need for the development of novel efficacious therapies. As melanoma is a highly therapy-refractory tumor, it demands effective therapeutic combinations. Suicide gene therapy has been proposed as a strategy for the treatment of intractable cancers and has been assayed in some clinical trials alone or in combination with other therapies. In this context, the *E* gene is another potentially interesting bacteriophage lysis gene for cancer therapy. In contrast to most double-stranded DNA phages, which generally encode two genes that elicit host cell lysis (endolysin and holing protein), the small single-stranded DNA phage fX174 has only one lysis gene.
Methods: To evaluate whether this *E* gene has a cytotoxic impact on melanoma cells *in vitro* and *in vivo* we selected the B16-F10 murine melanoma cell line as a model. We used a nonviral gene delivery approach (pcDNA3.1/E plasmid) to study the inhibition of melanoma cells' proliferation *in vitro* and direct intratumoral injection of pcDNA3.1/E complexed with jetPEI to deliver *E* cDNA to rapidly growing murine melanomas. The effect and mechanism action of the E protein in vitro and in vivo was studied by applying several viability (MTT), apoptosis and imagen diagnostics assays.
Results: We found that the *E* gene has both a strong antiproliferative effect in B16-F10 cells *in vitro* and induces an efficient decrease in melanoma tumor volume *in vivo* (90% in 15 days). Interestingly, the GFP-E fusion protein expressed in melanoma cells was located in the mitochondria. *In vitro* and *in vivo* analysis demonstrated significant functional and morphological mitochondrial alterations accompanied by a significant increase of cytochrome c and active caspase-3 and -9 in transfected cells, which suggests that tumoral cell death is mediated by the mitochondrial apoptotic pathway.
Conclusion: In summary, we have reported, for the first time, the ability of the *E gene* to induce the death of melanoma cells *in vitro* and *in vivo.* The successful use of this gene as a new anticancer gene therapy system may establish a role for it in cancer treatment.

9311 POSTER

MRI versus FDG-PET scan in patients with liver metastases from uveal melanoma: a prospective study with intraoperative confirmation

P. Mariani[1], C. Malhaire[2], V. Servois[2], S. Petras[2], S. Piperno-neumann[3], M.G. Berry[1], C. Levy-Gabriel[1], L. Lumbroso-Le Rouic[1], L. Desjardins[1], R.J. Salmon[1]. *[1]Institut Curie, Surgical Department, Paris, France; [2]Institut Curie, Radiological Department, Paris, France; [3]Institut Curie, Oncological Department, Paris, France*

Background: Resection of liver metastases is proposed to treat liver metastases of uveal melanoma (UM); microscopically complete (R0) resection of metastases improves median survival from 22 versus 9 months if incomplete surgery. The aim of this study was to compare the sensitivity of dynamic-enhanced MRI with FDG-PET in the pre-operative diagnosis of liver metastases UM.
Matherial and Methods: 15 consecutive patients (mean age 56 years (range 38−71)) underwent FDG-PET scan and liver MRI. All patients had suspected liver metastases following screening by hepatic US and/or CT scan. Extrahepatic metastatic disease was excluded by whole body CT scan and bone scintigraphy. MRI and FDG-PET were performed a mean of 19 days before surgery. Imaging findings were compared with surgical and histological findings on a lesional basis.
Results: 28 lesions were resected with 27 metastases being histologically proven. There were 9 (33.3%) lesions 10 mm. Sensitivity and positive predictive value were 66.7% and 94.7% for MRI compared to 40.7% and 100% on FDG-PET. The difference between the two methods was statisticaly significant ($p = 0.01$; Mac Nemar test). In the remaining 3 patients, diffuse miliary disease (>10 capsular lesions) was discovered intra-operatively, 2 of which had been suspected on pre-operative MRI.
Conclusions: In this study, MRI is superior to FDG-PET for the detection of hepatic metastasis of UM. Whilst in some cases miliary disease was suggested by MRI, preoperative confirmation remains imperfect so, when miliary disease is suspected, laparoscopy exploration prior to formal surgery is recommended.

