| | |
|---|---|
| **Protocol Title** | A clinico-pathological phase II study with translational elements to investigate the possible infective causes of non-Hodgkin lymphoma of the ocular adnexae with particular reference to Chlamydia species and the effects of MALT lymphoma treatment with tetracycline. |
| **Protocol Code** | IELSG27 |
| **Investigational Product** | Doxycycline |
| **Sponsor** | International Extranodal Lymphoma Study Group (IELSG)<br>Foundation for the Institute of Oncology Research (IOR)<br>Ospedale San Giovanni<br>Via Ospedale<br>6500 Bellinzona (Switzerland) |
| **Study Chairs** | Andrés J.M. Ferreri<br>IRCCS San Raffaele Scientific Institute, Milan, Italy<br>John Radford<br>Christie Hospital NHS Trust, Manchester, UK |
| **EudraCT Number** | 2006-005795-41 |
| **ClinicalTrial.Gov Number** | NCT01010295 |
| **Swissmedic Reference Number** | 2007 DR 2002 |
| **Development Phase** | II |
| **First Patient Enrolled on** | 09.11.2006 |
| **Last Patient Completed on** | 02.08.2012 |
| **Report Version and Date** | Version 1.0 – 18.09.2018 |

**CONFIDENTIAL**

This study was conducted in accordance with Good Clinical Practice (GCP), ICH Topic E6.
The information contained in this document is confidential and the property of the Sponsor. The information may not - in full or in part - be transmitted, reproduced, published, or disclosed to others than the applicable Competent Ethics Committee(s) and Regulatory Authority(ies) without prior written authorization from the Sponsor, except to the extent necessary to obtain informed consent from those who will participate in the study

# Synopsis

| | |
|---|---|
| SPONSOR | International Extranodal Lymphoma Study Group (IELSG) |
| NAME PRODUCT / INTERVENTION | Doxycycline |
| NAME OF ACTIVE PRINCIPLE | Doxycycline |
| PROTOCOL CODE | IELSG27 |
| PROTOCOL TITLE | A clinico-pathological phase II study with translational elements to investigate the possible infective causes of non-Hodgkin lymphoma of the ocular adnexae with particular reference to Chlamydia species and the effects of MALT lymphoma treatment with tetracycline |
| PRINCIPAL INVESTIGATORS | Dr. Andrés J.M. Ferreri — IRCCS San Raffaele Scientific Institute Milan (IT)<br>Dr. Luca Arcaini — IRCCS Policlinico S. Matteo Pavia (IT)<br>Dr. Caterina Stelitano — Azienda Ospedaliera Bianchi - Melacrino - Morelli Reggio Calabria (IT)<br>Dr. Francesco Zaja — Azienda Ospedaliera Universitaria Udine (IT)<br>Dr. Maria Elena Cabrera — Hospital del Salvador Santiago (CL)<br>Dr. Carlos Montalban — Hospital Ramon y Cajal Madrid (ES)<br>Prof. Emanuele Zucca — Oncology Institute of Southern Switzerland Bellinzona (CH) |
| STUDY SITES | Four Countries involved - Chile, Italy, Spain and Switzerland - and a total of 7 enrolling sites. |
| STUDY PERIOD | First Patient Enrolled 09.11.2006<br>Last Patient Enrolled 02.08.2010 |
| DEVELOPMENT PHASE | II |
| OBJECTIVES | <u>Primary Objective</u><br>To explore the role of antibiotic therapy in the treatment of Marginal zone B-cell lymphoma of the ocular adnexae (OAMZL) and correlate the treatment response to molecular genetic findings.<br><u>Secondary Objective</u><br>To comprehensively screen infectious agents associated with chronic eye infection. |
| STUDY DESIGN AND METHODOLGY | OAMZL is associated with Chlamydophila psittaci (Cp) infection. The prevalence of this infection has been assessed in retrospective, heterogeneous series, and bacteria eradication with doxycycline has been associated with lymphoma regression in half of patients, while prolonged contact with infected household animals may determine continuous reinfection and hinder therapeutic efficacy.<br>The IELSG27 is an international study on patients with lymphoproliferative disorders of the ocular adnexae aimed to |

| | |
|---|---|
| | investigate possibly related microorganisms, doxycycline activity and reinfection risk.<br>Patients with newly diagnosed, limited-stage lymphoproliferative disorders of the ocular adnexae were considered. The trial included two parts:<br>Part A – patients with OAMZL and measurable disease, eligible for treatment with tetracycline;<br>Part B – patients with any other lymphoproliferative disorders of the ocular adnexae or OAMZL ineligible for treatment with tetracycline.<br>At the study entry, lymphomatous tissue, conjunctival swab and peripheral blood mononuclear cells (PBMC) for all patients were assessed for several bacterial and viral infections.<br>Patients registered in Part A received one cycle of doxycycline 100 mg bid daily for 21 days, followed by clinical examination, conjunctival swab, PBMC evaluation, and MRI of the orbits at 3 and 12 months. Patient in CR or PR continued observation for a further 12 months.<br>Treatment for patients entered into Part B was not standardized, and was defined according to best current practice. |
| SUBJECT POPULATION | Number of Subjects Planned: 50 |
| | Number of Subjects Enrolled: 54 (34 Part A, 20 Part B)<br>Number of OAMZL Subjects: 47 (34 Part A, 13 Part B)<br><br>Number of Subjects for Each Analysis Population:<br>Response assessment (primary analysis): 34 (part A)<br>Cp eradication: 29 (Part A)<br>Prevalence of Chlamydial infection: 44 (31 part A, 13 Part B)<br>Safety: 34 (Part A)<br><br>Brief description of demographic and baseline characteristics<br>All patients enrolled in Part A, a total of 34 patients, had stage-I OAMZL with at least a measurable lesion.<br>The 20 patients enrolled in Part B had a stage-I OAMZL but they had no measurable lesion or they had other types of lymphoma or pseudotumor.<br>Among all patients with stage–I OAMZL, a preponderance of female (68%) and an excellent ECOG performance status score were observed (PS=0, 91 %); in addition, the single lesion was located in conjunctiva, orbit, lachrymal gland or both orbit and conjunctiva.<br><br>Brief description of subjects excluded from primary analysis population<br>No patients were excluded from primary analysis population: all 34 patients enrolled in Part A were treated and evaluated for response assessment.<br>Five out of 34 patients were excluded from analysis of Cp eradication because Cp DNA at diagnosis was absent or not available. |
| ELIGIBILITY CRITERIA | **Inclusion Criteria**<br>Part A: clinico-pathological evaluation<br>1. Age 18 years or over<br>2. Histologically confirmed marginal zone B-cell lymphoma of MALT-type<br>3. Lymphoma localised to the ocular adnexae (conjunctiva, lacrymal gland, orbit soft tissue, clinical stage IEA) following a CT scan of neck, thorax, abdomen and pelvis, bone marrow aspirate/trephine, full blood count and biochemical profile<br>4. No previous treatment, excepting RT for localised lymphoma of the contralateral eye |

| | |
|---|---|
| | 5. At least one measurable lesion<br>6. No systemic antibiotic therapy in the last three months<br>7. No other malignancy in the previous 5 years apart from appropriately treated basal cell carcinoma of the skin or carcinoma in situ of the cervix<br>8. In women with reproductive potential a willingness to use contraception from entry into the study for a period of 3 months<br>9. Written informed consent<br><br>Part B: pathological evaluation only<br>1. Age 18 years or over<br>2. Histologically confirmed non-Hodgkin's lymphoma or inflammatory condition ("orbital pseudo-tumour") of the ocular adnexae<br>3. Localised disease (clinical stage IEA) for patients with lymphoma following a CT scan of neck, thorax, abdomen and pelvis, bone marrow aspirate/trephine, full blood count and biochemical profile including creatinine, LDH, B2-microglobulin (optional) protein electrophoresis, ASAT/ALAT, bilirubin, alkalin phosphatase (not applicable in non-malignant inflammatory conditions)<br>4. No previous treatment, excepting RT for localised lymphoma of the contralateral eye<br>5. Written informed consent<br><br>**Exclusion criteria**<br>Part A: clinico-pathological evaluation<br>1. Pregnant or lactating women<br>2. Known allergy to tetracycline<br>3. Patients unwilling to comply with the requirements of follow-up as defined by this protocol<br>4. Myasthenia gravis (tetracycline can exacerbate muscle weakness)<br>5. Systemic lupus erythematous (tetracycline can exacerbate the condition)<br>6. Patients with large or rapidly enlarging tumours requiring immediate radiotherapy<br><br>Part B: pathological evaluation only<br>None |
| STUDY PRODUCTS / DOSE AND MODE OF ADMINISTRATION/ INTERVENTIONS | Patients enrolled in Part A of the study received one cycle of doxycycline 100 mg twice daily for 3 weeks.<br>Doxycycline is a broad-spectrum antibiotic, active against a wide range of Gram-positive and Gram-negative bacteria and certain other micro-organisms. It belongs to the therapeutic class of tetracyclines.<br>The route of administration is oral and the compound is provided in the form of tablets. |
| DURATION OF TREATMENT | Three weeks |
| STUDY ENDPOINTS | The primary endpoint of this trial was response rates and duration of response to tetracycline in patients with OAMZL.<br>The secondary endpoints were the identification of the infectious agents associated with OAMZL, the geographical variation in distribution of potential infective agents and molecular factors associated with response/non-response to tetracycline in OAMZL. |
| STATISTICAL METHODS | The design of the study was Gehan's two stage.<br>The first stage, stage I, had to enroll a sufficient number of patients in Part A so that 29 patients were Cp positive; if no responses were observed, the trial was to terminate.<br>If at least one lymphoma response was observed among the first 29 Cp positive patients of stage I, the second stage of accrual had to be activated and additional patients should be enrolled in order to |

| | |
|---|---|
| | estimate the true response rate to doxycycline with acceptable precision (≥ 10%+ 2 standard errors).<br>The maximum size of additional Cp positive patients to be added in the stage 2 was calculated to be 100.<br>On the basis of patients OAMZL Cp positive enrolled (29 out of 34 patients enrolled in Part A) and the response rate to doxycycline (overall response rate of 66%), the recruitment was closed after the enrollment of 54 patients.<br><br>As per UK requirement, in the protocol were introduced annual interim analyses of the accumulating data, that should have been reviewed by an Independent Data Monitoring Committee (IDMC). Moreover, on the basis of the collected data, an additional responsibility of IDMC was to decide if the study should proceed and, in this case, specify how many Chlamydia positive patients were needed.<br>Afterwards, UK sites declined to participate to the study and, as consequence, an IDMC was never established.<br>These responsibilities were taken over by the Study Chair in agreement with the Sponsor. |
| SUMMARY OF RESULTS | <u>Efficacy Results</u><br>On a total of 34 patients enrolled in Part A, 6 patients experienced a Complete response (CR) and 16 patients a Partial Response (PR) after doxycycline treatment; the Overall Response Rate (ORR) was 65%.<br>Cp DNA was detected in 89% of biopsies samples; 29 patients had Cp DNA in baseline swabs and/or blood samples and were evaluable for chlamydial eradication, which was achieved in 48% of patients at one year of follow-up.<br>Cp eradication was associated with improved ORR and a better 5-year Progression Free Survival (PFS).<br><br><u>Safety Results</u><br>The treatment was well tolerated and the incidence of non-serious adverse events was consistent with the expected AE profile of doxycycline. In particular, only few and mild gastro-intestinal symptoms were registered; no SAEs nor SUSAR occurred at whole.<br>Two deaths were registered; they both were unrelated to study treatment. |
| CONCLUSIONS | Cp infection is frequent in newly diagnosed OAMZL, and could be also present in other lymphoproliferative disorders of the ocular adnexae. First-line doxycicline is an active, safe and rational strategy in patients with limited-stage OAMZL, with lymphoma regression in 65% of cases. However, doxycycline failed to eradicate Cp infection in half of patients, with a negative impact on outcome. Further investigations aimed to identify better schedule of antibiotic administration and potential mechanism of resistance are warranted. |
| VERSION AND DATE OF THE REPORT | Version 1.0 – 18.09.2018 |

