Clinical Study Protocol

Protocol Title: Thymosin alpha 1 plus maintenance therapy with the Standard of Care (SoC) chemotherapy plus cisplatin (or carboplatin) in patients with metastatic Non-Small Cell Lung Cancer (NSCLC), EGFR wild type

Investigational Drug: Thymosin alpha 1 (ZADAXIN®) Lyophilisate for Solution for Injection

Protocol Number: SCI-Ta1-NSCLC-CHEMO P2-001

EudraCT number 2015-005605-36

Sponsor: SciClone Pharmaceuticals Italy.

Principal Investigator: Prof. Paolo Marchetti, Ospedale Sant'Andrea, Rome – Italy

Date: 8 December 2015

INVESTIGATOR AGREEMENT SIGNATURE PAGE

Protocol Title:Thymosin alpha 1 plus maintenance therapy with the Standard of Care (SoC) chemotherapy plus cispaltinum (or carboplatin) in patients with metastatic Non-Small Cell Lung Cancer (NSCLC), EGFR wild type

Original Protocol Issue Date:8 December 2015

I have read the attached protocol and appendices dated 8 December 2015 and agree to abide by all provisions set forth therein. I will provide copies of the protocol and other pertinent information to all individuals responsible to me who will assist with the study.

I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice and applicable EU regulations/guidelines set forth in Directive 2001/20/CE as well as Italian regulation (D.Lvo 211/2003).

Before study initiation, during the study if there are changes that affect my financial disclosure status, and after the study is completed, I agree to ensure that Financial Disclosure Statements will be completed by:

- Myself (including, if applicable, my spouse [or legal partner] and dependent children)
- My Subinvestigators (including, if applicable, their spouses [or legal partners] and dependent children)

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of SciClone Pharmaceuticals Italy.

The Sponsor will have access to source documentation from which case report forms have been completed.

_____

Signature of Principal Investigator Date (DD /MONTH / YYYY)

_____

Printed Name of Principal Investigator

PROTOCOL SYNOPSIS

Product	Thymosin alpha 1 (ZADAXIN®)lyophilisate for solution for injection
Title of Study	Thymosin alpha 1 plus maintenance therapy with the Standard of Care (SoC) chemotherapy plus cisplatin (or carboplatin) in patients with metastatic Non-Small Cell Lung Cancer (NSCLC), EGFR wild type
Study Protocol Number	SCI-Ta1-NSCLC-CHEMO P2-001
EudraCTNumber	2015-005605-36
Phase of Development	Phase II
Background and Rationale	Chemotherapy/cisplatin (or carboplatin) is the standard for ECOG PS 0-1 patients with metastatic non-small cell lung cancer that are negative for sensitizing EGFR mutation or ALK rearrangements. Continuation maintenance after 4 cycles of cisplatin and SoC chemotherapy for these patients is category 1 in current guidelines. A recent phase III randomized trial (PARAMOUNT) found that continuation maintenance therapy with chemotherapy (pemetrexed) increased both PFS and OS when compared with placebo. Even though significant, the survival gain was slight (pemetrexed vs placebo: 4.1 vs. 2.8 months and 13.9 vs. 11.0 months, for PFS and OS, respectively) and additional strategy to further improve outcome in this setting is still an unmet medical need. Thymosin alpha 1 is an immunomodulatory polypeptide that could potentially improve efficacy of treatment regimens for metastatic non small cell lung cancer without impacting toxicity. Thymosin alpha 1is produced endogenously by the thymus gland and augments T-cell–mediated immune responses by several mechanisms, including stimulation of T-cell differentiation and/or maturation, activation of natural killer and dendritic cells, and stimulation of proinflammatory cytokine release. In a randomized study of 22 patients with advanced non–small-cell lung cancer, patients given ifosfamide plus Thymosin alpha 1 (1 mg on

	days 8 through 11 and 15 through 18; 21-day cycles) plus low-dose IFN-α (3 MU on days 11 and 18) had an improved median TTP versus patients given ifosfamide alone (18 v 9 weeks; P = .006). Hematologic toxicity was reduced in the group given Thymosin alpha 1 versus the group given ifosfamide alone (grade 3 to 4 hematologic toxicity: 0% v 50%). SciClone intends to confirm this recent finding in an appropriately-powered, national, Phase II (randomized – open label)-controlled clinical trial.
Objectives	<u>Primary Objective</u> To evaluate the activity/efficacy in terms of PFS of Thymosin alpha 1 in patients with metastatic, non-small cell lung cancer (NSCLC), EFGR wild type, taking SoC chemotherapy as compared to SoC alone.
Study Design	Phase II, multi-center, open label, randomized, parallel group study to determine the activity/efficacy of Thymosin alpha 1 in patients with advanced EGFR wild type metastatic NSCLC taking SoC versus SoC alone. The study will be conducted in subjects with metastatic, NSCLC, EFGR wild type. Subjects with metastatic, NSCLC, EGRF wild type will be screened for EGFR mutation; in case of negative response, they will be screened for eligibility by the clinical center involved. Patients will be randomized to SoC or Thymosin alpha 1 plus SoC for treatment duration of 12 months. All patients will be followed for approximately 12 months or until the total number of PFS events required will be observed.
Sample Size	The trial is an event-driven trial, the total sample size is a function of both the event rate and the duration of follow-up. It is anticipated a total of approximately 140 patients will be enrolled in order to observe the required number of PFS events. However the sample size will be adjusted if needed to meet the required number of events
Countries involved	Italy

Study Period (years) **Target date of first subject enrolled to target date of last subject completed**	May, 2016 to Sep, 2019 (~40months, assuming 0.4 pts/site month and 7 actively enrolling sites)

Inclusion Criteria	✓ Age 18 years or older ✓ Histological or cytological confirmation of NSCLC EGRF wild type (either from initial diagnosis of NSCLC or subsequent biopsy). Only patients with available tissue samples will be enrolled ✓ Locally advanced or metastatic NSCLC, not amenable to curative surgery or radiotherapy ✓ Measurable disease by Response Evaluation Criteria in Solid Tumours (RECIST) in a lesion not previously irradiated or non-measurable disease ✓ Eastern Cooperative Oncology Group - performance status (ECOG-PS) 0-2 ✓ Absolute neutrophil count (ANC) > 1.5 x 10^9/liter (L) and platelets > 100 x 10^9/L ✓ Bilirubin level either normal or <1.5 x ULN ✓ AST (SGOT) and ALT (SGPT) <2.5 x ULN (≤ 5 x ULN if liver metastases are present) ✓ Serum creatinine <1.5 x ULN ✓ Effective contraception for both, male and female pts, if the risk of conception exists ✓ Recovery from all acute toxicities of prior therapies ✓ Provision of written informed consent

Exclusion Criteria	✓ Prior therapy with Thymosin alpha-1 ✓ Newly diagnosed central nervous system (CNS) metastases that have not yet been treated with surgery and/or radiation. Pts with previously diagnosed and treated CNS metastases or spinal cord compression may be considered if they have evidence of clinically stable disease (SD) (no steroid therapy or steroid dose being tapered) for at least 28 days ✓ Pregnancy or suspected pregnancy ✓ Any unresolved chronic toxicity from previous anticancer therapy that, in the opinion of the investigator, makes it inappropriate for the patient to be enrolled in the study ✓ Known severe hypersensitivity to study drugor any of the excipients of this product ✓ Other co-existing malignancies or malignancies diagnosed within the last 5 years with the exception of basal cell carcinoma or cervical cancer in situ ✓ Any evidence of clinically active interstitial lung disease (ILD) (patients with chronic, stable, radiographic changes who are asymptomatic or patients with uncomplicated progressive lymphangiticcarcinomatosis need not be excluded) ✓ As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g., unstable or uncompensated respiratory, cardiac, hepatic or renal disease) ✓ As judged by the investigator, any inflammatory changes of the surface of the eye ✓ Evidence of any other significant clinical disorder or laboratory finding that makes it undesirable for the patient to participate in the study

Drug Formulation:	Thymosin alpha 1 is contained, stored, and dispensed from individual tamper-proof glass vials with 1.6 mg Thymosin alpha 1 as a lyophilized cake containing 5% mannitol, buffered with phosphate to pH 6.4-7.3.Vials are reconstituted with 1 mL of supplied diluent (sterile water for injection), prior to subcutaneous (SC) administration.
Dose and Mode of Administration:	**Approximately 140 patients:** Arm A: 70 patients will receive Thymosin alpha 1 in 1mL SC injection five time a week (first four months); then two time a week for eight months. SoC chemotherapy and cisplatin (or carboplatin) for twelve months. Arm B: 70 patients (control group) will receive SoC chemotherapy and cisplatin (or carboplatin) for twelve months.
Duration of Treatment:	12 months
Randomization:	Patients will be randomized centrally through the automated 24-hour Interactive Web-based Response System (IWRS) system. Randomization will be stratified by centre.
Efficacy Endpoints:	**Primary endpoint** Time to progression free survival (PFS) **Secondary endpoint(s):** • time to Overall Survival (OS); • Quality of Life (QoL); • organ failure free days; • biomarkers of immunity and inflammation.
Safety Endpoints:	Adverse events, serious adverse events (SAEs),vital signs, electrocardiograms (ECGs) and laboratory parameters

Primary Statistical Methods:	The *intent-to-treat (ITT) set* includes all subjects who were randomized. The *per protocol* (PP) population is the subset of the ITT set that includes subjects who complete the study with no major protocol violations. The *safety analysis set* (SAF) is the population of all subjects who receive at least 1 dose of CTM. The primary anlaysis will use a time to event analysis that will compare the time to PFS between the group taking Thymosin alpha 1 + SoC and cisplatin (or carboplatin) andthe group taking SoCand cisplatin (or carboplatin) alone, using a one-sided Log-Rank Test. The Hazard Ratio andassociated 90% confidence interval would be calculated based on a Cox-Regression model. By using an exact single-stage trial design and considering that median PFS rate from the literature is 4.4 months (M0), the study will be looking for a relative increase of 79 % in the median PFS of study patients (M1 = 7.8 months). The hypothesis to be tested will be H0, M $\leq$ M0 v H1, M $\geq$ M1, where M will be the actual median PFS of enrolled patients. Assuming an exponential survival distribution, a constant hazard ratio of 1.79 (Group 1 exponential parameter, $\lambda 1$, of 0.1575 and a Group 2 exponential parameter, $\lambda 2$, of 0.0880), an 10% exponential dropout, a one-sided 5% type-I error, and 80% statistical power a minimum number of 70 patients in each group, with a total number of events of 73 is required to detect a 79% relative increase in the median PFS in treated patients when compared to a reference of 4.4 months. This effect was decided taking into consideration positive literature reports on pilot studies on NSCLC patients adding Thymosin alpha 1 to standard first line treatment. This assumes an accrual period of 8 months and a maximum follow up time of 26 months. nQuery Advisor + nTerim 7.0 was used to perform a sample size calculations. The primary endpoint of PFS will be calculated from the day of randomization to the day of death. PFS will be summarized by treatment group using the Kaplan-Meier method, and a comparison between treatment groups made using a Log Rank test. The hazard ratio will be estimated using a Cox Regression Model. The primary

	analysis will be conducted in the Intent-to-treat (ITT) analysis set, which will consist of all randomized patients. Descriptive statistics will be used to summarize the patient data at defined time-points. Toxicity will be reported by type, frequency and severity. Survival times will be estimated using the Kaplan-Meier method. The multivariate Cox model will be used to study variation in the PFS according to major baseline characteristics (age, sex, stage, histology, and treatment). Patients will be censored for overall survival at the last known alive date. Statistical analyses will be conducted using STATA/SE version 12·1 statistical software and SPSS software, version 13.0 (SPSS, Inc, Chicago, IL, USA).

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ABBREVIATIONS

ADR	Adverse drug reaction
AE	Adverse event
AFA	Afatinib
ALT	Alanine transferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
BAC	Bronchioloalveolar carcinoma
BL	Baseline
BSA	Body surface area
CI	Confidence interval
CNS	Central nervous system
CR	Complete response
CrCl	Creatinine clearance
CRF	Case report form
CSF	Colony stimulating factor
CT	Computed tomography
DCF	Data clarification form
DOC	Paclitaxel
EC	Ethics Committee
ECG	Electrocardiogram
ECOG-PS	Eastern Cooperative Oncology Group - performance status
EGFR	Epidermal growth factor receptor
EGFR+	EGFR protein expression
EGFR-	No EGFR protein expression
ERL	Erlotinib
FISH	Fluorescent in situ hybridisation
FISH+	High EGFR copy number
FISH-	Low EGFR copy number
G-CSF	Granulocite colony stimulating factor
GEF	Gefitinib
HR	Hazard ratio
ICH	International Conference on Harmonisation
IEC/IRB	Ethics committee/institutional review board
IHC	Immunohistochemical

ILD	Interstitial lung disease
INR	International normalised ratio
im	Intramuscular
iv	Intravenous
K-ras+	Presence of K-ras gene mutation
K-ras-	Absence of K-ras gene mutation
L	Liter
LD	Longest diameter
LDH	Lactate dehydrogenase
NCI-CTCAE	National Cancer Institute Common Toxicity Criteria for Adverse Events
NSAID	Nonsteroidal anti-inflammatory drugs
NSCLC	Non small cell lung cancer
OS	Overall survival
PCR	Polymerase chain reaction
PD	Progressive disease
PEM	Pemetrexed
PFS	Progression free survival
po	Per os
PR	Partialresponse
pts	Patients
QoL	Quality of life
RECIST	Response evaluation criteria in solid tumors
RR	Response rate
SAE	Serious adverse event
SD	Stable disease
SNP	Single nucleotide polymorphism
SUSAR	Suspected Unexpected Serious Adverse Reaction
TNM	Classification of malignant tumor
THY-α	Thymosin Alpha-1
TKI	Tyrosine kinase inhibitor
ULN	Upper limit of normal
WBC	White blood cell

1 INTRODUCTION AND STUDY RATIONALE

1.1 Introduction, background and rationale

Non Small Cell Lung Cancer (NSCLC) is the most common cause of cancer deaths worldwide and the development of more effective therapies remains challenging. Approximately 70% of NSCLC patients presents with advanced (stage III/IV) disease.

