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

E. Karapanagiotou[1], T. Pencavel[1], K. Twigger[1], D. Mansfield[1], V. Roulstone[1], K. Syrigos[2], K. Harrington[1]. *[1]The Royal Marsden NHS Trust − Institute of Cancer Research, Head and Neck Unit, United Kingdom; [2]Athens Medical School Sotiria Hospital Athens, Oncology Unit, Athens, Greece*

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

R. Leibowitz-Amit[1], H. Schayek[2], L. Zehavi[2], Y. Sidi[3], D. Avni[2]. *[1]Sheba Medical Center, Oncology, Tel Hashomer, [2]Sheba Medical Center, Laboratory of Molecular Cell Biology, Tel Hashomer, [3]Sheba Medical Center, Internal Ward C, Tel Hashomer, Israel*

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

A. Ladányi[1], J. Kiss[1], A. Mohos[2], B. Somlai[3], G. Liszkay[4], K. Gilde[4], Z. Fejos[4], I. Gaudi[5], J. Tímár[6]. *[1]National Institute of Oncology, Center of Surgical and Molecular Tumour Pathology, Budapest, [2]Semmelweis University, 1st Institute of Pathology, Budapest, [3]Semmelweis University, Institute of Dermato-Venerology, Budapest, [4]National Institute of Oncology, Department of Dermatology, Budapest, [5]National Institute of Oncology, National Cancer Registry, Budapest, [6]Semmelweis University, 2nd Institute of Pathology, Budapest, Hungary*

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).

Accordingly, high number of these cells provided significant survival advantage ($p = 0.0391$ and $p = 0.0136$ for intra- and peritumoral infiltration, respectively). Furthermore, combination of peritumoral B-cell density with the number of activated T lymphocytes identified patient subgroups with different disease outcome, which was most favorable in the case of high density, while very poor in the case of low density of both cell types. Multivariate survival analysis involving B-cell and activated T-cell densities alone and in combinations, as well as traditional prognostic factors, identified tumour thickness and $CD20^+/OX40^+$ cell density combination as significant independent prognostic factors.
Conclusions: Our results show correlation between low numbers of $CD20^+$ B lymphocytes and melanoma progression. Moreover, the density of these cells, especially in association with that of activated T lymphocytes, proved of prognostic significance, indicating a possible role of tumour-infiltrating B cells in antitumour immune response reflected in better outcome of the disease.

9326 POSTER

Cancer Chemopreventive Potential, Polyphenolic Contents and Antioxidant Activity of Prosopis Cineraria

P. Meena[1], A. Kumar[1]. [1]*Radiation & Cancer Biology Laboratory, Department of Zoology, Jaipur, India*

Background: Prosopis cineraria (L) Druce, commonly called as Khejri, widely distributed and used in Rajasthan, India, as folk medicine and food. **Materials and Methods:** Polyphenolic contents and free radical scavenging activity, in the stem bark, leaf, pod and flower of Prosopis cineraria, were measured. Cancer chemopreventive potential of Prosopis cineraria leaf extract was evaluated using two stage skin carcinogenesis model system. Male Swiss albino mice were divided into five groups; control, pre, peri, post and throughout treatment group.
Results: Total phenolic, flavonoid and flavonol contents were found significantly higher in leaf followed by flower, pod and stem bark. The leaf extract showed significant free radical scavenging activity followed by flower, pod and stem bark as evidenced by low IC_{50} for DPPH and high percentage inhibition of DPPH and ABTS. Correlation between polyphenolic contents and antioxidant activity suggest that polyphenolic constituents may be responsible for observed antioxidant activity. A significant reduction was observed in tumour incidence, tumour yield, tumour burden, weight and size of tumour in all treatment groups as compared to control. Average latent period was also increased significantly in all treatment groups. In biochemical estimation of mice skin, a significant increased was observed in GSH, SOD and CAT. Whereas significant decrease in LPO level was observed in all Prosopis cineraria treated groups as compared to control.
Conclusion: The results from the present study suggest significant polyphenolic contents, antioxidant activity and chemopreventive potential of Prosopis cineraria extract.

