measured at two months was not predictive of the PFS (p = 0.559). In univariate analysis, better PFS was correlated with early decrease in tumour density (p = 0.027), modified RECIST criteria (−15% size) (p = 0.017) and early decrease in tumour density normalized to liver ratio (p = 0.012). In multivariate analysis, Hazard ratio were 2.38 [1.41–4.03] (p = 0.004) for modified RECIST response criteria (−15% size) and 2.25 [1.32–3.84] (p = 0.001) for a tumour to liver ratio density response of more than 10%. We performed the same analysis in a control population (n = 70) treated in first line chemotherapy without bevacizumab for a metastatic colorectal cancer. Modified RECIST criteria evaluation measured at two months and changes in tumour density to liver ratio were not correlated with PFS (p = 0.139 and p = 0.299 respectively).
Conclusion: A change in tumour density to liver retio on CT combined with a modified response RECIST criteria seems to be an accurate method of assessing the PFS during the early stage of bevacizumab treatment. Theses findings should be validated in a prospective study.

References

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1403 POSTER DISCUSSION

Molecular Testing for BRAF V600 Mutations in Clinical Trials of the BRAF Inhibitor Vemurafenib (RG7204/PLX4032) in Metastatic Melanoma − a Comparison With Sanger Sequencing

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Background: The medical need for robust accurate companion diagnostic assays for selecting patients for targeted anti-cancer therapies is exemplified by the development of the selective BRAF inhibitor vemurafenib, which has shown significant activity in clinical trials of patients with BRAFV600 mutation-positive melanoma. We describe the analytic performance of an investigational PCR assay (**cobas**® 4800 BRAF V600 Mutation Test) designed to detect the V600E (1799 T>A) mutation, which was used to screen patients for the pivotal Ph II and Ph III trials of vemurafenib in metastatic melanoma.
Material and Methods: Samples from 477 eligible patients screened for the Phase II and Phase III trials were used to evaluate the positive (PPA), negative (NPA) and overall percent agreements (OPA) of the **cobas**® 4800 BRAF V600 Mutation Test with 2X bi-directional Sanger sequencing as a reference method for the detection of mutations in codon 600. The primary aim was to assess the agreement of the cobas® test and Sanger for the detection of the predominant V600E (1799 T>A) mutation. Samples with discordant PCR and Sanger results were subjected to "deep" sequencing with 454 GS-Titanium (454) to resolve discrepancies.
Results: A valid **cobas**® result was obtained for all 477 eligible patients. Sanger had a failure rate of 9.2% (44/477), leaving 433 evaluable samples for the agreement analysis. The PPA was 96.4% (215/223), and the NPA was 80% (168/210), with an OPA of 88.5%. Discordant resolution by 454 indicated that of 42 samples that were mutation-positive by the **cobas**® test and negative for the V600E mutation by Sanger, 17 samples were wild-type (15) or non-V600E (2) by Sanger but V600E-positive by 454 sequencing. In addition, 24 samples were V600K positive by both Sanger and 454 sequencing, and one sample had a rare GTG to GAC mutation by Sanger. For the 8 cobas® negative/Sanger V600E-positive discordant samples, the results by 454 were wild-type in 2 cases, V600K in 2 cases, V600E2 in 1 case, and V600E in 3 cases. The **cobas**® 4800 BRAF V600 Mutation Test detected 70% of the V600K mutations in this cohort.
Conclusions: The **cobas**® 4800 BRAF V600 Mutation Test 1) had a lower failure rate than Sanger; 2) was more sensitive in the detection of V600E mutations than Sanger, 3) and detected a majority of V600K mutations in the cohort. Robust, rapid and accurate molecular testing was achieved in these large multi-center clinical trial.