Despite the recent therapeutic progress the median survival of advanced disease rarely exceeds 8 month and less than 30% of pts is still alive at 12 months.

There is a consistent and important background of preclinical and clinical studies about Thymosin alpha 1 use in oncology aiming to highlight the importance of Thymosin alpha 1 as an immunotherapeutic tool to be used in combination with chemotherapy a concept that is not yet fully established in clinic.

The rationale of Thymosin alpha 1 in cancer treatment stems from the consideration that tumor progression is favored by a failure of the immune response and in turns induces immune suppression.

Thymosin alpha 1 is a naturally occurring thymic peptide of 28 aminoacids first described and characterized by Goldstein et al. (1,2).

Early included in the category of biological response modifiers his activity is related to the stimulation and/or maturation of various cells of the immune system. In fact many experimental data show that Thymosin alpha 1 induces the differentiation of human CD34 stem cells into CD3* 4 cells (3) and increases the ability of T cells to produce specific cytokines such as IFN-α, IFNγ, IL-2, IL-7, IL-10, IL-12 and IL-15 and regulates the expression of specific cytokine receptors such as high affinity IL-2 receptors (4,5).

Moreover Thymosin alpha 1 restores NK-cell activity in experimental models of immune suppression caused by chemotherapy radiation and tumor growth (6,7).

In several experimental preclinical murine tumor models such as Lewis Lung carcinoma(8,9) friend erythroleukemia (10), B16 melanoma (11), and DHD/K12 colorectal cancer liver metastasis models in rats the combined treatment of Thymosin alpha 1 with chemotherapy resulted in a powerful antitumor effect(12).

At the clinical level Chretien and Schulof showed for the first time the immune – restorative effect of Thymosin alpha 1 in lung cancer patients undergoing radiotherapy (13,14).

Many pilot studies performed in Italy in NSCLC with the addition of Thymosin alpha 1 to cisplatin/etoposide, and in melanoma with the addition of Thymosin alpha 1 to dacarbazine led to an improvement in response and immune parameters and to a decrease of toxicity (15-18).

The result obtained by these pilot studies encouraged further clinical evaluation.

A large randomized study involving a total of 488 patients with metastatic melanoma was performed by Maio et al. (17) for a better evaluation of the efficacy and safety of combining Thymosin alpha 1 with dacarbazine in these patients. The results of the study showed that the combined chemo - immunotherapeutic protocol induced an increase in median OS as well as in PFS, tumor response and duration of response. The authors concluded that potentiation of immune response with Thymosin alpha 1 in the absence of additional toxicity represents an important development toward improving clinical outcomes in patients with metastatic melanoma (17+expert opinion).

Multiple clinical studies on patients with NSCLC and treated with the combination of Thymosin alpha 1 and different chemotherapeutic regimens have been performed in China (19). They have been included recently in a systematic review and meta-analysis showing that the addition of Thymosin alpha 1 to cisplatin/vinorelbine induces an increase in overall tumor response rate (OR 1.86; 95% CI 1.08-3.20), tumor control rate (OR3.06; CI 1.36-6.88), 1-year survival rate (OR 3.05; CI 1.34-6.96), and quality of life (OR 3.39; CI 1.54-7.47).

The effects of the association of Thymosin alpha 1 with other treatment options were further investigated in patients affected by hepatocarcinoma (HCC). Trials comparing the safety and the efficacy of Thymosin alpha 1 plus Transcatheter Arterial Chemo Embolization (TACE) have been conducted in Italy, China and USA. (20-22)

In conclusion, in patients with unresectable HCC, TACE plus Thymosin alpha 1 resulted in numerically higher rates of survival and tumor response, including transplant candidacy, with fewer bacterial infections, than TACE alone.

The evidence accumulated in preclinical as well in clinical studies suggest that at least three factors contribute to render affective the chemo-immunotherapeutic control,

1. Direct action on tumor load

Chemotherapy reduces the tumor burden and, as a consequence, the quantity of tumor cells which could be eliminated by the stimulated immune effector cells.

Moreover the role of chemotherapy could be to release chemotactic molecules which attract specific cytotoxic lymphocyte, infiltrating at the tumor level.

The concentration of immune-specific effector cells in the area of tumor is an essential step for the immune control of tumor growth (8,9,11).

2. The increase of immune effector cell activity.

Many experimental data show that Thymosin alpha 1 induces T-cell maturation, increases the ability of T-cell to produce specific cytokines, activates DC system through the toll like receptors (TLRs), activates monocytes and tumor associated macrophages to a tumoricidal state via the enhancement of production of molecules (IL-I, TNF-α, reactive oxygen intermediates-ROI, nitric oxide-NO) inducing tumor cytotoxicity (23-25).

As a consequence of all the effects of Thymosin alpha 1 on immune cells is that, while activated DC and macrophages induce anti-tumor response and lead to more efficient antigen presentation, NK cells, together with CD4 helper Thymosin alpha 1and CD8 cytotoxic cells, acting in concert, lead to the killing of tumor cells.

Not less importantly, Thymosin alpha 1 increases lymphocytic infiltration to sites of disease and a correlation between tumor infiltrating lymphocytes and tumor prognosis has been documented.

3. Enhancement of tumor cell immunogenicity

Giuliani e al. (26) showed that treatment with Thymosin alpha 1 increases the expression of MHC class I surface molecules which present tumor antigen contributing to render tumor cells visible to immune effector cells and less prone to escape immune reactivity. Subsequent studies demonstrated that Thymosin alpha 1 is also capable of increasing expression of MHC Class II and other selected tumor specific antigens such as CEA, CSH275, DHD-K12 rat colon adenocarcinoma cell line, and Melan-A/Mart-I in a human melanoma cell line (27). Since the immune escape by tumor cells has been correlated with downregulation of tumor antigens and of antigen-presenting molecules, the new strategies for fighting tumors should be aimed at increasing both the activity of the immune-effector cells, through the modulation of cytokine expression, and the expression of specific tumor antigens at the level of tumor target cell.

In NSCLC, as in other solid tumors, patients undergo a consistent failure of the immune system and this state renders the tumor resistant to the therapy. The induction of immune tolerance within the tumor microenvironment is documented by the increased percentage of CD4+CD25 regulatory T cells (suppressive antitumor-specific Tregs) (28)and by the increase of CD123 BDCA2+pDCs. The latter observation might suggest that pDCs may be closely involved in the pathogenesis of NSCLC and may predict the progression of the disease (29). Indeed, patients with higher stages (III/IV) NSCLC had elevated levels of pDCs than those with lower stages (I/II).

Furthermore, CD123highBDCA2+HLA-DR+pDCs, key players in the regulation of the immune system and the differentiation of Treg, were recently shown significantly increased in NSCLC cases as compared to controls, (29).

Besides, it was reported that pDCs infiltrating NSCLC tissue are blocked at immature stage displaying low levels of costimulatory molecules and IFN-α secretion, thus suggesting that NSCLC cells might hamper the maturation of DCs, in turn, escaping an efficient immune response (30-32).

Employing an ex vivo multi-parametric multi-color flow cytometry analysis, here we propose to study whether the presence of Thymosin alpha 1 during the treatment of NSCLC patients, may impact on the proportion of circulating (proliferating versus apoptotic) $CD4^{pos}CD25^{bright}CD127^{low}FOXP3^{high}CD45RA^{low}$Treg and on $Lineage^{neg}CD123^{high}BDCA2^{+}HLA\text{-}DR^{+}$pDC frequency, thus affecting prognosis of disease.

Several mechanisms are involved in the ability of tumor cells to escape immune surveillance. The secretion of immune suppressive molecules may for instance weaken the host response against the tumor. Additionally, within tumor microenvironment, other molecules expressed in tumor, stromal, and endothelial cells can sustain tumor growth and spread. Among these molecules Galectin-1 (Gal-1), a β –galactoside binding secreted protein, is well recognized as modulator of essential biological processes in tumor cells such as angiogenesis, migration and metastasis (33). Moreover, Gal-1 is able to suppress tumor-specific immune response, maintaining an area of tumor immune privilege (34) as a consequence of the induction of T-cell apoptosis (35,36), favoring the expansion of T regulatory cells and contributing to their immunosuppressive activity, driving the differentiation of tolerogenic DC (37). Interestingly, the over expression of Gal-1 in NSCLC patients is associated with tumor progression and aggressiveness. In particular, tumor as well as stromal cells expression of Gal-1 represents an independent prognostic factor of poor outcome in NSCLC patients (38-40). Moreover, high serum levels of Gal-1 showed poor overall prognosis in these patients (Zhou et al, 2015). With

the present study we plan to investigate the impact of Thymosin alpha 1 on Gal-1 expression in NSCLC patients by the determination of serum levels before, during and after treatment, and the correlation with the response to therapy; data will be correlated with parameters of immune system activation/suppression to verify the ability of Thymosin alpha 1 to counteract the immunosuppressive state induced by tumor.

In addition, providing that Gal-I and Gal-9 are expressed on pDCs(41), and that both these immunoregulatory molecules are considered as prognostic factors in NSCLC (39), it would be interesting to investigate the expression and the balance of Gal-I and Gal-9 on pDCs of NSCLC patients and whether Thymosin alpha 1 treatment could regulate them.

Another aspect to take into account is that systemic inflammation driven by multiple pro-inflammatory cytokines is associated with cancer. In particular, the network of regulated upon activation normal T cell expressed and secreted (RANTES), IL-10 and IL-8 is dysregulated in NSCLC (42). Indeed, chronic inflammation is linked with the development of a number of solid cancers, including NSCLC. Tumor necrosis factor-α (TNF-α), being a tumor-promoting factor, is one of the major mediators of cancer-related inflammation, and it is linked to transformation, proliferation, angiogenesis and metastasis. Together with other inflammatory cytokines, TNF-α recruits additional inflammatory cells to the tumor microenvironment thus enhancing proliferation and survival of the transformed tumor cells (43). Beside TNF-α , IL-1β is also involved in cancer-related inflammation, by promoting angiogenesis and favoring a pro-metastatic environment. These cytokines in the tumor microenvironment promote migration and invasion and tumor host interactions. A hypoxic tumor microenvironment is a common feature of NSCLC, as is upregulation of vascular epithelial growth factor (VEGF), whose increased expression in alveolar epithelial cells contributes to the pathogenesis of inflammatory lung diseases and to the angiogenic phenotype of lung cancer (44). Finally, T cells have a central role in the regulation of systemic immune function, with a particular response and influence on cytokines (TNF-α, IL-1β, IL-2, IL-10 and interferon IFN-γ).

In the context of solid tumors, Yang et al.(45) demonstrated that Thymosin alpha 1 increases the percentage of Tregs and IL-1β, TNF-α, and IL-6 in vitro, in a population of gastric cancer patients.

In this study, we are planning to investigate the effect of Thymosin alpha 1 on the expression of pro-inflammatory cytokines and chemokines in plasma samples of NSCLC patients by using the Bio-plex system (Bio-Luminex).

1.1.1 Non-clinical Development

The initial non-clinical testing strategy for Thymosin alpha 1consisted of studies to confirm the pharmacodynamic effects, mechanism of action and potential side effects for pharmacovigilance. In addition, proof of concept studies were conducted for potential indications to investigate in the clinical setting. Non-clinical toxicology, absorption, distribution and metabolism studies were conducted to confirm the safety of Thymosin alpha 1 and to support licensure.

Pharmacokinetic (PK) studies were conducted with Thymosin alpha 1 in mice, rats, marmosets, and dogs by the SC route of administration, the intended clinical route.Studies demonstrated that Thymosin alpha 1 is rapidly absorbed and that kinetic parameters such as T_{max} and the serum half-life in various animal species are similar to that observed in man (see Investigator's Brochure).

Following SC administration of Thymosin alpha 1 to animals, the C_{max} was achieved in less than one hour; the serum half-life was also approximately one hour, suggesting rapid excretion; and there was no evidence of accumulation following repeated administration. Rapid absorption and rapid excretion of Thymosin alpha 1 are further substantiated by absence of retention of radioactivity in various tissues in distribution studies conducted in animals.These data demonstrate that there are no potential safety concerns related to kinetic parameters for repeated administration.

In vivo non-clinical toxicology studies have included single dose toxicity, 2-week, 13-week, and 26-week multiple-dose toxicity, special immune toxicity, and reproductive toxicity. Thymosin alpha 1 has also been tested for genotoxicity in *in vivo* and *in vitro* systems.In these studies, Thymosin alpha 1 has not demonstrated any drug-related adverse toxicity at the doses tested. A maximum tolerated dose has not yet been achieved in single or repeat-dose studies.

Non-clinical pharmacology and toxicology studies have been conducted with Thymosin alpha 1 in over 1000 animals of various species. In these studies, Thymosin alpha 1 has not demonstrated any drug-related adverse toxicity at any of the doses tested.

