



## Clinical trial results:

**A Single-blind, Randomized Phase I/II study of Pharmacokinetic and Pharmacodynamic investigation of Modufolin® (60 or 200 mg/m<sup>2</sup>) compared to Levoleucovorin (60 or 200 mg/m<sup>2</sup>) 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 fulfilled, 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 <sup>[1]</sup>                                   |
| 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                               |
| <b>Arm title</b>                       | 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.

|                    |                     |
|--------------------|---------------------|
| <b>Arm title</b>   | 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/m<sup>2</sup>] and was converted into number of milligrams using an automatic calculator for patient's body surface.

|                  |                           |
|------------------|---------------------------|
| <b>Arm title</b> | L-LV 60 mg/m <sup>2</sup> |
|------------------|---------------------------|

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/m<sup>2</sup>] and was converted into number of milligrams using an automatic calculator for patient's body surface.

|                  |                            |
|------------------|----------------------------|
| <b>Arm title</b> | L-LV 200 mg/m <sup>2</sup> |
|------------------|----------------------------|

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/m<sup>2</sup>] 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/m <sup>2</sup> | Modufolin 200 mg/m <sup>2</sup> | L-LV 60 mg/m <sup>2</sup> |
|--------------------------------|--------------------------------|---------------------------------|---------------------------|
| Started                        | 8                              | 8                               | 8                         |
| Completed                      | 8                              | 8                               | 7                         |
| Not completed                  | 0                              | 0                               | 1                         |
| Adverse event, non-fatal       | -                              | -                               | 1                         |

|                                       |                            |
|---------------------------------------|----------------------------|
| <b>Number of subjects in period 1</b> | L-LV 200 mg/m <sup>2</sup> |
|---------------------------------------|----------------------------|

|                          |   |
|--------------------------|---|
| 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

|   |  |
|---|--|
| <b>Statistical analysis title</b>   | Comparison of Methylene-THF conc. mucosa-60mg/m2 |
| Statistical analysis description:<br>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 <sup>[1]</sup>                             |
| P-value   | = 0.0034   |
| Method  | Wilcoxon (Mann-Whitney)                          |

Notes:

[1] - Exploratory

|   |   |
|---|---|
| <b>Statistical analysis title</b>   | Comparison of Methylene-THF conc. mucosa-200mg/m2 |
| Statistical analysis description:<br>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 <sup>[2]</sup>                              |
| 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 <sup>[3]</sup>               |
| 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 <sup>[4]</sup>                 |
| 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

|                                   |   |
|-----------------------------------|---|
| <b>Statistical analysis title</b> | 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 <sup>[5]</sup>               |
| P-value                                 | = 0.0124                           |
| Method                                  | Wilcoxon (Mann-Whitney)            |

Notes:

[5] - Exploratory

|                                   |  |
|-----------------------------------|--|
| <b>Statistical analysis title</b> | 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 <sup>[6]</sup>    |
| 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 <sup>[7]</sup>    |
| P-value                                 | = 0.8303                |
| Method                                  | Wilcoxon (Mann-Whitney) |

Notes:

[7] - Exploratory

|                                   |   |
|-----------------------------------|---|
| <b>Statistical analysis title</b> | 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 <sup>[8]</sup>                 |
| 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

|   |  |
|---|--|
| <b>Statistical analysis title</b>   | Comparison of Methyl-THF conc. in mucosa-60mg/m2 |
| Statistical analysis description:<br>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 <sup>[9]</sup>                             |
| P-value   | = 0.2246   |
| Method  | Wilcoxon (Mann-Whitney)                          |
| Notes:<br>[9] - Exploratory   |  |

|   |   |
|---|---|
| <b>Statistical analysis title</b>   | Comparison of Methyl-THF conc. in mucosa-200mg/m2 |
| Statistical analysis description:<br>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 <sup>[10]</sup>                             |
| P-value   | = 0.4309  |
| Method  | Wilcoxon (Mann-Whitney)                           |
| Notes:<br>[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:<br>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 <sup>[11]</sup>              |
| 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 <sup>[12]</sup>                |
| 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

|                                   |  |
|-----------------------------------|--|
| <b>Statistical analysis title</b> | 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 <sup>[13]</sup>              |
| P-value                                 | = 0.0058                           |
| Method                                  | Wilcoxon (Mann-Whitney)            |

Notes:

[13] - Exploratory

|                                   |   |
|-----------------------------------|---|
| <b>Statistical analysis title</b> | 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 <sup>[14]</sup>   |
| 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 <sup>[15]</sup>   |
| P-value                                 | = 0.1336                |
| Method                                  | Wilcoxon (Mann-Whitney) |

Notes:

[15] - Exploratory

|                                   |  |
|-----------------------------------|--|
| <b>Statistical analysis title</b> | 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 <sup>[16]</sup>                |
| 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 %

|   |                   |  |  |
|---|-------------------|--|--|
| <b>Non-serious adverse events</b>                     | 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<br>subjects affected / exposed<br>occurrences (all)  | 1 / 31 (3.23%)<br>1 |  |  |
| Nausea<br>subjects affected / exposed<br>occurrences (all)  | 1 / 31 (3.23%)<br>1 |  |  |
| Vomiting<br>subjects affected / exposed<br>occurrences (all)  | 2 / 31 (6.45%)<br>2 |  |  |
| Mouth haemorrhage<br>subjects affected / exposed<br>occurrences (all)   | 1 / 31 (3.23%)<br>1 |  |  |
| Dyspepsia<br>subjects affected / exposed<br>occurrences (all)   | 2 / 31 (6.45%)<br>2 |  |  |
| Respiratory, thoracic and mediastinal disorders<br>Dyspnoea<br>subjects affected / exposed<br>occurrences (all) | 1 / 31 (3.23%)<br>1 |  |  |
| Cough<br>subjects affected / exposed<br>occurrences (all)   | 1 / 31 (3.23%)<br>1 |  |  |
| Infections and infestations<br>Pneumonia<br>subjects affected / exposed<br>occurrences (all)                    | 2 / 31 (6.45%)<br>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 literature 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