# Table of Contents

# Abbreviations

| | |
|---|---|
| AE | Adverse Events |
| ALT | ALanine Transaminase |
| AST | ASpartate Transaminase |
| bid | Bis in die/twice a day |
| CI | Chlamydial infection |
| Cp | Chlamydophila psittaci |
| CR | Complete Response |
| CTC | Common Terminology Criteria |
| DNA | DeoxyriboNucleic Acid |
| EC | Ethics Committee |
| EDTA | EthyleneDiamineTetraacetic Acid |
| FPFV | First Patient First Visit |
| GCP | Good Clinical Practice |
| HBV | Hepatitis B Virus |
| HCV | Hepatitis C Virus |
| H pylori | Helicobacter pylori |
| ICH | International Conference on Harmonization |
| IELSG | International Extranodal Lymphoma Study Group |
| i.e. | Id est |
| IIT | Investigator Initiated Trial |
| IMP | Investigational Medicinal Product |
| ISF | Investigator Site File |
| LDH | Lactate DeHydrogenase |
| LR | Lymphoma Response |
| m | month |
| MALT | Mucose-Associated Lymphoid Tissue |
| mg | milligrams |
| ml | milliliter |
| MR | Magnetic Resonance |
| MRI | Magnetic Resonance Imaging |
| NCI | National Cancer Institute |
| NHL | Non-Hodgkin Lymphoma |
| OAL | Ocular Adnexae Lymphoma |
| OAMZL | Ocular Adnexal Marginal Zone Lymphoma |
| OS | Overall Survival |
| PBMC | Peripheral Blood Mononuclear Cell |
| PCR | Polymerase Chain Reaction |
| PD | Progressive Disease |
| PFS | Progression Free Survival |

| | |
|---|---|
| PI | Principal Investigator |
| p.o. | Per Os |
| PR | Partial Response |
| RR | Response Rate |
| RSI | Reference Safety Information |
| SAE | Serious Adverse Event |
| SAR | Serious Adverse Reaction |
| SD | Stable Disease |
| SEER | Surveillance, Epidemiology, and End Results (National Cancer Institute) |
| SOC | System Organ Class |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| TETR-PCR | Touchdown Enzyme Time Release - Polymerase Chain Reaction |
| TMF | Trial Master File |
| w/wks | weeks |

# 1. ETHICS

The study was conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki and with the International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice (GCP).
The study was reviewed and approved by the competent Ethics Committee (EC) of each participating site and by the Regulatory Authority of each involved Country.
A total of 4 countries were involved in the clinical trial and 7 enrolling sites.
The approved Clinical Study Protocol is presented in Appendix 1.1.
No amendments were issued during the course of the trial.
In Italy and Spain the Protocol version 1.0 dated February 2006 was approved. Instead 2 initial Protocols, version 1.0 dated Febrary 2006 and version 2.0 dated 2 August 2006, were submitted in Switzerland, since the Swiss Authorities requested preliminary corrections. The Swiss authorization was achieved with Protocol version 2.1 dated 24.11.2006. The same version was approved also in Chile.
EC and Regulatory Authority approvals were filed centrally and locally in the Trial Master File (TMF) and Investigator Site File (ISF), respectively, as required by GCP.
The submitted documentation included the patient information sheet and informed consent form.
The consent to participate in the study was obtained in writing from all patients (or patient's acceptable representative), prior to inclusion in the trial and after an adequate verbal and written full explanation regarding the objective and procedures of the trial and the possible risks involved. The consent form was to be personally signed and dated by the patient/legal representative and by the investigator conducting the informed consent procedure.
Patient information sheets and informed consent forms were prepared by the Principal Investigator (PI) of each institution following local regulatory and ethical requirements; these documents, including any subsequent revisions, were submitted and approved by the competent EC before their use. The right of a patient to refuse to participate or withdraw at any time from protocol treatment without giving reasons and without prejudicing further treatment was respected. A sample of the patient information sheet/informed consent form is provided in Appendix 1.2.
The clinician remained free to give alternative treatment to that specified in the protocol at any stage if it was in the patient's best interest.
A clinical trial-specific liability insurance was stipulated.

# 2. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The International Extranodal Lymphoma Study Group (IELSG) is the Sponsor of the trial.
IELSG was responsible for the overall project management of the study which was conducted in 7 enrolling centres, in 4 different Countries (Switzerland, Italy, Spain and Chile).

The Table 1 lists the administrative structure of the study.

**Table 1 Study Administrative Structure and Affiliations**

| | | |
|---|---|---|
| Study Chairs | Andrés J.M. Ferreri | IRCCS San Raffaele Scientific Institute<br>Department of Onco-Hematology<br>Milan – Italy |
| | John Radford | Cancer Research UK<br>Department of Medical Oncology<br>Christie Hospital NHS Trust,<br>Manchester – United Kingdom |
| Co-chairs | Emanuele Zucca | Oncology Institute of Southern Switzerland and Institute of Oncology Research<br>Bellinzona – Switzerland |
| | Franco Cavalli | Institute of Oncology Research,<br>Bellinzona – Switzerland |
| Pathology Coordinators | Maurilio Ponzoni<br>Claudio Doglioni | IRCCS San Raffaele Scientific Institute,<br>Pathology Unit<br>Milan – Italy |
| Molecular analysis coordinators | Riccardo Dolcetti<br>Massimo Guidoboni<br>Elisa Pasini | CRO-National Cancer Institute<br>Immunovirology and Biotherapy Unit<br>Aviano – Italy |
| | Francesco Bertoni | Institute of Oncology Research,<br>Bellinzona – Switzerland |
| | Ming-Qing Du | University of Cambridge<br>Department of Pathology<br>Cambridge – United Kingdom |
| Statisticians | Luciano Cascione | Institute of Oncology Research<br>Bellinzona - Switzerland |
| | David Ryder | Christie Hospital NHS Trust<br>Department of Medical Statistics<br>Manchester – United Kingdom |

Appendix 1.3 lists the investigators and their affiliations.
Appendix 1.4 contains the signatures of the Sponsor's representative and the Study Chair.

# 3. INTRODUCTION

Lymphoma arising in the ocular adnexae (OAL), namely in the conjunctiva, lachrymal gland, orbital fat, eyelid or lachrymal sac, represents 1-2% of all non-Hodgkin Lymphoma (NHLs) and 5-15% of all extranodal NHLs.[1,2]

The most common OAL, comprising approximately 80% of cases, is Marginal zone B-cell lymphoma (OAMZL) of MALT type, an indolent malignancy that usually arises after the fourth decade of age (median: 65 years), with a higher prevalence amongst females. Clinical presentation often consists of a single, slowly growing and painless mass that displaces the normal orbital structures, but sometimes patients have inflammatory-like signs and symptoms and a multifocal disease. About 25% of OAML involves conjunctiva, whilst intra-orbital lymphoma occurres in the remaining 75% of cases and can be associated with exophthalmos, palpable mass or nodule, eyelid ptosis, diplopia, epiphora and impaired ocular motility.[3]

OAMZL have an excellent prognosis, showing a 5-year overall survival more than 90% after different conventional approaches, including only observation, antibiotic therapy, radiotherapy, immunotherapy, chemoimmunotherapy, among others. [4] As a matter of fact, uniform guidelines are nowadays missing, hence the management remains heterogeneous.

Despite its rarity, OAMZL incidence is rapidly increasing, with annual rates more than 6% and with no evidence of peak, according to the analysis of population based incidence data from the National Cancer Institute Surveillance, Epidemiology, and End Results (SEER). [5]

Environment, occupational exposure, autoimmune disorders and infectious agents have been variously investigated as potential risk factors to justify the increasing incidence of OAMZL. Several studies have reported an increased incidence among individuals exposed to livestock or working with meat.[6-9]

In particular, different levels of evidence support a pathogenic association between Chlamydophila psittaci (Cp), the etiologic agent of psittacosis, and OAMZL.

Firstly, Cp DNA has been detected in 80% of lymphoma sample and 40% of peripheral blood monocyte/macrophages of patients with OAMZL.[10]

Then, chlamydial antigens have been detected in tumor tissue by immunohistochemistry and immunofluorescence and intact Cp within tumor-infiltrating macrophages or monocytes have been observed by electronic microscopy.[11]

Lastly, viable Cp from patients' conjunctival swabs and peripheral blood have been isolated in vitro.[12]

All these data fulfill the first and second of Koch's postulates (i.e., organism is found in the lesion of disease, and organism can be isolated and grown in vitro from disease lesions), which are still a useful benchmark in assessing the cause-effect relationship between an infectious agent and a disease.

Moreover, these evidences have provided the rationale for eradication of Cp infection with an antibiotic treatment. Thus, the antibiotic doxycycline has been investigated as targeted therapy firstly in a pilot study of 9 patients treated with 100 mg twice a day for 3 weeks,[13] then in a prospective multicenter phase II trial on 27 patients, with the same dose schedule.[14]

Doxycycline was preferred to other tetracyclines because of its fairly reliable absorption from the gastrointestinal tract, its long half-life (12 to 24 hours), its wide distribution in body tissues and fluids, and the fact that it can be administered to patients with renal insufficiency.

Lymphoma regression resulted in approximately 50% of patients, even in those who have experienced multiple treatment failures, have previously irradiated lesions, or have regional lymphadenopathies.[13,14]

These preliminary studies support the role of antibiotic therapy in OAMZL but may have included biases, mostly because they included a high proportion of patients with relapsed disease and registered after a variable follow-up duration.

Consequently, the IELSG27 study was designed to prospectively evaluate Cp prevalence and doxycycline activity as upfront treatment for patients with newly diagnosed stage I OAMZL.

