2. H3E-BP-JMIK Synopsis

Clinical Study Report Synopsis: Study H3E-BP-JMIK

Title of Study: An Exploratory, Prospective Phase 2 Study to Investigate Progression-Free Survival, Response and Overall Survival Seen with Pemetrexed/Cisplatin and the Role of Thymidylate Synthase Expression	
Number of Investigators: This multicenter study included 13 principal investigators.	
Study Centers: This study was conducted at 14 study centers in 2 countries (1 principal investigator involved in 2 different sites).	
Publications Based on the Study: None to date.	
Length of Study: Date of first patient enrolled: 07 April 2009 Date of last patient completed: 10 June 2011	**Phase of Development:** 2
Objectives: Primary: To determine the correlation (i.e. association) between thymidylate synthase (TS) expression and progression-free survival (PFS). Secondary: to determine the objective tumor response rate and overall survival (OS) rate at 18 months; to determine the level of concordance between local versus central histology review; to determine the biological characteristics of the more favorable (PFS $\geq$ 5.2 months) and less favourable (PFS < 5.2 months) outcome groups; to assess biomarkers relevant to the disease state and their correlation to clinical outcome.	
Study Design: This was an exploratory, single-arm Phase 2 study in patients with histologically confirmed non-squamous non-small cell lung cancer (NSCLC) of Stage IIIB or IV. Patients started first-line treatment with 4 cycles of pemetrexed plus cisplatin induction treatment. Patients without disease progression, that is with either complete response (CR), partial response (PR) or stable disease (SD), unconfirmed or confirmed, continued on pemetrexed maintenance treatment until disease progression or any other reason for discontinuation. All patients received folic acid and Vitamin B_{12} supplementation and prophylactic dexamethasone treatment throughout the entire treatment period. All patients were followed up until death or the end of study. The study was stopped 18 months after the last patient had started induction treatment.	
Number of Patients: Planned: 68 patients. Actual enrolled: 70 patients started induction treatment, 43 patients started maintenance treatment. Evaluable for efficacy (valid immunohistochemical [IHC]-based TS assessment): 60 patients.	
Diagnosis and Main Criteria for Inclusion: Male or female patients, $\geq$ 18 years of age, with histologically confirmed non-squamous non-small cell lung cancer (NSCLC) of Stage IIIB (with pleural effusion and/or positive supraclavicular lymph nodes) or IV, Eastern Cooperative Oncology Group (ECOG) performance status of 0-1 and an estimated life expectancy of $\geq$ 12 weeks. No previous systemic treatment for lung cancer was allowed. Previous palliative radiotherapy to non-target metastatic lesions was allowed to < 25% of the bone marrow. Patients had to have at least 1 unidimensionally measurable lesion meeting Response Evaluation Criteria in Solid Tumors (RECIST), and an adequate tumor biopsy specimen had to be available for TS assessment.	
Study Drug, Dose, and Mode of Administration: Induction phase (4 cycles): pemetrexed 500 mg/m^2, given as an intravenous (IV) infusion on Day 1 of each 21-day cycle, followed approximately 30 minutes later by cisplatin 75 mg/m^2, also given IV, along with supportive Vitamin B_{12}, folic acid and dexamethasone treatment. Maintenance phase: pemetrexed 500 mg/m^2, given IV on Day 1 of each 21-day cycle.	
Comparator, Dose, and Mode of Administration: Not applicable, this was a single-arm study.	
Duration of Treatment: Induction phase (all patients): up to 4 21-day treatment cycles. Maintenance treatment (patients with CR, PR or SD during induction phase): continued until disease progression or any other reason for discontinuation.	

