Clinical trial results:
A Single-blind, Randomized Phase I/II study of Pharmacokinetic and Pharmacodynamic investigation of Modufolin® (60 or 200 mg/m2) compared to Levoleucovorin (60 or 200 mg/m2) in tumour, adjacent mucosa and plasma for patients with colon cancer.

Summary

EudraCT number	2012-000522-22
Trial protocol	SE
Global end of trial date	05 July 2013

Results information

Result version number	v1 (current)
This version publication date	01 July 2016
First version publication date	23 November 2014

Trial information

Trial identification

Sponsor protocol code	ISO-CC-002

Additional study identifiers

ISRCTN number	-
ClinicalTrials.gov id (NCT number)	NCT01681472
WHO universal trial number (UTN)	-

Notes:

Sponsors

Sponsor organisation name	Isofol Medical AB
Sponsor organisation address	Biotech Center, Arvid Wallgrens Backe 20, Gothenburg, Sweden, SE-413 46
Public contact	Chief Scientific Officer, Isofol Medical AB, +46 (0)70 876 15 70, anders.vedin@isofolmedical.com
Scientific contact	Chief Scientific Officer, Isofol Medical AB , +46 (0)70 876 15 70, anders.vedin@isofolmedical.com

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:

Results analysis stage

Analysis stage	Final
Date of interim/final analysis	08 July 2014
Is this the analysis of the primary completion data?	Yes
Primary completion date	01 July 2013
Global end of trial reached?	Yes
Global end of trial date	05 July 2013
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

To compare [6R] 5,10-methylene-THF, 5-formyl-THF, 5-methyl-THF and THF concentration in the tumor tissue and adjacent mucosa for the different treatment groups

Protection of trial subjects:

The patients were treated as in routine care. No trial specific measures were put in place to protect the patients.

Background therapy: -

Evidence for comparator: -

Actual start date of recruitment	01 August 2012
Long term follow-up planned	No
Independent data monitoring committee (IDMC) involvement?	No

Notes:

Population of trial subjects

Subjects enrolled per country

Country: Number of subjects enrolled	Sweden: 32
Worldwide total number of subjects	32
EEA total number of subjects	32

Notes:

Subjects enrolled per age group

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	18
85 years and over	8

Subject disposition

Recruitment

Recruitment details:

Recruitment period:
Start: 06 September 2012
Stop: 20 June 2013

Pre-assignment

Screening details:

Potential patients were identified at various outpatient departments that refers patients to the study site. 35 patients had to be screened in order to identify 32 eligible patients. The reasons for screening failure were; performance status not fullfilled, presence of second primary malignancy, and medication intake not approved by the protocol.

Pre-assignment period milestones

Number of subjects started	32
Number of subjects completed	32

Period 1

Period 1 title	Overall trial (overall period)
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Single blind[1]
Roles blinded	Subject, Investigator, Monitor, Data analyst, Carer, Assessor

Blinding implementation details:

All roles in the study was blinded except the study nurse designated to prepare the study drug for the bolus injection who could not be blinded due to practical reasons. Study drug accountability and compliance was checked on regular basis by a monitor solely appointed for this check.

Arms

Are arms mutually exclusive?	Yes
Arm title	Modufolin 60 mg/m2

Arm description: -

Arm type	Experimental
Investigational medicinal product name	Modufolin
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Powder for solution for injection
Routes of administration	Intravenous bolus use

Dosage and administration details:

Study nurses trained in the Modufolin® reconstitution procedures prepared the study drug. Modufolin® was administered as an IV bolus injection after the patient was anesthetized for surgery. The study nurse obtained dose information for each patient from the randomization envelope. The dose was specified in the unit [mg/m2] and was converted into number of milligrams using an automatic calculator for patient’s body surface.

Arm title	Modufolin 200 mg/m2

Arm description: -

Arm type	Experimental

Investigational medicinal product name	Modufolin
Investigational medicinal product code	
Other name	
Pharmaceutical forms	Powder for solution for injection
Routes of administration	Intravenous bolus use

Dosage and administration details:

Study nurses trained in the Modufolin® reconstitution procedures prepared the study drug. Modufolin® was administered as an IV bolus injection after the patient was anesthetized for surgery. The study nurse obtained dose information for each patient from the randomization envelope. The dose was specified in the unit [mg/m2] and was converted into number of milligrams using an automatic calculator for patient's body surface.

