2 SYNOPSIS

Title of Trial: *Pilot study of the significance of PTEN expression in patients with hormone refractory prostate cancer (HRPC) treated with Cetuximab as monotherapy for 8 weeks, followed by Docetaxel plus Cetuximab*	
Investigator(s): Aristotelis Bamias Assistant Professor in Clinical Therapeutics University of Athens	
Trial Center(s): Oncology-Haematology Unit ALEXANDRA Hospital 80 Vasilissis Sofias Ave 115 28 Athens GREECE	
Publication (reference):	
Trial Period (years): 2	**Phase of Development: Premature terminated**
Objectives: A. *Primary objective*: Correlation of PTEN expression in biopsies from primary tumor and metastases with PSA response **B. *Secondary objectives:*** 1. Overall survival (OS) 2. Time to progression (TTP) 3. Objective response rate (RR) (RECIST) 4. Quality of life 5. Correlation of response with EGFR and pAkt expression 6. Toxicity 7. Correlation of PSA decline of at least 30% with PTEN loss	

8. Correlation of clinical responses not qualifying for PR with PTEN loss
Methodology: Patients with HRPC were screened, after signing informed consent, for PTEN loss in archive material from prostate biopsy. If no loss is found, the patient will undergo biopsy of a metastatic site or prostate. All patients were treated with weekly Erbitux for 8 weeks and subsequently were treated with 6 cycles of 3-weekly Docetaxel + weekly Erbitux. The total duration of treatment was 26 weeks and the duration of the study for each patient 29 weeks. The major objective was the PSA response and its correlation with PTEN expression. Other objectives were objective response rate, EGFR expression, TTP, OS, toxicity and quality of life
Number of Subjects: 16 patients with hormone refractory prostate cancer
Diagnosis and Main Criteria for Inclusion: METASTATIC HORMONE REFRACTORY PROSTATE CANCER (HRPC) 1. Histologically proven prostate cancer 2. PSA and/or clinical (including radiological) progression despite androgen deprivation (castration testosterone levels) (PSA progression:>=25% increase from baseline confirmed at least one week later) 3. Adequate antiadrogen withdrawal time (4 weeks for flutamide or nilutamide and 6 weeks for bicalutamide) 4. No prior chemotherapy 5. PSA at progression >= 20 ng/ml 6. ECOG PS 0-2 7. Adequate bone marrow, renal and liver function (platelets ≥ 100.000/ul, white blood cells ≥ 3.000/ul or neutrophil count ≥ 1.500/ul,, creatinine ≤ 1.5 mg/dl, hepatic transaminases ≤ 2.5 x ULN, serum total bilirubin ≤ 1.5 x ULN 8. Available archive material for PTEN screening or presence of lesions which could be biopsied (prostate, lymph nodes, liver, lung, soft tissue) 10. Signed written informed consent 11. Life expectancy >=3months 12. Age ≥ 18 years old

Test Product(s): ERBITUX

Dose and Mode of Administration, Batch Number(s): Weekly (initial dose 400mg/m2, subsequent doses 250mg/m2) Week 1-8 monotherapy, followed by combination with chemotherapy.

Mode of administration: i.v. infusion

Duration of Treatment: 26 weeks

Reference Therapy(ies), Dose and Mode of Administration, Batch Number(s):

Product: Docetaxel

Dosing schedule: 75 mg/m2 every 3 weeks

Mode of administration: i.v. infusion

Combination therapy starts on week 9.

Product: Prednisone

Dosing schedule: 10mg/day Mode of administration: PO

Product: GnRH-analogues should be continued as administered

Duration of Treatment: 18 weeks

Criteria for Evaluation:

Efficacy: The primary target variable will be PSA response in correlation with PTEN expression.

PSA estimation

Baseline, every 4 weeks during monotherapy with Erbitux, every 6 weeks during combination treatment, every 2 months after the completion of treatment

PTEN expression

PTEN loss will be assessed in archive material, with immunohistochemistry (± FISH), in paraffin embedded tissue, from prostate biopsy. If no loss is found, the patient will undergo biopsy of a metastatic site or prostate.