Variables: Efficacy: *Primary outcome:* Hazard ratio (HR) of association in Cox regression of PFS with TS IHC expression (H scores, range 0-300) in the nucleus and cytoplasm compartments (continuous variable). *Secondary outcomes:* Objective tumor response (RECIST 1.0 guidelines); PFS and OS; association between TS nucleus IHC H score (continuous) and OS; concordance between local and central histology review. *Exploratory biomarker research outcomes:* TS IHC expression in nucleus and cytoplasm (IHC H scores); TS mRNA expression (delta Cq levels) as evaluated by real-time quantitative polymerase chain reaction (qPCR); optimal cutpoints for TS IHC H scores and delta Cq levels to divide patients into low and high TS IHC expression/TS mRNA expression groups; association between TS IHC expression/TS mRNA expression in low and high expression groups (using the optimal cutpoint as well as different cutpoints along the IHC H score continuum) and PFS; exploratory gene expression profiles derived from a lung-cancer specific disease-specific array (DSA) gene expression microarray (LC-DSA™, ALMAC). Safety: Adverse events; study-drug related non-laboratory and laboratory toxicities based on Common Terminology Criteria for Adverse Events (CTCAE); laboratory tests (hematology and clinical chemistry); body weight; performance status (ECOG).

Statistical Evaluation Methods: Sample size: Monte Carlo simulations were used to determine a sample size that achieved a power of approximately 90% for detecting a significant association at a two-sided significance level of $\alpha = 0.05$. As a result, it was planned to recruit approximately 60 patients, to obtain 54 patients with valid TS assessments (assuming that TS assessment would fail in approximately 10% of the histological samples). Efficacy: All patients treated and with valid IHC-based TS assessments were included in the primary analysis and most secondary analyses ("patients evaluable for efficacy"). Tumor response and concordance between central and local histology reviews were evaluated for all patients treated. All statistical analyses were conducted at a significance level of $\alpha = 0.05$. *Primary analysis:* A Cox proportional hazards regression model was used to assess the association between TS IHC H scores (nucleus and cytoplasm) and PFS. The primary univariate model included PFS as dependent variable and the respective TS IHC H score as independent variable. For the primary correlative analysis, the TS IHC H score was included as continuous variable (HR per 1-unit increase in H score). The null hypothesis of no relationship between TS and PFS was tested using the Wald chi-square test. A supportive multivariate Cox regression analysis additionally adjusted for several covariates (smoking status, tumor stage, performance status; covariates of final model identified by forward selection process using a pre-specified significance level of $p = 0.2$). Time-dependent receiver operating characteristic (ROC) curves were used to explore the sensitivity and specificity of the TS nucleus IHC H score for predicting PFS. *Secondary analyses:* Overall best response and disease-control rates were reported including the respective 95% confidence interval (CI). Kaplan-Meier analyses were performed on the observed distribution of PFS and OS. For PFS and OS rates at specific time points, the point estimates and 95% CIs were reported. The association between TS IHC expression and OS was explored using Cox regression models corresponding to the primary analysis. The concordance between local and central histology review was evaluated descriptively. Spearman correlation analyses were applied to explore the consistency between different TS expression assessments (IHC H scores, qPCR delta Cq values). *Exploratory biomarker research:* Cox regression analyses were used to explore the association between dichotomous TS IHC H scores and PFS (HR presented for low versus high TS expression groups). The optimal cutpoint to divide patients into low and high TS IHC expression expression groups was identified using the maximal chi-square method; adjusted p-values were calculated using the formula of Miller and Siegmund, limiting the search to the central 80% of values. To explore the relationship between TS IHC expression and PFS along the continuum of TS IHC H score values, median PFS was plotted for the low and high TS IHC expression groups, using each IHC H score value in the continuum as a cutpoint. In addition, the Wald chi-square statistics from the Cox regression models were plotted against each dichotomized TS IHC H score. Corresponding analyses and plots were generated to explore the association between TS mRNA expression (qPCR delta Cq values) and PFS. Exploratory gene expression profiles (at gene and probeset level), looking at all genes/probesets on the DSA array and at a ranked list of approximately 100 top-ranked genes/probesets, were used for exploratory cluster analyses to identify patient clusters associated with PFS and TS nucleus IHC H scores. Safety: All patients treated were evaluated for safety. Treatment-emergent adverse events (TEAEs) and study-drug related CTCAE-toxicities were evaluated using descriptive statistics. Body weight and ECOG performance status data were listed.