Arm title	L-LV 60 mg/m2

Arm description: -

Arm type	Active comparator
Investigational medicinal product name	Isovorin
Investigational medicinal product code	
Other name	Levoleucovorin
Pharmaceutical forms	Solution for injection
Routes of administration	Intravenous bolus use

Dosage and administration details:

L-LV was administered as an IV bolus injection after the patient was anesthetized for surgery. The study nurse obtained dose information for each patient from the randomization envelope. The dose was specified in the unit [mg/m2] and was converted into number of milligrams using an automatic calculator for patient's body surface.

Arm title	L-LV 200 mg/m2

Arm description: -

Arm type	Active comparator
Investigational medicinal product name	Isovorin
Investigational medicinal product code	
Other name	Levoleucovorin
Pharmaceutical forms	Solution for injection
Routes of administration	Intravenous bolus use

Dosage and administration details:

L-LV was administered as an IV bolus injection after the patient was anesthetized for surgery. The study nurse obtained dose information for each patient from the randomization envelope. The dose was specified in the unit [mg/m2] and was converted into number of milligrams using an automatic calculator for patient's body surface.

Notes:

[1] - The number of roles blinded appears inconsistent with a single blinded trial. It is expected that there will be one role blinded in a single blind trial.
Justification: All roles in the study was blinded except the study nurse designated to prepare the study drug for the bolus injection who could not be blinded due to practical reasons. Modufolin is a powder for solution for injection and Isovorin a solution for injection. Study drug accountability and compliance was checked on regular basis by a monitor solely appointed for this check.

Number of subjects in period 1	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2
Started	8	8	8
Completed	8	8	7
Not completed	0	0	1
Adverse event, non-fatal	-	-	1

Number of subjects in period 1	L-LV 200 mg/m2

Started	8
Completed	8
Not completed	0
Adverse event, non-fatal	-

Baseline characteristics

Reporting groups	
Reporting group title	Overall trial
Reporting group description:	
All randomized patients	

Reporting group values	Overall trial	Total	
Number of subjects	32	32	
Age categorical			
Safety population			
Units: Subjects			
In utero	0	0	
Preterm newborn infants (gestational age < 37 wks)	0	0	
Newborns (0-27 days)	0	0	
Infants and toddlers (28 days-23 months)	0	0	
Children (2-11 years)	0	0	
Adolescents (12-17 years)	0	0	
Adults (18-64 years)	6	6	
From 65-84 years	18	18	
85 years and over	8	8	
Gender categorical			
Units: Subjects			
Female	16	16	
Male	16	16	

Subject analysis sets	
Subject analysis set title	Safety population
Subject analysis set type	Safety analysis
Subject analysis set description:	
All randomized patients who have received trial medication and from whom at least one (1) measurement has been obtained. Intention-to-treat population was equal to Safety Population (SP) in this study	
Subject analysis set title	Per protocol population
Subject analysis set type	Per protocol
Subject analysis set description:	
All randomized patients who completed the trial without any major deviations from the protocol procedures.	

Reporting group values	Safety population	Per protocol population	
Number of subjects	31	29	
Age categorical			
Safety population			
Units: Subjects			
In utero	0	0	
Preterm newborn infants (gestational age < 37 wks)	0	0	

Newborns (0-27 days)	0	0	
Infants and toddlers (28 days-23 months)	0	0	
Children (2-11 years)	0	0	
Adolescents (12-17 years)	0	0	
Adults (18-64 years)	6	6	
From 65-84 years	17	16	
85 years and over	8	7	
Gender categorical Units: Subjects			
Female	15	13	
Male	16	16	

End points

End points reporting groups

Reporting group title	Modufolin 60 mg/m2
Reporting group description: -	
Reporting group title	Modufolin 200 mg/m2
Reporting group description: -	
Reporting group title	L-LV 60 mg/m2
Reporting group description: -	
Reporting group title	L-LV 200 mg/m2
Reporting group description: -	
Subject analysis set title	Safety population
Subject analysis set type	Safety analysis
Subject analysis set description:	
All randomized patients who have received trial medication and from whom at least one (1) measurement has been obtained. Intention-to-treat population was equal to Safety Population (SP) in this study	
Subject analysis set title	Per protocol population
Subject analysis set type	Per protocol
Subject analysis set description:	
All randomized patients who completed the trial without any major deviations from the protocol procedures.	

