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Oral Abstract Session, Mon, 3:00 PM-6:00 PM

A study of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic tumors.

Roy S. Herbst, Michael S. Gordon, Gregg Daniel Fine, Jeffrey Alan Sosman, Jean-Charles Soria, Omid Hamid, John D. Powderly, Howard A. Burris, Ahmad Mokatrini, Marcin Kowanetz, Maya Leabman, Maria Anderson, Daniel S. Chen, F. Stephen Hodi; Yale University, New Haven, CT; Pinnacle Oncology Hematology, Scottsdale, AZ; Genentech, Inc., South San Francisco, CA; Vanderbilt-Ingram Cancer Center, Nashville, TN; Institut Gustave Roussy, Villejuif, France; The Angeles Clinic and Research Institute, Los Angeles, CA; Carolina BioOncology Institute, Huntersville, NC; Sarah Cannon Research Institute, Nashville, TN; Genentech Inc., South San Francisco, CA; Dana-Farber Cancer Institute, Boston, MA

Background: Tumor PD-L1 mediates cancer immune evasion. Therefore, inhibition of PD-L1 binding represents an attractive strategy to restore tumor-specific T-cell immunity. MPDL3280A, a human monoclonal antibody containing an engineered Fc-domain designed to optimize efficacy and safety, targets PD-L1, blocking PD-L1 from binding its receptors, including PD-1 and B7.1. **Methods:** A study was conducted with MPDL3280A administered IV q3w in pts with locally advanced or metastatic solid tumors, including 3+3 dose-escalation and expansion cohorts. ORR was assessed by RECIST v1.1 and includes u/cCR and u/cPR. **Results:** As of Jan 10, 2013, 171 pts were evaluable for safety. Administered doses include ≤ 1 (n=9), 3 (n=3), 10 (n=35), 15 (n=57) and 20 mg/kg (n=67). Pts in the dose-escalation cohorts did not experience DLTs. No MTD was identified. Pts had received MPDL3280A for a median duration of 127 days (range 1-330). 39% of pts reported G3/4 AEs, regardless of attribution. AEs of special interest included hepatitis, rash and colitis. No G3-5 pneumonitis was observed. MPDL3280A PK was linear at doses ≥ 1 mg/kg. 122 pts enrolled prior to Jul 1, 2012 were evaluable for efficacy. RECIST responses were observed in multiple tumor types including NSCLC, RCC, melanoma, CRC and gastric cancer. An ORR of 21% (25/122) was observed in nonselected solid tumors, including several pts who demonstrated tumor shrinkage within days of initiating treatment. Additional pts had delayed responses after apparent radiographic progression (not included in the ORR). Some responders demonstrated prolonged SD prior to RECIST responses. The 24-week PFS was 44%. Pts with PD-L1-positive tumors (from archival samples) showed an ORR of 39% (13/33) and a PD rate of 12% (4/33). In contrast, patients with PD-L1-negative tumors showed an ORR of 13% (8/61) and a PD rate of 59% (36/61). As of the cutoff date, all responses are ongoing or improving. Updated data will be presented. **Conclusions:** MPDL3280A was well tolerated, with no pneumonitis-related deaths. Durable responses were observed in a variety of tumors. PD-L1 tumor status appears to correlate with responses to MPDL3280A. PK supports q3w dosing at 15 mg/kg or fixed-dose equivalent. Clinical trial information: NCT01375842.

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Oral Abstract Session, Mon, 3:00 PM-6:00 PM

Biomarkers and associations with the clinical activity of PD-L1 blockade in a MPDL3280A study.

John D. Powderly, Hartmut Koeppen, F. Stephen Hodi, Jeffrey Alan Sosman, Scott N. Gettinger, Rupal Desai, Josep Tabernero, Jean-Charles Soria, Omid Hamid, Gregg Daniel Fine, Yuanyuan Xiao, Ahmad Mokatrín, Jenny Wu, Maria Anderson, Bryan A. Irving, Daniel S. Chen, Marcin Kowanetz; Carolina BioOncology Institute, Huntersville, NC; Genentech Inc., South San Francisco, CA; Dana-Farber Cancer Institute, Boston, MA; Vanderbilt-Ingram Cancer Center, Nashville, TN; Yale School of Medicine, New Haven, CT; Genentech, Inc., South San Francisco, CA; Vall d'Hebron University Hospital, Barcelona, Spain; Institut Gustave Roussy, Villejuif, France; The Angeles Clinic and Research Institute, Los Angeles, CA