# 4. OBJECTIVES AND ENDPOINTS

## 4.1 Objectives

### 4.1.1 Primary Objective

To explore the role of antibiotic therapy in the treatment of OAMZL and correlate the treatment response to molecular genetic findings.

### 4.1.2 Secondary Objective

To comprehensively screen infectious agents associated with chronic eye infection including Chlamydia, herpes simplex virus and adenovirus in OAMZL and various controls (other types of ocular adnexal lymphomas, inflammatory conditions, no known ocular disease) using molecular, immunohistological and serological tests with a view to identifying the agents associated with these lymphomas.

## 4.2 Endpoints

### 4.2.1 Primary Endpoint

The primary endpoint of this trial was to evaluate the response rates and the duration of response to tetracycline in patients with OAMZL.

Response was defined following the National Cancer Institute (NCI) standardized response criteria.[15]

### 4.2.2 Secondary Endpoints

- Identification of the infectious agents associated with OAMZL
- Geographical variation in distribution of potential infective agents associated with OAMZL in particular Chlamydia species.
- Molecular factors associated with response/non-response to tetracycline in OAMZL.

# 5. INVESTIGATIONAL PLAN

## 5.1 Overall Study Design and Plan

The IELSG27 represents an international multicentre phase II clinical trial.
The study was designed to elucidating the prevalence of Cp infection and assessing the efficacy of first-line doxycycline monotherapy in patients with newly diagnosed stage I OAMZL, since previous studies had shown the pathogenic role of this bacterium.
In particular, patients with suspected lymphoma of the ocular adnexae were subjected a diagnostic biopsy. If OAMZL was confirmed, the patients, after a full staging evaluation, entered the Part A or Part B of the study according to the following:

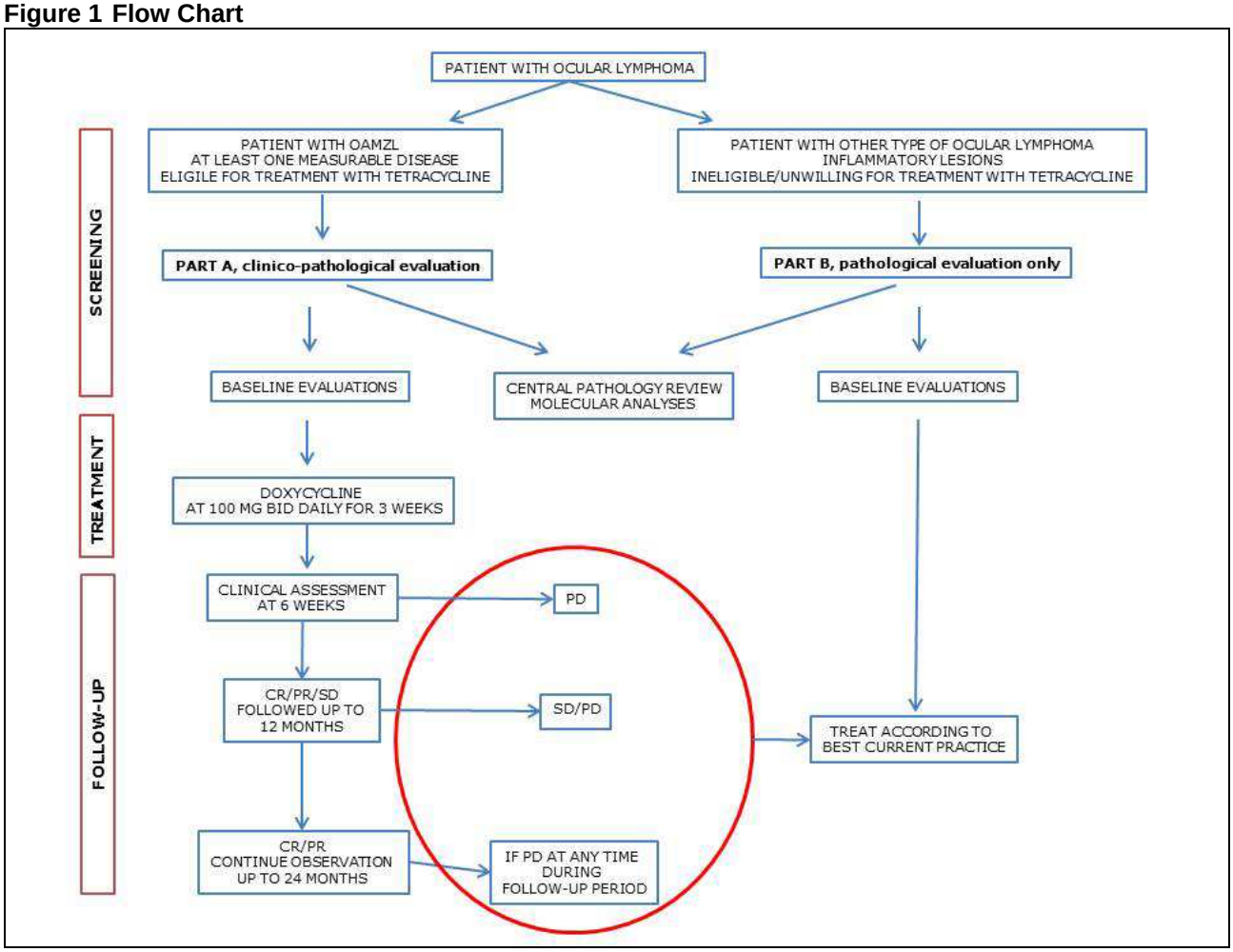
- Part A (phase II clinical trial, clinico-pathological evaluation), for patients eligible for treatment with tetracycline;
- Part B (pathologic evaluation only), for patients ineligible/unwilling for treatment with tetracycline.
  Of note, patients with other lymphoproliferative disorders, other types of ocular lymphoma or inflammatory lesions could enter Part B as well.

All the patients performed baseline evaluation, central pathology review and molecular analysis.
Patients enrolled in Part A received one cycle of doxycycline, 100 mg bid daily, for 3 weeks and then were assessed for response at 12 weeks from start of doxycycline. All patients who achieved Complete Response

(CR), Partial Response (PR) or Stable Disease (SD) proceeded up to 12 months (follow up period), with clinical observation every 2 months and an assessment for response at 12 months. Patients in CR or PR continued a clinical observation for a further 12 months. In this case clinical assessment were performed every 3 months and the assessment for response (MR scan) every 6 months. Patients in SD at the 12-months point or PD at any time, with or without a preceding remission, were treated according to the best current practice.
A total of 54 patients were enrolled because, on the basis of statistical consideration, a sufficient number of patients were to be recruited in Part A in order to have 29 Cp positive patients.
The diagnostic biopsies, after examination by the local pathologist, were centrally reviewed by 2 hematopathologists and diagnosis was established according to WHO classification.[15]
In addition, tumor biopsy, conjunctival swabs, and PBMCs obtained from each patient at the time of diagnosis and during clinical follow-up were centrally analyzed for 7 Chlamydiaceae infections.

As per UK requirement, in the protocol were introduced annual interim analyses of the accumulating data, that should have been reviewed by an Independent Data Monitoring Committee (IDMC). Moreover, on the basis of the collected data, an additional responsibility of IDMC was to decide if the study should proceed and, in this case, specify how many Chlamydia positive patients were needed.
Afterwards, UK sites declined to participate to the study and, as consequence, an IDMC was never established.
These responsibilities were taken over by the Study Chair in agreement with the Sponsor.
The study design predicted a first stage, stage 1, of 50 patients, sufficient number to guarantee 29 Cp positive patients.

**Figure 1 Flow Chart**



## 5.2 Discussion of Study Design

The Part A of the IELSG27 study represents the first international prospective phase II trial aimed at evaluating the Cp prevalence and antilymphoma activity of first-line doxycycline in patients with newly diagnosed stage I OAMZL.
The Part B is aimed at patients with non-OAMZL, inflammatory conditions or with OAMZL but ineligible to treatment with doxyxycline, where only the pathology will be evaluated.
The study was designed in 2 parts in order to optimise the access to pathological material in these rare diseases.

**Rationale for dose**
Doxycycline was preferred to other tetracyclines because of its fairly reliable absorption from the gastrointestinal tract, its long half-life (12 to 24 hours), its wide distribution in body tissues and fluids, and the fact that it can be administered to patients with renal insufficiency.
The dose of 100 mg twice daily was choosed on the basis of a previous prospective study where, with a 3 weeks schedule of treatment, half of patients with *Cp*-positive OAMZL showed a lymphoma regression after bacterial eradication.[14]

## 5.3 Selection of Study Population

Patients with suspected lymphoma of the ocular adnexae were eligible for this clinical trial.
After the usual diagnostic biopsy, they entered into Part A or B of the study as follows:
- Part A (clinico-pathological evaluation). Patients with OAMZL, measurable disease and eligible for treatment with tetracycline.
- Part B (pathological evaluation only). Patients with other types of ocular lymphoma, inflammatory lesions or ineligible/unwilling for treatment with tetracycline.

### 5.3.1 Inclusion criteria

Part A: clinico-pathological evaluation
1. Age 18 years or over
2. Histologically confirmed marginal zone B-cell lymphoma of MALT-type
3. Lymphoma localised to the ocular adnexae (conjunctiva, lacrymal gland, orbit soft tissue, clinical stage IEA) following a CT scan of neck, thorax, abdomen and pelvis, bone marrow aspirate/trephine, full blood count and biochemical profile
4. No previous treatment, excepting RT for localised lymphoma of the contralateral eye
5. At least one measurable lesion
6. No systemic antibiotic therapy in the last three months
7. No other malignancy in the previous 5 years apart from appropriately treated basal cell carcinoma of the skin or carcinoma in situ of the cervix
8. In women with reproductive potential a willingness to use contraception from entry into the study for a period of 3 months
9. Written informed consent

Part B: pathological evaluation only
1. Age 18 years or over
2. Histologically confirmed non-Hodgkin's lymphoma or inflammatory condition ("orbital pseudo-tumour") of the ocular adnexae
3. Localised disease (clinical stage IEA) for patients with lymphoma following a CT scan of neck, thorax, abdomen and pelvis, bone marrow aspirate/trephine, full blood count and biochemical profile including creatinine, LDH, B2-microglobulin (optional) protein electrophoresis, ASAT/ALAT, bilirubin, alkalin phosphatase (not applicable in non-malignant inflammatory conditions)
4. No previous treatment, excepting RT for localised lymphoma of the contralateral eye
5. Written informed consent

### 5.3.2 Exclusion criteria

Part A: clinico-pathological evaluation
1. Pregnant or lactating women
2. Known allergy to tetracycline
3. Patients unwilling to comply with the requirements of follow-up as defined by this protocol
4. Myasthenia gravis (tetracycline can exacerbate muscle weakness)
5. Systemic lupus erythematous (tetracycline can exacerbate the condition)
6. Patients with large or rapidly enlarging tumours requiring immediate radiotherapy

Part B: pathological evaluation only
None.

