than expected. This study confirms OS benefit of IPI in treatment-naïve metastatic melanoma.
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9319 POSTER

A Regional Review of Outcomes of Systemic Therapy in Patients With Metastatic Malignant Melanoma

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Background: The incidence of malignant melanoma (MM) has risen steadily over recent decades. NCI data from 2005–2007 have suggested that 1.93% of individuals born today in the US will develop melanoma at some stage [1]. Approximately 15% of patients with MM either present with metastatic disease or develop metastases during the course of their illness. Unfortunately, metastatic MM remains a challenge with limited treatment options and median overall survival (OS) reported as 6−9 months. Dacarbazine (DTIC) remains the standard first line treatment with published response rates of less than 10% and infrequent durable responses. We reviewed our data for the treatment of metastatic MM over a period of four years.
Material and Methods: Data from all patients with metastatic MM treated with systemic therapy outwith clinical trials from 2006 to 2009 were reviewed. Response rate was determined as per RECIST criteria.
Results: Sixty-four patients were treated with one or more lines of cytotoxic therapy. Median age was 62 years (range 23−82 years) with 53% males. Primary site of disease was skin in 75%, mucosal in 12.5%, ocular in 9.3% and nodal with occult primary in 3.1%. Visceral metastases were present in 75% of patients at the start of treatment, including pulmonary (39.6%) and hepatic (34.4%). All patients were screened for brain metastases: these were present in 26.5% of patients. ECOG performance status (PS) was 0 in 7.8%, 1 in 68.7%, 2 in 9.4% and undocumented in the remaining 14%. Patients without brain metastases received single agent DTIC first-line; those with brain metastases received temozolomide. Response rate was 7% for DTIC and 28% for temozolomide, with median progression free survival (PFS) of 2.1 and 2.4 months respectively. Seven patients who received DTIC are alive on follow-up, 2 have ongoing stable disease post-DTIC at 41 months and 18 months respectively. Second line therapy with vinblastine was given to 21 patients (32%), with a response rate of 9.5% and median PFS of 3.4 months. Median OS from initiation of therapy was 7.7 months for DTIC and 3.6 months for patients with brain metastases receiving temozolomide. PS of 2 was associated with shorter median OS (2.0 months).
Conclusions: Our results are comparable to those in published data [2]. MM is a disease with rising incidence and limited treatment options. These patients are best treated in the context of clinical trials as new targeted therapies are promising as future strategies.

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9320 POSTER

PolyMEL, a Polyepitope DNA Vaccine − Results From a Phase 1 Study for Metastatic Melanoma

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Background: Metastatic melanoma carries a poor prognosis. Although promising therapies are emerging, new approaches are still needed.
Materials and Methods: A phase I dose escalation study with PolyMEL, a DNA polyepitope vaccine encoding 8 nine-amino acid epitopes derived from 45 shared melanoma antigens, was devised. The objectives of this study were to establish the safety and feasibility of DNA vaccination with polyMEL, to determine the maximum tolerated dose, and to see whether polyMEL vaccination elicits a clinical anti-tumour response. Cohorts of 3 patients with stage IV melanoma who were either HLA-A1 or -A2 positive received 3 intramuscular injections of increasing doses of polyMEL DNA vaccination: 30 μg, 100 μg, 300 μg or 1000 μg/ injection.
Results: 15 patients were entered into the trial between October 2003 and September 2008. Two patients withdrew early from the study with clinical deterioration and were replaced, and a third was found to have brain metastases at baseline and did not receive study drug. 12 patients were therefore assessable for response, and 14 were assessable for toxicity. Nine patients had cutaneous melanoma and five had ocular melanoma. The vaccine was well tolerated, with no haematological or biochemical toxicity, and mainly grade 1 or 2 non-haematological toxicity. All 9 patients with cutaneous melanoma had progressive disease at the end of the study protocol. By contrast, 3 of the 5 patients with ocular melanoma had stable disease at the end of the study protocol (60% disease control rate), and 2 had progressive disease. Median progression-free survival was 64 and 102 days for patients with cutaneous or ocular primary melanoma respectively, and the respective median overall survival was 182 and 336 days.
Conclusions: Administration of polyMEL polyepitope vaccine at doses ranging from 30 μg to 1000 μg, given every 2 weeks to a total of 3 injections, is safe and associated with minimal toxicity. The tolerability of polyMEL should allow its combination with established or experimental agents in future studies. Patients with ocular melanoma had better outcomes compared with patients with metastatic cutaneous melanoma. This agent may therefore be of particular interest for patients with metastatic ocular melanoma, for whom no effective treatment exists.

