11 days for samples reconstituted by 4ºC water and 7 days for those reconstituted by 25ºC water. To evaluate how freezing can affect the physical stability, we studied the characteristics of the suspension before and after freezing (−20ºC, 48 hours) and thawing at room temperature. The sedimentation kinetics was studied by following the decrease of absorbance at 500 nm versus time. The aspect of crystals, after filtration on a 0.22 μm filter, was studied by scanning electron microscopy.
Results: The degradation of AZC followed a biphasic kinetic with a rapid initial phase strongly depending on the temperature (% of remaining AZC at 25ºC: 93.2%; 4ºC: 95.9%). Using water at 25ºC, the rate of initial degradation is higher than using cold water (0.336% hr^{-1} vs 0.162% hr^{-1}). However, regardless of the initial conditions of reconstitution, the total degradation was less than 5% after 7 days if reconstituted vial was immediately stored at 4ºC. After storage at −20ºC, no degradation of AZC was observed. The physical characteristics of suspension were not modified: sedimentation rate (4ºC: 144 s; −20ºC: 152 s); identical size and shape of crystals.
Conclusion: If syringes are stored at 4ºC immediately after reconstitution, the use of iced water permits only to slow the initial degradation step but is not essential since the total degradation remains inferior to 5% after 7 days for both reconstitution temperatures. Therefore, the in-use stability period of AZC suspension is higher than recommended by the manufacturer. Freezing should permit long term storage of the suspension without any physical and chemical alterations.

Poster Discussion Presentations (Mon, 26 Sep, 08:00−09:00)

Biomarkers / Imaging

1400 POSTER DISCUSSION

Interest of CHOI and Modified CHOI Criterion for Evaluation of Metastatic Renal Cell Carcinomas (mRCC) Patients Treated With Everolimus

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Background: Because tumour response may be underestimated by RECIST as new targeted therapies can induce more necrosis than tumour shrinkage, we studied whether CHOI and modified CHOI (mCHOI) criterion might be valuable to assess everolimus efficacy.
Materials and Methods: We, retrospectively, reviewed the computed tomography (CT) of 70 mRCC patients (pts) enrolled in the French centers participating to the randomized, double-blind, multicenter phase III study comparing Everolimus vs placebo (RECORD-1). In this trial, the primary endpoint was PFS, based on RECIST criteria assessed on CT performed at baseline and every two months. We investigated CT until first progression according to CHOI criteria where partial response (PR) was defined as $\geqslant$10% decrease in tumour size OR $\geqslant$15% decrease in attenuation; and according to mCHOI criteria where partial response (PR) was defined as $\geqslant$10% decrease in tumour size AND $\geqslant$15% decrease in attenuation. Attenuation was measured on region of interest covering at least $\frac{3}{4}$ of the surface area of the targeted lesions on the CT sections where the largest diameter could be measured.
Results: Because of renal impairment that precluded contrast injection and lesions that could not correctly be assessed for attenuation, only 50 pts were eligible for analysis. Among them 19 were in the placebo arm and 31 treated by Everolimus. PFS were 2.8 and 6 months ($p < 0.005$), respectively. In the placebo group, CHOI criteria identified 47% of PR compared to non-responders with significant differences for PFS (3.6 vs 2.0 months $p < 0.01$, respectively), while mCHOI criteria found 0% of PR.
In the Everolimus group, 55% of pts were considered PR and 45% non responders according to CHOI criteria without significant differences for PFS (6.0 and 5.9 months, respectively) while mCHOI found 26% PR compared to 74% non-responders without significant differences for PFS (7.4 and 5.5 months, $p = 0.13$, respectively).
Conclusion: The use of CHOI or mCHOI criterion could not discriminate PFS between responders or non-responders pts treated with Everolimus. In the placebo arm, CHOI criteria identified a subgroup of pts with spontaneous necrosis associated with a longer PFS.

