



## Clinical trial results:

### Chemopreventive action of Mesalazine on colorectal cancer: a pilot study for an "in vivo" evaluation of the molecular effects on -catenin signaling pathway

#### Summary

EudraCT number	2012-001351-40
Trial protocol	IT
Global end of trial date	17 June 2016

#### Results information

Result version number	v1 (current)
This version publication date	23 September 2017
First version publication date	23 September 2017

#### Trial information

##### Trial identification

Sponsor protocol code	MES-CT01
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##### Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT02077777
WHO universal trial number (UTN)	-

Notes:

#### Sponsors

Sponsor organisation name	SOFAR SPA
Sponsor organisation address	via Firenze, 40, Trezzano Rosa, Italy, 20060
Public contact	Dipartimento Medico, SOFAR SPA, +39 02 909362291, laura.patrucco@sofarfarm.it
Scientific contact	Dipartimento Medico, SOFAR SPA, +39 02 909362291, laura.patrucco@sofarfarm.it

Notes:

#### Paediatric regulatory details

Is trial part of an agreed paediatric investigation plan (PIP)	No
Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial?	No
Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial?	No

Notes:

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**Results analysis stage**

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Analysis stage	Final
Date of interim/final analysis	16 June 2017
Is this the analysis of the primary completion data?	No

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Global end of trial reached?	Yes
Global end of trial date	17 June 2016
Was the trial ended prematurely?	No

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Notes:

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**General information about the trial**

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Main objective of the trial:

The primary objective of this study was to obtain "in vivo" confirmation that mesalazine induces the gene expression of  $\mu$ -protocadherin and other genes related to the  $\beta$ -catenin signaling pathway in biopsies of normal colonic mucosa collected at the beginning and at the end of the study through molecular analysis (quantitative RT-PCR). Signal variation from baseline was evaluated analyzing the number of times it varies.

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Protection of trial subjects:

The study will be performed in accordance with the Declaration of Helsinki (see Appendix 5) approved by the 18th World Medical Assembly (WMA) General Assembly in Helsinki-Finland, June 1964 and amended by the

29th WMA, Tokyo-Japan, October 1975;

35th WMA in Venice-Italy, October 1983;

41st WMA in Hong Kong, September 1989;

48th WMA, Somerset West, Republic of South Africa, October 1996

52nd WMA, Edinburgh, Scotland, October 2000; and the 59th WMA General Assembly, Seoul, Korea, October 2008.

It is mandatory that all considerations about protection of human subjects are carried out in accordance with the Declaration of Helsinki.

The study descriptions was submitted to the IEC before study start.

All patient received all the information about the study and they gave their written acceptance through informed consent signature.

Sponsor provided a full insurance coverage.

All personal data complied with local law for privacy protection. All data recorded has been coded.

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Background therapy: -

Evidence for comparator: -

Actual start date of recruitment	03 January 2013
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

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Notes:

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**Population of trial subjects**

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**Subjects enrolled per country**

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Country: Number of subjects enrolled	Italy: 21
Worldwide total number of subjects	21
EEA total number of subjects	21

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Notes:

<b>Subjects enrolled per age group</b>	
In utero	0
Preterm newborn - gestational age < 37 wk	0
Newborns (0-27 days)	0
Infants and toddlers (28 days-23 months)	0
Children (2-11 years)	0
Adolescents (12-17 years)	0
Adults (18-64 years)	6
From 65 to 84 years	15
85 years and over	0

## Subject disposition

### Recruitment

Recruitment details:

Study Period: 1 January 2013 (first enrollment); 11 March 2016 (last completed). 1 investigational centre in Italy.