Summary:

Patient disposition and baseline characteristics: A total of 70 patients with NSCLC of non-squamous histology were enrolled and started pemetrexed/cisplatin induction treatment (54.3% male, median age 65.1 years, 78.6% with adenocarcinoma, 90.0% Stage IV disease). Of these, 43 patients (61.4%) without disease progression continued into the maintenance phase. The most common reasons for discontinuations were progressive disease (PD; induction treatment 18.6%, maintenance treatment 34.3%) and non-fatal adverse events (induction treatment 11.4%, maintenance treatment 24.3%). A total of 60 patients had a valid IHC-based TS assessment and were included in the primary analysis.

Efficacy:
Primary analysis: In the primary univariate Cox regression analysis, a statistically significant association between TS nucleus IHC H scores and PFS was observed (p<0.0001; HR per 1-unit increase in H score 1.015 [95% CI 1.008, 1.021]), indicating that lower TS expression levels in the nucleus were associated with longer PFS. The association between TS cytoplasm IHC H scores and PFS was less pronounced, but also statistically significant (Table JMIK 2.1). Results of the supportive, multivariate Cox regression analysis which included additional covariates were similar to those of the univariate analysis. Time-dependent ROC curves revealed that none of the TS IHC H scores actually measured showed a high true positive rate (sensitivity) and a low false positive rate (1 – specificity) for predicting PFS.

Table JMIK 2.1. Association between TS IHC H Scores (Nucleus and Cytoplasm) and PFS, Primary Univariate and Supportive Multivariate Cox Regression Analysis
Patients Evaluable for Efficacy (N = 60)

	Hazard ratio (95% CI)	p-value (Wald test)
TS nucleus IHC H score		
Hazard ratio per 1 unit increase	1.015 (1.008, 1.021)	<0.0001
TS cytoplasm IHC H score		
Hazard ratio per 1 unit increase	1.007 (1.001, 1.012)	0.0134

Cox regression analysis: Included PFS as dependent variable and TS IHC H scores as independent variable. TS IHC H scores were included in the model as continuous variable (hazard ratios presented per 1 unit increase; scores ranged from 0-300).
Abbreviations: CI = confidence interval; IHC = immunohistochemical; N = number of patients with valid TS assessment; PFS = progression-free survival; TS = thymidylate synthase.

Secondary Clinical Efficacy Results: All 70 patients enrolled were evaluated for tumor response. The overall response rate was 30.0% (95% CI 19.62, 42.13%; best response: 0 CR, 21 PR). In addition, 23 patients achieved a best response of SD, for an overall disease control rate of 62.9% (95% CI 50.48, 74.11%). Median PFS was 5.5 months (95% CI 3.9, 6.9 months) at 18 months of follow-up after the last patient had started treatment, median OS was 9.6 months (95% CI 7.3, 15.7 months). As for PFS, the association between continuous TS nucleus IHC H score and OS was statistically significant in the univariate analysis (HR per 1-unit increase in H score 1.015; 95% CI 1.008, 1.022; p<0.0001), and the multivariate analysis showed similar results. The histological diagnosis obtained locally and the centralized histology review of tumor diagnosis yielded a concordance rate of 0.786 (95% CI 0.671, 0.875).

Exploratory biomarker analysis: Using the maximal chi-square method, the optimal cutpoint for dividing patients into low and high TS expression groups was identified as a TS nucleus IHC H score of 70 and as a TS cytoplasm IHC H score of 100, on the H score scale ranging from 0-300. The optimal cutpoint for dividing patients into low and high TS mRNA expression groups was identified as a TS qPCR delta Cq value of –1.30. When the patient population was dichotomized at the identified optimal cutpoint into low and high expression groups, there was a statistically significant association (adjusted p-value <0.05) indicating that a low TS IHC expression in the nucleus was associated with longer PFS (Table JMIK 2.2). The association between TS nucleus IHC H scores and PFS was statistically significant (unadjusted p-value <0.05) across a wide range of cutpoints. The associations between TS IHC expression in the cytoplasm with PFS and between TS mRNA expression with PFS were less pronounced, but showed the same direction as for the TS IHC expression in the nucleus (Table JMIK 2.2). Overall, the association between TS nucleus and cytoplasm IHC H scores was statistically significant (Spearman correlation coefficient 0.41, p=0.001), as were the associations between TS IHC H scores and TS qPCR delta Cq values. The latter correlation was higher for the TS nucleus IHC H score (–0.62, p<0.001) than for the TS cytoplasm IHC score (–0.29, p=0.030).