Conservatively, the marmoset no observable effect level (NOEL) of 1 mg/kg represents a 50-fold safety margin above the typical clinical and commercial dose (on body weight basis) of about

0.02 mg/kg (human dose of 1.6 mg). As the rodent studies were conducted at higher doses, and as these doses were also nontoxic, they provide a 1,000-fold safety margin. A similar safety margin is found from comparison of actual maximal concentration (C_{max}) and area under the curve (AUC) data. For example, extrapolation of human clinical PK data in adult volunteers provided values of 32 ng/mL for C_{max} and 175 ng.hr/mL for AUC at the clinical dose of 1.6 mg; marmoset dosing at 1 mg/kg leads to C_{max} of 1,220 ng/mL and AUC of 4050 ng.hr/mL, 40- and 30-fold safety margins, respectively. Therefore, the absence of any significant target organ toxicity in animal studies, including absence of mutagenic/teratogenic potential, provides a sufficient safety margin for the current Thymosin alpha 1 dose regimen of 1.6 mg twice daily for seven days.

1.1.2 Summary of Human Experience

1.1.2.1 Pharmacokinetic Studies

The pharmacokinetics of Thymosin alpha 1 in humans has been evaluated in two studies in healthy subjects and one study in cancer patients. These studies are summarized below and more details can be found in the Investigator's Brochure.

Single-dose and multiple-dose pharmacokinetic studies were conducted in healthy volunteers. Doses ranging from 0.8 to 6.4 mg were evaluated in single-dose studies and daily doses of 1.6 and 3.2 mg were evaluated in multiple-dose studies of 5 or 7 days' duration. Thymosin alpha 1 is rapidly absorbed with a T_{max} of approximately 2 hours. A dose-proportional increase was present in serum levels for C_{max} and AUC. The C_{max} values were 39, 63, 85 and 130 ng/mL and the AUC values were 124, 261, 314 and 679 ng.hr/mL, respectively, at 0.8, 1.6, 3.2, and 6.4 mg. Serum levels returned to basal levels by 24 hours after administration. The serum half-life was approximately 2 hours, and there was no evidence of accumulation following multiple administrations. The urine excretion accounted for up to approximately 50% to 60% of the single dose and 24% following multiple doses.

A multiple-dose study in healthy volunteers (at doses of 1.6, 8, and 16 mg twice weekly for 4 weeks) revealed doses to be well tolerated. A preliminary evaluation of serum drug levels of Thymosin alpha 1 indicated a dose-proportional increase; the approximate C_{max} levels were 30, 180, and 310 ng/mL at doses of 1.6, 8, and 16 mg, respectively. Peak levels occurred at 1 to 2 hours, and there was no evidence of accumulation.

In a PK study in lung cancer subjects, the subjects in the loading dose treatment arm showed similar results as above. Plasma levels returned to basal levels within 24 hours of administration and there was no evidence of accumulation.

1.1.2.2 Safety and Effectiveness in Humans

1.1.2.2.1 Summary of Safety

A summary of significant safety findings seen with use in humans is provided in Table 1.1 below, and shows that Thymosin alpha 1 has a clinically acceptable safety profile (further details can be found in the Investigator's Brochure). The majority of adverse experiences have been assessed as mild to moderate in severity, and consisted primarily of injection-site pain (includingburning) and erythema, as well as fever (pyrexia), nausea, and flu-like symptoms.

Clinical experiences involving the use of Thymosin alpha 1 for any indication, regardless of sponsorship, are included in the safety files or safety database (if provided by SciClone).

Human trials have demonstrated a high degree of safety for Thymosin alpha 1. Reported adverse events include abdominal pain, alopecia, alanine aminotransferase (ALT) elevation, asparagine aminotransferase (AST) elevation, anorexia, back pain, depression, dysgeusia, epistaxis, fatigue, headache, hematuria, injection site erythema, injection site pain, leucopenia, malaise, nausea, neutropenia, nipple pain, peripheral edema, pruritus, pyrexia, rectal hemorrhage, tremor, and weakness. Reported serious adverse events (SAEs) include acute pancreatitis, anemia, aplasia, atrial fibrillation, cryptococcal infection, dehydration, depression, diabetes mellitus, gastritis, hemoglobin decreased, hemorrhage, jaundice, peripheral edema, peripheral ischemia, pulmonary embolism, rash, retrobulbar neuritis, subileus, thrombocytopenia, and transient ischemic attack. Most of these SAEs occurred in single incidence.

A summary of safety findings seen with Thymosin alpha 1 use in humans is shown in Table 1.1, which includes all known information from over 75 studies conducted under US corporate INDs, physician INDs, local clinical experience trials in all countries, and marketed use.

Table 1.1: Integrated Summary of Thymosin alpha 1 Use in Humans

Subject Type	Number of Subjects[1]	Dose Range(mg)[2]	Duration/ Frequency	Route	Adverse Experiences Reported as Related to Thymosin alpha 1
Normal volunteers (single-dose, multiple-dose, bioequivalence)	98	0.8-16	QD up to 7 days; BIW up to 15 weeks	s.c.	Pain at the injection site, which was mild and resolved after less than 30 minutes
Cancer (mostly melanoma, HCC, NSCLC; also gastric, breast, pancreatic, stomach, colon, rectal	1,006	1.0-6.4	QD up to 3 months; BIW up to 1 year	s.c.	Subject deaths attributed to underlying disease; nipple pain reported as related; AEs no greater in treated versus control subjects; possibly related AEs and SAEs
Acute infections (severe sepsis; fungal, viral, or bacterial infections)	872	1.6-3.2	BID up to 12 days; QD for up to 16 weeks	s.c.	None reported
Hepatic viral disease (CHB; CHC)	1,969	1.0-3.2	QD up to 2 weeks; TIW up to 6 months; BIW up to 12 months	s.c.	Subject deaths attributed to underlying disease; abnormal renal function was seen in 3 subjects and abdominal pain, anemia, fever, hernia, and pancreatitis was seen in 2 subjects; most AEs possibly related were in lower frequency in treated groups; rash reported as related in 2 subjects; nervous system SAEs seen in 6 subjects (2%) 2 subjects had TSH abnormalities; 1 subject attempted suicide; 1 subject gave birth to a baby with esophageal atresia; 1AE, nipple pain, was considered related
HIV	58	0.4-3.2	QD up to 10 weeks; BIW up to 1 year	s.c.	None reported
Vaccine augmentation (influenza vaccine in geriatric subjects; influenza and hepatitis vaccine in hemodialysis subjects)	416	1.6-6.4	Once per week for 2 weeks; BIW up to 6 weeks	s.c.	Minor local discomfort at the site of injection
DiGeorge anomaly, primary immunodeficiency	11	40 ug/kg – 1.6	BIW up to 6 months	s.c.	None reported
Total subjects treated with Thymosin alpha 1	**4,430**				

[1] Total estimated number of subjects treated with Thymosin alpha 1 in clinical investigational studies. Thymosin alpha 1 (ZADAXIN) is commercially available in certain countries in Asia, the Middle East and Latin America, but this table includes only persons treated in clinical studies outlined in the IB

[2] When appropriate, doses were converted to mg per person, using a range of 1.6-1.7 m^2 per person

Abbreviations: AZT = azidothymidine; BIW = twice per week; CHB = chronic hepatitis B; CHC = chronic hepatitis C; HCC = hepatocellular carcinoma; HIV = human immunodeficiency virus; IFN = interferon; i.m. = intramuscular; m = meter; mg = milligram; s.c. = subcutaneous; NSCLC = non-small cell lung cancer; Thymosin alpha 1 = thymosin alpha 1; TIW = three times per week; QD = every day; wks = weeks; yr = year

Thus far, the accumulated animal and human data, including toxicology data and pharmacokinetic data, do not suggest any special considerations for future clinical trials.

1.1.2.2.2 Efficacy Studies

Summary

Clinical trials of Thymosin alpha 1 as a primary or an adjunctive therapy indicate that it may have applications in a variety of indications. Thymosin alpha 1 has been studied as a primary treatment for severe sepsis; for viral or bacterial diseases, such as CHB, CHC, HIV; as an adjunct treatment for cancers, such as HCC, NSCLC, and melanoma; and as an enhancement to both hepatitis B and influenza vaccines in immune-depressed individuals. Thus far, clinical exposure to Thymosin alpha 1 includes over 75 trials (including numerous local country trials), and in post-marketing use in over 230,000 patients. Studies have been sponsored by SciClone Pharmaceuticals, Inc. (Foster City, CA; SciClone), Schering-Plough SPKK (SciClone's licensee in Japan), Alpha 1 Biomedicals (previous sponsor for US studies, Washington DC), SclavoS.p.A. (licensee in Italy), Sigma-tau S.p.A. (licensee in Italy), and Hoffmann-LaRoche (original sponsor for Thymosin alpha 1 cancer trials). In addition, a large number of subjects have been treated with Thymosin alpha 1 under physician-sponsored INDs.

Controlled clinical efficacy studies with Thymosin alpha 1 are tabulated in Table 1.2 below.

Table 1.2: Controlled Clinical Efficacy Studies with Thymosin alpha 1

Type of Study Study Identifier	Study Design and Type of Control	Test Product; Dosage Regimen; Route of Administration	Number of Subjects	Duration of Treatment
Non-small cell lung cancer				
US Phase 2 Schulof	Randomized, placebo controlled,comparison of 2 regimenspost-radiotherapy	Thymosin alpha 1 • 0.9 mg/m^2 SC BIW for up to 1 year (or relapse) • 0.9 mg/m^2 SC for 14 days, then BIW for up to 1 year (or relapse) Placebo Placebo SC for 14 days, then BIW for up to 1 year (or relapse)	41 total 15 Thymosin alpha 1 BIW 13 Thymosin alpha 1 loading dose then BIW 13 placebo BIW	Up to 1 year
Hepatocellular carcinoma				
US Phase 2 THYMOSIN ALPHA 1-HCC-2K1001	Combination with TACEcompared to TACE alone	Thymosin alpha 1 - ZADAXIN 1.6 mg 5 times a week for up to 6 months	28 total 14 Thymosin alpha 1 + TACE 14 TACE alone	Up to 6 months
Metastatic malignant melanoma				
Europe Phase 2 ST1472-DM-01-012	Multi-center,randomized,open-label,dose-ranging,combination with DTIC or DTIC plus IFN-alpha,comparison to DTIC plus IFN-alpha	Thymosin alpha 1 - ZADAXIN • 1.6 mg, SC • 3.2 mg, SC • 6.4 mg, SC Days 8-11 and 15-18 of each 28-day cycle for up to 6 cycles	488 total 97 at 1.6 mg Thymosin alpha 1 plus IFN/DTIC 97 at 3.2 mg Thymosin alpha 1 plus IFN/DTIC 98 at 6.4 mg Thymosin alpha 1 plus IFN/DTIC 99 at 3.2 mg Thymosin alpha 1 plus DTIC 97 IFN/DTIC	Up to 6 months
Severe sepsis				
China Wu Study[1] NCT 00711620	Multi-center,randomized,single-blind,controlled	Thymosin alpha 1 - ZADAXIN 1.6 mg SCtwice daily for 5 days, then once per day for 2 days Control Normal saline	367 total 183 Thymosin alpha 1 184 control	7 days

Chronic hepatitis B				
Taiwan Phase 3 SC92-1A	Multi-center,randomized, controlled ,open label	Thymosin alpha 1 - ZADAXIN 1.6 mg SC BIW for 6 or 12 months	158 total 51 Thymosin alpha 1 (6 months) 54 Thymosin alpha 1 (12 months) 53 untreated control	Up to 1 year
US Phase 3 Mutchnick	Multi-center,randomized,double-blind,placebo-controlled	Thymosin alpha 1 1.6 mg SC BIW for 6 months Placebo Placebo SC BIW for 6 months	99 total 50 Thymosin alpha 1 49 placebo	6 months
Italy Phase 2 Andreone	Randomized,controlled, comparison to IFN-alpha	Thymosin alpha 1 0.9 mg/m^{2}SC BIW for 6 months IFN-alpha 5 million units SC TIW for 6 months	48 total 17 Thymosin alpha 1 16 IFN-alpha 15untreated control (historical)	6 months
US Phase 2 Mutchnick	Randomized,double-blind,placebo-controlled	Thymosin alpha 1 • 0.9 mg/m^{2}SCBIW for 6 months • 1.2 mg/m^{2}SCBIW for 6 months Placebo Placebo SC BIW for 6 months	20 total 12 Thymosin alpha 1 8 placebo	6 months
Chronic hepatitis C				
Italy Phase 3 ST1472-DM-03-004	Multi-center,randomized, double-blind,placebo-controlled,combination treatment with PEG-IFN-alpha and ribavirin	Thymosin alpha 1 - ZADAXIN 1.6 mg SC BIW for 48 weeks Placebo Placebo SC BIW for 48 weeks	553 total 275 Thymosin alpha1 277 placebo	48 weeks
US Phase 3 THYMOSIN ALPHA 1-CHC-2K0803a (non-cirrhotic subjects)	Multi-center,randomized,double-blind,placebo-controlled,combination treatment with PEG-IFN-alpha	Thymosin alpha 1 - ZADAXIN 1.6 mg SC BIW for 48 weeks Placebo Placebo SC BIW for 48 weeks	534 total 269 Thymosin alpha1 265 placebo	48 weeks