9321 POSTER

Prognostic Factors in a Cohort of Dacarbazine Treated Patients for Metastatic Melanoma

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Background: Incidence and mortality of melanoma in Europe is increasing. Metastatic melanoma (mM) has poor prognosis with 5-year survival rates of 15%. Dacarbazine (DTIC) is considered a standard treatment for mM despite response rates of 10−20% and median response duration 3−6 months. Our purpose was to identify prognostic factors associated with improved survival in mM patients (pts) treated with DTIC.
Methods: Retrospective cohort study, in a Portuguese cancer centre, of mM pts treated with DTIC as 1st line systemic treatment. Survival time was defined as a difference between the start date of DTIC and death. Pts alive were censored on the date they were last seen in the clinic. Potential prognostic variables were assessed in univariate analysis with a log rank test and in multivariate analysis through a Cox proportional hazards model.
Results: From 2005 through 2009, 109 pts with mM were treated with DTIC in our centre. Median age was 58 years (range 20−77), 45% were male and 60% had an ECOG performance status of 0. Visceral metastases were present in 66% of pts.
Half of the patients completed at least 4 cycles of DTIC and 27% completed $\geqslant$ 6 cycles. A relative dose-intensity of DTIC $\geqslant$90% was achieved in 77%. Main reason for treatment interruption was disease progression (68%). Serious adverse events occurred in 19% and 2 pts died on treatment due to unknown causes. Overall response rate was 32% and median duration of response was 3 months (m). Median overall survival was 6 m (95CI: 4.1−7.9).
In univariate analysis, normal LDH was associated with improved survival (median survival: 9 m *vs* 4 m; $p = 0.005$) as was ECOG status 0 *vs* $\geqslant$ 1 (median survival: 8 m *vs* 4 m; $p = 0.013$). Patients with visceral metastases tended to have a worst prognosis (median survival 5 m *vs* 7 m), however this was not statistically significant ($p \geqslant 0.05$). Age and gender had no impact on survival. In multivariate analysis, normal LDH was the only factor associated with increased survival (HR, 0.50; [95CI, 0.28–0.93]).
Conclusion: Of the known prognostic factors in metastatic melanoma only LDH had a significant impact on survival. The small sample size limits the power of our study which may explain our inability to identify other probably less discriminating prognostic factors. The strong prognostic information conveyed by LDH should be incorporated in patient stratification in trials of new treatment for metastatic melanoma.

9322 POSTER

Immunological and Biological Changes and Their Correlation With Clinical Response and Survival During Ipilimumab in Metastatic Melanoma Compassionate Use Program

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Background: Recentely FDA has approved ipilimumab at 3 mg/kg as first and second line of therapy in patients with metastatic melanoma. This is sustained by an impact on overall survival in this setting of patients of 10.1 months. Anyway no clinical parameter has been consistentely found to be a surrogate or a predictive marker for response to ipilimumab therapy

and only a few immunologic changes (absolute lymphocytes value) have been demonstrated. **Patients and Methods**: From June 2010 to date we have been treating in the Compassionate Use Program for ipilimumab at 3 mg/kg fifty pretreated metastatic melanoma patients. 35 out of 50 patients (70%) completed all four doses and were considered evaluable for clinical response, toxicity and for sieric changes of LDH and RCP (reactive C protein), and time to progression (TTP). For RCP evaluation we defined 3 categories: <5 mg/dl for normal values, ⩾5<8 for high values and ⩾ 8 to indicate very high values. According the immunological and biological assessment we have collected PBMC and sera of these patients. Blood draw was performed at week 0, 4, 7, 10 and 12. PBMC were thawed and labeled with FoxP3-AlexaFlour488/CD4-Pe-Cy/CD25-Pe (Kit Biolegend). Labeled cells were analyzed using a FACSAriaII (Becton Dickinson). We have also studied serum cytokines (IL-10, IL-6 and TGF-b) and auto-Ab (as Anti DS-Dna, Anti-Tg, ,ANA), that were measured using enzyme-linked immunosorbent assays.
Results: In this setting of patients, we found in 30/35 (85%) of them a good correlation between the increase of LDH and CRP, and the worsening of clinical response. For patients [17/35(48%)] with a rapid progressive disease not responsive to ipilimumab, we found that the percentage of Treg increased during the treatment (median: 1.8%; range 1−2.6%); this increase was not influenced by development of autoimmunity. In the responsive patients group [18/35(51%)] the values of Treg remained stable at 0.50% [(10/18 (55%)], while the remaining group [8/18(45%)] decreased of 0.10% per cycle. At moment, no changes in sieric cytokines and antibodies have been found.
Conclusion: LDH and RCP seems to be predictive parameters of response to ipilimumab. Moreover, very preliminary data shows a relationship between the increase of the circulating Treg cell percentage and a bad response to ipilimumab. Further studies are necessary to verify this data.