### 5.3.3 Removal of patients from therapy or assessment

The following were considered predetermined reasons for removing patients from the study:
- Progressive Disease (PD) at any time;
- Unacceptable toxicity dictating cessation of treatment;
- Change in patient's conditions such that the Investigator believed that patient's safety might be compromised by treatment or that it was in the best interest of the patient to stop treatment;
- Withdrawal of consent; if a patient decided to discontinue completely his/her study participation and did not authorize the Sponsor to collect further information about his/her disease status, no further attempts were to be made to collect additional data;
- Non-compliance by the patient with protocol requirements
- Patient lost to follow-up. If a patient did not return for scheduled visits, every effort was to be made to re-establish contact. In any circumstance, every effort was to be made to document patient outcome.

Patients in PD at any time, as well as those in stable disease at 12 months follow up, were treated according to current best clinical practice (local radiotherapy in most cases).

## 5.4 Treatments

### 5.4.1 Treatments Administered

Patients enrolled in Part A of the study received only one cycle, lasted 3 weeks, of doxycycline 100 mg twice daily.
The treatment should commence within 2 weeks of registration.

### 5.4.2 Identity of investigational product

Doxycycline is an antibiotic, primarily bacteriostatic; it is active against a wide range of Gram-positive and Gram-negative bacteria and certain other micro-organisms. It is believed to exert its antimicrobial effect by the inhibition of protein synthesis. The therapeutic class of doxycycline, tetracycline, is broad-spectrum antibiotic.
The route of administration is oral and the compound is provided in the form of tablets.
Contraindications to doxycycline are allergy to tetracycline, myasthenia gravis and systemic lupus erythematosus.
Doxycycline is commercially available worldwide and has had a market approval since November 1969.
The study drug was provided by each participating institute, which could choose the manufacturer/brand because in the study protocol the product was defined only by active substance.

### 5.4.3 Method of Assigning Patients to Treatment Groups

According to the protocol, patients were assigned to an unique treatment group with doxycycline.
Patients with OAMZL and eligible for treatment with doxycycline entered Part A of the study, that formally represents the clinical trial, and received doxycycline 100 mg twice daily for 3 weeks.

Patients with any other lymphoprolipherative disorders of ocular adnexae or ineligible for treatment with tetracycline were enrolled into Part B. They were treated according to normal practice and were not considered for outcome assessment as they were investigated for pathological evaluation only.

### 5.4.4 Selection and timing of doses in the study

Patients received one cycle of doxycycline 100 mg administered orally twice daily for 3 weeks, regardeless of their chlamydial infection status.
The patients had to avoid taking milk one hour before and one hour after each dose of tetracycline as milk decrease drug absorption.
The dose was choosed on the basis of a previous prospective study where, with the same schedule of treatment, half of patients with *Cp*-positive OAMZL showed a lymphoma regression after bacterial eradication.[14]

### 5.4.5 Blinding

No blinding procedures were used in this study.

### 5.4.6 Prior and Concomitant Therapy

All concomitant medications had to be entered into the CRFs.
Therapies considered necessary for the patient's well-being could be given at the discretion of the investigator, i.e. chronic treatments for concomitant medical conditions, as well as agents required for life-threatening medical problems, analgesics etc.
Before treatment start, the only permitted therapy was radiotherapy for localised lymphoma of the contralateral eye. Of note, patients could not receive antibiotic therapy in the 3 months preceeding enrollment.
During treatment, patients were asked to avoid antacids for one hour before and one hour after each dose of doxycycline.
Lastly, patients could not received any concomitant cytotoxic, antibiotic or steroid therapy in the period from the start of doxycycline to the maximum response assessment.

### 5.4.7 Treatment compliance

Records of study medication used, actual dose (mg per day), duration of therapy, as well as reasons for treatment discontinuation were to be kept during the study and recorded in the CRF.

Compliance with the study treatment was monitored directly by each investigator (by patient's interview and drug accountability) and centrally by the review of the CRF.

## 5.5 Efficacy and Safety Variables and Measurement Procedures

### 5.5.1 Study Flow Chart

Planned assessments and study procedures are summarized in Table 2.

**Table 2 Schedule of Events for Part A and Part B**

| **Week (w) or month (m)** | **Entry** | **w6** | **w12** | **m5** | **m7** | **m9** | **m12** | **m15** | **m18** | **m21** | **m24** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Part A (clinico-pathological evaluation** | | | | | | | | | | | |
| Tissue block[1] | + | | | | | | | | | | |
| Conjunctival swab[1] | + | | + | | | | + | | | | + |
| Serum (2-3mls) [1] | + | | + | | | | + | | | | + |
| PBMC (5mls in EDTA) [1] | + | | + | | | | + | | | | + |
| Baseline laboratory[2] | + | | | | | | | | | | |
| Clinical assessment | + | + | + | + | + | + | + | + | + | + | + |
| Clinical Photograph | + | | + | | + | | + | | + | | + |
| MR scan orbits | + | | + | | + | | + | | + | | + |
| **Week (w) or month (m)** | **Entry** | **w6** | **w12** | **m5** | **m7** | **m9** | **m12** | **m15** | **m18** | **m21** | **m24** |
| **Part B (pathological evaluation only)** | | | | | | | | | | | |
| Tissue block[1] | + | | | | | | | | | | |
| Conjunctival swab[1] | + | | + | | | | + | | | | + |
| Serum (2-3mls) [1] | + | | + | | | | + | | | | + |
| PBMC (5mls in EDTA) [1] | + | | | | | | | | | | |

Footnotes for Schedule of Events

[1] These specimens were sent to the central laboratories

[2] Complete blood counts, creatinine, bilirubin, LDH, beta-2 microglobulin, alkaline phosphatase, AST, ALT, protein electrophoresis were mandatory for baseline evaluations.

In addition, the protocol recommended serology for HCV and HBV, breath test or serology for H. pylori.

**Baseline evaluations for Part A and Part B**

- Clinical examination of the affected eye and identification of tumour site (conjunctival, lacrimal, retro-bulbar)
- Photography of the affected eye
- MR scan of the orbits (within 1 month before study entry)
- Complete blood counts, creatinine, bilirubin, LDH, beta-2 microglobulin, alkaline phosphatase, AST, ALT, protein electrophoresis. Serology for HCV and HBV, breath test or serology for H. pylori were recommended but not mandatory.
- Collection and shipment to the central laboratiories of the following specimens:
  - Conjunctival swab for detection of microbial DNA
  - 2-3ml serum for serological analysis
  - 5ml whole blood collected in EDTA tube for Chlamydiae detection in PBMC
  - Paraffin embedded tumour tissue for central histology review and copy of local histopathology report

**Follow up for Part A**

A first clinical assessment ro exclude PD was scheduled at 6 weeks from start of doxycycline, followed by a revaluation with MR scan at 12 weeks (i.e. 3 months) to formally assess response. Patients in CR o PR or SD were then observed clinically every 2 months and a further MR scan was performed at 12 months.

Thereafter, follow up for patients in CR/PR was scheduled with 3 monthly clinical assessment and 6 monthly MR scans until 24 months from start of doxycycline.

Patients who experienced SD at 12 months follow up or PD at any time, were treated according to best clinical practice, that in the most cases was local radiotherapy.

Here below are reported the investigations that were scheduled at each follow up time-point from start of treatment with doxycycline:

**6 weeks**
- Clinical examination of the affected eye

**12 weeks**
- Clinical examination of the affected eye, including photography
- MR imaging
- Conjunctival swab
- Peripheral blood samples (serum and PBMC) for detection of microbial DNA.

**5 months**
- Clinical examination of the affected eye

**7 months**
- Clinical examination of the affected eye, including photograph
- MR imaging

**9 months**
- Clinical examination of the affected eye

**12 months**
- Clinical examination of the affected eye
- MR imaging
- Conjunctival swab
- Peripheral blood samples (serum and PBMC) for detection of microbial DNA.

**15 months**
- Clinical examination of the affected eye

**18 months**
- Clinical examination of the affected eye, including photograph
- MR imaging.

**21 months**
- Clinical examination of the affected eye

**24 months**
- Clinical examination of the affected eye, including photograph
- MR imaging
- Conjunctival swab
- Peripheral blood samples (serum and PBMC) for detection of microbial DNA.

**Follow up for Part B**

After registration in Part B, biopsy material was collected and submitted for central pathology review; in addition, conjunctival swabs and blood were taken at registration and screened for Chlamydia. Further to this, patients were treated according to current best practice and no follow up were scheduled.

### 5.5.2 Efficacy Variable(s)

#### 5.5.2.1 Response Rate

Doxycycline activity, in terms of overall lymphoma response rate (complete and partial responses), was the primary end point for patients enrolled in Part A.
According to protocol, response assessment was performed at 3 and 12 months from doxycycline treatment and included clinical evaluation, photography of the affected eye and magnetic resonance imaging (MRI) of the orbits.

Response were defined following the National Cancer Institute standardized response criteria,[16] dated 1999 and reported in the Tables 3 and 4 below.

**Table 3 Standard Cheson Criteria – Response Category (1999)**

| **Complete Response (CR)** |
|---|
| 1. Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of those biochemical abnormalities (eg, lactate dehydrogenase [LDH]) definitely assignable to NHL.<br>2. All lymph nodes and nodal masses must have regressed to normal size (< 1.5 cm in their greatest transverse diameter for nodes >1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter before treatment must have decreased to <1 cm in their greatest transverse diameter after treatment, or by more than 75% in the sum of the products of the greatest diameters (SPD).<br>3. The spleen, if considered to be enlarged before therapy on the basis of a CT scan, must have regressed in size and must not be palpable on physical examination. However, no normal size can be specified because of the difficulties in accurately evaluating splenic and hepatic size. For instance, spleens thought to be of normal size may contain lymphoma, whereas an enlarged spleen may not necessarily reflect the presence of lymphoma but variations in anatomy, blood volume, the use of hematopoietic growth factors, or other causes. The determination of splenic volume or splenic index by CT scan are cumbersome and not widely used. Any macroscopic nodules in any organs detectable on imaging techniques should no longer be present. Similarly, other organs considered to be enlarged before therapy due to involvement by lymphoma, such as liver and kidneys, must have decreased in size.<br>4. If the bone marrow was involved by lymphoma before treatment, the infiltrate must be cleared on repeat bone marrow aspirate and biopsy of the same site. The sample on which this determination is made must be adequate (≥20 mm biopsy core). Flow cytometric, molecular, or cytogenetic studies are not considered part of routine assessment to document persistent disease at the present time. These studies should only be incorporated into trials examining important research questions. |
| **Complete Response/unconfirmed (CRu)** |
| Patients who fulfill criteria 1 and 3 of CR, but with one or more of the following features:<br>1. A residual lymph node mass greater than 1.5 cm in greatest transverse diameter that has regressed by more than 75% in the SPD. Individual nodes that were previously confluent must have regressed by more than 75% in their SPD compared with the size of the original mass.<br>2. Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia). |
| **Partial Response (PR)** |
| 1. ≥50% decrease in SPD of the six largest dominant nodes or nodal masses. These nodes or masses should be selected according to the following features: (a) they should be clearly measurable in at least two perpendicular dimensions, (b) they should be from as disparate regions of the body as possible, and (c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.<br>2. No increase in the size of the other nodes, liver, or spleen.<br>3. Splenic and hepatic nodules must regress by at least 50% in the SPD.<br>4. With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease.<br>5. Bone marrow assessment is irrelevant for determination of a PR because it is assessable and not measurable disease; however, if positive, the cell type should be specified in the report, eg, large-cell lymphoma or low-grade lymphoma (ie, small, lymphocytic small cleaved, or mixed small and large cells).<br>6. No new sites of disease. |
| **Stable Disease (SD)** |
| Defined as less than a PR but is not progressive disease. |
| **Relapsed disease (applicable to CR, CRu)** |
| 1. Appearance of any new lesion or increase by ≥50% in the size of previously involved sites.<br>2. ≥50% increase in greatest diameter of any previously identified node greater than 1 cm in its short axis or in the SPD of more than one node. |

| Progressive disease (applicable to PR, non-responders) |
|---|
| 1. ≥50% increase from nadir in the SPD of any previously identified abnormal node for PRs or nonresponders.<br>2. Appearance of any new lesion during or at the end of therapy. |