Background: PD-L1, which is highly expressed on tumors, is a ligand of PD-1, an inhibitory receptor present on activated T cells. PD-L1 expression is regulated by intrinsic (mutations) and adaptive (tumor infiltrating T cells) mechanisms. MPDL3280A, a human monoclonal antibody containing an engineered Fc-domain designed to optimize efficacy and safety, targets PD-L1, blocking PD-L1 from binding its receptors, including PD-1 and B7.1. **Methods:** A Ph I study was conducted with MPDL3280A administered IV q3w in pts with locally advanced or metastatic solid tumors. The expansion cohort required available tumor tissue. In addition, the study included a serial biopsy cohort with tumor sampling prior to and during treatment. Tumor samples were analyzed by IHC and a Genentech immunochip measuring ≈90 immune-related genes to characterize the tumor immune microenvironment at baseline (BL) and/or during MPDL3280A treatment. Further, blood-based biomarkers and circulating immune cell subsets were serially measured. **Results:** Pretreatment tumor samples were available for IHC from 112 pts and for immunochip from 96 pts. In addition, 23 pts had paired BL and on-treatment samples. Blood-based biomarkers were evaluated in 76 pts. Elevated BL PD-L1 expression by IHC was associated with response to MPDL3280A, and coordinated expression of PD-L1 and CD8+ T-cells was observed. Furthermore, a T-cell gene signature (including CD8, IFN γ and Granzyme-A) was associated with treatment response. On treatment, responding tumors showed increasing PD-L1 expression and a Th1-dominant immune infiltrate, providing evidence for adaptive PD-L1 upregulation. Nonresponders showed minimal tumor CD8+ T-cell infiltration and an absence of T-cell activation (measured by Granzyme-A and Perforin expression). Additionally, a subpopulation of pts exhibited changes in circulating cytokines (IFN γ) and activated T-cell subsets (HLADR+Ki67+). **Conclusions:** PD-L1 tumor expression and T-cell gene signature correlate with response to MPDL3280A. MPDL3280A therapy led to T-cell reactivation and restored antitumor immunity. These data provide mechanistic insights into immunotherapy and support pt selection for treatment with MPDL3280A monotherapy. Clinical trial information: NCT01375842.

Nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients with advanced solid tumors: Survival and long-term safety in a phase I trial.

Suzanne Louise Topalian, Mario Sznol, Julie R. Brahmer, David F. McDermott, David C. Smith, Scott N. Gettinger, Janis M. Taube, Charles G. Drake, Drew M. Pardoll, John D. Powderly, Richard D. Carvajal, Jeffrey Alan Sosman, Michael B. Atkins, Scott J. Antonia, David R. Spigel, Donald P. Lawrence, Georgia Kollia, Ashok Kumar Gupta, Jon M. Wigginton, F. Stephen Hodi; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD; Yale Cancer Center, New Haven, CT; Beth Israel Deaconess Medical Center, Boston, MA; University of Michigan Comprehensive Cancer Center, Ann Arbor, MI; Yale University, New Haven, CT; Johns Hopkins School of Medicine, Baltimore, MD; Carolina BioOncology Institute, Huntersville, NC; Memorial Sloan-Kettering Cancer Center, New York, NY; Vanderbilt University Medical Center, Nashville, TN; Georgetown University Lombardi Comprehensive Cancer Center, Washington, DC; H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; Sarah Cannon Research Institute; Tennessee Oncology, Nashville, TN; Massachusetts General Hospital Cancer Center, Boston, MA; Bristol-Myers Squibb, Princeton, NJ; Dana-Farber Cancer Institute, Boston, MA