US Phase 3 THYMOSIN ALPHA 1-CHC-2K0804 (compensated subjects)	Multi-center,randomized,double-blind,placebo-controlled,combination treatment with PEG-IFN-alpha	Thymosin alpha 1 - ZADAXIN 1.6 mg SC BIW for 48 weeks Placebo Placebo SC BIW for 48 weeks	527 total 264 Thymosin alpha1 263 placebo	48 weeks
US Phase 3 Sherman	Multi-center,randomized,double-blind, double-placebo-controlled,combination treatment with IFN-alpha	Thymosin alpha 1 - ZADAXIN 1.6 mg SC BIW for 6 months plus IFN Placebo Placebo SC BIW for 6 months plus IFN or plus IFN placebo	110 total 36 Thymosin alpha 1 37 placebo 37 double placebo	6 months
Human immunodeficiency virus infection				
Italy Phase 2 2445	Multi-center,randomized,open-label, combination with IFN-alpha and AZT	Thymosin alpha 1 2 mg SC BIW for 12 months	92 total 31 Thymosin alpha 1 plus AZT/IFN-alpha 23 AZT/IFN-alpha 38 AZT	12 months
Vaccine enhancement				
Europe Pilot ST1472-DM-09-005 (Patients on chronic dialysis with ESRD)	Single site, randomized,open-label, dose-ranging, adjuvant to influenza vaccination (2009 H1N1 vaccine)	Thymosin alpha 1 - ZADAXIN • 3.2 mg, SC • 6.4 mg, SC On Day -7 and Day 0 (vaccine on Day 0)	121 total 40 at 3.2 mg Thymosin alpha 1 plus vaccine 42 at 6.4 mg Thymosin alpha 1 plus vaccine 39 vaccine	One week
US Study (Elderly subjects)	Single site,randomized,double-blind, placebo-controlled, adjuvant to influenza vaccination (1987 trivalent vaccine)	Thymosin alpha 1 • 0.9 mg/m^2SCBIW 8 doses • 0.9 mg/m^2SCBIW 4 doses plus 4 doses placebo Placebo Placebo BIW for 8 doses	330 total 111 at 8 doses Thymosin alpha 1 110 at 4 doses Thymosin alpha 1 plus 4 doses placebo 109 at 8 doses placebo	1 month

US Study (Patients on chronic dialysis with ESRD)	Single site,randomized,double-blind,placebo-controlled,adjuvant to hepatitis B vaccination (Heptavax vaccine)	Thymosin alpha 1 • 0.9 mg/m^{2}SC BIW for 5 injections after each of 3 vaccinations Placebo Placebo BIW for 5 injections after each of 3 vaccinations	23 total 11 Thymosin alpha 1 12 placebo	3 months

Abbreviations:AZT = azidothymidine; BIW = twice per week; DTIC = dacarbazine; IFN = interferon; m = meter; mg = milligram; PEG = polyethylene glycol-modified; SC = subcutaneous; Thymosin alpha 1= Thymosin alpha 1; TACE = transarterial chemoembolization; TIW = three times per week

Abbreviations:APACHE II = Acute Physiology and Chronic Health Evaluation; ICU = intensive care unit; ID = study identification; mHLA = monocyte histocompatibility antigen; PI = protease inhibitor; QD = every day; Thymosin alpha 1= thymosin alpha 1; vs = versus

Summary of pre-clinical and clinical use of Thymosin alpha 1 in Lung Cancer.

Soon after the discovery of the immune-reconstituting potential of Thymosin alpha 1 the idea of the possible use in cancer therapy came out. Several pre/clinical studies have shown the anti-tumor activity of Thymosin alpha 1 when used in combination with chemotherapeutic agents. Amongst these studies, very interesting results was obtained in murine tumor model such Lewis Lung Carcinoma (3LL).

Moody et. Al (47) have shown that Thymosin alpha 1 inhibited the growth of NSCLC in vitro and in vivo suggesting that Thymosin alpha 1 directly interacts with NSCLC cells.

The effectiveness of a combined treatment protocol with cyclophosphamide (CY) followed by Thymosin alpha 1 plus interferon in the regression of 3LL murine tumor is clearly shown.

The effectiveness of this combined protocol was evident on the long-term survival in a high percentage of animals. The same combination therapy strongly stimulated natural killer activity and cytotoxicity against 3LL tumor cells.

Histological analysis demonstrated a strong increase of tumor-infiltrating lymphocytes in the same group when compared to the other treatments (8).

In a subsequent study was demonstrated that combined administration of Thymosin alpha 1 plus Interleukin-2 after CY treatment induced complete tumor regression.

Depletion of immune cells using either total-body or antibodies directed against T cells (anti-CD4 and CD8 or NK cells population) induced a loss of anti-tumor effect. It is worth mentioning that the tumor regression was induced without the appearance of toxicity or side effects. Histological analysis of the tumor revealed a high member of infiltrating lymphoid cells surrounding area of necrosis (9).

At the clinical level Schulof at al. (14) performed the first randomized trial to evaluate the immunorestorative properties of Thymosin alpha 1 in patient with lung cancer.

The radiotherapy induced immuno -suppression and a T cell lymphopenia characterized by an evident impairment of T cell function. Patients treated with Thymosin alpha 1 exhibited a normalization of T cell function. Moreover, Thymosin alpha 1 treatment was associated either significant improvements in relapse-free and overall survival (14).

A systematic review and metanalysis of randomized controlled trials of Thymosin plus Cisplatin with vinorelbine (NP) or gemcitabine (GP) for non small cell lung cancer was performed by Jin Jiang et al. (19).

10 randomized controlled trials including 724 patients were eligible for metanalysis. Compared with an NP treatment alone, Thymosin plus NP could increase overall response rate, tumor control rate and 1 year compared with a GP program alone Thymosin plus GP could improve ORR, TCR, QoL and increased the function of immune system. As a consequence Thymosin plus NP/GP is a better choice for patients with advanced NSCLC than NP/GP alone.

A phase II study was performed to evaluate the clinical and immunological effects of a regimen of cisplatin and etopside combined with Thymosin alpha 1 and low/dose of interferon α in the treatment of patients with advanced NSCLC.

Median survival was 12,6 month, 24 were the responses (2 complete, 22 partial). Overall the treatment was well tolerated.

In a phase II controlled trial in NSCLC the combined treatment with Thymosin alpha 1 and low–dose interferon α after ifosfamide is able to enhance response rate, and to induce a difference with chemotherapy treated patients statistically significant. Toxicity was drastically reduced in patients treated with combination therapy (19).

Taken together the preclinical data on murine tumor lung 3LL and clinical data a NSCLC show an effective role of Thymosin alpha 1 in strongly potentiating an anti/tumor response. The data suggest that combined treatment of chemotherapy and Thymosin alpha 1 is fundamental.

Many factors exposed in the introduction of this protocol, explain the synergistic mechanism underlying this treatment.

New knowledges about the immunosuppressive environment induced by the NSCLC such as Galectin-1 further push to the use of Thymosin alpha 1 with the aim of counteract the failure of immune response present in NSCLC. In fact Thymosin alpha 1 may inactivate Gal-1 (data not published, personal communication). But a critical point is represented by dosage of Thymosin alpha 1. In mice comparatively to human the selected dosage is higher than in human; furthermore, pre-clinical data show that an increasing dosage of Thymosin alpha 1 increase its own efficacy (11). Even at clinical level, the Maio study has shown an increased response and better PFS with dosage of 6,4mg and 3,2 mg of Thymosin alpha 1. Overall Thymosin alpha 1 is

well tolerated, also for prolonged administration (five time a week for six months)(21). For these reasons, we would like to study Thymosin alpha 1 treatment in this particular setting of patients, through two step period (5 administration/week for 4 months followed by 2 administration/week for 8 months).

1.1.2.3 Standard of Care (SoC)

Platinum doublets are the standard of care for the treatment of patients with goodperformance status (ECOG 0 or 1), advanced or metastatic, previously untreated NSCLC patients who lack an EGFR sensitizing (activating) mutation and ALK translocation. These platinum doublets include a platinum compound in combination with gemcitabine,pemetrexed, docetaxel, or bevacizumab. These combinations have demonstrated OS gain, improved quality of life, and control of disease related symptoms compared tosingle agent regimens, with several combinations demonstrating similar efficacy.

Approval for gemcitabine in combination with cisplatin was granted based upon dataobserved in phase 3 randomized controlled trial of gemcitabine in combination with cisplatinwhich demonstrated a significant improvement in response rate (RR) (30.4% compared with 11.1%, respectively; P < .0001), median TTP disease (5.6 months comparedwith 3.7 months, respectively; P = .0013), and OS (9.1 months compared with7.6 months, respectively, P = .004), compared to single agent cisplatin.

A randomized phase 3 study of gemcitabine + cisplatin vs gemcitabine + carboplatin (1200mg/m(2) on days 1 and 8, followed on day 1 by cisplatin 80 mg/m(2) or carboplatin AUC=5, each for a maximum of 6 cycles or until disease progression or unacceptable toxicity), inpreviously untreated stage IIIB and IV NSCLC subjects demonstrated overall response rates,median time to disease progression, response duration and survival of 41/29%, 5.87/4.75 months, 7.48/5.15 months, and 8.75/7.97 months for the gemcitabine/cisplatin and gemcitabine/carboplatin arms, respectively. Toxicity profiles were similar across the twocombinations with the only significantly different grade 3/4 toxicities including nausea and vomiting in the gemcitabine + cisplatin combination and thrombocytopenia in the gemcitabine + carboplatin combination. The comparable efficacy and toxicity between these two combinations indicate that either platinum in combination with gemcitabine is acceptable for the treatment of advanced or metastatic, previously untreated NSCLC.

In addition, the phase 3 trial proposed will offer the choice of pemetrexed in combination with either carboplatin or cisplatin for a maximum cycles followed by optional pemetrexed maintenance as well as carboplatin in combination with paclitaxel followed by optional pemetrexed maintenance. Pemetrexed will only be permitted in subjects with NSCLC non squamous histologies, since both the FDA and EMA approval is limited to this histology. The approval was based upon the phase 3, non inferiority trial in first line, advanced NSCLC subjects that compared the OS of pemetrexed plus cisplatin to gemcitabine plus cisplatin. Overall survival for the cisplatin/pemetrexed was non inferior to the cisplatin/gemcitabine combination with

median survivals of 10.3 months in both treatment arms (adjusted hazard ratio 0.94 (95 percent CI: 0.84, 1.05)). The median PFS was 4.8 and 5.1 months for the pemetrexed + cisplatin and gemcitabine + cisplatin arms, respectively (adjusted hazard ratio 1.04 (95 percent CI: 0.94,1.15)). The overall RR were 27.1 percent and 24.7 percent for the pemetrexed + cisplatin and gemcitabine + cisplatin arms, respectively.

Further gains in OS have been achieved with the administration of pemetrexed maintenance. A phase 3, double-blind study of maintenance pemetrexed plus best supportive care (BSC) versus placebo plus BSC was conducted in subjects with NSCLC whose disease had not progressed following 4 cycles of cisplatin + pemetrexed induction. Subjects were randomized 2:1 to either pemetrexed + BSC or placebo + BSC, which were continued until disease progression or toxicity. The median OS for subjects in the intent-to-treat (ITT) group was 13.4 months for subjects receiving pemetrexed and 10.6 months for those receiving placebo (hazard ratio (HR) of 0.79 (95 percent CI: 0.65, 0.95, p=0.012)). Median OS was15.5 months versus 10.3 months for subjects with non-squamous NSCLC receiving pemetrexed or placebo, respectively (HR of 0.70 (95 percent CI: 0.56, 0.88)). The median OS in subjects with squamous cell NSCLC receiving pemetrexed was 9.9 months versus 10.8 months for those receiving placebo (HR of 1.07 (95 percent CI: 0.77,1.50)). These findings have established pemetrexed maintenance following completion of six cycles of a platinum doublet induction as a safe and efficacious regimen in patients with advanced or metastatic non-squamous NSCLC.

A meta-analysis of randomized controlled clinical trials compared chemotherapy regimens containing either cisplatin or carboplatin in combination with third generation antineoplastic agents including docetaxel, paclitaxel, and gemcitabine. Cisplatin containing regimens were associated with a median survival of 9.1months and a 1-year survival probability of 37%, while carboplatin containing regimens wereassociated with a median survival of 8.4 months and a 1-year survival probability of 34%.

These data support the interchangeable use of carboplatin or cisplatin in combination with standard of care antineoplastic agents and should be offered as options forthe treatment of advanced or metastatic NSCLC patients that have not previously received systemic cytotoxic therapies for their metastatic disease.

The platinum containing regimen of carboplatin in combination with paclitaxel will also beincluded as a control chemotherapy option. Multiple phase 3 studies have demonstrated similar efficacy for carboplatin plus paclitaxel compared to other platinum doublets, including ECOG 1594. This is especially significant given that treatment options arelimited for squamous histologies.

Bevacizumab containing regimens will be included among the SOC treatment.VAiL, a pivotal phase 3, demonstrated improved progression free survival of both a lowand high dose of bevacizumab in combination with gemcitabine and cisplatin. Overall survival was not statistically significant. E4599, a pivotal phase 3, demonstrated improved overall survival of bevacizumab in combination with carboplatin and paclitaxel.

The above data support multiple platinum containing chemotherapy comparators as control options and include paclitaxel, gemcitabine, and pemetrexed containing platinum regimens.

2 OBJECTIVES

2.1 Primary Objective

Primary Objective

To evaluate the activity/efficacy in terms of PFS of SoC chemotherapy with cisplatin (or carboplatin) and either Pemetrexed, Paclitaxel, Gemcitabine, Vinorelbine or Bevacizumab + Thymosin alpha 1 in patients with advanced EGFR wild type NSCLC as compared to a control group of patients receiving SoC chemotherapy alone.

3 STUDY DESIGN

Phase II, multi-center, open label, randomized, parallel group study to determine the activity/efficacy of Thymosin alpha 1 in patients with advanced EGFR wild type metastatic NSCLC taking SoC chemotherapy (cisplatin (or carboplatin) and either Pemetrexed, Paclitaxel, Gemcitabine, Vinorelbine, Bevacizumab) versus SoC chemotherapy alone.