**Table 4 Standard Response Criteria for Non-Hodgkin's Lymphoma**

| Response Category | Physical Examination | Lymph Nodes | Lymph Node Masses | Bone Marrow |
|---|---|---|---|---|
| CR | Normal | Normal | Normal | Normal |
| CRu | Normal<br>Normal | Normal<br>Normal | Normal<br>>75% decrease | Indeterminate<br>Normal or indeterminate |
| PR | Normal<br>Normal<br>Decrease in liver/spleen | Normal<br>> 50% decrease<br>> 50% decrease | Normal<br>>50% decrease<br>>50% decrease | Positive<br>Irrelevant<br>Irrelevant |
| Relapse/progression | Enlarging liver/spleen<br>New sites | New or increased | New or increased | Reappearance |

### 5.5.2.2 Identification of the infectious agents associated with OAMZL

**Chlamydial infection prevalence**
Assessment of chlamydial infection was performed by different Polymerase Chain Reaction (PCR) protocols in DNA extracted from formalin-fixed, paraffin-embedded diagnostic specimens. Chlamydial infections were also investigated in DNA extracted from conjunctival swabs and PBMCs collected after trial registration.
All participating centres should send two 10µm sections of formalin-fixed and paraffin-embedded ocular biopsy, together with conjunctival swabs and peripheral blood samples from each case, to the reference laboratory for molecular analysis immediately after collection.
Swabs and PBMCs were collected again at 3 and 12 months from doxycycline treatment.
Tumor biopsy, conjunctival swabs, and PBMCs obtained from each patient at the time of diagnosis and during clinical follow-up were analyzed for 7 Chlamydiaceae (Cp, C trachomatis, C pneumoniae, C abortus, C caviae, C felis, and C suis) by PCR amplification of 3 different regions of bacterial genome, specific for the 16S-23S spacer rRNA, the omp-A porin, and the GroEL chaperonin (HSP-60).[17-19] A multiplex touchdown enzyme time-release PCR (TETR-PCR) assay, designed to simultaneously detect C trachomatis, C pneumoniae, and Cp DNA at bacterial loads lower than one infection-forming unit, was performed according to a previously published protocol with few modifications.[17]
Cp infection was also detected in single PCR assays using different primers.
For all patients investigated, at least 3 independent PCR amplifications were performed per tissue, swab, and PBMC sample. Samples were considered positive for chlamydial infection when chlamydial DNA was amplified in at least two independent experiments.
PCR products were analyzed by electrophoresis, and DNA fragment size was quantified by image analysis. The specificity of PCR products was confirmed by sequencing.

**Chlamydial infection eradication**
Patients with chlamydial DNA detected in DNA extracted from conjunctival swabs and PBMCs collected after trial registration were considered assessable for bacterial eradication, that was defined as the disappearance of chlamydial DNA in post-treatment samples. Bacteria eradication results did not modify therapeutic programs.

**Herpes simplex virus and adenovirus screening**
All DNA samples from ocular biopsy, conjunctival swabs and peripheral blood, collected at the time of diagnosis, should have been further investigated for presence or absence of herpes simplex virus type 1 and 2, and adenovirus type 8 and 19.
The promoter of these analysis was Professor Ming-Qing Du' (Cambridge). Since UK declined to join the these analysis were not performed.

**Geographical variation**
The study aimed at assessing the impact of the geographical area on the association of potential infective agents in particular Chlamydial species, associated with OAMZL.

During the study conduction, it became evident that an analysis of geographical variation could not have been carried out.
As matter of facts, the population was not geographically distributed: UK, Northern Europe and North America declined to participate and, above all, most of patients were included by a single italian site that alone enrolled 85% of patients (46 out of 54).

**Molecular factors**
All cases of MALT lymphomas and diffuse large B cell lymphoma (DLBCL) should have been investigated for specific translocations by FISH in order to correlate the genetic aberrations with OAMZL response to antibiotic therapy, and to examine the value of these genetic aberrations in prediction of the treatment response and disease prognosis.
In addition, selected cases should have been subjected to array Comparative Genomic Hybridisation (aCGH). The aim was to identify the genetic lesions important for development of ocular MALT lymphoma, particularly those negative for the above chromosomal translocations, and their high-grade transformation. Since no sufficient material was collected, these analysis were not performed.

### 5.5.3 Safety Variables

Even if safety was not an objective of this clinical trial, patients were instructed by the investigator to report the occurrence of any AE; the investigator, hence, had to report to the Sponsor all AEs with a suspected relationship to the study drug and all SAEs.

**Adverse Events**

An AE was defined as any untoward medical occurrence in a patient or a clinical trial subject administered a medicinal product and which did not necessarily have causal relationship with the use of the product. An adverse event could therefore be any unfavorable and unintended sign (e.g. an abnormal laboratory finding), symptom, or diagnosis temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Any untoward medical occurrence, which occurred outside the period of subject follow-up defined in the protocol, was not considered an AE. Symptoms or medically significant laboratory or instrumental (e.g., by electrocardiography) abnormalities of a pre-existing condition had not to be considered an AE. However, occurrence of new symptoms, laboratory or instrumental abnormalities, as well as worsening of pre-existing ones, were to be considered AEs.

**Tumor progression**

In this trial, the general concept of “tumor progression” had not to be reported as an AE, while the specific symptoms of disease worsening, if any, had to be reported as AEs.

**Abnormal Laboratory Findings**

Any abnormal laboratory findings, including uncomplicated and asymptomatic abnormal laboratory findings, were to be considered adverse events and were to be collected, graded, and reported in the CRF. In addition, laboratory abnormalities fulfilling the SAE criteria or requiring any action such as study treatment modifications or therapeutic measures, were also to be considered as adverse events.

**Serious Adverse Events**

An adverse event that met one or more of the following criteria/outcome was classified as serious:
- Resulting in death
- Life-threatening, i.e., an event which, in the view of the Investigator, places the subject at immediate risk of death from the event as it occurred (it does not include an event which hypothetically might have caused death if it were more severe)
- Requiring inpatient hospitalization or prolongation of existing hospitalization
- Resulting in persistent or significant disability/incapacity, where disability was defined as a substantial disruption of a person’s ability to conduct normal life functions, either reported or defined as per clinical judgment
- Was a congenital anomaly/birth defect (if exposure to product just before conception or during pregnancy resulted in an adverse outcome in the child)
- Any other important medical event, that could not result in death, be life-threatening, or require hospitalization, but based upon appropriate medical judgment, it could jeopardize the subject and could require medical or surgical intervention to prevent one of the outcomes listed in the points above. Any new malignancy other than a relapse of the current tumor was included in this cathegory.

A non-serious adverse event was any adverse event that did not meet the criteria listed above or the outcome could not be determined with the information provided.

According to the protocol, Grade 4 haematological toxicity and Grade 4 mucositis were exempted from SAE reporting.

**Reporting Procedures**

The AE/SAE reporting period began upon signing of informed consent and ended after 30 days after the treatment administration.
All AEs/SAEs occurred to the patients during the reporting period had to be reported to the Sponsor, **whether or not the event was considered related to the study medication**.
**In addition**, any known SAE suspected to be related to the study treatment occurring after the defined reporting period, had also to be reported to the Sponsor.
Any SAE was to report to the Sponsor within 24 hours of its awareness by completing and sending by fax the "SAE Report form".
The SAE outcome had to be reported within 2 weeks after definitive assessment by completing the "SAE Follow-up form".
The investigator was responsible for organizing any supplementary investigation of SAEs based on the clinical judgement on the likely causing factors, including further opinion from a specialist in the field of the adverse event. If a patient dies, any post mortem finding including histopathology must be provided.

**Recording Adverse Events in the Case Report Forms**

The Investigator assessed the relationship between each AE and the investigational medication and reported any relevant toxicity on the CRF.
Each AE with a suspected relationship to study drug had to be reported once for cycle, at the worst CTC grade.

**Grading of Adverse Event Severity**

In this study the severity of the adverse events was to be graded by the investigator according to the Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0 of the US National Cancer Institute.

### 5.5.4 Drug Concentration Measurements

Drug concentration measurements were not foreseen.

### 5.5.5 Appropriateness of Measurements

Not applicable.

## 5.6 Data Quality Assurance

Data were recorded on source documents, and entered into the CRF by the responsible personnel at the site. The investigator or her/his designee had to review and sign the CRFs to verify their accurancy and authenticity.

Data management was performed by the Sponsor who reviewed the data for completeness and logical consistency.
To ensure the collection of accurate, consistent and reliable data, the pathology review was centralized. In fact, after examination by the local pathologist, the diagnostic biopsies were centrally reviewed by 2 hematopathologists and diagnosis was established according to WHO classification.

The molecular analysis were also centralized and were able to screen 7 Chlamydiacee infection in all ocular biopsies, conjunctival swabs and peripheral blood obtained from each patient at the time of diagnosis and, for patients enrolled in the clinical trial, during follow-up.

Blinded investigators and clinicians assessed respectively Cp prevalence/ eradication and tumor response.

The trial sites might have been also subject to review by the competent Ethics Committees, to quality assurance audits performed by the Sponsor and/or to inspection by regulatory authorities. For this purpose the investigator/institution had to guarantee direct access to source documents to auditors and inspectors.

