9308 POSTER

Activity of ipilimumab at 10 mg/kg in patients with advanced melanoma is independent of baseline prognostic factors

D. Schadendorf[1], J. Wolchok[2], B. Neyns[3], C. Lebbé[4], K. Harmankaya[5], C. Verschraegen[6], K. Chin[7], V. de Pril[8], A. Hoos[9], M. Maio[10]. *[1]University Hospital of Essen, Department of Dermatology, Essen, Germany; [2]Memorial Sloan-Kettering Cancer Center, Department of Medicine, New York, USA; [3]Universitair Ziekenhuis, Department of Oncology, Brussels, Belgium; [4]Saint-Louis Hospital, Department of Dermatology, Paris, France; [5]Medical University of Vienna, Department of Dermatology, Vienna, Austria; [6]University of New Mexico, Cancer Research and Treatment Center, Albuquerque, USA; [7]Bristol-Myers-Squibb, Global Clinical Research Oncology, Wallingford, USA; [8]Bristol-Myers Squibb, Global Biometrics & Science, Braine l'Alleud, Belgium; [9]Bristol-Myers Squibb, Global Clinical Research Oncology, Wallingford, USA; [10]University Hospital of Siena, Department of Oncology, Siena, Italy*

Background: Several factors predict a poor prognosis in patients (pts) with advanced melanoma, including advanced stage of disease, age 60+, elevated serum levels of lactate dehydrogenase (LDH), and lack of a prior response to therapy. Ipilimumab is a fully human monoclonal antibody against cytotoxic T-lymphocyte antigen-4, yet the impact of prognostic factors on ipilimumab activity in these pts is unknown.
Methods: In this pooled analysis of two completed Phase II studies, prognostic factors in previously treated advanced melanoma pts administered/randomized to ipilimumab at 10 mg/kg were explored (CA184−008, N = 155 and CA184−022, N = 72). Ipilimumab was given every 3 weeks (Q3W) ×4 (induction); eligible pts could receive ipilimumab Q12W starting at W24 (maintenance). Response was based on modified World Health Organization criteria. Each variable was analyzed separately; LDH levels were not capped for study entry, and pts were stratified by normal and elevated (>1× upper limit of normal [UNL]) levels.
Results: No statistically significant association between disease control rate (DCR; complete or partial response plus stable disease) or median overall survival (OS) and several baseline prognostic factors was obtained (Table).

Prognostic factor	Endpoint	
	DCR (%)* [95% CI]	Median OS, months [95% CI]
Age		
<65 yrs	25.2 [18.5−32.9]	11.6 [9.5−16.3]
⩾65 yrs	32.9 [22.5−44.6]	7.6 [5.1−16.3]
M stage		
M0	33.3 [9.9−65.1]	21.9 [10.2−NR]
M1a	41.0 [25.6−57.9]	15.7 [10.2−NR]
M1b	26.4 [15.3−40.3]	15.4 [9.3−17.9]
M1c	23.6 [16.4−32.1]	6.6 [5.1−12.2]
Response to prior therapy		
Yes	32.5 [18.6−49.1]	11.6 [5.7−18.4]
No	26.7 [20.5−33.7]	10.7 [7.7−15.4]
LDH, all M stages		
Normal	29.7 [21.4−39.1]	15.7 [10.2−18.4]
Elevated	25.9 [18.2−34.8]	7.0 [4.4−12.2]
LDH, M1c stage only†		
Normal	28.6 [15.7−44.6]	15.0 [6.31−NR]
Elevated	21.0 [12.7−31.5]	4.8 [3.4−8.57

NR, not reached; *In study 022, pts treated with ipilimumab 0.3 mg/kg (control arm) had a DCR of 13.7% [6.8−23.8] (all M stages); †Of the total 227 pts, 123 (54%) had M1c stage disease, with 42 having normal LDH and 81 having elevated LDH (of which 32 had LDH 2× UNL).

Conclusions: Despite their previously identified prognostic value in the general advanced melanoma population, no factor had a statistically significant effect on ipilimumab activity.