Safety: Toxicity was assessed according to NCI CTC (Version 3):

http://ctep.cancer.gov/reporting/ctc.html at each treatment visit. Dose modifications were applied according to study protocol.

Serious Adverse Events (SAEs) were reported within 24 hours. A SAE is defined as any untoward medical occurrence at any dose that:

- Results in death
- Is life-threatening
- Requires hospitalization or prolongs existing hospitalization
- Results in persistent or significant disability/incapacity

Results in congenital anomaly/birth defect

Statistical Methods: The initial design of the study provided PTEN expression (positive vs. negative) correlations with PSA response (yes vs. no), objective responses (yes vs. no) and baseline characteristics (such as baseline PSA, disease sites, Gleason score), using the Fisher's exact test.

Median TTP and OS was planned to be calculated using the Kaplan-Meier method. The impact of PTEN expression (loss vs. no loss), and baseline characteristics (such as baseline PSA, disease sites, Gleason score, ECOG PS) on TTP and OS planned to be assessed using the log rank test and the independent significance of these factors was planned to be assessed using a multivariate Cox regression analysis.

However, due to the premature discontinuation of the study, the performed analysis was just descriptive.

Summary and Conclusions:

Subject Disposition: The cohort of the study (till its premature discontinuation) consisted of 16 male patients with histologically proven metastatic hormone refractory prostate cancer (HRPC).

Demographics and Baseline Characteristics: The mean age of patients was 71.9 years (range 48-89 years). The mean height of patients was 170.4 cm , the mean weight was

78 kg and the mean body surface area (BSA) was 1.9 m^2. The 31 % of the patients were current smokers, 38% were ex smokers and 31% were nonsmokers.

According to the medical history, 11 of the patients had concomitant diseases. Specifically, 4 patients (25%) had diabetes mellitus and were taking hypoglycemic drugs, 5 (31%) were hypertensive under treatment, 2 of them (13%) had history of coronary artery disease and had a coronary artery bypass graft. One patient suffered from Chronic Obstructive Pulmonary Disease (COPD) and 2 had also malignancy in colon.

The mean Gleason score from the histopathological biopsies was 8 (range from 7 to 9) and 88% (14/16) of patients had secondary bone metastases. The baseline mean PSA was 103 (range from 10.79 to 509) and the mean time from androgen discontinuation was 120 days. Archive material was obtained in all but 1 patient. In the available results so far, PTEN loss was found in no case. Material from metastases was available in 4 patients, since the remaining 12 patients refused to undergo biopsy of metastatic lesions. PTEN expression was studied in 3 of these cases and no loss was found. Due to the almost universal finding of no PTEN loss, no correlation with PSA decline was possible. More refined analysis is under way.

The mean value for white blood cells (WBC) was 7190 (63% neutrophil), for haematocrit was 37.2% and for platelets was 258.000. The mean values for creatinine, bilirubin, SGOT and SGPT were 1.02 mg/dl, 0.63 mg/dl, 26 U/ml and 22 U/ml respectively.

Finally the majority of patients were at Grade 0 or 1 in ECOG performance status (88) and the mean value at the EORTC QLQ-C30 (questionnaire for quality of life) at the beginning of study was 56.

Efficacy Results: The study was prematurely terminated due to withdrawal of Erbitux in Greece. The mean starting dose was 714 mg (400mg/m^2) and the mean subsequent dose was 470 mg (250 mg/m^2). Two of the 16 patients discontinued the cetuximab therapy due to safety reasons. The consecutive monthly measurements of PSA showed a rise of the mean PSA from 103 to 154 and 162.7 at the 1st and 2nd month of cetuximab use respectively. However, PSA decline was noted in 2 patients with Erbitux monotherapy. From the 3rd month the mean value of PSA was significantly decreased (83, 44 and 47 at the 3rd, 5th and 6th month of cetuximab plus docetaxel, p=0.03).