Primary: Tissue concentration of [6R]-5,10-methylene-THF in adjacent mucosa

End point title	Tissue concentration of [6R]-5,10-methylene-THF in adjacent mucosa
End point description:	
End point type	Primary
End point timeframe:	
Biopsies for analysis were collected at one time-point, i.e. during surgery.	

End point values	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2	L-LV 200 mg/m2
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	7	8	6	8
Units: pmol/g				
arithmetic mean (standard deviation)	3241 (± 2137)	5606 (± 4441)	816 (± 218)	1535 (± 527)

End point values	Per protocol population			
Subject group type	Subject analysis set			
Number of subjects analysed	29			
Units: pmol/g				
arithmetic mean (standard deviation)	2921 (± 3094)			

Statistical analyses

Statistical analysis title	Comparison of Methylene-THF conc. mucosa-60mg/m2

Statistical analysis description:

An exploratory evaluation of the mucosa concentrations of [6R]-5,10-methylene-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 60 mg/m2 v L-LV 60 mg/m2
Number of subjects included in analysis	13
Analysis specification	Pre-specified
Analysis type	other[1]
P-value	= 0.0034
Method	Wilcoxon (Mann-Whitney)

Notes:

[1] - Exploratory

Statistical analysis title	Comparison of Methylene-THF conc. mucosa-200mg/m2

Statistical analysis description:

An exploratory evaluation of the mucosa concentrations of [6R]-5,10-methylene-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 200 mg/m2 v L-LV 200 mg/m2
Number of subjects included in analysis	16
Analysis specification	Pre-specified
Analysis type	other[2]
P-value	= 0.0019
Method	Wilcoxon (Mann-Whitney)

Notes:

[2] - Exploratory

Primary: Tissue concentration of [6S]-5-THF in tumor

End point title	Tissue concentration of [6S]-5-THF in tumor

End point description:

End point type	Primary

End point timeframe:

Biopsies for analysis were collected at one time-point, i.e. during surgery.

End point values	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2	L-LV 200 mg/m2
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	7	8	6	8
Units: pmol/g				
arithmetic mean (standard deviation)	2219 (± 1995)	4175 (± 3127)	933 (± 598)	1329 (± 720)

End point values	Per protocol population			
Subject group type	Subject analysis set			
Number of subjects analysed	29			
Units: pmol/g				
arithmetic mean (standard deviation)	2247 (± 2273)			

Statistical analyses

Statistical analysis title	Comparison of THF concentration in tumor-60mg/m2

Statistical analysis description:

An exploratory evaluation of tumor concentrations of [6S]-5-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 60 mg/m2 v L-LV 60 mg/m2
Number of subjects included in analysis	13
Analysis specification	Pre-specified
Analysis type	other[3]
P-value	= 0.0538
Method	Wilcoxon (Mann-Whitney)

Notes:

[3] - Exploratory

Statistical analysis title	Comparison of THF concentration in tumor-200mg/m2

Statistical analysis description:

An exploratory evaluation of tumor concentrations of [6S]-5-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 200 mg/m2 v L-LV 200 mg/m2
Number of subjects included in analysis	16
Analysis specification	Pre-specified
Analysis type	other[4]
P-value	= 0.0313
Method	Wilcoxon (Mann-Whitney)

Notes:

[4] - Exploratory

Primary: Tissue concentration of [6S]-5-THF in adjacent mucosa

End point title	Tissue concentration of [6S]-5-THF in adjacent mucosa

End point description:

End point type	Primary

End point timeframe:

Biopsies for analysis were collected at one time-point, i.e. during surgery.

End point values	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2	L-LV 200 mg/m2
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	7	8	6	8
Units: pmol/g				
arithmetic mean (standard deviation)	3481 (± 3926)	5099 (± 3927)	626 (± 439)	1333 (± 852)

End point values	Per protocol population			
Subject group type	Subject analysis set			
Number of subjects analysed	29			
Units: pmol/g				
arithmetic mean (standard deviation)	2744 (± 3260)			

Statistical analyses

Statistical analysis title	Comparison of THF conc in adjacent mucosa-60mg/m2

Statistical analysis description:

An exploratory evaluation of mucosa concentrations of [6S]-5-THF was done respectively for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 60 mg/m2 v L-LV 60 mg/m2
Number of subjects included in analysis	13
Analysis specification	Pre-specified
Analysis type	other[5]
P-value	= 0.0124
Method	Wilcoxon (Mann-Whitney)