Background: Blockade of programmed death-1 (PD-1), a co-inhibitory receptor expressed by activated T cells, can overcome immune resistance and mediate tumor regression (Topalian et al., NEJM 2012). Here we present long-term safety and efficacy outcomes from a phase I study of nivolumab, a PD-1 blocking mAb, in patients (pts) with advanced solid tumors. **Methods:** Pts enrolled between 2008-2012 received nivolumab (0.1–10 mg/kg IV Q2W) during dose escalation and/or cohort expansion. Tumors were assessed by RECIST 1.0 after each 4-dose cycle. Pts received ≤12 cycles until unacceptable toxicity, confirmed progression, or CR. **Results:** 304 pts with non-small cell lung cancer (NSCLC, n=127, squamous and nonsquamous), melanoma (MEL, n=107), renal cell (RCC, n=34), colorectal (n=19) or prostate cancer (n=17) were treated. Durable ORs (CR/PR) were observed in MEL, NSCLC and RCC (Table); in 54 responders with ≥1 yr follow-up, 28 lasted ≥1 yr. Median OS in these heavily pretreated pts (47% with 3-5 prior systemic therapies) compared favorably with expected outcomes as of July 2012. Drug-related AEs (any grade) occurred in 72% (220/304) and G3/G4 AEs in 15% (45/304) of pts. Drug-related pneumonitis occurred in 3% (10/304), including G3/G4 in 1% (3/304), resulting in 3 deaths early in the trial, which led to increased clinical monitoring and an emphasis on management algorithms. Nivolumab-related pneumonitis characteristics and management will be summarized. Updated survival and safety data from Feb 2013 (≥1 yr follow-up all pts) will be presented, including OS at 3 yr. **Conclusions:** Nivolumab produced sustained survival with a manageable long-term safety profile in advanced MEL, NSCLC and RCC, supporting its ongoing clinical development in controlled phase III trials with survival endpoints. Clinical trial information: NCT00730639.

Tumor type	Dose, mg/kg	ORR, No. pts (%)	OS median, months (95% CI)	OS rate, % ^a (95% CI); pts at risk, n	
				1 Yr	2 Yr
MEL	0.1–10	33/106 (31)	16.8 (12.5–NR)	61 (52–71); 50	44 (33–56); 24
NSCLC	1–10	20/122 (16)	9.6 (7.4–13.7)	43 (33–53); 24	32 (18–47); 4
RCC	1 or 10	10/34 (29)	>22	70 (55–86); 22	52 (32–72); 7

^aOS estimates after 1 yr reflect censoring and shorter follow-up for pts enrolling later in the study. NR = Not reached.

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Oral Abstract Session, Mon, 3:00 PM-6:00 PM

Peripheral and tumor immune correlates in patients with advanced melanoma treated with combination nivolumab (anti-PD-1, BMS-936558, ONO-4538) and ipilimumab.

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Background: Nivolumab and ipilimumab are fully human monoclonal antibodies that block the immune checkpoint receptors PD-1 and CTLA-4, respectively. In a multi-cohort, phase I study of nivolumab/ipilimumab combination therapy in melanoma patients (pts), objective response rates up to 47% were observed (NCT01024231). Putative predictive biomarkers from peripheral blood (PB) or tumor, including tumor PD-L1 expression, absolute lymphocyte count (ALC) and PB myeloid derived suppressor cells (MDSC) were evaluated. Pharmacodynamic changes in activated and effector T cells were also assessed. **Methods:** Tumor PD-L1 membrane expression was assessed in archival FFPE specimens by immunohistochemistry (28-8 PD-L1 antibody). ALC was measured in serial PB samples; changes in the percentage, number and phenotype of activated CD4⁺ and CD8⁺T cells and MDSC were characterized by flow cytometry. **Results:** PD-L1 expression was seen in 37% (10/27) of pts, using a cut-off of 5% tumor cell membrane staining. Objective responses (OR) were seen in pts with both PD-L1 negative (8/17) and PD-L1 positive (4/10) tumors. Relative to baseline, a rise in ALC was not detected, but phenotypic changes in PB T-cell subsets, including increases in the percentage of CD4 and CD8 expressing HLA-DR, ICOS and/or Ki67 were seen with combination therapy. Low ALC (<1.0 at wk 6-7) did not preclude OR as 3 of 12 pts with low ALC responded. Of pts evaluated, OR with ≥80% reduction in tumor burden at 12 wk were seen in pts with a low frequency of pretreatment PB MDSC (3/7) but no OR were seen in pts with high MDSC (0/6). **Conclusions:** In this small subset of pts, OR were seen independent of PD-L1 or ALC status in contrast to prior observations with nivolumab or ipilimumab, respectively. Thus, the immune response generated by combination therapy may have unique features compared to either monotherapy. The relationship between frequency of PB MDSC and reduction in tumor burden will be further explored. Further efforts in this study and in future phase III randomized studies will investigate these and other phenotypic changes in immune cell populations and their relationship to patterns of clinical activity. Clinical trial information: NCT01024231.