### Pre-assignment

Screening details:

Patients screened n.: 21; Patients screening failure No: 0; Patients Randomized: 21; Safety Population: 21; Per protocol population: 18

### Period 1

Period 1 title	Overall trial (overall period)
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Not blinded

### Arms

Are arms mutually exclusive?	Yes
<b>Arm title</b>	5-ASA Group

Arm description:

Patients treated with Mesalazine 2,4 g/die for 3 months

Arm type	Experimental
Investigational medicinal product name	Mesalazine
Investigational medicinal product code	
Other name	Pentacol 800
Pharmaceutical forms	Tablet
Routes of administration	Oral use

Dosage and administration details:

1 tablet three times a day

<b>Arm title</b>	No Treatment Group
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Arm description:

Patients not treated

Arm type	No intervention
No investigational medicinal product assigned in this arm	

Number of subjects in period 1	5-ASA Group	No Treatment Group
Started	11	10
Completed	10	10
Not completed	1	0
Consent withdrawn by subject	1	-

## Baseline characteristics

### Reporting groups

Reporting group title	5-ASA Group
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Reporting group description:

Patients treated with Mesalazine 2,4 g/die for 3 months

Reporting group title	No Treatment Group
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Reporting group description:

Patients not treated

Reporting group values	5-ASA Group	No Treatment Group	Total
Number of subjects	11	10	21
Age categorical			
Adult patients (at least 18 years old)			
Units: Subjects			
In utero	0	0	0
Preterm newborn infants (gestational age < 37 wks)	0	0	0
Newborns (0-27 days)	0	0	0
Infants and toddlers (28 days-23 months)	0	0	0
Children (2-11 years)	0	0	0
Adolescents (12-17 years)	0	0	0
Adults (18-64 years)	4	2	6
From 65-84 years	7	8	15
85 years and over	0	0	0
Gender categorical			
Patients of either sex			
Units: Subjects			
Female	7	3	10
Male	4	7	11

## End points

### End points reporting groups

Reporting group title	5-ASA Group
Reporting group description:	
Patients treated with Mesalazine 2,4 g/die for 3 months	
Reporting group title	No Treatment Group
Reporting group description:	
Patients not treated	

### Primary: Gene expression levels of $\mu$ -protocadherin and other genes

End point title	Gene expression levels of $\mu$ -protocadherin and other genes
End point description:	
Molecular analysis (with quantitative RT-PCR) of gene expression levels of $\mu$ -protocadherin and other related proteins: Protocadherin 19, Protocadherin 24, Cadherin E, TCF7L2, c-myc, Cyclin D1, P21waf1, VEGF, CD44, Met, KLF4 e CEBP- $\alpha$ .	
End point type	Primary
End point timeframe:	
3 months after visit 1	

End point values	5-ASA Group	No Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	8	10		
Units: N. of signal variations from baseline				
arithmetic mean (standard deviation)	1.03 ( $\pm$ 0.52)	1.07 ( $\pm$ 0.39)		

### Statistical analyses

Statistical analysis title	Efficacy Results
Statistical analysis description:	
This pilot study was not formally powered to assess effect and sample size was related to feasibility. The results obtained thus do not have inferential validity. The focus was on descriptive statistics of gene expression of $\mu$ -protocadherin and other genes related to the $\beta$ -catenin signaling pathway evaluated in terms of the number of signal variations from baseline and was analyzed as mean value between right and left colon as well as separately for right and left colon.	
Comparison groups	5-ASA Group v No Treatment Group
Number of subjects included in analysis	18
Analysis specification	Pre-specified
Analysis type	other <sup>[1]</sup>
P-value	$\leq$ 0.05
Method	ANOVA

Notes:

[1] - Since this was a pilot study and sample size was related to feasibility, a very small number of patients were enrolled into the study: therefore, all the efficacy results are interpreted only with a descriptive meaning.