Notes:

[5] - Exploratory

Statistical analysis title	Comparison of THF conc in adjacent mucosa-200mg/m2

Statistical analysis description:

An exploratory evaluation of mucosa concentrations of [6S]-5-THF was done respectively for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 200 mg/m2 v L-LV 200 mg/m2

Number of subjects included in analysis	16
Analysis specification	Pre-specified
Analysis type	other[6]
P-value	= 0.0039
Method	Wilcoxon (Mann-Whitney)

Notes:

[6] - Exploratory

Primary: Tissue concentration of [6S]-5-methyl-THF in tumor	
End point title	Tissue concentration of [6S]-5-methyl-THF in tumor

End point description:

End point type	Primary

End point timeframe:

Biopsies for analysis were collected at one time-point, i.e. during surgery.

End point values	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2	L-LV 200 mg/m2
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	7	8	6	8
Units: pmol/g				
arithmetic mean (standard deviation)	1882 (± 1204)	4396 (± 1858)	1904 (± 918)	3574 (± 2415)

End point values	Per protocol population			
Subject group type	Subject analysis set			
Number of subjects analysed	29			
Units: pmol/g				
arithmetic mean (standard deviation)	3047 (± 2000)			

Statistical analyses

Statistical analysis title	Comparison of Methyl-THF conc in tumor-60mg/m2

Statistical analysis description:

An exploratory evaluation of the tumor concentrations of [6S]-5-methyl-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene’s test.

Comparison groups	Modufolin 60 mg/m2 v L-LV 60 mg/m2

Number of subjects included in analysis	13
Analysis specification	Pre-specified
Analysis type	other[7]
P-value	= 0.8303
Method	Wilcoxon (Mann-Whitney)

Notes:

[7] - Exploratory

Statistical analysis title	Comparison of Methyl-THF conc in tumor-200mg/m2

Statistical analysis description:

An exploratory evaluation of the tumor concentrations of [6S]-5-methyl-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 200 mg/m2 v L-LV 200 mg/m2
Number of subjects included in analysis	16
Analysis specification	Pre-specified
Analysis type	other[8]
P-value	= 0.3184
Method	Wilcoxon (Mann-Whitney)

Notes:

[8] - Exploratory

Primary: Tissue concentration of [6S]-5-methyl-THF in adjacent mucosa

End point title	Tissue concentration of [6S]-5-methyl-THF in adjacent mucosa

End point description:

End point type	Primary

End point timeframe:

Biopsies for analysis were collected at one time-point, i.e. during surgery.

End point values	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2	L-LV 200 mg/m2
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	7	8	6	8
Units: pmol/g				
arithmetic mean (standard deviation)	2066 (± 1517)	2494 (± 765)	1216 (± 455)	3667 (± 2043)

End point values	Per protocol population			
Subject group type	Subject analysis set			
Number of subjects analysed	29			
Units: pmol/g				
arithmetic mean (standard deviation)	2450 (± 1583)			

Statistical analyses

Statistical analysis title	Comparison of Methyl-THF conc. in mucosa-60mg/m2

Statistical analysis description:

An exploratory evaluation of the mucosa concentrations of [6S]-5-methyl-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 60 mg/m2 v L-LV 60 mg/m2
Number of subjects included in analysis	13
Analysis specification	Pre-specified
Analysis type	other[9]
P-value	= 0.2246
Method	Wilcoxon (Mann-Whitney)

Notes:

[9] - Exploratory

Statistical analysis title	Comparison of Methyl-THF conc. in mucosa-200mg/m2

Statistical analysis description:

An exploratory evaluation of the mucosa concentrations of [6S]-5-methyl-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 200 mg/m2 v L-LV 200 mg/m2
Number of subjects included in analysis	16
Analysis specification	Pre-specified
Analysis type	other[10]
P-value	= 0.4309
Method	Wilcoxon (Mann-Whitney)

Notes:

[10] - Exploratory

Primary: Tissue concentration of [6S]-5-formyl-THF in tumor

End point title	Tissue concentration of [6S]-5-formyl-THF in tumor

End point description:

End point type	Primary

End point timeframe:

Biopsies for analysis were collected at one time-point, i.e. during surgery.