Table JMIK 2.2. Association between TS IHC Expression or TS mRNA Expression (Low and High Expression Groups) and PFS, Cox Regression Model

	Low expression group	High expression group	HR[a] (95% CI)	p-value[b]
TS nucleus IHC H score (N = 60)				
Optimal cutpoint for IHC H score	< 70	≥ 70	0.283 (0.155, 0.516)	0.0015
Number of patients per group	40	20		
Median PFS, months (95% CI)	7.1 (5.7, 8.3)	2.6 (1.3, 4.1)		
TS cytoplasm IHC H score (N = 60)				
Optimal cutpoint for IHC H score	< 100	≥ 100	0.425 (0.235, 0.769)	0.0902
Number of patients per group	25	35		
Median PFS, months (95% CI)	6.6 (5.5, 12.9)	4.1 (2.7, 5.9)		
TS qPCR delta Cq value (N = 61)				
Optimal cutpoint for delta Cq value[c]	≥ –1.3	< – 1.3	0.398 (0.221, 0.719)	0.0500
Number of patients per group	41	20		
Median PFS, months (95% CI)	6.6 (5.2, 8.1)	3.2 (1.7, 4.4)		

Note: High Cq values (and high delta Cq levels) correspond to low RNA expression levels, and vice versa.

[a] Hazard ratio > 1 indicates that higher TS expression levels were associated with shorter PFS, or increasing hazards with low TS (mRNA) expression compared to high TS (mRNA) expression.

[b] Adjusted p-value as the asymptotic probability of the observed maximum chi-square statistic calculated with formula of Miller and Siegmund, limiting the search to the central 80% of values.

[c] High delta Cq levels correspond to low RNA expression levels, and vice versa.

Cox regression analysis: Included PFS as dependent variable and low or high TS mRNA expression as explanatory variable.

Abbreviations: CI = confidence interval; delta Cq = difference in cycle thresholds, quantitative measure of differential gene expression identified by qPCR; IHC = immunohistochemical; HR = hazard ratio; N = number of patients; qPCR = quantitative polymerase chain reaction; PFS = progression-free survival; TS = thymidylate synthase.

Exploratory gene expression profiling: Gene expression profiles (at gene and probeset levels of the DSA microarray) were explored in 51 of the 70 patients (72.9%) who had samples with at least 1 evaluable marker result. The exploratory cluster analyses were most informative when looking at the gene level. Based on the ranked biomarker list which included approximately 100 top-ranked biomarkers plus a few specific genes known to be relevant for the TS pathway (RRM2, ABCC3, CDT1, MYB, CES1), 2-4 clusters were observed at the gene level. The 3-cluster analysis provided clinically relevant PFS patterns for 2 of the 3 clusters. One cluster (N=11) identified good performers (long median PFS of 13.0 months, 90.9% of patients in the more favorable PFS outcome group, and 90.9% of patients with low TS nucleus IHC H score). One cluster identified poor performers (short median PFS of 2.4 months, 88.2% of patients in the less favorable PFS outcome group, and 64.7% of patients with high TS nucleus IHC H score).

Safety:

Of all 70 patients treated, 51 (72.9%) received all 4 cycles of pemetrexed plus cisplatin induction therapy, and 43 (61.4%) started pemetrexed maintenance therapy for a maximum of 24 cycles (1 patient). Overall, patients received median 7 cycles of pemetrexed and 4 cycles of cisplatin. Median relative dose intensity was 93.0% for pemetrexed and 97.6% for cisplatin.