9312 POSTER

Antitumor responses to ipilimumab in advanced melanoma are not affected by systemic corticosteroids used to manage immune-related adverse events (irAEs)

J. Grob[1], O. Hamid[2], J. Wolchok[3], M. Maio[4], B. Neyns[5], L. Thomas[6], V. de Pril[7], R. Ibrahim[8], S. O'Day[9], C. Lebbé[10]. *[1]Hopital Sainte Marguerite, Service de Dermatologie, Marseille, France; [2]The Angeles Clinic and Research Institute, Neuro-Oncology Clinic, Santa Monica, USA; [3]Memorial Sloan-Kettering Cancer Center, Department of Medicine, New York, USA; [4]University Hospital of Siena, Department of Oncology, Siena, Italy; [5]Universitair Ziekenhuis, Department of Oncology, Brussels, Belgium; [6]Claude Bernard University, Department of Dermatology, Lyon, France; [7]Bristol-Myers Squibb, Global Biometrics & Science, Braine l'Alleud, Belgium; [8]Bristol-Myers Squibb, Global Clinical Research Oncology, Wallingford, USA; [9]The Angeles Clinic and Research Institute, Medical Oncology, Santa Monica, USA; [10]Saint-Louis Hospital, Department of Dermatology, Paris, France*

Background: The monoclonal antibody ipilimumab overcomes peripheral immune tolerance by blocking cytotoxic T-lymphocyte antigen-4. The irAEs associated with ipilimumab primarily affect the skin, gastrointestinal (GI) tract, liver, and endocrine systems. Specific treatment guidelines to manage irAEs were incorporated into protocols within the ipilimumab clinical trial program for advanced melanoma, which include the use of high-dose steroids for grade 3−4 diarrhea/colitis to reduce the incidence of life-threatening complications, e.g. GI perforation. The current analyses were undertaken to determine if steroids affect ipilimumab antitumor responses.
Methods: A total of 283 advanced melanoma patients (pts) were treated in the Phase II studies CA184008, 022, and 007 with ipilimumab administered at 10 mg/kg every 3 weeks (Q3W) $\times 4$ (induction); eligible pts could continue to receive maintenance ipilimumab Q12W from Week 24. Tumor assessments were first carried out at Week 12 (end of induction period). Response was evaluated using modified World Health Organization (mWHO) criteria and novel immune-related response criteria (irRC). [1]
Results: Of 283 pts, 119 received steroids for the treatment of irAEs (Table). Eighty-three pts (29.3%) achieved disease control (DC) by mWHO criteria [complete/partial response (CR/PR), or stable disease (SD) $\geqslant$12 weeks], for which 43 received steroids. Fifteen of the 43 pts achieved CR or PR (2 pts received steroids after response only; 1 pt received steroids prior to response only; 12 pts received steroids before and after response). Of the 43 pts, 25 (58.1%) maintained DC wheres 26 of 40 (65.0%) who did not receive steroids maintained DC. Similar results were obtained using irRC.

	Achieved DC		Maintained DC		Progressive disease (PD)/Lost DC
	CR/PR	SD	CR/PR	SD	
mWHO criteria					
Steroid use (n = 119)	15	28	12	13	94
No steroid use (n = 164)	11	29	8	18	138
irR criteria (irRC)					
Steroid use (n = 119)	16	36	12	23	84
No steroid use (n = 164)	16	35	13	21	30

Conclusions: When the severity of irAEs requires steroids, there is no evidence that their use precludes the development of an antitumor response to ipilimumab, or adversely affects responses once achieved.

References

[1] Hodi FS, et al. J Clin Oncol 2008; 26 (May 20 suppl): abstr 3008.

9313 POSTER

NEMO-binding domain peptide induces apoptosis in human melanoma cells: an effect associated to inhibition of constitutive NF-kappaB activation

M. Tersigni[1], G. Belardo[2], M. Napolitano[3], P.A. Ascierto[4], A. Ialenti[1], A. Ianaro[1]. *[1]University of Naples Federico II - Faculty of Pharmacy, Department of Experimental Pharmacology, Naples, Italy; [2]University of Rome Tor Vergata, Department of Biology, Rome, Italy; [3]Clinical Immunology National Tumor Institute, Fondazione Pascale, Naples, Italy; [4]Medical Oncology and Innovative Therapy National Tumor Institute, Fondazione Pascale, Naples, Italy*

Background: melanoma is the most aggressive form of skin cancer. Recent studies have identified key signalling pathways important in promoting melanoma tumorigenesis. One such important target is the Nuclear Factor-κB (NF-κB) pathway. *In vitro* studies have shown that IKK is constitutively active in human melanoma cells as compared to

normal melanocytes, leading to NF-κB activation which in turn regulates the expression of anti-apoptotic proteins such as the inhibitor of apoptosis (IAP) proteins, survivin as well as Bcl-2 like proteins. These events are major molecular mechanisms for melanocytes transformation [1]. It has been found that a short cell-permeable peptide spanning the IKKβ NEMO binding domain (NBD), named NBD peptide, disrupted the association of NEMO with IKKs *in vitro* and blocked TNFα-induced NF-κB activation *in vivo* [2]. In the present study we investigated the effect of the NBD peptide on IKK/NF-κB signalling pathway and survival of several human melanoma cell lines (A375, WM115, SK-Mel-5).