## 5.7 Statistical Methods Planned in the Protocol and Determination of Sample Size

### 5.7.1 Statistical and Analytical Plans

The main objectives of this study were to establish the prevalence of chlamydial infections and define bacteria eradication and anti-lymphoma activity of doxycycline as exclusive first-line treatment for patients with newly diagnosed stage I OAMZL. Doxycycline activity, in terms of overall lymphoma response rate, complete and partial responses, was the primary end point.
The design of the study was Gehan's two stage, suitable not only for estimating the response rate but also for providing for early termination if the drug shows insufficient antitumor activity.
Comparison of discrete variables was performed by $\chi 2$ or Fisher's exact test. Progression-free survival (PFS) was calculated from the date of start of treatment to relapse, progression, or death or to the last date of follow-up. Survival curves were generated using the Kaplan-Meier method and compared by log-rank test. All probability values were two sided.
The protocol established annual interim analyses of the accumulating data, that should had been reviewed by an Independent Data Monitoring Committee (IDMC). In addition, the IDMC should be responsible for the activation of stage 2 Gehan's design.

### 5.7.2 Determination of Sample Size

Primary endpoint, on which the sample size had been calculated, was the doxycycline activity, in terms of overall lymphoma response rate (complete and partial response).
Statistics was based on Gehan's 2 stage design.
The first stage had to enroll a sufficient number of patients in Part A so that 29 patients Cp positive were available for evaluation of response.
If at least one lymphoma response was observed among the first 29 patients of the first stage, the second stage of accrual had to be activated and additional patients should be enrolled in order to estimate the true response rate to doxycicline with acceptable precision of 10%, i.e. +¨2 standard error =10%.
The maximum size of additional Cp positive patients to be added in the stage 2 was calculated to be 100.

## 5.8 Changes in the Conduct of the Study or Planned Analyses

### 5.8.1 Protocol amendments

No amendments were issued during the course of the trial.
In Italy and Spain the Protocol version 1.0 dated February 2006 was approved. Instead 2 initial Protocol, version 1.0 dated Febrary 2006 and version 2.0 dated 2 August 2006, were submitted in Switzerland, since the Swiss Authorities requested preliminary corrections. The Swiss authorization was achieved with Protocol version 2.1 dated 24.11.2006. The same version was approved also in Chile.

### 5.8.2 Changes in the Statistical Plan

A total of 34 patients were enrolled in the first stage. Twenty-nine of them were Cp positive OAMZL. Nineteen of the 29 patients achieved an objective response (ORR 66%, 95% CI, 50% to 82%). Consequently, the true response rate obtained was >10 %, according the the statistical consideration of the protocol. Therefore, it was not necessary to activate the second stage of accrual.

# 6. STUDY PATIENTS

## 6.1 Disposition of Patients

Between 09.11.2006 and 02.08.2010, a total of 7 centres were involved from 4 different countries (Chile, Italy, Spain and Switzerland), see Table 5 below for details.
Fifty-four patients with newly diagnosed lymphoproliferative disorders of the ocular adnexae were enrolled, 34 patients in Part A and 20 in Part B.

**Table 5 Patients Enrolled by Investigational Countries**

| Country | Centres per Country | Enrolled patients (n=54) |
|---|---|---|
| Chile | 1 | 1 |
| Italy | 4 | 49 |
| Spain | 1 | 1 |
| Switzerland | 1 | 3 |

All patients enrolled in Part A had stage-I OAMZL and were treated with doxycycline as per protocol.
There were no treatment withdrawal, neither due to PD neither due to AEs.
All the 34 enrolled patients of Part A entered the follow up phase and 20 of them completed the study as per protocol. Fourteen patients stopped the follow up due to relapse (11 cases) and lost to follow up (3 cases).
Twenty patients were enrolled in Part B because 13 cases had a stage-I OAMZL but no measurable lesion, 4 cases concerned other types of lymphoma (2 DLBCLs and 2 follicular lymphomas) and 3 cases were pseudotumor.

A summary of patient disposition and reasons for withdrawal from treatment and ending study is provided in Table 6.

**Table 6 Patient Disposition**

| | n | % |
|---|---|---|
| **PART A +PART B (n=54)** | | |
| **Patient Enrolment and Treatment** | | |
| Screened | 54 | 100 |
| Enrolled | 54 | 100 |
| - Enrolled in Part A | 34 | 63 |
| - Enrolled in Part B | 20 | 37 |
| Patients with stage I OAMZL (n=54) | 47 | 87 |
| - Enrolled in Part A (n=34) | 34 | 100 |
| - Enrolled in Part B (n=20) | 13 | 65 |
| **PART A ONLY (n=54)** | | |
| Treated and Completing Study Treatment | **34** | **100** |
| **Reason for Treatment Withdrawal** | | |
| Treatment Completed as per Protocol | 34 | 100 |
| Tumor Progression | 0 | 0 |
| Adverse Event<br>IMP-Related<br>IMP-Unrelated | <br>0<br>0 | <br>0<br>0 |
| **Total Off Treatment** | **0** | **0** |
| **Reason for Ending Study** | | |
| Follow Up Completed as per Protocol | 20 | 59 |
| Consent Withdrawal | 0 | 0 |
| Lost to Follow Up | 3 | 9 |
| Death | 0 | 0 |
| Relapse (median follow up at 37 months) | 11 | 32 |
| **Total Off Study** | 14 | 41 |

## 6.2 Protocol Deviations

No major protocol violations were observed in the study.

## 6.3 Demographic and other Baseline Characteristics

Table 7 reports a summary of critical demographic and baseline characteristics of the 47 patients, out of the 54 enrolled, who presented a stage I OAMZL.
According to disease peculiarities, the study enrolled a preponderance of female (68%); in addition, the whole population had an excellent ECOG performance status score, with PS=0 accounting for 91 %.

The single lesion, as per stage I definition, was located in conjunctiva (23 cases), orbit (14 cases), lachrymal gland (5 cases) or both orbit and conjunctiva (5 cases).

Twenty-five patients reported a history of prolonged exposure to household animals, especially dogs, cats, and small birds; 6 of them also lived in rural areas or had a professional exposure to potentially *Cp*-infected animals. Seven patients reported household animal exposure even during and after doxycycline treatment.

**Table 7 Demographic and other Baseline Characteristics (Patients with confirmed stage I OAMZL)**

| | n=47 | |
|---|---|---|
| | **n** | **%** |
| **Age (years)** | | |
| Median<br>(range) | 60<br>(24-83) | - |
| < 65 – years | 27 | 57 |
| ≥ 65 – years | 20 | 43 |
| **Sex** | | |
| Female | 32 | 68 |
| Male | 15 | 32 |
| **Performance status (ECOG)** | | |
| 0 | 43 | 91 |
| 1 | 4 | 9 |
| **Sites of disease** | | |
| Conjunctiva | 23 | 49 |
| Orbit | 14 | 29 |
| Lachrymal gland | 5 | 11 |
| Orbit & Conjunctiva | 5 | 11 |
| **Other infections** | | |
| *Helicobacter pylori* infection | 10 | 21 |
| HCV infection | 4 | 8 |
| HBV infection | 3 | 6 |
| HBV + HCV infection | 1 | 2 |

## 6.4 Data Sets Analyzed

The IELSG27 study enrolled a total of 54 patients between November 2006 and August 2010.
Different data sets were analyzed, depending on the objective and on the availability and quality of material submitted for central analysis.
All the 34 subjects who entered Part A represented the population for analysis of overall intention-to-treat, response assessment and safety.
Biopsy material sufficient for PCR analysis was available for 44 patients (31 in Part A; 13 in Part B): this population was analyzed for the prevalence of Chlamydial infection.
Only 29 out of the 34 patients enrolled in Part A were assessed for Cp eradication, because material at diagnosis was not available for 3 patients and no Cp DNA was detected in swab and/or PBMCs of 2 further patients.
Table 8 summarizes the above described analysis populations.

**Table 8 Analysis Populations**

| | Part A | Part B | Overall |
|---|---|---|---|
| Enrolled | 34 | 20 | 54 |
| Patients with confirmed stage I OAMZL | 34 | 13 | 47 |
| | | | |
| Intention-To-Treat Population | 34 | na | 34 |
| Per-Protocol (Efficacy-evaluable) Population Response assessment | 34 | na | 34 |
| Per-Protocol (Efficacy-evaluable) Population Cp eradication | 29 | na | 29 |
| Prevalence of Chlamydial infection | 31 | 13 | 44 |
| Safety-Evaluable Population | 34 | na | 34 |

Seven of the 29 patients with OAMZL assessable for eradication reported prolonged contact with household animals during and after doxycycline treatment.
Eradication was not correlated with animal exposure; it was achieved in two of the seven patients with post-therapy animal contact and in 12 of the 22 patients without animal exposure.

**Table 9 Correlation with Animal Exposure and Eradication in the 29 Cp Positive OAMZL Patients.**

| Animal exposure and eradication | n |
|---|---|
| Prolonged exposure to household animals | 7 |
| Eradication achieved | 2 out of 7 |
| No animal exposure | 22 |
| Eradication achieved | 12 out of 22 |

# 7. MEASUREMENTS OF TREATMENT COMPLIANCE AND EXPOSURE

## 7.1 Treatment Compliance

Dose delays, reductions and withdrawals were performed according to local clinical practice and medical judgment.

## 7.2 Treatment Exposure

All the 34 patients entered in Part A received doxycycline 100 mg twice daily for 3 weeks and completed the planned treatment.

# 8. EFFICACY EVALUATION

## 8.1 Analysis of Efficacy

The ORR, primary endpoint of the study, was achieved in large excess compared with the expected sample size estimation.
On a total of 34 patients enrolled in Part A, a lymphoma regression after treatment was complete in 6 patients and partial in 16, with an ORR of 65% (95% CI, 49% to 81%); 11 patients had SD, and 1 had PD.
A summary of response rate is provided in Table 10.

**Table 10 Response Rate after Doxycycline**

| | All patients in Part A (n=34) | |
|---|---|---|
| **Response** | **n** | **%** |
| Complete Response (CR) | 6 | 18 |
| Partial Response (PR) | 16 | 47 |
| Stable Disease (SD) | 11 | 32 |
| Progressive Disease (PD) | 1 | 3 |
| Overall Response Rate (CR - PR) | 22 | 65 |

**Chlamydial infection prevalence**

Biopsy material sufficient for PCR analyses was available for 31 out of the 34 patients enrolled in Part A and for 13 out of the 20 patients enrolled in Part B.

Cp was the only Chlamydiacea recognized in this series and its DNA was detected in 29 out of 31 patients (94%) in Part A and 10 (77%) in Part B (see table 11).

**Table 11 Chlamydiaceae Infections**

| | Part A (n=31) | | Part B (n=13) | | Total (n=44) | |
|---|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | **n** | **%** |
| **C psittaci** | 29 | 94 | 10 | 77 | 39 | 89 |
| **C pneumoniae**<br>**C trachomatis**<br>**C caviae**<br>**C felis**<br>**C abortus**<br>**C suis** | 0 | 0 | 0 | 0 | 0 | 0 |

Among the 39 patients with Cp-positive biopsy, Cp DNA was detected in 97% of conjuntival swabs (n=38) and in 69% of PMBC samples (n=27) collected at diagnosis.