Phase I study of margetuximab (MGAH22), an FC-modified chimeric monoclonal antibody (MAb), in patients (pts) with advanced solid tumors expressing the HER2 oncoprotein.

Howard A. Burris, Giuseppe Giaccone, Seock-Ah Im, Todd Michael Bauer, Jane B. Trepel, Jeffrey L. Nordstrom, Hua Li, David A Carlin, Jan E. Baughman, Stanford Stewart, Yung-Jue Bang; Sarah Cannon Research Institute; Tennessee Oncology, Nashville, TN; National Cancer Institute, Bethesda, MD; Seoul National University Hospital, Seoul, South Korea; MacroGenics, Inc., Rockville, MD; MacroGenics, Inc., South San Francisco, CA

Background: The anti-HER2 MAb, trastuzumab (H), has proven effective for HER2+ breast cancer (BC) and gastroesophageal cancer (GEC). H's mechanism of action is incompletely understood, but evidence indicates ADCC is important. MGAH22 is an Fc-modified chimeric MAb which preserves the antigen binding properties of H, and exhibits enhanced binding to the activating low affinity Fcγ receptor, CD16A, and diminished binding to the inhibitory low affinity Fcγ receptor, CD32B. Preclinical data indicate MGAH22 is more potent than H. **Methods:** 34 pts with HER2 positive (2+ or 3+ by IHC) tumors have been treated: 19 in 5 dose-escalation cohorts (0.1 - 6.0 mg/kg qw x 4); 15 in 6.0 mg/kg expansion. Tumor types include: BC (10), GEC (13), colon (5), lung (2), salivary (1), ampulla of Vater (1), endometrium (1), bladder (1). **Results:** MGAH22 was well tolerated. Most common adverse events (AEs) were Grade 1-2 constitutional symptoms and infusion-related reactions. Related AEs ≥ Grade 3 were limited to a single infusion reaction, 2 episodes of brief lymphopenia confounded by steroids, and transient worsening anemia. No cardiac toxicities were observed. Dose escalation was halted at 6 mg/kg, well above the preclinically predicted minimally effective dose (0.1 mg/kg). Antitumor activity has been observed at all dose levels, including partial responses (PRs) and long times to progression (≥ 5 mo). Two PRs lasting 3.5 and 5.5 mo were observed among the 10 BC pts (both had failed prior H and lapatinib). 4/13 GEC pts experienced stable disease lasting a median of 3.6 mo (range 1.5–5.3), all but one previously failing anti-HER2 treatment. **Conclusions:** Margetuximab is well tolerated with promising activity in pts with BC and GEC who have failed prior HER-2 therapies and in pts with HER2+ tumors for which H is considered ineffective. A phase II trial in relapsed or refractory HER2 2+, nonamplified BC is underway. Clinical trial information: NCT01195935.

Benefit	Tumor type	IHC/FISH	Prior H	Prior lapatinib	MGAH22 dose >mg/kg
cPR	BC	3+/non-A	X	X	3.0
	BC	3+/A	X	X	6.0
uPR	Salivary gland	3+/A			1.0
SD ≥ 5 mos.	BC	3+/A	X	X	0.1
	GEC	neg/non-A		X	3.0
SD with tumor marker decline	GEC	neg/non-A		X	3.0
	GEC	2+/non-A			6.0
	GEC	3+/A	X	X	6.0
	GEC	3+/A	X		6.0
	BC	3+/A	X	X	0.1

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Oral Abstract Session, Mon, 3:00 PM-6:00 PM

Randomized phase II clinical trial of the anti-HER2 (GP2) vaccine to prevent recurrence in high-risk breast cancer patients: A planned interim analysis.