Materials and Methods: viability was determined by vital-dye exclusion assay. For the electrophoretic mobility shift assay, aliquots of whole-cell extracts were incubated ^{32}P-labeled κB DNA probe and were analyzed by non-denaturing 4% polyacrylamide gel electrophoresis. A375 cells were stained with 7-AAD and incubated at 4°C until analysis on the flow cytometer. The active form of caspase-3 was measured by FACScalibur cytometer using the PE-conjugated anti-human-active caspase-3 monoclonal antibody. Proteins electrophoretic analyses was performed via immunoblotting.

Results: we report that NBD peptide is able to inhibit the proliferation of all human melanoma cell lines used as compared to normal human melanocytes. Inhibition of cell growth was associated with direct inhibition of i) constitutive IKK activity; ii) NF-κB DNA-binding activation, iii) induction of apoptosis in all cell lines tested. Using as a model the A375 melanoma cell line, we show that inhibition of IKK/NF-κB signalling pathway by NBD peptide leads to down-regulation of the expression of several NF-κB-dependent antiapoptotic gene products and to the activation of caspase-3 as confirmed by the cleavage and consequently inactivation of poly (ADPribose) polymerase (PARP-1) known as the best marker of this process.

Conclusions: our studies indicate that selective inhibition of IKK/NF-κB activation can be an effective strategy for challenging melanoma.

References

[1] Amiri K.I. and Richmond A. 2005. *Cancer and Metastasis Rev* 24:301−313.
[2] May M. *et al*. 2000. *Science* 289:1550−1554.

9314 POSTER

Impact of ipilimumab on the health-related quality of life (HRQL) of patients with previously treated unresectable stage III or IV melanoma

D. Revicki[1], S. Kotapati[2], I. Villanueva[2]. *[1]United BioSource Corporation, Center for Health Outcomes Research, Bethesda, USA; [2]Bristol-Myers Squibb, Global Health Outcomes, Wallingford, USA*

Objective: The anti-CTLA-4 monoclonal antibody, ipilimumab, induces anti-tumor activity that has resulted in significant survival benefit in many patients with advanced melanoma. We evaluated the impact of ipilimumab treatment on patient-reported functioning and symptoms in patients with unresectable stage III or IV melanoma.

	022			008
	0.3 mg/kg (n = 44)	3 mg/kg (n = 44)	10 mg/kg (n = 46)	10 mg/kg (n = 102)
Score				
Physical function	−8.0	−10.2	−7.4	−8.1
Role function	−17.1	−19.7	−9.1	−11.9
Emotional function	1.7	−0.6	−1.2	−3.5
Cognitive function	−1.5	−4.9	−7.6	−7.6
Social function	−10.2	−7.6	−9.4	−7.6
Global health	−9.7	−11.5	−10.0	−7.0
Fatigue	10.9	15.2	5.6	9.5
Nausea/vomiting	8.3	6.1	3.3	3.8
Pain	8.3	14.8	2.5	−0.8
Dyspnea	9.1	7.6	6.5	4.3
Insomnia	−6.1	3.8	4.4	2.0
Appetite loss	10.6	12.4	8.2	4.6
Constipation	−1.5	2.3	−4.4	8.7
Diarrhea	12.9	2.3	4.4	11.3

For the function and global health scales, improvements are indicated by positive scores. For symptom scales, improvements are indicated by negative scores.

