="6">6 countries
Austria/ Germany/ Netherlands/ Poland/ Israel/ Sweden</td></tr>
<tr><td>PATIENTS / GROUPS</td><td colspan="6">1080 Patients will be assessed for eligibility
540 randomized patients in 3 groups: 180 patients per group
Randomization ratio 1:1:1</td></tr>
<tr><td>INCLUSION CRITERIA</td><td colspan="6">• Proven tumor-free (including patients in which the polyps are removed endoscopically) carriers of a germline pathologic mutation on one of the MMR genes including MLH1, MSH2 (including EpCAM) and MSH6
• Male or female subjects with the age > 25 years
• Females who have been post-menopausal more than one (1) year or females of childbearing potential using a highly efficient method of contraception with less than 1% failure rate (i.e. oral hormonal contraceptives, hormone implants, hormone injections, sterilization, hormonal or copper intrauterine device, sterilized/vasectomized partner, or diaphragm in combination with a condom, spermicide or birth control pills) or should agree to abstain from heterosexual activity during treatment period. Females of childbearing potential must have a negative pregnancy test at screening and before randomization.
• Signed written informed consent prior to inclusion in the study</td></tr>
<tr><td>EXCLUSION CRITERIA</td><td colspan="6">• Presence of colorectal endoscopically non-removable benign neoplasia (patient can be included if the adenoma is removed)
• Carriers of germline mutations in PMS2
• Patients with history of stage 3 and 4 colorectal cancer (CRC) are excluded
• Presence of metastatic disease
• Regular use of acetylsalicylic acid (ASA or aspirin): daily use of ≥100mg in more than 3 continuous months within the last year
• Regular use of NSAIDs or COX-2 inhibitors: daily use in more than 3 continuous months within the last year
• Hypersensitivity to 5-ASA
• Patients after total or subtotal colectomy
• Colorectal surgery within the previous 6 months
• Unwillingness to participate or who is considered incompetent to give an informed consent
• Pregnant or breastfeeding women
• Participation in another clinical study investigating another IMP within 3 months prior to screening</td></tr>
</table>

	• *Renal insufficiency (GFR <30ml/min/1.73m^2)* • *Severe liver disease or liver failure (elevation of liver enzymes above 3xULN)* • *Current or history of serious psychiatric disorder or alcohol/drug abuse that in the opinion of the investigator may impact the assessment of IMP safety andefficacy or protocol adherence* • *Prior history of myocarditis or pericarditis. Other severe acute or chronic medical condition (such as severe chronic lung (COPD, including asthma), kidney or heart diseases) or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or ability to comply with study procedures, IMP administration and, in the judgment of the investigator, would make the subject inappropriate for entry into this study*
STUDY PERIODS	**Screening phase:** ➢ *A colonoscopy with a white light high resolution colonoscope such as Olympus 180 or higher has to be performed. A minimum of 3 pictures has to be documented (appendiceal orifice, cecum with ileocoecal valve, rectum with inversion of scope). A biopsy of the colon ascendens and the rectum will be taken (not applicable for the Netherlands. If a neoplastic lesion is identified, it should be removed by snare polypectomy. Complete removal must be documented by at least 2 pictures (before and after removal) and histological confirmation.* ➢ *Blood and stool will be sampled at the primary colonoscopy in selected centres (i.e. at the sites of national coordinators).* ➢ *Medical history and vital signs, concomitant medication, physical examination, body weight and height have to be assessed.* ➢ *Blood samples for hematology, serum chemistry and (if applicable) pregnancy test will be done.* **Treatment phase: 24 months per patient** ➢ *Once daily intake of either two tablets mesalamine (5-ASA) (1200mg each), or one tablet mesalamine (5-ASA) (1200mg) with one tablet matching placebo, or two tablets placebo.* ➢ *Site visits every 3 months to collect empty containers, compliance check, check renal function (blood creatinine) and safety reports, concomitant medication, physical examination and vital signs have to be assessed.* ➢ *Telephone interviews every 6 weeks in between the visits for compliance and safety check.* ➢ *Colonoscopy for observation will be performed annually or every two years according to patient's treatment plan; Neoplasia found via colonoscopy will be (endoscopically or surgically) resected and analyzed by the local pathologist. A second analysis will be performed at the pathology department in Poland.* ➢ *Additionally for German sites, liver parameters (ALAT, ASAT, bilirubin and alkaline phosphatase) will be determined from blood at every site visit to examine patients liver function. These laboratory assessments are performed for*

	patient security only and will not be documented in the eCRF, except for screening, randomization and end of treatment visits. Bilirubin levels are never documented in the eCRF. All data should be documented in patients' source data. **End of Treatment:** ➢ *At the end of treatment (after 24 months +/- 1 month of treatment) a colonoscopy will be performed with biopsies (normal tissue of ascending colon and rectum – not applicable for the Netherlands), blood and stool sampling for 5-ASA compliance and biomarker analysis (in selected centres). The serum and stool samples will be stored at -20°C; the biopsies will be fixed in formaldehyde and embedded in paraffin (not applicable for the Netherlands).* ➢ *Blood creatinine for estimation of GFR.* ➢ *Concomitant medication, physical examination and vital signs have to be assessed.* ➢ *Blood samples for hematology and serum chemistry will be done.* **Phone Follow-up visit:** *4 years after the end of treatment a Phone Follow-up visit is planned to collect recent colonoscopy data and analyze the long-term effect of 2-years treatment with 5-ASA.*
	Mesalamine (5-ASA) *1200mg or 2400mg daily dose* ***Placebo***
CONCOMITANT MEDICATION	***Not allowed concomitant medication:*** • *Regular use of NSAIDs or COX-2 inhibitors:* more than 3 doses a week NSAIDs and COX-2 inhibitors. • *Regular use of ASA (aspirin):* more than 3 doses (>100mg) a week. *Other concomitant medication is allowed*.
	Interactions with other medicinal products have to be considered by the investigator as reported in the SmPC.
STATISTICAL METHODOLOGY	***Primary endpoint:*** • *Occurrence of any colorectal neoplasia (both benign and malignant tumors) between groups is described by absolute frequencies and percentages with 95 % confidence intervals.* A logistic regression is used to assess differences between active treatment and placebo for the occurrence of any colorectal neoplasia, adjusted for country and history of cancer before randomization. Treatment effects are assessed by odds-ratios and corresponding 95 % confidence intervals. ***Secondary endpoints:*** • The number of colorectal neoplasia (both benign and malignant tumors)per patient will be tested between groups by an analysis of variance, adjusting for country and

	history of cancer before randomization. In case of non-normally distributed residuals a suitable transformation to achieve normal distribution is considered. • *The tumor progress in the 4 or*dered stages will be tested between groups by a chi-square trend test stratified for country and history of cancer before randomization and modelled by an ordinal logistic regression. • *The dependence of treatment effects on history of colorectal cancer, sex and patients age (<45 years and ≥45 years) will be assessed by modelling interactions between these factors and treatment in the corresponding regression models.* • *Differences between high and low dose ASA for the occurrence of colorectal neoplasia, tumor multiplicity and tumor progression will be analysed by the same methods as for the comparison between ASA and placebo.* • *Safety data are described and compared between groups in an exploratory manner.* *All tests are two-sided and a significance level of 5 % is used.*