End point values	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2	L-LV 200 mg/m2
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	7	8	6	8
Units: pmol/g				
arithmetic mean (standard deviation)	57 (± 48)	100 (± 47)	512 (± 259)	3611 (± 3899)

End point values	Per protocol population			
Subject group type	Subject analysis set			
Number of subjects analysed	29			
Units: pmol/g				
arithmetic mean (standard deviation)	1144 (± 2499)			

Statistical analyses

Statistical analysis title	Comparison of Formyl-THF in tumor-60mg/m2

Statistical analysis description:

An exploratory evaluation of the tumor concentrations of [6S]-5-formyl-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 60 mg/m2 v L-LV 60 mg/m2
Number of subjects included in analysis	13
Analysis specification	Pre-specified
Analysis type	other[11]
P-value	= 0.0034
Method	Wilcoxon (Mann-Whitney)

Notes:

[11] - Exploratory

Statistical analysis title	Comparison of Formyl-THF in tumor-200mg/m2

Statistical analysis description:

An exploratory evaluation of the tumor concentrations of [6S]-5-formyl-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 200 mg/m2 v L-LV 200 mg/m2
Number of subjects included in analysis	16
Analysis specification	Pre-specified
Analysis type	other[12]
P-value	= 0.0009
Method	Wilcoxon (Mann-Whitney)

Notes:

[12] - Exploratory

Primary: Tissue concentration of [6S]-5-formyl-THF in adjacent mucosa

End point title	Tissue concentration of [6S]-5-formyl-THF in adjacent mucosa

End point description:

End point type	Primary

End point timeframe:

Biopsies for analysis were collected at one time-point, i.e. during surgery.

End point values	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2	L-LV 200 mg/m2
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	7	8	6	8
Units: pmol/g				
arithmetic mean (standard deviation)	42 (± 18)	82 (± 77)	1403 (± 640)	5456 (± 3963)

End point values	Per protocol population			
Subject group type	Subject analysis set			
Number of subjects analysed	29			
Units: pmol/g				
arithmetic mean (standard deviation)	1843 (± 3130)			

Statistical analyses

Statistical analysis title	Comparison of Formyl-THF conc. in mucosa-60mg/m2

Statistical analysis description:

An exploratory evaluation of mucosa concentrations of [6S]-5-formyl-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 60 mg/m2 v L-LV 60 mg/m2
Number of subjects included in analysis	13
Analysis specification	Pre-specified
Analysis type	other[13]
P-value	= 0.0058
Method	Wilcoxon (Mann-Whitney)

Notes:

[13] - Exploratory

Statistical analysis title	Comparison of Formyl-THF conc. in mucosa-200mg/m2

Statistical analysis description:

An exploratory evaluation of mucosa concentrations of [6S]-5-formyl-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 200 mg/m2 v L-LV 200 mg/m2

Number of subjects included in analysis	16
Analysis specification	Pre-specified
Analysis type	other[14]
P-value	= 0.0014
Method	Wilcoxon (Mann-Whitney)

Notes:

[14] - Exploratory

Primary: Tissue concentration of [6R]-5,10-methylene-THF in the tumor

End point title	Tissue concentration of [6R]-5,10-methylene-THF in the tumor

End point description:

End point type	Primary

End point timeframe:

Biopsies for analysis were collected at one time-point, i.e. during surgery.

End point values	Modufolin 60 mg/m2	Modufolin 200 mg/m2	L-LV 60 mg/m2	L-LV 200 mg/m2
Subject group type	Reporting group	Reporting group	Reporting group	Reporting group
Number of subjects analysed	7	8	6	8
Units: pmol/g				
arithmetic mean (standard error)	2393 (± 1920)	4725 (± 2210)	959 (± 417)	1871 (± 1159)

End point values	Per protocol population			
Subject group type	Subject analysis set			
Number of subjects analysed	29			
Units: pmol/g				
arithmetic mean (standard error)	2596 (± 2100)			

Statistical analyses

Statistical analysis title	Comparison of Methylene-THF conc in tumor-60 mg/m2

Statistical analysis description:

An exploratory evaluation of the tumor concentrations of [6R]-5,10-methylene-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene’s test.