Francois Trappey, John S. Berry, Timothy J Vreeland, Diane F. Hale, Alan K. Sears, Sathibalan Ponniah, Sonia A. Perez, Guy T. Clifton, Michael Papamichail, George Earl Peoples, Elizabeth Ann Mittendorf; Brooke Army Medical Center, San Antonio, TX; Cancer Vaccine Development Program, United States Military Cancer Institute, USUHS, Bethesda, MD; St. Savas Cancer Hospital, Athens, Greece; Department of Surgery, Brooke Army Medical Center, Fort Sam Houston, TX; Cancer Immunology and Immunotherapy Center, Athens, Greece; The University of Texas MD Anderson Cancer Center, Houston, TX

Background: A prospective, randomized, multi-center, placebo-controlled, single-blinded, phase II trial was designed to evaluate the safety and clinical efficacy of GP2, a HER2-derived peptide vaccine, in breast cancer patients. **Methods:** Clinically disease-free, node-positive or high-risk node-negative patients (pts) with any level of HER2 expression were enrolled after standard of care therapy. HLA-A2+ pts were randomized to receive GP2 + GM-CSF (VG) or GM-CSF alone (CG). HLA A2- controls from a parallel arm of the study were also eligible for evaluation, the extended CG (ECG). Pts receive 6 monthly intradermal inoculations (R0-R6) during the primary vaccine series followed by four boosters every 6 mos. Immune responses (IR) were measured by delayed type hypersensitivity (DTH) at R0 and R6. This planned interim analysis was performed at 24 months median follow-up. **Results:** We have currently enrolled 172 pts (46, VG; 43, CG; 83 extended CG). There are no differences between groups with respect to age, rate of node positivity, tumor grade, tumor size, ER/PR status, and HER2 over-expression (all $p > 0.05$). Maximum local toxicity (tox) was similar between the two groups (grade (Gr) 1 and 2: VG 93%, CG 98%; Gr 3: VG 2%, CG 1%). Maximum systemic tox was also similar between the groups (Gr 1 and 2: VG 91%, CG 85%). No Gr 3 systemic tox has been reported. The most frequent systemic reactions are fatigue, headache, and myalgias. IR to GP2 has been robust. DTH is increased from R0 to R6 in the VG (3.0 ± 0.98 to 21.5 ± 4.04 mm, $p < 0.01$) vs. the smaller increase in CG (2.6 ± 0.89 to 6.0 ± 1.6 mm, $p = 0.01$). VG DTH at R6 is significantly higher than the CG (21.5 vs 6.0 mm, $p < 0.01$). The recurrence rate (RR) is decreased in the VG vs CG (4.3% vs. 11.6%, $p = 0.41$) and VG vs ECG (4.3% vs 9.5%, $p = 0.41$). In pts with HER2-overexpressing (IHC3+ or FISH+) tumors, the RR is decreased in the VG (0% vs 5% CG, $p = 0.28$). For TNBC (HER2 low, ER/PR-) pts, the RR is reduced in the VG vs ECG (0% vs 10.6%, $p = 0.251$). **Conclusions:** The GP2 vaccine is safe and the minimal toxicity is comparable between the VG and CG, suggesting that it is due to GM-CSF. Robust in vivo immune response has correlated with a $>50\%$ reduction in breast cancer recurrences in the VG. Clinical trial information: NCT00524277.

Novel cancer vaccines in combination with oxaliplatin-based chemotherapy as first-line therapy in advanced colorectal cancer: A randomized phase II study.