1404 POSTER DISCUSSION

Baseline (BL) IL-6, IL-8, and VEGF as Predictive and Prognostic Markers for Overall Survival (OS) in Metastatic Renal Cell Carcinoma (mRCC) Patients (pts) Treated in a Phase III Trial of Pazopanib (PAZO) Versus Placebo (PL)

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Background: PAZO is a multi-kinase inhibitor approved for the treatment of mRCC. Analysis of BL plasma cytokine and angiogenesis factors (CAFs) in Phase II and III studies identified candidates (HGF, IL-6, IL-8, TIMP-1, VEGF, E-Selectin and OPN) that significantly correlated with PFS for pts receiving PAZO (Tran ASCO 2010, #4522). IL-8, and OPN were found to be prognostic and IL-6 was both prognostic and predictive (Liu, GU,ASCO 2011 #334).
Material and Methods: OS (Sternberg ESMO 2010 #LBA22) of pts with mRCC from the Phase III randomized PL-controlled trial (NCT00334282) was correlated to BL plasma CAF. Markers associated with clinical outcome in the PL arm were considered prognostic and those identifying groups receiving relative different degrees of benefit from PAZO compared to PL were predictive. Samples (254 P; 53 PL) were analyzed by SearchLight Protein Array. CAFs were correlated to OS. A 6 factor angiogenic signature (IL6, IL8, HGF, OPN, TIMP1, and VEGF) was generated by hierarchical clustering (UPGMA, Euclidean distance). Pts were stratified into signature high and low groups to test for correlation to OS.
Results: TIMP1 was significant as a prognostic marker for OS (PL p = 0.013, PAZO arm p < 0.0001). Increased IL6 (Interaction (IA) p = 0.010), IL8 (IA p = 0.012), and VEGF (IA p = 0.013) were predictive of greater OS benefit for PAZO and OPN (IA p = 0.057) showed borderline significance. High HGF was associated with shorter OS in PAZO arm (p = 0.003) but not in PL arm (p = 0.178). The 6 angiogenic factor signature was both predictive for OS (IA p = 0.033) and prognostic.
Conclusions: Results suggest that BL plasma IL-6, IL8, VEGF and OPN (borderline) were both predictive and prognostic markers for OS, in which pts with higher levels of these BL CAFs showed a greater benefit from PAZO treatment. BL TIMP1 was prognostic for OS. High HGF was associated with shorter OS in the PAZO but not PL arms suggesting HGF may be associated with PAZO resistance. The novel 6 angiogenic signature profile showed predictive and prognostic value. BL plasma CAF markers, if validated, could be useful tool in determining prognosis and evaluating individualized therapeutic response to PAZO and other VEGF inhibitors in mRCC pts.
Trial Sponsor: GSK

1405 POSTER DISCUSSION

Cyp2C19*2 Polymorphism Predicts Benefit of Adjuvant Tamoxifen in ER Positive Postmenopausal Breast Cancer Patients

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Background: Polymorphisms in tamoxifen metabolizing enzymes are associated with variation in response. Much attention has focused on cyp2D6, the main enzyme involved in conversion of tamoxifen to endoxifen. However, a meta-analysis showed that cyp2D6 polymorphisms do not greatly affect benefit of tamoxifen. Much less is known about the association between tamoxifen benefit and variants of cyp2C19, which is involved in 4-OH-tamoxifen formation. The cyp2C19*2 variant has a minor allele frequency of 13% in Caucasians and has previously been associated with increased breast cancer survival rates in incident tamoxifen users. The aim of our study was to investigate the association between the CYP2C19*2 and CYP2D6*4 genotypes and benefit of adjuvant tamoxifen.
Material and Methods: From 1982 until 1994 a randomized clinical trial was conducted in The Netherlands, studying the benefit of adjuvant tamoxifen (TAMOX-trial). Patients were randomized among tamoxifen (1 to 3 years) versus no adjuvant therapy. None of the patients received adjuvant chemotherapy. Median follow-up of this series is 9.6 years. In total 1662 patients were included. We recollected tissue blocks with sufficient material of 739 patients. Genotyping for CYP2D6*4 and CYP2C19*2 was performed, using Taqman allelic discrimination, and results were correlated with recurrence free interval (RFI) in estrogen receptor (ER) positive patients. Multivariate Cox proportional hazard models, including