Approximately *140* patients (Arm A: approx 70 SoC chemotherapy + Thymosin alpha 1, Arm B: approx 70 SoC chemotherapy alone) will be enrolled with Thymosin alpha 1 1.6 mg subcutaneously five time a week for four months following by Thymosin alpha 1 1.6 mg subcutaneously two time a week for eight months plus SoC chemotherapy with cisplatin (or carboplatin), and either Pemetrexed, Paclitaxel, Gemcitabine,Vinorelbine or Bevacizumab. Control group will be treated with SoC with cisplatin (or carboplatin), and either Pemetrexed, Paclitaxel, Gemcitabine,Vinorelbine or Bevacizumab alone.

Recruitment duration: Approximately 8 months

Length of treatment: 12 months.

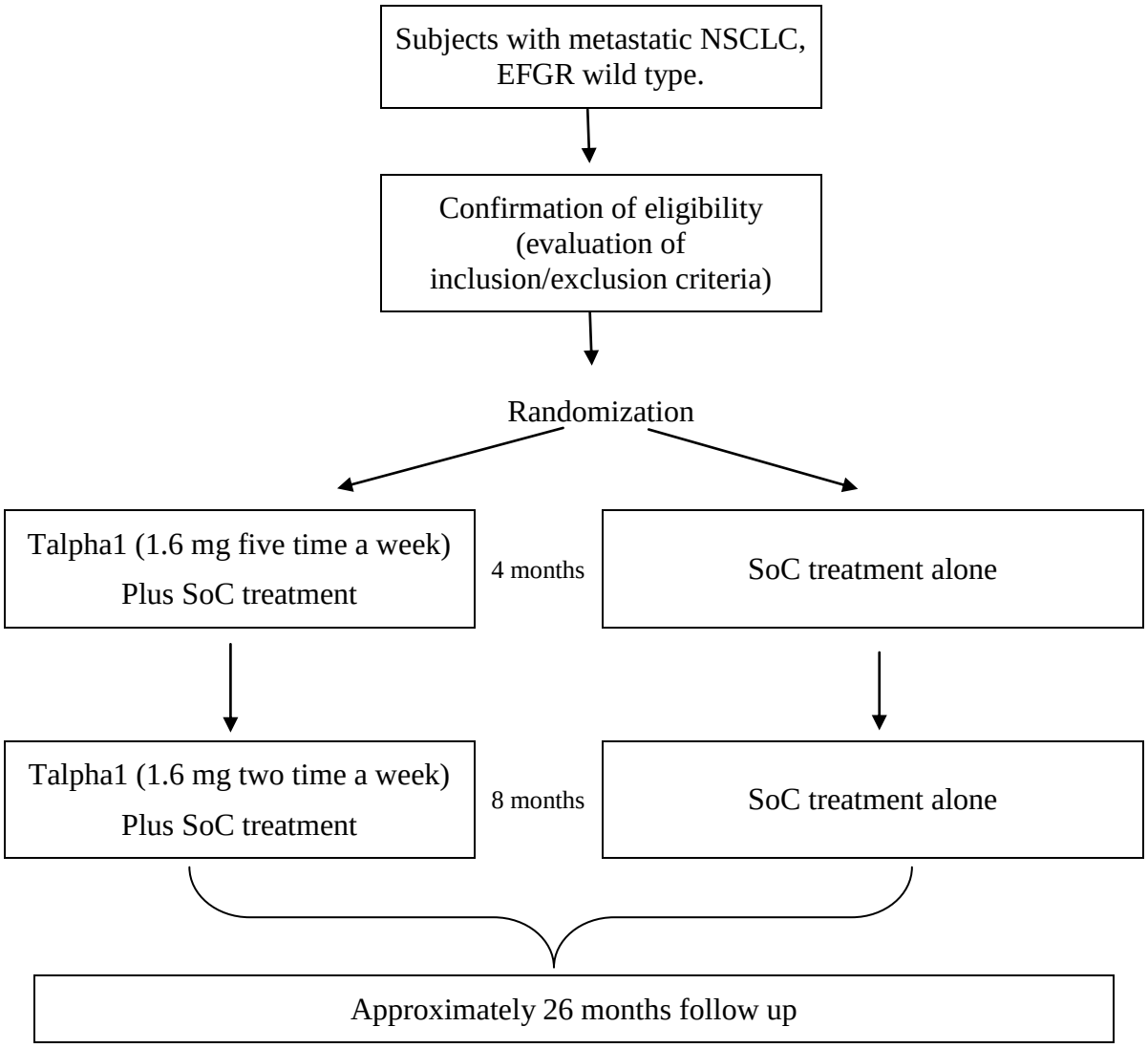
Follow-up for PFS: Approximately 24 months from last patient enrolled

Follow-up for survival: Approximately 26 months from last patient enrolled

Total study duration: Approximately 40 months

A schema outlining the study design is presented in Figure 3-1.

Figure 3-1: Study Design Schema



4 ENDPOINTS

4.1 Primary Endpoint

To assess the activity/efficacy in terms of Progression-Free Survival (PFS) of Thymosin alpha 1+ SoC chemotherapy/cisplatin (or carboplatin), Pemetrexed, Paclitaxel, Gemcitabina, Vinorelbine or Bevacizumab in patients with advanced EGFR wild type NSCLC as compared to a control group of patients receiving SoC chemotherapy/cisplatin (or carboplatin), Pemetrexed, Paclitaxel, Gemcitabina, Vinorelbine or Bevacizumab alone.

4.2 Secondary and Exploratory Efficacy Endpoints

- time to Overall Survival (OS);
- Quality of Life (QoL);
- organ failure free days;
- biomarkers of immunity and inflammation.

4.3 Other measures:

- Adverse events (AEs), chemistry, hematology, coagulation, ECG intervals and vital sign data.
- Biological markers (in particular immunological markers, circulating immune cells); cytokines and inflammatory factors: TNF-α IL-1β, IL-2, IL-6, IL10, IFNγ and VEGF, $CD4^{+}CD25^{high}CD127^{-}FoxP3^{+}$, CBC, D-dimer, fibrinogen, ultrasensitive C-reactive protein; Thymosin and Galectin levels determination.

5 STUDY POPULATION

5.1 Inclusion Criteria

- ✓ Age 18 years or older
- ✓ Histological or cytological confirmation of NSCLC (may be from initial diagnosis of NSCLC or subsequent biopsy). Only patients with available tissue samples will be enrolled
- ✓ Locally advanced or metastatic NSCLC, not amenable to curative surgery or radiotherapy
- ✓ Measurable disease by Response Evaluation Criteria In Solid Tumours (RECIST) in a

lesion not previously irradiated or non-measurable disease

- ✓ Eastern Cooperative Oncology Group - performance status (ECOG-PS) 0-2
- ✓ Absolute neutrophil count (ANC) > 1.5 x 10^9/liter (L) and platelets > 100 x 10^9/L
- ✓ Bilirubin level either normal or <1.5 x ULN
- ✓ AST (SGOT) and ALT (SGPT) <2.5 x ULN (≤ 5 x ULN if liver metastases are present)
- ✓ Serum creatinine <1.5 x ULN
- ✓ Effective contraception for both, male and female pts, if the risk of conception exists
- ✓ Recovery from all acute toxicities of prior therapies
- ✓ Provision of written informed consent.

5.2 Exclusion Criteria

- ✓ Prior therapy with Thymosin alpha-1
- ✓ Newly diagnosed central nervous system (CNS) metastases that have not yet been treated with surgery and/or radiation. Pts with previously diagnosed and treated CNS metastases or spinal cord compression may be considered if they have evidence of clinically stable disease (SD) (no steroid therapy or steroid dose being tapered) for at least 28 days
- ✓ Pregnancy or suspected pregnancy
- ✓ Any unresolved chronic toxicity from previous anticancer therapy that, in the opinion of the investigator, makes it inappropriate for the patient to be enrolled in the study
- ✓ Known severe hypersensitivity to a study drugor any of the excipients of this product
- ✓ Other co-existing malignancies or malignancies diagnosed within the last 5 years with the exception of basal cell carcinoma or cervical cancer in situ
- ✓ Any evidence of clinically active interstitial lung disease (ILD) (patients with chronic, stable, radiographic changes who are asymptomatic or patients with uncomplicated progressive lymphangiticcarcinomatosis need not be excluded)
- ✓ As judged by the investigator, any evidence of severe or uncontrolled systemic disease (e.g., unstable or uncompensated respiratory, cardiac, hepatic or renal disease)
- ✓ As judged by the investigator, any inflammatory changes of the surface of the eye
- ✓ Evidence of any other significant clinical disorder or laboratory finding that makes it undesirable for the patient to participate in the study

5.3 Removal of Subjects

In accordance with the Declaration of Helsinki, subjects have the right to withdraw from the study at any time for any reason. The Investigator and Sponsor also have the right to withdraw subjects from the study. Subjects may be removed from the study for the following reasons:

- Adverse events
- At the request of the Investigator or Sponsor
- Subject unwilling or unable to comply with study protocol (noncompliance)
- Termination of the study by the Sponsor

If the reason for removal of a subject from the study is an adverse event (AE), the specific event and any related test results will be recorded on the electronic CRF (eCRF). The subject will be followed until the AE resolves, becomes chronic, or the subject is lost to follow up. If a subject dies, the date of the last dose of clinical trial material (CTM) and all observations collected up to the time of study termination will be recorded on the eCRF as will be the reason for death.

If a subject or the Investigator chooses to discontinue CTM, the Investigator should inquire whether the subject will agree to participate in remaining study evaluations.

If a subject is removed from the study at the request of the Sponsor or Investigator, the date of the last dose of CTM and all observations collected up to the time of termination will be recorded on the eCRF along with the reason for termination, and scheduled safety evaluations and follow-up examinations will be conducted, if possible.

The date that a subject discontinues and the reason for discontinuation will be recorded in the eCRF. Subjects who discontinue after randomization will not be replaced. If a subject withdraws consent from the study during treatment, follow-up visit study procedures should be conducted.

If a subject fails to return for follow up visits, a reasonable effort should be made to contact him/her to determine the reason for the failure to return. Three telephone attempts, including the date and time, should be documented in the subject's medical chart or study source documents. If there is no response to the telephone calls, a certified letter should be sent. After these efforts have been exhausted, the subject can be identified as lost to follow-up in the eCRF.

6 STUDY TREATMENT

6.1 Identity of Clinical Trial Material

Thymosin alpha 1 (ZADAXIN®) is contained, stored, and dispensed from individual tamper-proof glass vials with 1.6 mg Thymosin alpha 1 as a lyophilized cake containing 5% mannitol, buffered with phosphate to pH 6.4 -7.3. Vials are reconstituted with 1 mL of diluent (sterile Water for Injection), prior to subcutaneous (SC) administration.

6.2 Labeling and Packaging

Thymosin alpha 1 (ZADAXIN®) lyophilisate for solution for injection is a sterile, white to off-white lyophilized powder, containing Thymosin alpha 1 manufactured by solid-phase peptide synthesis.

The CTM intended for investigational use should be stored in a locked area with limited access, refrigerated between 2°C and 8°C.

6.3 Study Drug Accountability

The Investigator or designee is responsible for ensuring adequate accountability of all used and unused CTM. This includes acknowledgement of receipt of each shipment of CTM (quantity and condition), and documentation of dispensing, returns, and destruction of CTM. Dispensing records will document quantities of CTM received. Quantities dispensed will be documented, including kit number, date dispensed, subject identification number, and the initials of the person dispensing the CTM.

Throughout the course of the study, the Sponsor or its designee will perform study drug accountability and provide instructions for adequate CTM disposal. Study drug accountability records must be readily available for inspection by the Sponsor or its designee and for inspection by regulatory authorities at any time. The Investigator will not allow CTM to be given to any subject not randomized into the study or any unauthorized person.

It is the responsibility of the site personnel to maintain adequate CTM dispensing records.

6.4 Method of Assigning Subjects to Treatment

The subjects who meet all inclusion and exclusion criteria will be randomized 1:1 to receive 1.6 mg Thymosin alpha 1 (ZADAXIN) in 1 mL doses to be delivered five times a week via SC injection for 4 months followed by 1.6 mg Thymosin alpha 1 (ZADAXIN) in 1 mL doses to be delivered two times a week via SC injection for 8 months plus SoCchemotherapy withcis-platinum (or carboplatin) (experimental group) or SoC chemotherapy cis-platinum (or carboplatin) alone (control group). Patients will be randomized centrally through the automated 24-hour Interactive Web-based Response System (IWRS) system and will be stratified by centre.

6.5 Selection of Doses

Thymosin alpha 1 will be add to chemotherapy Standard of Care treatment and it will be administered SC at doses of 1.6 mg to be given:

a) five times a week for the first 4 months *and*

a) two times a week for the following 8 months.

This dose is supported by safety studies which show that Thymosin alpha 1 is well tolerated; in particular, no safety signals were seen in studies in severe sepsis (1.6 mg Thymosin alpha 1 twice daily evaluated in 402 subjects),severe acute pancreatitis (3.2 mg twice daily for 7 days in 12 subjects), or hematopoietic stem cell transplant (1.6 mg Thymosin alpha 1every day for 16 weeksin 30 subjects). No safety signals were seen in studies which enrolled subjects with: acute respiratory disease due to cytomegalovirus (CMV) or pneumonia, chronic obstructive pulmonary disease, severe lung infections, ESRD requiring hemodialysis, chronic infections (hepatitis B, hepatitis C, HIV/AIDS), cancer and those being treated with chemotherapy, or elderly subjects.