Comparison groups	Modufolin 60 mg/m2 v L-LV 60 mg/m2

Number of subjects included in analysis	13
Analysis specification	Pre-specified
Analysis type	other[15]
P-value	= 0.1336
Method	Wilcoxon (Mann-Whitney)

Notes:

[15] - Exploratory

Statistical analysis title	Comparison of Methylene-THF conc in tumor-200mg/m2

Statistical analysis description:

An exploratory evaluation of the tumor concentrations of [6R]-5,10-methylene-THF was done for differences between treatments using Kruskal-Wallis and Wilcoxon tests as it was found that the assumption of normality was violated. Differences in variability between treatment groups were assessed using Proc GLM and Levene's test.

Comparison groups	Modufolin 200 mg/m2 v L-LV 200 mg/m2
Number of subjects included in analysis	16
Analysis specification	Pre-specified
Analysis type	other[16]
P-value	= 0.0074
Method	Wilcoxon (Mann-Whitney)

Notes:

[16] - Exploratory

Adverse events

Adverse events information

Timeframe for reporting adverse events:

The recording of AEs started in connection with the start of the treatment and continued until the patient had completed the end of study visit.

Adverse event reporting additional description:

SAEs occurring after the end of study visit at day 5 were to be reported if a relationship to study drug was suspected.

Assessment type	Systematic

Dictionary used

Dictionary name	MedDRA
Dictionary version	15

Reporting groups

Reporting group title	Safety population

Reporting group description: -

Serious adverse events	Safety population		
Total subjects affected by serious adverse events			
subjects affected / exposed	5 / 31 (16.13%)		
number of deaths (all causes)	1		
number of deaths resulting from adverse events	0		
Injury, poisoning and procedural complications			
Anastomotic leak			
subjects affected / exposed	1 / 31 (3.23%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Postoperative wound complication			
subjects affected / exposed	1 / 31 (3.23%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Cardiac disorders			
Acute myocardial infarction			
subjects affected / exposed	1 / 31 (3.23%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		
Cardiac arrest			

subjects affected / exposed	1 / 31 (3.23%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 1		
Respiratory, thoracic and mediastinal disorders			
Pulmonary embolism			
subjects affected / exposed	1 / 31 (3.23%)		
occurrences causally related to treatment / all	0 / 1		
deaths causally related to treatment / all	0 / 0		

Frequency threshold for reporting non-serious adverse events: 0.03 %

Non-serious adverse events	Safety population		
Total subjects affected by non-serious adverse events			
subjects affected / exposed	12 / 31 (38.71%)		
Investigations			
Hepatic enzyme increased			
subjects affected / exposed	1 / 31 (3.23%)		
occurrences (all)	1		
Vascular disorders			
Flushing			
subjects affected / exposed	1 / 31 (3.23%)		
occurrences (all)	1		
Hypotension			
subjects affected / exposed	1 / 31 (3.23%)		
occurrences (all)	1		
Cardiac disorders			
Atrial fibrillation			
subjects affected / exposed	4 / 31 (12.90%)		
occurrences (all)	4		
General disorders and administration site conditions			
Pyrexia			
subjects affected / exposed	1 / 31 (3.23%)		
occurrences (all)	1		
Gastrointestinal disorders			

Abdominal pain subjects affected / exposed occurrences (all) Nausea subjects affected / exposed occurrences (all) Vomiting subjects affected / exposed occurrences (all) Mouth haemorrhage subjects affected / exposed occurrences (all) Dyspepsia subjects affected / exposed occurrences (all)	1 / 31 (3.23%) 1 1 / 31 (3.23%) 1 2 / 31 (6.45%) 2 1 / 31 (3.23%) 1 2 / 31 (6.45%) 2		
Respiratory, thoracic and mediastinal disorders Dyspnoea subjects affected / exposed occurrences (all) Cough subjects affected / exposed occurrences (all)	 1 / 31 (3.23%) 1 1 / 31 (3.23%) 1		
Infections and infestations Pneumonia subjects affected / exposed occurrences (all)	 2 / 31 (6.45%) 2		

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
05 July 2012	Initially two strata (one morning stratum and one afternoon stratum) were defined in the protocol. Prior to enrollment of first patient in the trial the protocol was amended and the afternoon stratum was deleted due to logistic reasons in order to optimize the recruitment rate and consequently the overall trial duration.
09 October 2012	The initial protocol defined and interim analysis after 16 patients had completed the protocol. An additional interim analysis after 32 patients was added.
28 June 2013	The list of genes to be analyzed was updated. Recent litterature data (post study start) indicated that the initial list of genes had to be updated in order to include all specific genes with the highest probability of impact on the metabolism of LV and methylene-THF.

Notes:

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

None reported