Five patients (7.1%) who died during treatment or within 30 days after discontinuation of study drug were recorded as fatal adverse events in the study database. One death occurred due to toxicity possibly related to study drug (neutropenic sepsis during Cycle 3 of induction phase). One patient died of pneumonia (not related to study drug) during pemetrexed maintenance treatment. One patient died of intracranial tumor hemorrhage which was reported as a serious TEAE, but disease progression was documented as reason for discontinuation. Two patients were documented as deaths due to study disease within 30 days after discontinuation of study drug. A total of 41 patients (58.6%) reported at least 1 serious treatment-emergent adverse event (serious TEAE), 24 patients (34.3%) had at least 1 serious TEAE possibly related to study drug. The most common serious TEAEs were nausea (10.0%), vomiting (10.0%), lower respiratory tract infections (8.6%), non-cardiac chest pain (7.1%) and diarrhea (7.1%). In total, 25 patients (35.7%) had at least 1 adverse event leading to discontinuation; these were treatment-emergent in 23 patients (32.9%). Fatigue and TEAEs related to renal dysfunction lead to discontinuation most frequently; no other TEAEs lead to discontinuation in more than 1 patient. In terms of study-drug related CTCAE toxicities, 50.0% of patients had at least 1 Grade 3/4 non-laboratory toxicity, with fatigue (18.6%), nausea (10.0%), diarrhea (10.0%) and vomiting (8.6%) reported most frequently. There was 1 study-drug related Grade 5 laboratory toxicity (the patient with neutropenic sepsis mentioned above), and 24 patients (34.3%) experienced at least 1 study-drug related Grade 3/4 toxicity, most frequently neutropenia (14.3%), anemia (12.9%) and thrombocytopenia (7.1%).

Conclusions:

The following conclusions can be drawn from this single-arm, exploratory study of patients with advanced, non-squamous NSCLC who received 4 cycles of induction treatment with pemetrexed and cisplatin, followed by pemetrexed continuation maintenance treatment until disease progression:

- A median PFS of 5.5 months from the start of induction treatment was observed for all evaluable patients, including those who did not receive continuation maintenance treatment. 60 of the 70 patients treated had a valid TS IHC assessment (and were evaluable for analysis). The scientific study hypothesis that there is an association between TS expression and PFS was met: An association between TS IHC expression in the nucleus and PFS was observed in the patient population studied, suggesting that lower TS expression in the nucleus was associated with prolonged PFS [HR per 1-unit increase in H score 1.015, 95% CI 1.008, 1.021; $p<0.0001$).
- Using the identified optimal IHC H score cutpoint (maximal chi square method), low TS IHC expression in the nucleus was associated with longer PFS.
- The association between TS IHC expression in the cytoplasm and PFS and the association between TS mRNA expression and PFS was less pronounced, but showed the same direction as the TS IHC expression in the nucleus.

- This hypothesis-driven study suggests that TS expression may be explored as potential prognostic marker of clinical outcome of pemetrexed treatment. Further studies are required to confirm the putative association between nuclear TS IHC expression and PFS.
- The objective tumor response rate was 30.0% (no CR, 21 PR), the overall disease control rate including SD was 62.9%. Median PFS was 5.5 months and median OS 9.6 months (OS rate at 18 months 37.1% for all 70 patients treated). These data are within the expected range when compared to previous studies of first-line pemetrexed induction and maintenance treatment in similar patient populations.
- The review of the local diagnosis by the central reviewer yielded 78.6% agreement.
- The exploratory analysis associating microarray gene expression profiles to TS IHC expression in the nucleus and PFS indicated that patient clusters with clinically relevant PFS and TS expression patterns may be observed ("good performers" with long median PFS, more favorable PFS outcome group [PFS $\geq$ 5.2 months] and low TS nucleus IHC expression; "poor performers" with short median PFS, less favorable PFS outcome group [PFS < 5.2 months] and high TS nucleus IHC expression).
- Toxicities were consistent with the known safety profile of pemetrexed.