No Cp DNA was present in the corresponding swabs or PBMCs of patients with Cp-negative biopsy.

**Table 12 Cp DNA Detection in Swabs and PMBC at Diagnosis in Cp-positive Biopsy Patients**

| | Part A (n=29) | | Part B (n=10) | | Total (n=39) | |
|---|---|---|---|---|---|---|
| | **n** | **%** | **n.** | **%** | **n** | **%** |
| **Swabs** | 28 | 97 | 10 | 100 | 38 | 97 |
| **PMBC** | 20 | 69 | 7 | 70 | 27 | 69 |

**Chlamydial infection eradication**

The 29 patients with Cp-positive biopsy enrolled in the Part A were assessable for Cp eradication since DNA of this agent was detected in swabs (n = 9), PBMCs (n = 1), or both (n = 19) at diagnosis (Table 13).

**Table 13 Cp DNA at Diagnosis in the 29 Cp positive Patients assessable for Eradication.**

| | Patients assessable for Cp eradication (n=29) | |
|---|---|---|
| | **n** | **%** |
| Swabs | 9 | 31 |
| PBMC | 1 | 3.4 |
| Swabs and PBMC | 19 | 65.6 |

At 3 months from doxycycline, Cp eradication was achieved in 14 assessable patients (48%); at 12 months, Cp DNA presence reoccurred in swabs from 3 patients, in whom eradication had been indicated at 3 months.

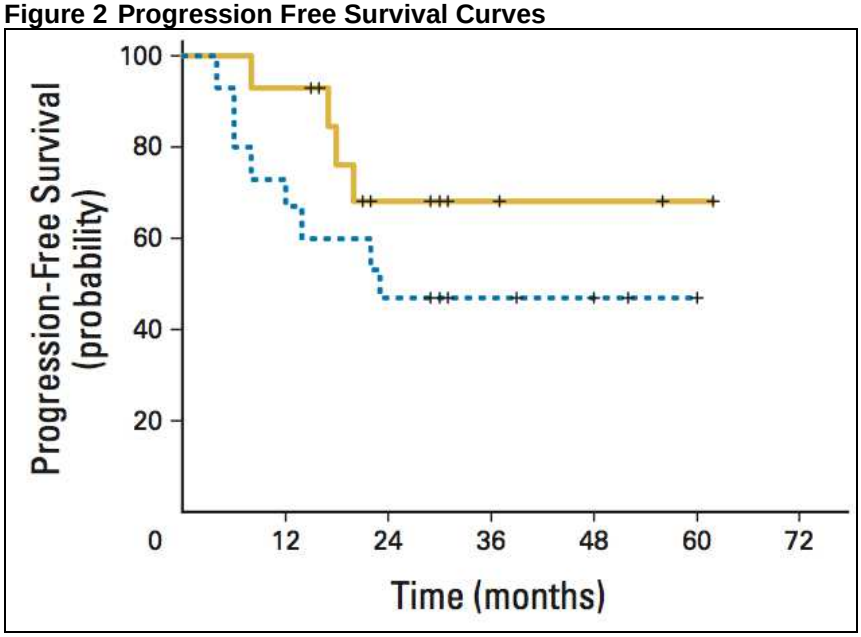
Twelve of the 14 Cp-eradicated patients achieved an objective lymphoma regression, whereas only seven of the 15 non–Cp-eradicated patients exhibited tumor response (86% v 47%; p = 0.02).
Nineteen out of the 29 patients with Cp-positive OAMZL achieved an objective response (overall response rate, 66%; 95% CI, 50% to 82%).
Conversely, the 2 patients with Cp-negative OAMZL had no clinical benefit from doxycycline; 1 patient achieved a short-lived PR (5 months), and the other had SD for 11 months (Table 14).

**Table 14 Chlamidial DNA in Tumor, Swabs and PBMCs, Eradication and Response in Part A Patients.**

| **Chlamydial DNA** | | | **Chlamydial eradication** | **Lymphoma response** |
|---|---|---|---|---|
| **Tumor** | **Swabs** | **PBMC** | | |
| Positive (29) | Positive (28) | Positive (19) | Eradicated (7) | CR (1), PR (6) |
| | | | Not eradicated (12) | CR (3), PR (4), SD (4), PD (1) |
| | | Negative (9) | Eradicated (6) | CR (1), PR (3), SD (2) |
| | | | Not eradicated (3) | SD (3) |
| | Negative (1) | Negative (1) | Eradicated | PR (1) |
| Negative (2) | Negative (2) | Negative (2) | Not parametrable (2) | PR (1), SD (1) |
| NA (3) | NA (3) | NA (3) | Not parametrable (3) | CR (1), PR (1), SD (1) |

No significant association between lymphoma response and site of disease (conjunctival vs orbital), animal exposure, or concomitant infections (H pylori or viral hepatitis) was observed.
At a median follow-up of 37 months (range, 15 to 62 months), 20 patients remained failure (progressive disease or relapse) free, with a 5-year PFS ± standard deviation of 55% ± 9%. Fifteen of the 22 patients with responsive lymphoma were relapse free at the time of analysis, with a median time to progression more than 30 months (range, 8 to >62 months). Importantly, none of the patients who achieved CR experienced relapse after 36 to 60 months. Tumor failure occurred in 4 of the 14 Cp-eradicated patients and in 8 of the 15 non–Cp-eradicated patients, with 5-year PFS of 68%± 13% and 47%± 13% (P= 0.11), respectively.
Lymphoma failure was invariably limited to the primary tumor site.
In the Figure 2 are reported the curves of patients registered in Part A and divided according to Cp eradication. Successful Cp eradication (solid line) was associated with better PFS.

**Figure 2 Progression Free Survival Curves**



### 8.1.1 Statistical/Analytical Issues

#### 8.1.1.1 Adjustments for Covariates

The selection of a sufficient number of patients in Part A, so that 29 chlamydia-positive patients were assessable, was planned.
Patients older that 18 years, with an histologically confirmed OAMZL of stage IA and without any previous treatment, except for radiotherapy of the controlateral eye, were baseline characteristics for Part A.
Patients with stage IA OAMZL, but without measurable or parametrable lesion or patients with inflammatory condition ("pseudotumour") were instead baseline characteristics for Part B.
As secondary endpoint, the study aimed at assessing the impact of the geographical area on the association of potential infective agents, in particular Chlamydial species, associated with OAMZL.
During the study conduction, it became evident that the population was not geographically distributed: most of patients were included by a single italian site that alone enrolled 85% of patients (46 out of 54). As consequence, this secondary analysis could not have been carried out.

#### 8.1.1.2 Handling of Dropouts or Missing Data

No dropouts or missing data were observed.

#### 8.1.1.3 Interim Analyses and Data Monitoring

As per UK requirement, during the drafting of the protocol were introduced annual interim analyses of the accumulating data, that should have been reviewed by an Independent Data Monitoring Committee (IDMC).
Moreover, responsibility of IDMC was also to decide, on the basis of the collected data during the first stage, if the study had to proceed. Afterwards, since the UK sites declined to participate to the study, it was established that it were the Study Chair and the Sponsor to assess the data, specifying how many Chlamydia positive patients were needed to be enrolled.

The Sponsor did not establish a specific monitoring plan for this study, but directly performed data management and reviewed the data for completeness and logical consistency.

#### 8.1.1.4 Multiple Comparison/Multiplicity .

Not applicable.

### 8.1.2 Drug Dose, Drug Concentration, and Relationships to Response

Not applicable.

## 8.2 Efficacy Conclusions

This trial proves that doxycycline is a rational and active first-line treatment for patients with stage I Cp-positive OAMZL and that lymphoma regression is consequent to Cp eradication.

In particular, lymphoma regression after doxycycline treatment was complete in 6 patients and partial in 16, with an ORR of 65% that largely exceed the primary end-point of the trial. In addition, 29 patients with Cp DNA in baseline samples were evaluable for chlamydial eradication, which was achieved in 48% of patients at one year of follow-up.

Cp eradication was associated with improved ORR and a better PFS.

# 9. SAFETY EVALUATION

No safety objectives were identified for this clinical trial.

## 9.1 Adverse Events (AEs)

All 34 patients who entered into Part A are included in the safety analysis.
As per protocol, only AEs with a suspected relationship to the study drugs and all SAEs, whether or not considered drug related, were collected.
Doxycycline treatment was well tolerated and no unexpected side effects were recorded.
Only 4 out of 34 patients, corresponding to 12% of the population, presented with an AE related to the study drug. In particular, 4 mild gastro-intestinal symptoms (Grade 1), expected and common after doxycycline treatment, were registered.
There were not patients who reported severe AEs or SAEs.

**Table 15 Overall Summary of Treatment-Emergent Adverse Events (Treated Patients – Part A)**

| | n=34 |
|---|---|
| No. patients with at least one AE | 4 |
| No. patients with at least one severe (CTCAE Grade ≥3) AE | 0 |
| No. patients with at least one SAE | 0 |

All treatment-emergent AEs, considered at least possibly related to doxycycline use, are presented in the Table 16.

**Table 16 Treatment-Emergent AEs by Maximum CTC Grade Experienced by Patients in Part A**

| Adverse Events | n=34 | | | |
|---|---|---|---|---|
| | G 1 | % | G > 1 | % |
| Nausea/vomiting | 3 | 9 | 0 | 0 |
| Stomatitis/mucositis | 1 | 3 | 0 | 0 |

## 9.2 Deaths, Serious Adverse Events, and other Significant Adverse Events

### 9.2.1 Deaths

At a median follow up of 37 months (range, 15 to 62 months), only 2 deaths were recorded in Part A.
Both deaths were unrelated to study treatment.
No cases of high-grade transformation or lymphoma –related death occurred.
A summary of deaths observed in Part A is reported in Table 17.

**Table 17 Deaths Occurred in Part A**

| Number of Patients | Sex | Age (years) | Days since First Dose | Days since Last Dose | Cause of Death |
|---|---|---|---|---|---|
| 2 | F | 80 | 583 | 562 | Ictus cerebri |
| 7 | F | 74 | 318 | 297 | Unknown |

### 9.2.2 Serious Adverse Events

No Serious Adverse Events were reported at whole during the study conduction.

### 9.2.3 Other Significant Adverse Events

There were not adverse events that led to treatment withdrawal or dose reduction.

### 9.2.4 Narratives of Deaths, Other Serious Adverse Events and Other Significant Adverse Events

This section contains only the narratives of 2 deaths occurred to the patients enrolled in the Part A during the whole study.
No SAEs or other significant AEs were reported at whole.