PK studies show that there is no accumulation of Thymosin alpha 1 and the $T_{1/2}$ is only 2 hours.

Support for the safety of the proposed dosing regimen is also seen in the nonclinical safety studies, which showed no adverse effects in rodents (mice and rats) at doses up to 20 mg/kg/day for acute dosing or 6 mg/kg/day for 6 months repeat dosing; and no adverse effects were seen in marmosets dosed at 1 mg/kg/day for 6 months of repeat dosing. Conservatively, the marmoset NOEL of 1 mg/kg/day represents a 25–fold safety margin over dosing of 1.6 mg twice daily.

6.6 Concomitant therapy

All concomitant therapies, including anaesthetic agents, vitamins, homeopathic/herbal remedies, nutritional supplements, must be recorded in the patient's chart during the screening and treatment period, starting from the date of signature of informed consent, and ending at the Follow-Up Visit (FU). Any concomitant therapy usedfrom the time a subject signs an ICF through the months 4 or 12 must be recorded on the eCRF. In addition, for any SAEs that require medication for treatment, those medications must be recorded on the concomitant medications page. The medication name, dose, route of administration, start and stop dates and indication for use must be recorded. Additional experimental anti-cancer treatment and/or standard chemo-, immunotherapy, or radiotherapy (other than palliative radiotherapy for symptom control) is not allowed concomitantly with the administration of trial treatment.

7 ASSESSMENTS

7.1 Activity/efficacy assessments

In addition to 12 month all-cause mortality, the following assessments will be collected and recorded:

- SOFA and APACHE II scores (Month1,2,3,4,5,6,7,8,9,10,11 and 12)
- Incidence of secondary diseases/malignancies
- ICU length of stay
- Hospital length of stay
- Hospital re-admissions
- Organ failure-free days
- Quality of Life (QoL – EORTC QLQ-C30; EORTC LC13; EQ-5D 3L)
- CD4, CD8, CD4/CD8, NK, Treg cell count, cytokines

Assessment for secondary diseases/malignancies will be based on documentation in the eCRF. Sites will be required to record all diseases/malignancies occurring from 48 hours after enrollment until Month 12 of the study.

QoL will be evaluated with the EORTC QLQ-C30; EORTC LC13; EQ-5D 3L.

"Organ failure-free days" is defined as the days in which a study subject is alive and free of organ failure after study entry.

Samples will be collected and stored for future analysis of biomarkers,including TNF-a, IFNγ,IL-1β, IL-2, IL-6, IL-10, VEGF and anti-Thymosin alpha 1 antibodies.

The study will use a central laboratory for conducting CD4, CD8, CD4/CD8, NK, and Treg cell count, and for storing the samples for future analysis of cytokines (see Appendix A, Appendix B and specific Informed Consent on leftover).

7.2 Safety Assessments

Safety will be assessed by collecting and recording AEs, vital signs, ECGs, and laboratory parameters.

The clinical laboratory will indicate if laboratory values are out of normal ranges. The Investigator must assess all abnormal clinical laboratory results for clinical significance in a timely fashion. A notation of clinically significant (CS) or not clinically significant (NCS), with initials and date, will be documented on the respective laboratory report next to any abnormal value. An abnormal laboratory value of clinical significance will be considered and documented as an AE if, in the opinion of the Investigator, it is clinically significant and not considered part of the clinical condition.

Severity of AEs and laboratory abnormalities will be assessed according to the NCI-CTC AE Version 4 criteria.All AEs of ≥ Grade 4 severity will be considered SAEs (see Section 9.1 for the definition of *adverse event*, and Section 9.7 for the definition of *serious adverse event*). The Investigator will follow proper AE and SAE reporting procedures.

Vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate, will be measured and recorded at the baseline visit and every month until the end of the study. The timing of administration of all doses of CTM and evaluation of vital signs will be recorded on the CRF for all subjects on each day of treatment.

Electrocardiographic monitoring will be conducted with a 12-lead electrocardiogram (ECG) before the first dose of CTM on Day 0, and every Month hours after the first dose of CTM on those days.

Pregnancy will be evaluated by means of urine human chorionic gonadotropin (HCG) measurements at screening. Subjects will agree, as outlined in the ICF, to use appropriate contraception methods for up to 28 days after the last CTM dose.

8 STUDY PROCEDURES

8.1 Schedule of Events

A schedule of study procedures is located in Appendix A.

8.2 Screening

The following screening observations and procedures will be completed prior to randomization:

- Obtain a signed ECs-approved informed consent (ICF)
- Collect demographics information
- Obtain medical history
- Perform physical examination
- Assess a subject against the inclusion/exclusion criteria, including blood/ urine BHCG for subjects of child-bearing potential
- Confirm eligibility
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Conduct 12-lead ECG
- Perform ECHO or MUGA
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Determine Charlson co-morbidity and SOFA scores
- Perform Tumor assessment

All questions related to subject eligibility should be directed to the clinical center.

8.3 Randomization

Once eligibility has been confirmed, central randomization will be used. Randomization will be stratified by centre. Patients will be randomized into 1 of the 2 armsthrough the automated 24-hour Interactive Web-based Response System (IWRS) system.

8.4 Cycle1 - Study Procedures

If the screening lab test are within one week of range period, they could be consider valid also for T0.

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Hematology profile: complete blood count (CBC)
    - Chemistry profile: blood urea nitrogen (BUN), creatinine, glucose, sodium, potassium, chloride, phosphorus, albumin, total bilirubin, alkaline phosphatase, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), asparagine aminotransferase (AST), γ-glutamyltransferase (gGT), serum amylase, lipase, uric acid, creatinine kinase(CK), total proteins, protein electrophoresis, high sensitivity-C reactive protein (hs-CRP), CEA, CYFRA.
    - Coagulation assays: prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR), Fibrinogen, D-Dimer.
    - Urine analysis (pH, protein, glucose, ketones, occult blood, leukocyte esterase, nitrite, HCG for subjects of child-bearing potential)
- Draw samples for shipment to central lab:
    - Whole blood for storage 4.0 ml in EDTA
    - Whole blood for storage 7 ml in lithium heparin
    - Serum for storage 10 ml
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Administer first CTM dose

8.5 Cycle 2 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer, CEA CYFRA
- Draw samples for shipment to central lab:
    - Whole blood for storage 4.0 ml in EDTA
    - Whole blood for storage 7 ml in lithium heparin
    - Serum for storage 10 ml
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Tumor assessment

8.6 Cycle 3 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer

- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF

8.7 Cycle 4 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer, CEA, CYFRA
- Draw samples for shipment to central lab:
    - Whole blood for storage 4.0 ml in EDTA
    - Whole blood for storage 7 ml in lithium heparin
    - Serum for storage 10 ml
- BHCG (urine/blood) for subjects of child-bearing potential
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Quality of Life evaluation
- Tumor assessment

8.8 Cycle 5 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status

- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF

8.9 Cycle 6- Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer, CEA, CYFRA
- Draw samples for shipment to central lab:
    - Whole blood for storage 4.0 ml in EDTA
    - Whole blood for storage 7 ml in lithium heparin
    - Serum for storage 10 ml
- HCG (urine/blood) for subjects of child-bearing potential
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Tumor assessment

8.10 Cycle 7 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF

8.11 Cycle 8 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- BHCG (urine/blood) for subjects of child-bearing potential
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF

8.12 Cycle 9 - Study Procedures

- Perform physical examination

- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer, CEA, CYFRA
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Tumor assessment

8.13 Cycle 10 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Qulity of Life evaluation

8.14 Cycle 11 - Study Procedures

- Perform physical examination

- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF

8.15 Cycle 12 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer, CEA, CYFRA
- Draw samples for shipment to central lab:
    - Whole blood for storage 4.0 ml in EDTA
    - Whole blood for storage 7 ml in lithium heparin
    - Serum for storage 10 ml
- HCG (urine/blood) for subjects of child-bearing potential
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Tumor assessment

8.16 Cycle 13 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Quality of Life evaluation

8.17 Cycle 14 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF

8.18 Cycle 15 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Tumor assessment

8.18 Cycle 16 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- HCG (urine/blood) for subjects of child-bearing potential
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Quality of Life evaluation

8.19 Cycle 17 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF

8.20 Cycle 18 - Study Procedures

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer, CEA, CYFRA
- Draw samples for shipment to central lab:
    - Whole blood for storage 4.0 ml in EDTA
    - Whole blood for storage 7 ml in lithium heparin
    - Serum for storage 10 ml
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF

- Tumor assessment

8.21 In case of "early termination" of the study

- Perform physical examination
- Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate
- Perform an ECOG performance status
- Draw blood for local laboratory measurements:
    - Complete blood cell count
    - Inflammation parameters: albumin, total proteins, high sensitivity-C reactive protein (hs-CRP), Fibrinogen, D-Dimer, CEA, CYFRA
- Draw samples for shipment to central lab:
    - Whole blood for storage 4.0 ml in EDTA
    - Whole blood for storage 7 ml in lithium heparin
    - Serum for storage 10 ml
- HCG (urine/blood) for subjects of child-bearing potential
- Record any concomitant medications
- Record AEs and SAEs that have occurred since the time the subjects signed the ICF
- Perform Tumor assessment
- Quality of Life evaluation

9 ADVERSE EVENT REPORTING

Throughout the study, AEs will be recorded in the source documents and transcribed onto the appropriate pages of the eCRF regardless of whether the AEs are considered study treatment-related. All AEs with onset dates after the date the subject signed the ICF through the Month 12 will be recorded as an AE on the eCRF. Conditions existing prior to signing the ICF will be recorded as part of the subject's medical history. All SAEs with onset dates after the subject

signs the ICF through the Month 12 must be recorded following the guidelines in Sections 9.4, 9.8, and 9.11. To avoid confusion, AEs should be recorded in standard medical terminology.

The Investigator is responsible for assessing the relationship of AEs to CTM (see Section 9.3).

9.1 Adverse Event Definition

An *adverse event* is any untoward medical occurrence in a subject administered an investigational product and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (for example, an abnormal laboratory finding), symptom or disease temporally associated with the use of an investigational product, whether or not considered related to this investigational product. This includes an event that emerges during treatment having been absent pre-treatment or an event that worsens relative to the pretreatment state. Recurrent symptoms of a chronic preexisting condition are not considered AEs, unless they occur in a worse or unexpected pattern during CTM administration.

9.2 Assessing Severity of Adverse Events

The severity of AEs will be designated as mild (Grade 1), moderate (Grade 2), severe (Grade 3), life-threatening (Grade 4), or fatal (Grade 5), per NCI-CTCAE,Version 4. If an event is not specifically addressed in the NCI-CTCAE, use Table 9.1.

Table 9.1: Adverse Event Severity

Grade	Definition
Mild – Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention notindicated
Moderate – Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)[1]
Severe – Grade 3	Severe or medically significant, but not immediately lifethreatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[2]
Life-threatening – Grade 4	Life-threatening consequences; urgent intervention indicated
Death – Grade 5	Death related to AE

[1] *Instrumental ADL* refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

[2] *Self-care ADL* refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not being bedridden.

9.3 Assessing Relationship of Adverse Event to Study Treatment

All AEs will be categorized by the Investigator with respect to their possible relationship to study treatment. The relationship betweenstudy treatment and the AE may be considered related or not related. The criteria for each category are listed below:

- Related: When there is a reasonable possibility that the study treatment caused the reported AE
- Not related: When there is no reasonable possibility thatthe study treatment caused the reported AE

9.4 Recording of Adverse Events

All AEs with onset dates after the subject signed the ICF through the Month 12 will be recorded on the eCRF. All AEs must be recorded on the appropriate eCRF, regardless of their severity or relationship to the CTM. All AEs that meet the seriousness criteria (Section 9.7) should also be recorded on the SAE Report Form. All SAEs must be reported to the SciClone Drug Safety Department within the timeline stated in Section 9.8.

All clinical events are to be recorded, including both observed (such as any reaction at injection sites) and volunteered problems, complaints, or symptoms.The need to capture this information does not depend upon the clinical event'sassociation with the use of the CTM. Adverse events resulting from concurrent illnesses, reactions to concurrent medications, or clinically significant progression of disease states are also to be recorded.

9.5 Adverse Event Recording Period

AEs will be collected and recorded on the eCRF by the Investigator from the time the ICF is signed by the subject through Month 12 follow-up visit.

9.6 Adverse Event Follow-up Period

All subjects with AEs will be followed until the events resolve, stabilize, become chronic, the subject completes the study, or the subjectis lost to follow-up.

9.7 Serious Adverse Event Definition

A *serious adverse event* (SAE) is defined as any AE occurring at any dose regardless of relationship to CTM that results in any of the following outcomes:

- Death
- Is life-threatening (does not include a reaction that, had it occurred in a more severe form, might have caused death)
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability or incapacity
- Congenital anomaly or birth defect
- Other significant medical events

In addition, all AEs with NCI-CTCAE Version 4 severity of Grade 4 or Grade 5 should be reported as SAEs. Important medical events that may not result in death, be life-threatening or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent oneof the outcomes listed above.

Complications that occur during hospitalization are AEs. If a complication prolongs a hospitalization, it is a SAE.

As used in this study, the term *inpatient hospitalization* is defined as formal admission to a hospital for medical reasons.This may or may not be overnight.

When a SAE occurs in a subject receiving a product not licensed by the Sponsor as a control or concomitant medication, the SAE must be processed and reported as if it were a product of the Sponsor.