## Secondary: DNA Depurination

End point title	DNA Depurination
End point description:	
Quantification of number of AP sites per 100,000 DNA bp	
End point type	Secondary
End point timeframe:	
3 months after Visit 1	

End point values	5-ASA Group	No Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	8	10		
Units: number of AP sites per 100,000 DNA bp				
arithmetic mean (standard deviation)	2.91 ( $\pm$ 1.25)	2.64 ( $\pm$ 0.8)		

## Statistical analyses

Statistical analysis title	Efficacy results
Statistical analysis description:	
The molecular analysis of the depurination levels of DNA considering mean values did not reveal any differences between treatment groups at Visit 2 for AP (/100,000 bp)	
Comparison groups	5-ASA Group v No Treatment Group
Number of subjects included in analysis	18
Analysis specification	Pre-specified
Analysis type	other <sup>[2]</sup>
P-value	$\leq$ 0.05
Method	t-test, 2-sided

Notes:

[2] - Since this was a pilot study and sample size was related to feasibility, a very small number of patients were enrolled into the study: therefore, all the efficacy results are interpreted only with a descriptive meaning.

## Secondary: DNA Oxidation

End point title	DNA Oxidation
End point description:	
Quantification of nanograms of 8-OhdG (8hydroxyguanine) per micrograms of DNA	
End point type	Secondary
End point timeframe:	
3 months after Visit 1	

End point values	5-ASA Group	No Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	8	10		
Units: nanograms				
arithmetic mean (standard deviation)	0.03 (± 0.01)	0.03 (± 0.01)		

## Statistical analyses

Statistical analysis title	Efficacy results
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Statistical analysis description:

The molecular analysis of the oxidation considering mean values between right and left colon, right colon only, and left colon only, did not reveal any differences between treatment groups at Visit 2 for AP (/100,000 bp) and ng 8-OhdG (/µg of DNA).

Comparison groups	5-ASA Group v No Treatment Group
Number of subjects included in analysis	18
Analysis specification	Pre-specified
Analysis type	other <sup>[3]</sup>
P-value	≤ 0.05
Method	t-test, 2-sided

Notes:

[3] - Since this was a pilot study and sample size was related to feasibility, a very small number of patients were enrolled into the study: therefore, all the efficacy results are interpreted only with a descriptive meaning.

## Secondary: Cells expressing Histone H2Axy

End point title	Cells expressing Histone H2Axy
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End point description:

Percentage of cells expressing Histone H2Axy evaluated by immunohistochemical analysis

End point type	Secondary
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End point timeframe:

3 months after visit 1

End point values	5-ASA Group	No Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	8	10		
Units: percentage				
arithmetic mean (standard deviation)	0 (± 0)	0 (± 0)		

## Statistical analyses

Statistical analysis title	Efficacy results
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Statistical analysis description:

The percentage of cells expressing Histone H2Axy by immunohistochemical analysis did not present any substantial between-group differences, with Histones H2Axy values always equal to 0%.

Comparison groups	5-ASA Group v No Treatment Group
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Number of subjects included in analysis	18
Analysis specification	Pre-specified
Analysis type	other <sup>[4]</sup>
P-value	≤ 0.05
Method	Wilcoxon (Mann-Whitney)

Notes:

[4] - Since this was a pilot study and sample size was related to feasibility, a very small number of patients were enrolled into the study: therefore, all the efficacy results are interpreted only with a descriptive meaning.

### Secondary: Cells expressing Caspase-3

End point title	Cells expressing Caspase-3
End point description:	
Percentage of cells expressing Caspase-3 evaluated by immunohistochemical analysis	
End point type	Secondary
End point timeframe:	
3 months after visit 1	

End point values	5-ASA Group	No Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	8	10		
Units: percentage				
arithmetic mean (standard deviation)	0 (± 0)	0 (± 0)		

### Statistical analyses

Statistical analysis title	Efficacy results
Statistical analysis description:	
The percentage of cells expressing Caspase-3 by immunohistochemical analysis did not present any substantial between-group differences, with Caspase-3 values always equal to 0%.	
Comparison groups	5-ASA Group v No Treatment Group
Number of subjects included in analysis	18
Analysis specification	Pre-specified
Analysis type	other <sup>[5]</sup>
P-value	≤ 0.05
Method	Wilcoxon (Mann-Whitney)

Notes:

[5] - Since this was a pilot study and sample size was related to feasibility, a very small number of patients were enrolled into the study: therefore, all the efficacy results are interpreted only with a descriptive meaning.