1401 POSTER DISCUSSION

Choi Response Criteria for Prediction of Clinical Outcome in Patients With Metastatic Renal Cell Carcinoma Treated With Targeted Therapies

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Background: Anticancer treatment efficacy is measured by decrease in tumour size and standardized according to the Response Evaluation Criteria in Solid Tumours (RECIST). With the advent of new targeted therapies, necrosis and cavitation rather than shrinkage were described as first response to treatment. The purpose was to evaluate whether early assessment of tumour shrinkage alone (RECIST criteria) or combined with changes in tumour density (Choi criteria) better predict clinical outcome in patients with metastatic renal cell cancer (mRCC).
Patients & Methods: In this retrospective multicenter study we included 47 patients with mRCC treated with a tyrosine kinase and/or mTor inhibitor and for whom at least two CT scans (baseline and follow-up) with measurable lesions were available. CT scans were blinded and analyzed centrally according to RECIST and Choi criteria. Patients were categorized according to both criteria into complete and partial response (CR/PR), stable disease (SD) and progressive disease (PD). A dichotomisation into responders (CR or PR) and non-responders (SD or PD) was conducted. The response to therapy was compared with clinical outcome including progression free survival (PFS) and overall survival (OS). Differences in survival of responders and non-responders were assessed with log-rank tests and Cox proportional hazards models.
Results: According to RECIST criteria, 8 patients were responders and 26 patients non-responders, whereas to Choi criteria, 17 were responders and 17 non-responders.
Responders had higher PFS and OS according to Choi criteria (log-rank test $p = 0.001$ and $p = 0.023$, respectively) than according to RECIST criteria ($p = 0.404$ and $p = 0.055$, respectively). Based on Cox proportional hazards models adjusted with prior treatment with interferon and the time between diagnosis and start of therapy, the hazard ratios for responders vs. non-responders according to Choi criteria were 0.25 for PFS (95% CI 0.10–0.61, $p = 0.002$) and 0.33 for OS (95% CI 0.12–0.90, $p = 0.030$) as opposed to the hazard ratios according to RECIST criteria of 0.59 for PFS (95% CI 0.21–1.65, $p = 0.313$) and 0.34 for OS (95% CI 0.10–1.17, $p = 0.087$).
Conclusion: Using Choi criteria in evaluating mRCC patients treated with targeted therapies will change response evaluation and better correlates with PFS and OS compared to using RECIST criteria.

1402 POSTER DISCUSSION

CT Evaluation of the Response of Colorectal Liver Metastasis After Bevacizumab Treatment − a Density Quantitative Analysis Correlated With Patient Outcome

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Context: The standard criteria used to evaluate tumour response, the Response Evaluation Criteria in Solid Tumours (RECIST), were developed to assess tumour shrinkage after cytotoxic chemotherapy and may be limited (1) in assessing response to biologic agents, which have a cytostatic mechanism of action.
Purpose: To validate novel tumour response criteria based on tumour size and density early changes observed on computed tomography (CT) in patients with colorectal liver metastases treated with bevacizumab-containing chemotherapy regimens.
Material and Methods: We performed a centralized review of the 145 patients included in ACCORD 13 prospective clinical trial (NCT00423696). Seventy one patients were excluded of the analysis because of the absence of liver metastasis (n = 19), images data not available or incomplete (n = 30), CT delay time not respected (n = 7), CT acquisition protocol not respected (n = 15). The final study population was 74 patients treated by FOLFIRI + Bevacizumab (n = 46) or XELIRI + Bevacizumab (n = 28), with a median follow up of 34.1 months [2.8–47.5 months]. Tumour size (RECIST) and density were determined objectively using a semi- automatic segmentation tools (Myran®, Intrasense) at the portal phase. We analyzed changes in tumour size and density, in patient who underwent a CT scan before and 2 months after starting treatment.
Results: There was no significant difference between the entire clinical trial population study (ACCORD 13) and the analysed patient group in terms of age, sex, PFS and OS. The RECIST response (PR or CR)

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

[1] Association of computed tomography morphologic criteria with pathologic response and survival in patients treated with bevacizumab for colorectal liver metastases. Chun YS, Vauthey JN, Boonsirikamchai P, Maru DM, Kopetz S, Palavecino M, Curley SA, Abdalla EK, Kaur H, Charnsangavej C, Loyer EM. JAMA. 2009 Dec 2;302(21):2338−44.

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