9.8 Reporting of Serious Adverse Events

For any SAEs, regardless of causal relationship, the Investigator must inform the **SciClone Safety Department – QPPV: Dott. Marco Anelli – Telephone +39-0258318521 / 3423455117 Fax: 0258318583** immediately (within 24 hours of knowledge of the event by the Investigator or designee) by faxing the completed SAE Report Form to the number indicated on the SAE Report Form. The study site will receive a faxed confirmation from the SciClone Safety Department following receipt of the SAE Report Form. All follow-up information collected for an initial

report of a SAE must also be reported to the SciClone Safety Department within 24 hours of receipt by the Investigator or designee. If discussion is required regarding treating the subject or completing the SAE Report Form, the Study Monitor should be contacted.

All SAE forms must be faxed to the SciClone Safety Department at Fax: 0258318583.

The SAE information to be recorded may include the following:

- The onset of any new SAE or the worsening of an observation from the time the subject signed the ICF through the Month 12 follow-up visit
- The specific type of reaction in standard medical terminology
- The duration of the clinical event (start and stop dates)
- The clinical event's severity (Grade 1, 2, 3, 4, or 5)
- Seriousness criteria
- Relevant diagnostic test results and examination results
- CTM dosing schedule
- Indications and dosing schedules of concomitant medications
- Relationship of the SAE to the CTM (see Section 9.3)
- Management of CTM administration and other action taken to alleviate the clinical events
- Past medical and surgical histories and concurrent diseases

9.9 Serious Adverse Event Recording Period

The Investigator will collect and record SAEs from the time the subject signs the ICF through the Months 12 visit.

9.10 Serious Adverse Event Follow-up Period

All subjects who experience SAEs will be followed until the events resolve, stabilize, become chronic,the subject completes the study, or the subject islost to follow-up.

9.11 Regulatory Reporting of Adverse Events

The Sponsor will determine whether the SAE must be reported to the Competent Authority within 7 or 15 days. If reportable, the Sponsor or its designee will report the event to the Comptent Authority. The Investigators will report all SAEs to theirECs, as required. Adverse events will be reported to regulatory authorities in compliance with local and regional law and established guidance by the Sponsor or its designee. The format of the reports will be dictated by national requirements.

Copies of all IND Safety Reports will be distributed to all active sites.Copies of all IND Safety Reports must be submitted to the corresponding EC by each Investigator, in accordance with local or central EC requirements and procedures. Copies of each IND Safety Report will be kept in the Investigators' files (and adequate documentation of this filing will be provided to the Sponsor), including documentation that their IRB has been notified of each SAE, as required.

9.12 Pregnancy

Although pregnancy is not considered a SAE, it is the responsibility of the Investigator or their designee to report any pregnancy in a subject or subject's partner (spontaneously reported to them) that occurs after the first CTM dose is received and up to 28-days (4 weeks) after the last CTM dose administration. The Investigator should report to the SciClone Safety Department all pregnancies within 24 hours of becoming aware, using the SAE Report Form. In addition, the Investigator or designee should record the pregnancy information on the Pregnancy eCRF. All subjects who become pregnant must immediately discontinue CTM, and the Investigator should counsel the subject about the risk of continuing with the pregnancy and the possible effects on the fetus. The subject must be followed until outcome of the pregnancy. If the pregnancy continues to term, health/outcome of the infant up to 8 weeks of agemust be reported to the Sponsor or designee.

Any complication of pregnancy should be reported as an AE or SAE, as appropriate.

10 DATA MANAGEMENT

A validated electronic clinical data management system will be used for capturing and managing data from the study. Data will be entered into the eCRFs following the eCRF Completion Guidelines.

The Investigator should ensure the accuracy, completeness and timeliness of the data recorded on the eCRFs. Data recorded on the eCRF will be consistent with source documents. Any discrepancies must be explained or resolved.

Completed eCRFs will be reviewed by the Sponsor's monitoring staff or the Sponsor's designee. An eCRF will be completed for each randomized subject.

Some laboratory tests will be performed at a central laboratory. Central laboratory data will be transferred electronically to the Data Management Center at predefined intervals during the study. The central laboratory will provide the Data Management Center with a complete and clean copy of the data, accompanied by a Quality Control statement.

11 STATISTICAL METHODS

This section outlines the statistical analysis strategy and procedures for the study.If, after the study has begun, changes are made to primary and/or key secondary hypotheses, or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9).Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, along with an explanation as to when and why they occurred, will be listed in the Clinical Study Report (CSR) for the study.Post hoc exploratory analyses will be clearly identified in the CSR.

11.1 Sample Size Considerations

Assuming an exponential survival distribution, a constant hazard ratio of 1.79 (Group 1 exponential parameter, $\lambda 1$, of 0.1575 and a Group 2 exponential parameter, $\lambda 2$, of 0.0880), an 10% exponential dropout, a one-sided 5% type-I error, and 80% statistical power a minimum number of 70 patients in each group, with a total number of events of 73 is required to detect a 79% relative increase in the median PFS in treated patients when compared to a reference of 4.4 months. This effect was decided taking into consideration positive literature reports on pilot studies on NSCLC patients adding Thymosin alpha 1 to SoC first line treatment. This assumes an accrual period of 8 months and approximate follow up time of 26 months. nQuery Advisor + nTerim 7.0 was used to perform a sample size calculations.

11.2 Definitions of Study Populations for Analysis

The *intent-to-treat (ITT)set* includes all subjects who were randomized. Subjects in this population will be analyzed according to the treatment group to which they were randomized. All efficacy analyses will be based on this assignment to treatment group.

The *per protocol* (PP) population is the subset of the ITT set that includes subjects who complete the study with no major protocol violations; major protocol violations will be described in the Statistical Analysis Plan that will be approved prior to unblinding. The primary and secondary analyses will be repeated on this population to assess the effect of compliance.

The *safety analysis set* (SAF) is the population of all subjects who receive at least 1 dose of CTM. Subjects in this population will be analyzed by the treatment received. All safety analyses will be based on this population.

11.3 Baseline Characteristics and Demographic Variables

All demographic (age, sex, race) and baseline characteristic data (e.g., disease factors, baseline clinical measurement values, previous and concomitant illnesses) will be summarized by treatment group and prespecified treatment sub-group (i.e. Pemetrexed, Paclitaxel, Gemcitabine, Vinorelbine, Bevacizumab) for the ITT, SAF and the PP population. For continuous data, summaries will include the number of observations, mean, standard deviation, median, 25th and 75th quantiles, and minimum and maximum values. For categorical data, frequency counts and percentages will be reported.

11.4 Efficacy Analysis

11.4.1 Primary efficacy analysis

The primary efficacy endpoint is an evaluation of the time to PFS in the two treatment arms of the Intent-to-Treatment (ITT) population.

The primary analysis will compare the time to PFS between Thymosin alpha 1 + SoC to SoC using a one-sided Log-Rank Test. The Kaplan-Meier estimator will be used to display the PFS distributions of the SoC chemotherapy+Thymosin alpha 1 treatment group and SoC chemotherapy alone treatment group. In addition, a Cox-Regression model with SoC treatment as a covariate will be used to estimate the Hazard Ratio and associated 90% confidence interval.

Patients will be censored for progression-free survival at the last clinical assessment, or after loss to follow-up, whichever occurred first.

11.4.2 Sub-Groups

Cox regression will be used to assess the influence of pre-specified sub-groups (i.e. Pemetrexed, Paclitaxel, Gemcitabine, Vinorelbine, Bevacizumab) on the primary activity/efficacy endpoint. For each sub-group (i.e Pemetrexed, Paclitaxel, Gemcitabine, Vinorelbine, Bevacizumab), a Cox regression model with treatment, sub-group and the treatment by sub-group interaction will be fit to the data. The p-value for the interaction and the HazardRatio with 90% confidence intervals, within each sub-group will be reported.

11.4.3 Secondary efficacy analysis

The secondary efficacy endpoints will be analyzed without adjustment for multiplicity.

The mortality rate due to any cause will be analyzed using the same methods as described above for the primary endpoint. Patients will be censored for overall survival at the last known alive date.

The change from baseline for the QoL score will be summarized by SoC treatment group (pre-specified SoC sub-group) and time point. The QoL scores will be analyzed using a repeated measures mixed effects model with terms for baseline, treatment, time and the treatment by time interaction. An unstructured covariance structure for each subject, nested within SoC treatment group (pre-specified SoC sub-group), will be used (assuming missing to be missing at random). For each time point, the lsmeans (adjusted means) and standard error of the lsmeans for each SoC treatment group (pre-specified SoC sub-group) will be reported. In addition, the lsmeans treatment difference, 95% confidence interval for the SoC treatment difference and two-sided p-value for the treatment difference will be reported.

The change from baseline in biomarkers will be summarized by SoC treatment group (pre-specified SoC sub-group) and time point. Methods analogous to those described above for the QoL score will be used to analyze the data.

11.4.4 Secondary statistical analysis

Inter-correlation (in particular immunological markers, circulating immune cells, cytokines and inflammatory factors) with basic clinical variables (histotype, gender, age, smoking habit) will be tested using Pearson's or Spearman's correlation test, as appropriate.

The association of biological markers levels with basic clinical variables will be tested fitting appropriate multivariate Cox regression models with co-linearity control. The best fitting models will be compared using AIC.

11.5 Safety Analysis

All safety analyses will be presented using the safety analysis set. No formal hypothesis testing will be conducted.

11.5.1 Adverse Events

Adverse events will be coded using MedDRA, version 17.0. All AEs that occur after the first dose of study medication (Treatment Emergent) will be summarized using frequency counts and percentages. Summaries will be presented by treatment group using the MedDRA level hierarchy (system organ class, high level group term, high level term, and preferred term) as follows;

- Overall (i.e., regardless of severity or relationship to treatment)
- By severity grade (mild, moderate, severe, or life threatening)
- By relationship to study medication (potential relationship to study treatment or unlikely/no relationship to study treatment) according to the mapping scheme below:
    - Potentially related:will include all AEs with a relationship rating of "definite", "probably" or "possibly"
    - Unlikely/not related:will include all AEs with a relationship rating of "unlikely" or "unrelated"

Unless otherwise specified, at each level of patient summarization in reporting the incidence of the AEs, a subject will be counted once if the subject reported one or more events. If more than one occurrence of an event is reported, the event of the worst severity or the worst-case relationship assessment will be summarized.

AEs leading to premature discontinuation of study drug and SAEs will also be summarized by treatment group and relationship.AEs leading to premature discontinuation of study drug will be defined as any AE with an action taken regarding study medication equal to "permanently discontinued."

11.5.2 Laboratory Parameters, ECG Intervals and Vital Signs

Descriptive summary statistics for chemistry, hematology, coagulation, ECG intervals and vital sign data, by time point, will be presented by SoC treatment group (pre-specified SoC sub-group). For change from baseline summaries, subjects with an undefined change from screening, because of missing data, will be excluded.

Shift tables, showing individual subject changes from baseline, will be presented for each parameter, by treatment group (pre-specified SoC sub-group) and time point.

11.6 Concomitant Medications

Concomitant medications will be coded using the WHO Drug Dictionary.

Concomitant medications will be summarized by WHO Drug Dictionary drug class and preferred term for each treatment group.

12 ETHICS

12.1 Good Clinical Practice

The Investigator will ensure that this study is conducted in full compliance with the principles of the Declaration of Helsinki (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), ICH guidelines, in particular ICH GCP E6, and with the laws and regulations of the country in which the research is conducted, whichever affords the greatest protection to the study subject. Because this study is conducted in Italy, the Investigator will ensure adherence to the basic principles of Good Clinical Practice, as outlined, for example, in 21 CFR §312.50 and 312.56 and to the European Directive 2001/20/CE and Italian Decree n.211/2003.

12.2 Ethics Committee

The protocol and ICF for this study must be reviewed and approved by an appropriate Ethics Committee (EC) before subjects are randomized into the study. It is the responsibility of the Investigator to assure that all aspects of the ethical review are conducted in accordance with the requirements of all regulatory authorities. A letter documenting the EC approval, for each study site,that specifically identifies the protocol by number and title, must be received by the Sponsor prior to initiation of the study at each study site.

12.3 Subject Information and Informed Consent

In accordance with EU regulatory and local ethics committee requirements, before study procedures are performed, subjects will be informed about the study and required to sign the EC-approved ICF. This form will be signed after adequate explanation of the aims, methods, objective and potential hazards of the study and prior to undertaking any study-related procedures. No subject is to be screened or treated until an ICF has been obtained. The signed ICF will be retained with the study records. Each subject will also be given a copy of his or her signed ICF at the screening visit. A separated informed consent should be signed to allow the storage of the left over sample into the facilities of the Interinstitutional Multidisciplinary Biological Bank (BioBIM) of the Research Center of the IRCCS San Raffaele Pisana, Rome.

12.4 Data and Safety Monitoring Committee

An independent DSMB will be constituted before the start of the study. The DSMB will supervise the ethical performance of the study, review safety data arising during the study, and review the interim analysis of safety and efficacy according to the procedures described in the DSMB Charter. The Charter will describethe following activities:

- Committee membership
- Committee responsibilities
- Frequency and content of meetings
- Procedures for the review and assessment of the cumulative safety and efficacy data
- Criteria for determining whether to recommend stopping the study for either overwhelming benefit or unacceptable harm

The DSMB will be composed of 3 or more members who will have relevant experience in the treatment of subjects with NSCLC and the conduct of randomized clinical trials. At least one member will be a statistician. None of the members will be Investigators in the study.

Any AE that leads to a subject's treatment termination, and any SAE a subject experiences during the study, will be regularly reported to the DSMB, as specified in the Charter. The DSMB may request and receive the treatment code for any subject in the case of these safety events.