9327 POSTER

Surface Enhanced Raman Spectroscopy Used for the Identification and Characterization of Melanoma Mice Skin Tissues

A. Falamas[1], C. Dehelean[2], V. Chis[3], S. Cinta Pinzaru[3]. [1]*Babes-Bolyai University, Faculty of Physics, Cluj Napoca,* [2]*"Victor Babes" University of Medicine and Pharmaceutics, Faculty of Pharmacy, Timisoara,* [3]*Babes-Bolyai University, Faculty of Physics, Cluj-Napoca, Romania*

Raman spectroscopy has currently become a powerful vibrational technique largely used to probe the molecular composition of biological tissues [1]. Raman spectra provide information on molecular vibrations leading thus to the possibility of highly specific fingerprinting of the molecular structure and biochemical composition of cells and tissues. In the past two decades there has been a renewed interest in Raman techniques due to the discovery of surface-enhanced Raman scattering (SERS) effect. Briefly, the usually weak Raman signals can be greatly enhanced when Raman scattering takes place on molecules at the surface or in very close vicinity to gold or silver nanoparticles. The SERS effect is mainly employed for the investigation of the molecular species adsorbed on noble metal nanoparticles. Recently, SERS was applied in the study of biological cells and tissue and proved great potential for a wide variety of applications in areas where nucleic acid identification is involved and could lead to the development of detection methods that minimize the time, expense, and variability of preparing samples. [2].
This work is intended to provide the latest investigations of our group in the field of spectroscopy applied in study of biological systems. Mice specimens employed in this study were inoculated with B16 melanoma cells and autopsy samples were collected at different stages of malignancy. The samples immersed in formalin solution mixed with silver colloidal nanoparticles were submitted to spectroscopic investigations which revealed that the Ag nanoparticles penetrated the tissues and enhanced the signals especially from nucleic acids [3]. These observations proved the fact that Raman and SERS are capable to investigate biological systems and to diagnose the samples at an early stage of cancer.
Inclusion complexes of pentacyclic triterpenes found in the outer bark of the birch tree, such as betulin and betulinic acid were used to create pharmaceutical formulations which were tested on the diseased mice, since betulin proved to have benefic effects in skin treatments and closed inhibitory activity on proliferation of tumour cells [4, 5]. First results demonstrating the activity of these inclusion complexes formulations will be presented.
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9328 POSTER

Radiosensitized Secondary Brain Melanoma Treatment

L. Bloznelyte-Plesniene[1], D. Sendiuliene[1]. [1]*Oncology Institute of Vilnius University, Department of Laser and Photodynamic Therapy, Vilnius, Lithuania*

Background: A high level of porphyrins has been found in the melanoma tissue therefore sensitized melanomas treatment is promising. Melanoma is the third most common cancer causing brain metastases. The aim of this work was to investigate the possibilities of sensitized secondary brain melanoma treatment using derivatives of hematoporphyrin as a radiosensitizer.
Material and Methods: From 2000 to 2010 a total of 30 patients with secondary brain melanoma underwent radiosensitized treatment (RST). 27 (90%*)* of the patients had advanced metastatic disease outside the brain and 24 (80%) of them had multiple brain metastasis. MRI and/or CT examination revealed single brain metastatic lesion in 6 patients; 2−5 metastases in 8 patients; 6−19 metastases in 11 patients and 20 or more metastases in the remaining 5 patients. Hematoporphyrin derivative was injected intravenous; 24, 48 and 72 hours after an injection of the sensitizer tumours were irradiated with gamma rays 2 Gy at a time from radioactive ^{60}Co (the full dose of the course was 6 Gy). 7 patients underwent a single course of radiosensitized treatment, for the rest treatment was repeated after 1−12 months. There was a control group, which consisted of all 29 patients with malignant brain melanoma treated at the Oncology Institute of Vilnius University from 2000 to 2010 (except for those who were treated with addition of RST).
Results: The effectiveness of RST was already noticeable in the course of treatment. Especially rapid effect was observed in the patients who were in a critical condition. 6 out of those 8 patients began to walk, to speak and even to read within two weeks. Nausea disappeared in 9 patients and headaches disappeared in 14 patients immediately after radiosensitized treatment. CT or MRI examinations conducted after RST courses revealed regression of a tumour in 24 patients. Complete regression of all treated tumours was observed in 3 patients. Two patients are alive and well for more than 140 months and 8 months. A significant response − regression of more than 50% of all brain metastases and remission of the disease for over 6 months was established in 11 patients. A partial response was observed in 10 patients with malignant brain tumours. For the rest 6 patients treatment was ineffective. The median survival of patients treated with addition of radiosensitized treatment was 9 months. Comparing it with the 3.5 months median survival of the control group patients it was statistically significant longer ($P < 0.05$). The Karnofsky performance scale index increased immediately in 23 patients following RST treatment.
Conclusion: Radiosensitized tumour treatment improves the survival of patients with secondary brain melanoma statistically significantly.