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Background: A phase I cancer vaccination trial using five novel HLA-A*24-binding peptides derived from not only three oncoantigens, RNF43 (ring finger protein 43), TOMM34 (34 kDa translocase of the outer mitochondrial membrane), and KOC1 (IMP-3; IGF-II mRNA binding protein 3) but also antiangiogenic cancer vaccine targeting VEGFR1 (vascular endothelial growth factor receptor 1) and VEGFR2 had revealed safety and immunogenicity in advanced colorectal cancer (CRC) as presented at 2011 ASCO Oral Abstract Session (No. 2510). We further performed a phase II trial to evaluate the benefit of the cancer vaccination in combination with oxaliplatin-based chemotherapy as first-line therapy. **Methods:** Ninety-six chemotherapy naïve CRC pts were enrolled to evaluate primarily response rates (RR), and secondarily OS and PFS. Each of the five peptides (3 mg each) was mixed with 1.5 ml of IFA and subcutaneously administered weekly for 12 weeks and after then biweekly. Chemotherapy was performed simultaneously as mFOLFOX6 or XELOX with/without bevacizumab. All enrolled pts had received the therapy without knowing HLA-A status double-blindly, and the HLA genotype were key-opened at analysis point and then, the endpoints are evaluated between HLA-A*2402 positive and HLA-A*2402 negative group. **Results:** Between February 2009 and November 2012, a total of 96 pts were enrolled in this study. The cutoff date for the main analysis was January 31, 2013 (median duration of follow-up of 26.5 months). mFOLFOX6 and XELOX were administered to 93 and 3 pts, respectively. Bevacizumab was used for 5 pts. RR, the primary study end point, was 61.5% (CR 1, PR 58, SD 33, PD 4). It seemed superior as compared to other reports. The median duration to reach the best responses (14 weeks; range 8-69) was surprisingly long and indicated the delayed effect of vaccination. PFS and OS were 8.2 m and 20.7 m, respectively. The HLA genotype will be key-opened at March 2013 and the endpoints will be presented between HLA-A*2402 positive and negative group at the meeting. **Conclusions:** The phase II cancer vaccine therapy demonstrated the promising response, and warrants further clinical studies. Clinical trial information: UMIN000001791.

Effect of algenpantucel-L immunotherapy for pancreatic cancer on anti-mesothelin antibody (Ab) titers and correlation with improved overall survival.

Gabriela R. Rossi, Jeffrey M Hardacre, Mary Frances Mulcahy, Mark S. Talamonti, Jennifer Carrie Obel, Caio Max S. Rocha Lima, Howard Safran, Heinz-Josef Lenz, E. Gabriela Chiorean, Nicholas N. Vahanian, Charles J. Link, NLG0205; NewLink Genetics, Ames, IA; University Hospitals Case Medical Center, Cleveland, OH; Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, IL; Kellogg Cancer Center NorthShore University Health System, Evanston, IL; NorthShore University HealthSystem, Evanston, IL; Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL; Brown University Oncology Research Group, Providence, RI; University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA; Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN

Background: Hyperacute rejection of tissues expressing the carbohydrate $\alpha(1,3)\text{Gal}$ xenoantigen is a potent innate immune defense mechanism that was leveraged to treat resected pancreatic cancer patients by immunization with genetically modified allogeneic tumor cells expressing αGal moieties (algenpantucel-L). We propose that adding algenpantucel-L to SOC adjuvant therapy may improve survival and induce immunological biomarkers that positively correlate with improved median overall survival (OS). **Methods:** Open-label, multicenter phase II study evaluating algenpantucel-L+SOC (RTOG-9704: gemcitabine + 5-FU+XRT) for resected pancreatic cancer patients. Endpoints: 1° disease-free survival (DFS) at 1 year; 2° OS, toxicity and immunologic analysis. Biomarkers were evaluated including total IgG, complement, CA19-9 levels, anti- αGal Ab, anti-CEA Ab, and anti-membrane-bound recombinant mesothelin (MSLN) Ab. **Results:** Patients received gemcitabine with 5-FU modulated radiation therapy plus algenpantucel-L. The primary endpoint, 12-month DFS, was 62% and 12-month OS was 86%. All evaluable patients have been in follow-up for ≥ 3 years. We now report OS rates at 3 years of 39% and DFS of 26% at 3 years. Evaluable patients (n=64) were tested for the induction of anti-MSLN Ab where $\geq 25\%$ increase in the anti-MSLN Ab compared to baseline was considered significant ($p<0.001$). Twenty of 64 patients (31%) had increased anti-MSLN Ab. Patients responding with anti-MSLN Ab had a median OS of 42 months compared to 20 months for patients without sero-conversion. The positive correlation between increased anti-MSLN Ab and improved median OS was statistically significant ($p=0.027$). **Conclusions:** The addition of algenpantucel-L to SOC for resected pancreatic cancer may improve survival. In 20/64 patients, algenpantucel-L-induced anti-MSLN Ab responses that correlates with improved survival (median OS 42 vs 20 months). Immunological monitoring of algenpantucel-L immunotherapy with this biomarker is feasible and might predict patient response to therapy. A multi-institutional, phase III study is currently underway (ClinicalTrials.gov NCT01072981). Clinical trial information: NCT00569387.