12.5 Financial Disclosure by Principal Investigators and Sub-Investigators

The Investigators and all Subinvestigators (inclusive of family members) provide documentation of their financial interest or arrangement with the Sponsor or proprietary interests in the study drug being studied. This documentation must be provided prior to the participation of the Investigator and any Subinvestigator. The Investigator and Subinvestigator agree to notify the Sponsor of any change in reportable interests during the study and for 1 year following completion of the study. Study completion is defined as the date that the last subject has completed the protocol-defined activities.

13 STUDY MANAGEMENT AND ADMINISTRATION

13.1 Regulatory Documentation

The Investigator is responsible for forwarding the following documents to the Sponsor or its designee for review prior to study initiation:

- An up-to-date curriculum vitae for each individual named on the Trial Staff Form
- Signed and dated signature page for the protocol or current amendment(s), if applicable
- Original signed and dated Financial Disclosure form for each Investigator and Subinvestigator
- Original executed, signed, and dated Investigator Agreement
- Written notification (copy) to the Investigator from the EC, approving the protocol, informed consent form and amendment(s), if applicable
- Documentation that state and local regulations have been met, if these are not included in the items above

The Investigator will promptly report to the EC all changes in research activity and all unanticipated problems involving risks to human subjects and will not make any changes in the research without EC approval except where necessary to eliminate immediate hazards to the subjects.

Documentation of all study amendment activity should be forwarded to the Sponsor. The Investigator must also report to the EC at least yearly and within 3 months after completion, termination or discontinuation of the study. Continuing review must be documented in writing from the EC.

In compliance with the European and Italian legislation, the sponsor will register this trial with the EudraCT and OsSC Data Registries.

13.2 Protocol Modifications

Amendments to the protocol will be subject to the same requirements as the original protocol. The Sponsor will submit protocol modifications according to the applicable regulations.

In situations requiring a departure from the protocol, the Investigator or other physician in attendance should contact the Study Monitor by telephone. If possible, this contact will be made before implementing any departure from the protocol.In all cases, contact with the Study Monitor must be made as soon as possible in order to discuss the situation and agree on an appropriate course of action.The source documents and the eCRF will document all protocol departures and the circumstances behind them.

13.3 Data Quality Control and Quality Assurance

The study will be monitored and managed in accordance with ICH GCP E6.

13.4 Monitoring

Investigative sites will be monitored by the Sponsor's monitoring staff or designee at appropriate intervals to ensure satisfactory enrollment rate, data recording and protocol adherence. The Investigator and staff are expected to cooperate and provide for review all relevant study documentation requested at each site visit.

13.5 Audit and Inspection

The Investigator will agree to receive periodic quality assurance audits conducted by the Sponsor's clinical quality assurance personnel or designee.In addition, the Investigator will agree to inspections by the Competent Authority and other regulatory agencies to the extent permitted by law. Auditors will have direct access to all relevant study documentation.

13.6 Source Documents

The Investigator must allow the Competent Authority and other regulatory agencies, individuals delegated by the EC, or the Sponsor or its designee to have access to all the original documentation of the study, including the ICFs signed by the subjects enrolled into the study and the relevant subject medical files. The individuals who are allowed access to the documentation must take every reasonable precaution to keep the identity of the subjects and the proprietary information of the Sponsor as confidential information in accordance with relevant applicable legislation.

13.7 Case Report Forms

Electronic case report forms (eCRFs) will be supplied by the Sponsor or its designee and should be handled in accordance with the provided instructions. All eCRFs should be filled out completely by authorized study personnel.

The Investigator should ensure the accuracy, completeness and timeliness of the data recorded on the eCRF. Data recorded on the eCRF will be consistent with source documents. Any discrepancies must be explained or resolved.

Completed eCRFs will be reviewed by the Sponsor's monitoring staff or designee. An eCRF will be completed for each randomized subject.

13.8 Premature Termination of the Study

The sequence of events that will determine premature termination of the study are: (a) evaluations of several study subjects as aggregate data,(b) determination of the likelihood that the AEs are related to CTM, (c) if the severity of study-related AE(s) is judged extreme, and (d) emergence of unexpected SAEs.

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the investigators and the regulatory authority (ies) of the reason(s) for termination or suspension. The EC also will be promptly informed by the Sponsor or Investigator and provided with the reason(s) for the termination or suspension, as specified by the applicable regulatory requirement(s).

13.9 Publication and Presentation Policy

The results of this study will be published and/or presented to the EudraCT data bank as well as at scientific meetings in a timely manner. Any publication of study results will be a collaborative effort between the Sponsor and the investigator(s). All manuscripts or abstracts will be reviewed and approved in writing by the Sponsor prior to submission.

13.10 Archiving and Data Retention

According to law in force, an Investigator shall retain adequate records for the study including copies of each subject's eCRFs, medical records, laboratory reports, ICFs, study drug accountability records, safety reports, information regarding subjects who were withdrawn and any other pertinent data. All records are to be retained by the Investigator for a period of 7 years following the date of study termination. The records will be available for copying and inspection if requested by regulatory authorities. To avoid any possible errors, the Investigator will notify the Sponsor in the event of accidental loss or destruction of any study records or CTM.

If the responsible Investigator retires, relocates or for other reasons withdraws from the responsibility of keeping records, custody must be transferred to a person who will accept the responsibility. The Sponsor will be notified in writing of the name and address of the new custodian 30 days in advance of the transfer.

13.11 Confidentiality

The Investigator must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. The subjects' identification code and the subjects' initials should be recorded on any form submitted to the Sponsor, the Sponsor's designee or EC, unless it is forbidden to identify the subject by name and/or initials. Where it is forbidden to indicate the subjects' name and/or initials, only the subjects' identification code and the subjects' date of birth should be recorded.

The Investigator must keep a screening log showing codes, names, and addresses for all screened subjects and all subjects randomized into the trial.

The Investigator agrees that all information received from the Sponsor, including but not limited to the Investigator Brochure, this protocol, eCRFs and any other study information remain the sole and exclusive property of the Sponsor during the conduct of the study and thereafter. This information is not to be disclosed to any third party (expect with prior written consent from the Sponsor). The Investigator further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of the study site to any third party or otherwise into the public domain.

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ANNEXES

15. APPENDIX A: FLOW CHART

		TREATMENT CYCLE																	
Phase II: **140 patients**	Screening*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Informed consents[1]	X																		
Demographics	X																		
Medical History	X																		
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs and weight	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG performance status	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12 Lead Digital ECG[2]	X																		
ECHO or MUGA[3]	X																		
Haematology and biochemistry[4]	X§	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Tumor markers	X	X			X		X			X			X						X
Immunity[5]		X	X		X		X						X						X
Cytokine, Thymosin, Galectin levels determination		X	X		X		X						X						X
Urinalysis[6]	X						X								X				X
Urine/blood β-HCG[7]	X				X				X				X				X		
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Tumor assessment[8]	X			X			X			X			X			X			X
Quality of Life evaluation	X	X			X			X			X			X			X		
Study drug administration																			
Cisplatin (carboplatin) +Pemetrexed		X	X	X	X	X	X	Pemetrexed maintenance											
Cisplatin (carboplatin) +Gemcitabine ± Bevacizumab		X	X	X	X	X	X	Bevacizumab maintenance											
Cisplatin (carboplatin) + Paclitaxel ± Bevacizumab		X	X	X	X	X	X	Bevacizumab maintenance											

In case of "early termination" of the study

Perform physical examination; Record vital signs, including temperature, systolic and diastolic blood pressures, heart rate, and respiration rate; Perform an ECOG performance status; Draw blood for local laboratory measurements:

- Hematology profile: complete blood count (CBC)
- Chemistry: Blood Urea Nitrogen (BUN), creatinine, glucose, albumin, total proteins, protein electrophoresis, lactate dehydrogenase (LDH) serum amylase, lipase, uric acid, creatinine kinase (CK), alkaline phosphatase, aspartate amino transferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), γ-glutamyltransferase (γGT), total bilirubin, sodium, potassium, chloride, hs-CPRP, CEA, CYFRA
- Coagulation: prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR), Fibrinogen, D-Dim
- Immunology profile: Characterization of the % of regulatory T cells $CD4^{pos}CD25^{bright}CD127^{low}FOXP3^{high}CD45RA^{low}$; Cytokine; Thymosin and Galectin levels determination.

Record any concomitant medications; Record AEs and SAEs that have occurred since the time the subjects signed the ICF; Perform Tumor assessment; Quality of Life evaluation

Note:

* If the screening lab test are within one week of range period, they could be consider valid also for Cycle 1; the screening visits are identical for all patients but have been included on both flow charts for clarity.

1 Written informed consents must be obtained before any protocol specific screening assessments are performed.

2 A 12-lead resting digital electrocardiogram (ECG) will be performed at screening and then at any other timepoint if clinically indicated.

3 ECHO or MUGA will be performed at screening and then at any other timepoint if clinically indicated.

4 Laboratory tests performed at enrollment(§) should include the following:

Haematology: Haemoglobin, Red Blood Cell (RBC), White Blood Cell (WBC) and platelet count

Chemistry Electrolytes: Sodium, potassium, chloride

Liver function tests: Alkaline phosphatase, aspartate amino transferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), γ-glutamyltransferase (γGT), total bilirubin

Renal function parameters: Blood Urea Nitrogen (BUN), creatinine

Inflammatory parameters: hs-CPRP, total proteins, protein electrophoresis

Coagulation: Prothrombin time (PT), partial thromboplastin time (PTT), international normalized ratio (INR), Fibrinogen, D-Dimer

Other: Glucose, albumin, lactate dehydrogenase (LDH) serum amylase, lipase, uric acid, creatinine kinase (CK), CEA, CYFRA,

Urinalysis: pH, protein, glucose, blood/erythrocytes, leucocytes, nitrite*;* in case of pathological finding further evaluation should be performed and results documented

All post screening laboratory testing must be performed before every cycle as clinical practice, and should include:

Haematology profile, albumin, total proteins, hs-CRP, D-Dimer, Fibrinogen. CEA and CYFRA will be tested at indicated timepoints

5 At cycles 1, 2, 4, 6, 12 and 18 withdrawal of

1 Whole blood in EDTA containing tube (3.5 ml blood)

1 Whole blood in lithium heparin containing tubes (7 ml blood).

1 Serum (10 ml blood)

For immunological profile and thymosin and Galectin levels determination

6 Urinalysis will be performed at Screening and at any other timepoint only if clinically indicated.

7 Urine/blood β-HCG will be performed at Screening and every three cycles. Proteinuria should be tested in patient assigned to the Bevacizumab treatment as clinical practice.

8 Tumor assessment (CT scan with contrast IV) performed usually every 6 weeks or according to normal practice; brain MRI performed at screening)

16 APPENDIX B: METHODS

Sample preparation

Blood samples will be obtained early in the morning, after an overnight fast and a rest period of at least 20 minutes. Blood will be withdrawn from each consenting participant, without stasis, from the antecubital vein using a 20 G needle. Whole blood samples for haematological evaluation will be collected in EDTA containing tubes and freshly evaluated. Serum samples will be obtained from non-anticoagulated samples left to clot at RT and then centrifuged for 10 min at 1,500g. Serum and plasma samples will be aliquoted, coded and stored at -80°C for batch analysis. Storage conditions will be carefully maintained, and all aliquots will be limited to one freeze-thaw cycle, thus ensuring no decline of coagulation activity due to long-time storage analyses will be immediately frozen at -80°C until processing.

Cytokine assay

Serum TNF-α, IL-1 β, IL-2, IL-6, IL-10, interferon IFN-γ and VEGF will be detected by enzyme-linked immunosorbent assays (ELISA) with commercially available kits according to the manufacturer's instructions. All samples will be tested in duplicate

PBMCs preparation

PBMCs will be isolated by Ficoll density gradient separation, washed three times, and cryopreserved in liquid nitrogen at a concentration of $1–2 \times 10^7$ cells/mL until assayed.

Flow cytometry analysis

Cryopreserved PBMCs will be analyzed by 5-color flow cytometry for phenotypic characterization of Tregs, according to Vergati et al. Cells will be resuspended in staining buffer (PBS containing 3% fetal bovine serum) and stained for 30 min at 4°C with FITC-conjugated anti-CD4, PECy7- conjugated anti-CD25, PE-conjugated or PerCp-Cy5.5-conjugated anti-CD127, and PE conjugated anti-CTLA-4. FoxP3 intracellular staining will be performed on cells stained with anti-CD4, anti-CD25, and anti-CD127. Cells will be fixed and permeabilized

using a fix/perm kit (eBioscience; San Diego, CA, USA) according to the manufacturer's instructions, then labeled with APC-conjugated anti-FoxP3 antibody (236A/E7 clone) (eBioscience) or its isotype control antibody (eBioscience), as a negative control. Flow cytometry will be performed on a Becton–Dickinson LSRII (BD Biosciences); 1×10^5 cells will be acquired and data analyzed using DiVa software (BD Biosciences). To determine the percentage of Tregs, lymphocytes will be gated by plotting forward versus side scatter. The CD4+ population will be gated first, followed by the CD25+CD127− and FoxP3+ populations. Thus, CD4+CD25high T-cell enrichment CD4+CD25high T cells will be enriched using a CD4+CD25+ Treg isolation kit (Miltenyi Biotec; Auburn, CA, USA), with modifications to the manufacturer's instructions. CD4+CD25high T cells will be enriched by a method described by Yokokawa et al. [7]. CD4+ T cells will be negatively enriched by LD column, and positive selection for CD25+ T cells will be done on the negatively selected CD4+ T cells. The CD25+ fraction will be collected by eluting the cells twice through a magnetic separation column for further enrichment of CD4+CD25high T cells.