### Secondary: Cells expressing Ki-67

End point title	Cells expressing Ki-67
End point description:	
Percentage of cells expressing Ki-67 evaluated by immunohistochemical analysis	
End point type	Secondary
End point timeframe:	
3 months after visit 1	

End point values	5-ASA Group	No Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	8	10		
Units: percentage				
arithmetic mean (standard deviation)	12.75 (± 3.76)	16.7 (± 4.1)		

## Statistical analyses

Statistical analysis title	Efficacy results
Statistical analysis description:	
The percentage of cells expressing Ki-67 by immunohistochemical analysis did not present any substantial between-group differences.	
Comparison groups	5-ASA Group v No Treatment Group
Number of subjects included in analysis	18
Analysis specification	Pre-specified
Analysis type	other <sup>[6]</sup>
P-value	≤ 0.05
Method	t-test, 2-sided

Notes:

[6] - Since this was a pilot study and sample size was related to feasibility, a very small number of patients were enrolled into the study: therefore, all the efficacy results are interpreted only with a descriptive meaning.

## Secondary: Cells expressing µ-protocadherin

End point title	Cells expressing µ-protocadherin
End point description:	
Percentage of cells expressing µ-protocadherin evaluated by immunohistochemical analysis	
End point type	Secondary
End point timeframe:	
3 month after visit 1	

End point values	5-ASA Group	No Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	8	10		
Units: score	100	100		

## Statistical analyses

Statistical analysis title	Efficacy results
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**Statistical analysis description:**

The percentage of cells expressing  $\mu$ -protocadherin by immunohistochemical analysis did not present any substantial between-group differences, with  $\mu$ -protocadherin score resulted to be equal to 2+ in both groups of patients at both Visits.

Comparison groups	5-ASA Group v No Treatment Group
Number of subjects included in analysis	18
Analysis specification	Pre-specified
Analysis type	other <sup>[7]</sup>
P-value	$\leq 0.05$
Method	Wilcoxon (Mann-Whitney)

**Notes:**

[7] - Since this was a pilot study and sample size was related to feasibility, a very small number of patients were enrolled into the study: therefore, all the efficacy results are interpreted only with a descriptive meaning.

## Adverse events

### Adverse events information<sup>[1]</sup>

Timeframe for reporting adverse events:  
within 24 hours for Serious adverse event

Assessment type	Systematic
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### Dictionary used

Dictionary name	MedDRA
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Dictionary version	16.1
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### Reporting groups

Reporting group title	5-ASA Group
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Reporting group description:

Patient treated with Mesalazine

Reporting group title	Not Treated Group
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Reporting group description:

Patients Not treated

Notes:

[1] - There are no non-serious adverse events recorded for these results. It is expected that there will be at least one non-serious adverse event reported.

Justification: Only one patient in the treatment Group had an adverse event that was serious. No other adverse events (serious or non-serious) were observed.

Serious adverse events	5-ASA Group	Not Treated Group	
Total subjects affected by serious adverse events			
subjects affected / exposed	1 / 11 (9.09%)	0 / 10 (0.00%)	
number of deaths (all causes)	0	0	
number of deaths resulting from adverse events	0	0	
Gastrointestinal disorders			
Perforation			
subjects affected / exposed	1 / 11 (9.09%)	0 / 10 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	

Frequency threshold for reporting non-serious adverse events: 0 %

Non-serious adverse events	5-ASA Group	Not Treated Group	
Total subjects affected by non-serious adverse events			
subjects affected / exposed	0 / 11 (0.00%)	0 / 10 (0.00%)	

## More information

### Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
30 October 2014	The presence "Diverticular disease/diverticular colitis" does not contribute to the definition of the study endpoints while is a critical point in the selection of patients, with impact on the duration of the trial. Study duration was amended to prolong the enrollment phase.

Notes:

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### Interruptions (globally)

Were there any global interruptions to the trial? No

### Limitations and caveats

None reported