**Patient # 2**
**Part A – doxycycline**
**Death: ictus cerebri**
A 78 year-old female patient with a histologically confirmed MALT lymphoma of left orbit.
The patient was enrolled in Part A on 10.11.2006 and started doxycycline treatment on 14.11.2006 at the study dose of 100 mg/bid.
The treatment was completed after 3 weeks as per protocol, without any reported AE or dose modifications.
Response assessment after 12 weeks confirmed SD.
A first PD occurred during the follow up on 21.06.2007, after 7 months from treatment start.
Then, as per protocol, she was treated according to normal practice and a second line therapy consisting in clarythromycin started on 21.06.2007.
The patient died of ictus cerebri, unrelated to doxycycline treatment, on 19.06.2008.

**Patient # 77**
**Part A – doxycycline**
**Death: unknown**
A 74 years-old patient with a histologically confirmed MALT lymphoma of right orbit.
The patient was enrolled in Part A on 30.11.2006 and started doxycycline treatment on 15.12.2006 at the study dose of 100 mg/bid.
The treatment was completed after 3 weeks as per protocol, without any reported AE or dose modification.
Due to a concomitant disease, unrelated to lymphoma, but conditioning inability to attend planned visits, the patient was never evaluated for response and was lost to follow up.
She was hospitalized in an hospice and suddenly died on 29.10.2007. Cause of death remains unknown.

## 9.3 Clinical Laboratory Evaluation

No laboratory test abnormalities were reported as toxicities over the treatment period.

### 9.3.1 Individual Patient Changes

No analysis of individual patient changes was done.

## 9.4 Vital Signs, Physical Findings and other Observations Related to Safety

During the study no significant changes were reported by the treated patients in vital signs or physical examination.

## 9.5 Safety Conclusions

During the whole study period, no safety findings have been observed that necessitated any action to be taken with regard to the conduct of the clinical trial. The treatment was well tolerated and the incidence of

non-serious adverse events was consistent with the expected AE profile of doxycycline. In particular, only 4 patients with mild gastro-intestinal symptoms were registered. No SAEs nor SUSAR occurred during the whole period of the .
Two deaths were registered and both of them were unrelated to study treatment.
There are no important safety issues which impact on the risk benefit profile of the study programme.

# 10. DISCUSSION AND OVERALL CONCLUSIONS

OAMZL is a rare disease with a pathogenesis related to exposure to Chlamydia psittaci, the etiologic agent of psittacosis, a human infection caused by contact to infected animals.
First-line therapy in localized disease is not standardized even if radiotherapy is the most adopted. Considering the indolent behavior of this disease and the documented causal relationship with Cp infection, the IELSG27 study was designed to assess the efficacy of doxycycline, the antibiotic of choice to treat Cp infections, given as monotherapy in newly diagnosed stage I OAMZL patients. In this way, radiotherapy and chemotherapy side effects could be avoided.
The primary end point (overall response rate) of the trial was achieved in large excess in comparison with figures predicted during sample-size estimation. Moreover, response quality after doxycycline was excellent; the 6 patients who achieved CR did not experience relapse at 36 to 60 months, and patients with lymphoma regression but residual lesion on MRI (i.e., PR) were successfully managed with a watchful waiting strategy. Importantly, no cases of high-grade transformation or lymphoma-related death occurred in this trial. The treatment was well tolerated and the incidence of adverse events was as expected with doxycycline.
Consistently, 86% of patients who achieved successful Cp eradication attained a major and durable lymphoma regression, with a reduced risk of relapse, whereas outcome was significantly poorer in non–Cp-eradicated patients. These findings also confirm the critical role played by Cp in maintaining OAMZL growth. Overall, these activity data are similar, or even superior, to those reported with chemotherapy and radiotherapy, suggesting that upfront doxycycline should be proposed for patients with stage IE Cp-positive OAMZL to obtain durable remission and avoid adverse effects and sequelae of more intensive treatments.
This trial also showed an almost complete concordance between Cp detection on tumor tissue and conjunctival swab therefore proved that Cp infection can be monitored through this minimally invasive and simple procedure. In parallel with conjunctival swabs, infection should be assessed in PBMCs, because this test provides additional information useful in predicting lymphoma dissemination and recurrence.
Unlike gastric marginal zone lymphoma, OAMZL cannot be repeatedly biopsied to monitor disease and infection, and thus, periodic assessment on swabs and PBMCs represents an attractive alternative to test for Cp infection and eradication. Execution of these assays once per year during follow-up could be a useful approach to drive antibiotic re-treatment in the case of Cp reoccurrence. In the present study, half of the assessable patients displayed Cp persistence after doxycycline treatment, which was associated with poorer results. This may be explained by reinfection from an external source (infected animal) or antibiotic failure. The present trial does not support an effect of prolonged animal exposure on outcome, which remains to be verified in ad hoc studies. Likewise, the almost unknown mechanisms of resistance of Cp to doxycycline should be investigated in future studies.
In conclusion, the results of this trial allow doxycycline to switch from a valid salvage strategy to a rational, safe, and effective first-line treatment for patients with stage I Cp-positive OAMZL. Lymphoma regression after doxycycline treatment is consequent to Cp eradication, which can easily be monitored on conjunctival swabs and PBMCs.

# 11. ACKNOWLEDGEMENTS

We are grateful to all the investigators, nursing and staff involved in this study and, most importantly, to the patients who willingly agreed to participate.

The IELSG27 study was supported in part by a grant from the Association for Cancer Research and an unrestricted grant from Oncosuisse (Swiss Cancer League; ICPOCS-02062-03-2007).

# 12. REFERENCES

1. Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. Cancer 1972 Jan;29(1):252e60.
2. Fung CY, Tarbell NJ, Lucarelli MJ, Goldberg SI, Linggood RM, Harris NL, et al. Ocular adnexal lymphoma: clinical behavior of distinct World Health Organization classification subtypes. Int J Radiat Oncol Biol Phys 2003 Dec;57(5):1382e91.
3. Ferreri AJ, Dolcetti R, Du MQ et al. Ocular adnexal MALT lymphoma: an intriguing model for antigen-driven lymphomagenesis and microbial-targeted therapy. Ann Oncol 2008; 19: 835–46.
4. Martinet S, Ozsahin M, Belkac_emi Y, Landmann C, Poortmans P, Oehlere C, et al. Outcome and prognostic factors in orbital lymphoma: a rare cancer network study on 90 consecutive patients treated with radiotherapy. Int J Radiat Oncol Biol Phys 2003 Mar 15;55(4):892e8.
5. Moslehi R, Devesa SS, Shairer C, Fraumeni Jr JF. Rapidly increasing incidence of ocular non-hodgkin lymphoma. J Natl Cancer Inst 2006 Jul 5;98(13): 936e9.
6. Amadori D, Nanni O, Falcini F, Saragoni A, Tison V, Callea A, et al. Chronic lymphocytic leukaemias and non-Hodgkin's lymphomas by histological type in farming-animal breeding workers: a population case-control study based on job titles. Occup Environ Med 1995 Jun;52(6):374e9.
7. Svec MA, Ward MH, Dosemeci M, Checkoway H, De Roos AJ. Risk of lymphatic or haematopoietic cancer mortality with occupational exposure to animals or the public. Occup Environ Med 2005 Oct;62(10):726e35.
8. Fritschi L, Johnson KC, Kliewer EV, Fry R, Canadian Cancer Registries Epidemiology Research Group. Animal-related occupations and the risk of leukemia, myeloma, and non-Hodgkin's lymphoma in Canada. Cancer Causes Control 2002 Aug;13(6):563e71.
9. Dolcetti R, Serraino D, Dognini G, Govi S, Crocchiolo R, Ghia P, et al. Exposure to animals and increased risk of marginal zone B-cell lymphomas of the ocular adnexae. Br J Cancer 2012 Feb 28;106(5):966e9.
10. Ferreri AJ, Guidoboni M, Ponzoni M, De Conciliis C, Dell'Oro S, Fleischhauer K, et al. Evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. J Natl Cancer Inst 2004 Apr;96(8):586e94.
11. Ponzoni M, Ferreri AJ, Guidoboni M, et al: Chlamydia infection and lymphomas: Association beyond ocular adnexal lymphomas highlighted by multiple detection methods. Clin Cancer Res 14: 5794-5800, 2008
12. Ferreri AJ, Dolcetti R, Dognini GP, et al: Chlamydophila psittaci is viable and infectious in the conjunctiva and peripheral blood of patients with ocular adnexal lymphoma: Results of a single-center prospective case-control study. Int J Cancer 123: 1089-1093, 2008
13. Ferreri AJ, Ponzoni M, Guidoboni M, et al: Regression of ocular adnexal lymphoma after chlamydia psittaci– eradicating antibiotic therapy. J Clin Oncol 23:5067-5073, 2005
14. Ferreri AJ, Ponzoni M, Guidoboni M, et al: Bacteria-eradicating therapy with doxycycline in ocular adnexal MALT lymphoma: A multicenter prospective trial. J Natl Cancer Inst 98:1375-1382, 2006
15. Swerdlow SH, Campo E, Harris NL, et al (eds): WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. Lyon, France, IARC Press, 2008
16. Cheson BD, Horning SJ, Coiffier B, et al: Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas: NCI-sponsored international working group. J Clin Oncol 17:1244, 1999
17. Madico G,Quinn TC,Boman J,et al:Touchdown enzyme time release-PCR for detection and identification of Chlamydia trachomatis, C. pneumoniae, and C. psittaci using the 16S and 16S-23S spacer rRNA genes. J Clin Microbiol 38:1085-1093, 2000
18. Tong CY, Sillis M: Detection of chlamydia pneumoniae and Chlamydia psittaci in sputum sam- ples by PCR. J Clin Pathol 46:313-317, 1993
19. Hill JE, Goh SH, Money DM, et al: Characterization of vaginal microflora of healthy, nonpregnant women by chaperonin-60 sequence-based methods. Am J Obstet Gynecol 193:682-692, 2005
20. Ben Simon GJ, Cheung N, McKelvie P, et al: Oral chlorambucil for extranodal, marginal zone, B-cell lymphoma of mucosa-associated lymphoid tissue of the orbit. Ophthalmology 113:1209-1213, 2006
21. Ejima Y, Sasaki R, Okamoto Y, et al: Ocular ad- nexal mucosa-associated lymphoid tissue lymphoma treated with radiotherapy. Radiother Oncol 78:6-9, 2006

# 13. TABLES AND FIGURES REFERRED TO THE TEXT

In this section are reported all tables and figures mentioned/presented in Sections 6-9 of the report.

# 14. LIST OF APPENDICES

1.0 Study Information

- 1.1 Protocol and Protocol Amendments
- 1.2 Sample of Informed Consent Form
- 1.3 List and Description of Investigators
- 1.4 Signatures of Sponsor's Representative and Study Chair
- 1.5 Audit Certificates (if available)
- 1.6 Publication Based on the Study (if available)
- 1.7 Publications and Supporting Documentation Referenced in the Report

2.0 Patient Data Listings

- 2.1 Major Protocol Deviations
- 2.2 Patients Excluded from the Efficacy Analysis