9323 POSTER

Enhanced in Vitro and in Vivo Cytotoxicity of Combined Vaccinia Virus Strain GLV-1h68 and Chemotherapy in Melanoma

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Background: Monotherapies in cancer treatment have only shown modest activity and short-lived disease control. Adaptive genetic alterations in tumours lead to treatment resistance. Hence, it is now widely accepted that the future development of virotherapy will occur as a part of combination with chemotherapeutic drugs. The purpose of this study was to test combination treatment of oncolytic vaccinia virus strain GLV-1h68 and cisplatin in human skin melanoma cells *in vitro* and *in vivo*.
Methods: In vitro cytotoxicity of GLV-1h68 given alone and combined with cisplatin was assessed by colorimetric and tissue culture infectious dose 50-based assays. Viral replication alone and combined with chemotherapy was tested by viral plaque assays and real-time PCR. Interactions between the agents were evaluated using combination index analysis. Mechanism of cell kill was assessed using western blotting and probed for cleaved caspases-3. The combination treatment of GLV-1h68 and cisplatin was assessed in one tumour model *in vivo*.
Results: GLV-1h68 cytotoxicity was seen in all melanoma cell lines tested. Combination of GLV-1h68 and cisplatin yielded increased cytotoxicity and combination index analysis revealed synergy between virus and chemotherapy at combinations of 1 or 2-times the half maximal inhibitory concentration of each agent. Combination treatment significantly increased apoptosis in tumour cells relative to either single-treatment. Increased cell kill was not due to increased viral replication in combination treatment. *In vivo* study using xenograft tumours (A375) established in female CD1 nude mice showed statistically significant enhanced activity in terms of overall survival of the combination treatment compared to either treatment alone ($P < 0.05$).
Conclusions: Combining vaccinia virus strain GLV-1h68 with cisplatin synergistically enhances cytotoxicity in melanoma in *in vitro* and *in vivo*. These data may provide the direct basis for the design of translational clinical trials.

9324 POSTER

Abberant Regulation of Nerve Growth Factor Receptor (NGF-R) by Micro-RNAs in Melanoma − Mechanisms and Implications

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Background: Metastatic melanoma is a devastating disease with limited therapeutic options. Micro-RNAs (miRNAs) are small RNA molecules with important roles in post-trascriptional gene expression regulation that have recently been implicated in cancer. We previously showed that the expression of miRNAs from a large cluster on human chromosome 14q32 is significantly down-regulated in melanoma, and that epigenetic modifications can partly lead to re-expression of some miRNAs from this cluster. A recent publication demonstrated that only human melanoma cells expressing nerve growth factor receptor (NGF-R) were capable of initiating melanoma in nude mice, suggesting that NGF-R is a melanoma 'stemness' factor. Bio-informatic analysis revealed that several miRNAs from the chromosome-14 cluster, among them mir-377, could potentially target NGF-R.
Materials and Methods: Melanoma cell lines were stably transfected with mir-377, and the expression of NGF-R mRNA and protein was assessed by qRT-PCR and western blot, respectively. A luciferase reporter assay using the 3'UTR of the NGF-R was performed to study whether mir-377 negatively regulates the mRNA of NGF-R.
Results: NGF-R was not detected in normal melanocytes but was detected in benign nevi and in melanoma cell lines and samples. In contrast, mir-377 was detected in normal melanocytes and in nevi but not in melanoma samples or cell lines. Stable expression of mir-377 in two melanoma cell lines led to a significant decrease in the level of both NGF-R mRNA and protein. Reporter assays using the luciferase gene attached to the 3'UTR of NGF-R showed that luciferase expression is decreased following over-expression of mir-377, indicating that NGF-R is a true target of mir-377.
Conclusions: Our work demonstrates that mir-377 targets NGF-R, a membrane receptor recently implicated in melanoma tumorigenesis. Our results suggest that down-regulation of mir-377 leads to a significant increase in the levels of NGF-R during the transformation process of normal melanocytes. Such increased expression of NGF-R may contribute to the melanocytes' ability to propagate and even metastasize. We are currently studying the biological implications of mir-377 silencing and NGF-R expression in melanoma cells using a battery of biological assays. We are also assessing whether epigenetic modifications can lead to re-expression of mir-377, thus potentially reverting, at least to some extent, the tumorigenic and metastatic behavior of melanoma cells.

9325 POSTER

Prognostic Impact of B-Cell Infiltration in Melanoma

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Background: Studies on the prognostic importance of tumour-infiltrating lymphocytes have mainly focused on T cells while little is known about the possible role of tumour-infiltrating B cells, although their presence has been documented in various tumour types.
Material and Methods: We investigated the prevalence of B lymphocytes expressing CD20 by immunohistochemistry in primary cutaneous melanoma samples of 106 patients, and analyzed in relation to clinico-pathological parameters, tumour progression (>5 years follow-up), and patients' survival.
Results: We found that the majority of samples contained a significant amount of infiltrating B cells, localized predominantly to the peritumoral areas. In most cases $CD20^+$ lymphocytes were dispersed in the stroma surrounding tumour deposits; B cells organized in follicle-like aggregates were also observed in 26% of the samples. The amount of B lymphocytes significantly correlated with the density of activated ($CD25^+$ or $OX40^+$) T cells. The intensity of infiltration by $CD20^+$ lymphocytes did not show correlation with the thickness of the tumours, while the presence of B-cell aggregates was observed more frequently in thick melanomas. Both intra- and peritumoral infiltration by $CD20^+$ lymphocytes was more pronounced in nonmetastatic or lymph node metastatic tumours, compared to visceral metastatic ones ($p = 0.0309$ and $p = 0.0055$, respectively).