Methods: Data from a randomized, double-blind, fixed dose phase II trial (022) and a single-dose, open label phase II clinical study (008) were used for these analyses. In study 022, pts were randomly assigned to treatment with 0.3 mg/kg (n = 73), 3 mg/kg (n = 72) or 10 mg/kg (n = 72) at weeks 1, 4, 7 and 10. In study 008 all pts (n = 155) were treated with 10 mg/kg at weeks 1, 4, 7, and 10. HRQL was assessed using the EORTC Quality of Life Questionnaire C30 which measures physical, role, emotional, social and cognitive functioning, global health status, and 8 symptoms (pain, nausea/vomiting, fatigue, dyspnea, appetite loss, insomnia, diarrhea, constipation). HRQL data were collected at weeks 1, 4, 7, and 12. Baseline to endpoint changes were interpreted through mean change in scores as "no change" (0), "a little" (5−10 points), "moderate" (10−20 points) and "very much" (>20).

Results: HRQL completion rates were 77% and 80% at week 12. Most observed changes were in the "no change" to "a little" range across the treatment groups. Mean baseline to 12-week changes in HRQL scores for studies 022 and 008 are reported (Table). The best overall response profile of HRQL outcomes was demonstrated with 10 mg/kg.

Conclusions: These findings show that ipilimumab 10.0 mg/kg has a little impact on functional and symptom domains suggesting that relevant aspects of HRQL are maintained in this advanced stage melanoma population.

9315 POSTER

Ipilimumab in pretreated metastatic uveal melanoma patients: safety and clinical efficacy

R. Danielli[1], P. Queirolo[2], A. Testori[3], R. Plummer[4], E. Razi[5], V. Chiarion Sileni[6], L. Calabrò[1], A.M. Di Giacomo[1], R. Ridolfi[7], M. Maio[8]. *[1]Azienda Ospedaliera Universitaria Senese, Medical Oncology and Immunotherapy Unit, Siena, Italy; [2]National Institute for Cancer Research, Medical Oncology, Genoa, Italy; [3]European Institute of Oncology, Melanoma and Sarcoma Division, Milan, Italy; [4]Northern Institute for Cancer Research Newcastle University, Newcastle upon Tyne, United Kingdom; [5]Hygeia Hospital, First Deparment of Medical Oncology, Athens, Greece; [6]Istituto Oncologico Veneto-IRCCS, Medical Oncology Unit, Padua, Italy; [7]Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Somatic Cell Therapy Unit, Meldola-Forlì, Italy; [8]Azienda Ospedaliera Universitaria Senese-Istituto Toscano Tumori, Medical Oncology and Immunotherapy Unit, Siena, Italy*

Background: The anti-CTLA-4 monoclonal antibody ipilimumab induces anti-tumor responses in cutaneous metastatic melanoma (MM) patients (pts). However, no data are available on the clinical effectiveness of ipilimumab treatment in uveal MM pts. We report the European (7 Institutions) experience utilizing ipilimumab in uveal MM pts.

Materials and Methods: Fourteen stage IV pts (8 male, 6 female), median age 57 (30−76) years, ECOG performance status 0−1, with uveal MM progressing to 2 median (1−4) previous therapies for metastatic disease received ipilimumab within a compassionate use program. Thirteen pts had history (1) or evidence (12) of liver metastases, 2 of brain metastases and 3 of elevated (>1× upper limit of normal [ULN]) LDH values. In the induction phase (IF) pts received ipilimumab (10 mg/Kg i.v.) q3 weeks (wks) ×4 cycles; after a 12 wks rest, treatment was repeated q12 wks in the maintainance phase (MF). Tumor assessment (TA) *per* modified World Health Organization criteria was evaluated at baseline, at week (wk) 12 and wk 24, than every 12 wks. Adverse Events (AE) and immune related AE (irAE) were collected according to Common Terminology Criteria for Adverse events version 3.0.

Results: All pts received at least one ipilimumab dose, and 10/14 completed the IF. The remaining 4 pts were withdrawn for early disease progression, while 4 pts entered the MF and are still on treatment. TA at wk 12 showed partial response (PR) in 1/10 or stable disease (SD) in 3/10 pts; TA at wk 24 showed PR and SD in 1/4 and 3/4 pts, respectively, with a clinical benefit (SD+PR) of 29%. As previously reported for cutaneous MM, slow, steady decline in tumor volume and appearance of new lesions with subsequent shrinking has been observed. No grade 3/4 AE and irAE were reported. Median overall survival is 32 (2−52) wks.

Conclusions: Ipilimumab administration in pretreated uveal MM pts is feasible and safe. A sizeable proportion of treated pts experienced clinical benefit with extended survival. These data, thought preliminary and in a limited number of pts, suggest that uveal MM might represent a promising indication for ipilimumab treatment to be further investigated.