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Changes in peripheral blood absolute lymphocyte count (ALC) may guide patient selection for continued treatment with ipilimumab

S. Aamdal[1], J.D. Wolchock[2], J. Weber[3], O. Hamid[4], S. O'Day[5], H. Schmidt[6], D. Berman[7], S.D. Chasalow[8]. *[1]Norwegian Radium Hospital, Oncology, Oslo, Norway; [2]Memorial Sloan-Kettering Cancer Center, Oncology, New York, USA; [3]Comprehensive Melanoma Research Center at the H. Lee Moffitt Cancer Center and Research Institute, Oncology, Tampa, USA; [4]The Angeles Clinic and Research Institute, Neuro-Oncology, Santa Monica, USA; [5]The Angeles Clinic and Research Institute, Medical Oncology and Immunotherapy, Santa Monica, USA; [6]Aarhus University Hospital, Oncology, Aarhus, Denmark; [7]Bristol-Myers Squibb, Global Clinical Research, Princeton, USA; [8]Bristol-Myers Squibb, Global Biometrics and Science, Pennington, USA*

Background: The anti-CTLA-4 monoclonal antibody, ipilimumab, induces durable antitumor responses in patients (pts) with advanced melanoma. However, a reliable marker of ipilimumab activity has not been identified. We evaluated whether changes in peripheral blood ALC are associated with ipilimumab disease control.
Methods: Peripheral ALC from routine safety labs were collected from 533 pts with unresectable stage III or IV melanoma treated with ipilimumab in four Phase II studies. Ipilimumab was given every 3 weeks (Q3W) ×4; eligible pts could continue to receive ipilimumab Q12W at Week 24. ALC was first analyzed in studies CA184007, 008, and 022 combined (ipilimumab at 0.3, 3, or 10 mg/kg), and then analyzed for confirmation in the separate study, CA184004 (ipilimumab at 3 or 10 mg/kg). Using modified World Health Organization criteria, response-evaluable pts (n = 444) were classified as those who achieved disease control (DC; defined here as complete or partial response, or stable disease through Week 24) and those who did not achieve DC.
Results: In combined analyses from studies CA184007, 008, and 022 (n = 379), pts who achieved DC had a greater mean rate of ALC change (slope) than pts who did not achieve DC ($P = 0.0013$); in these 3 studies, no pt with a negative ALC slope over the induction period achieved DC (Table). These associations were confirmed in study CA184004 (n = 65): pts who achieved DC had a greater mean slope ($P = 0.00042$), and only 1 pt with a (slightly) negative ALC slope achieved DC (Table). Further analyses will determine if there is a potential association between changes in ALC and overall survival.

Dose (mg/kg)	Group	N	Mean slope (1000 cells/μL/week)	Standard deviation of slope	Fraction negative slope
Studies 007, 008, 022 pooled					
0.3	DC achieved	0	NA	NA	NA
	DC not achieved	47	−0.005	0.024	0.60
	Unknown	7	0.019	0.029	0.43
3	DC achieved	6	0.043	0.039	0
	DC not achieved	39	0.023	0.057	0.21
	Unknown	9	0.022	0.048	0.22
10	DC achieved	49	0.086	0.051	0
	DC not achieved	197	0.054	0.077	0.18
	Unknown	25	0.077	0.091	0.20
Study 004					
3	DC achieved	6	0.030	0.030	0.17
	DC not achieved	21	−0.019	0.068	0.52
	Unknown	5	0.028	0.026	0
10	DC achieved	6	0.153	0.124	0
	DC not achieved	23	0.030	0.063	0.30
	Unknown	4	−0.036	0.172	0.50

Conclusions: The positive association of a greater average rate of increase in ALC with DC at 10 mg/kg supports this dose for ipilimumab studies. A negative ALC slope could possibly be used to identify those pts in which ipilimumab therapy should be discontinued as they are unlikely to achieve DC.

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The cytotoxic activity of the phage E protein suppress the growth of murine B16 melanomas in vitro and in vivo

R. Ortiz Quesada[1], J.c. Prados Salazar[1], C. Melguizo Alonso[1], A.r. Rama Ballesteros[1], C. Velez[1], O. Caba[2], A. Amat[2], F. Hita[2], H. Boulaiz[1], A. Aranega[1]. *[1]University of Granada, Center of Medical Research (CIBM), Granada, Spain; [2]University of Jaen, Department of Health Sciences, Jaen, Spain*

Background: Melanoma represents only 4% of all skin cancers but nearly 80% of total skin cancer deaths, predominantly because of metastatic spread. Apart from surgery, the treatment options for melanoma, particularly metastatic melanoma, are relatively limited and emphasize the

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.

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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.

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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

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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