At the end of the follow up period 8 patients (50%) were alive. The median survival during the follow up period was 24 months. The low values of PSA remained at the end of follow up period (mean value for PSA: 54).

In the field of quality of life the 1st month after Erbitux administration the EORTC QLQ-C30 score rose to 69 (from 54 at baseline) but in the 2nd to 6th month of Erbitux administration the score was significantly decreased (50 at 2nd month, 48 at 4th month and 39 at 6th month from Erbitux administration). Due to missing cases it was not possible to calculate the statistical significance of this finding.

Safety Results: From the total study cohort, three patients discontinued cetuximab therapy. The first one discontinued after the second infusion due to high fever with tremor during both infusions and the second patient due to sensory neuropathy 2 months after starting the cetuximab. The third one self-discontinued treatment and did not attend follow up visits.

The other severe adverse effects which presented during the therapy and the follow up period were the follows:

- Four patients experienced fever (one at the first infusion , two at the second infusion

and one at the fifth infusion of cetuximab). One of these patients presented fever and chest infection Grade III which led to hospitalisation for 6 days. At the time of the event, the patient had received 5 cycles of Erbitux. As a result of the events antibiotic i.v. therapy was given for one week. Two patients developed urinary tract infection. The first patient had presented with urinary retention which led to hospitalisation, where urethral obstruction was diagnosed, 2 months after initiation of therapy with cetuximab. Urethral Obstruction resolved and the patient was discharged from the hospital.

The second patient was diagnosed with urinary tract infection following a positive urine culture test for enterobacter aerogenes during his treatment with Docetaxel+cetuximab. The patient was treated initially with Amoxicillin –Clavulanic Acid for 3 days but following antibiogram results, where enterobacter aerogenes showed resistance to this antibiotic, the treatment changed and the patient received ciprofloxacin. One week after antibiotic initiation the patient became febrile, grade II with clinical signs of urinary tract infection. The patient refused hospitalization and 24 hours later the patient developed septic shock and one day later he died.

- One patient presented with acute kidney failure after the 5th infusion of Erbitux which was caused, according to the cystoscopy, from uretral obstruction due to the prostate tumor. The patient died after 2 weeks of hospitalization in the Nephrology Department.
- One patient presented with Acute Respiratory Distress upon having a meal 2 months after initiation of therapy. His condition deteriorated rapidly and the patient died from a nonmedically confirmed cause of death (possible cause of death: aspiration of ingested food). Autopsy or lab tests were not performed
- Seven patients experienced transient rash but one experienced progressively worsening widespread rash which required hospitalization in a Dermatology Clinic and delay of cetuximab and docetaxel for 2 doses.
- Two patients experienced transient loss of consciousness. In the first of them hydrocephalus was diagnosed from the brain CT, and the patient was hospitalized in the Neurosurgery Department without discontinuation of therapy. The other one suffered from sinus node dysfunction and a permanent pacemaker was implanted.

Conclusions: The number of patients included in the study was small due to the premature discontinuation of the study. At the end of follow up, 50% of the patients were alive after treatment with cetuximab and docetaxel. The median survival has been 24 months, which is well within the expected survival for this group. The administration of cetuximab as monotherapy did not reduce the level of PSA, but the combination therapy with docetaxel and cetuximab significantly reduced the level of PSA and this reduction remained at the end of follow up period.

The drug was relative well tolerated, as two patients (12.5%) discontinued the drug due to adverse effects and one presented with acute renal failure. Conclusive results regarding

the correlation of PTEN expression with the PSA response are not yet available. In conclusion this study (which was prematurely terminated) does not support the hypothesis that PTEN expression in biopsies from primary tumor and metastases are correlated with PSA response.
Date of Report / Version No.: 23/7/2013 –Version 1.0 Final