First-in-human phase I study of CetuGEX, a novel anti-EGFR monoclonal antibody (mAb) with optimized glycosylation and antibody dependent cellular cytotoxicity.

Walter Fiedler, Cristiana Sessa, Luca Gianni, Sara Cresta, Henning Schulze-Bergkamen, Jens Weidmann, Sara De Dosso, Anna Tessari, Marc Oliver Salzberg, Hans Baumeister, Antje Danielczyk, Bruno Dietrich, Johannes Nippgen, Steffen Goletz; Hubertus-Wald University Cancer Center, Hamburg, Germany; Ospedale Regionale Bellinzona e Valli, Bellinzona, Switzerland; San Raffaele Scientific Institute, Milan, Italy; Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; National Center for Tumor Diseases, University of Heidelberg, Heidelberg, Germany; Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; Glycotope GmbH, Berlin, Germany

Background: Epidermal growth factor receptor (EGFR) is a validated target in cancer. EGFR antagonists in clinical use do not exploit the full potential of this target. CetuGEX is an IgG1 mAb against EGFR. Fully human and optimized glycosylation lead to a 10- to 250-fold improvement of ADCC-mediated tumor cell killing in all FcγRIIIa allotypes and lack of immunogenic carbohydrate-chains, compared to cetuximab. **Methods:** Eligible patients with advanced solid tumors, progressing after standard treatment, were enrolled into this phase I, first-in-human, multicenter, single agent dose escalation trial. PK, PD and immunological parameters were assessed. Endpoints were safety and tolerability and secondarily pharmacokinetics, immunogenicity and anti-tumor activity. **Results:** 41 patients were treated on a q1w (8 dose levels from 12 to 1,370 mg flat dose), or q2w (990 mg flat) schedule. 25 pts had received at least 8 weekly doses (per protocol population [PP]). The most frequently observed drug-related AE were nausea (20%), vomiting (20%), hypertension (20%), almost all low grade and acneiform dermatitis (25%), only grade 1 or 2. Infusion-related reactions (IRR), virtually restricted to the first infusion, were associated with cytokine secretion: IL-6, IL-8, TNFα, IFNγ and IP-10 as marker of macrophage activation. Optimization of infusion scheme and premedication reduced IRRs in severity and frequency from 76% to 57% mainly of low grade. Blood NK cells were reduced as sign of redistribution. Activity was seen over all dose levels. One patient with NSCLC achieved a complete response. One patient with metastatic colorectal cancer had a partial response, another 2 patients with esophageal and gastric cancer without measurable disease at study entry had marked improvement of symptoms and normalization of tumor markers. Additional 15 pts had stable disease lasting from 8 weeks to over a year, including several minor responses, leading to a clinical benefit rate of 46% (19/41) in the overall and 76% (19/25) in the PP population. PK supports q1w and q2w dosing. **Conclusions:** CetuGEX shows clear signs of activity and acceptable toxicity. Phase II will soon be initiated. Clinical trial information: NCT01222637.

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Poster Discussion Session (Board #1), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Highlighting the challenge of delayed overall survival (OS) curve separation in immunotherapy clinical trials.**

Savanna D. Steele, Song Wang, Alan Solinger, Austin J. Combest, Marie-Edith Anne Bonnetterre, Dirk J. Reitsma; PPD Inc., Wilmington, NC

Background: Cancer immunotherapy holds great potential but can elicit delayed responses, unlike chemotherapy. A delay in the separation of OS curves (between the control and treatment arms) has led to difficulty in achieving adequate power for OS analysis (Hoos A, *Ann Oncol.* 2012). Since combining immunotherapies, such as an anti-CTLA4 and an anti-PD-1, may increase the percentage of subjects achieving durable responses (Curran MA, *PNAS.* 2010), we sought to determine how adding a complementary immunologic agent to the currently-approved ipilimumab would impact delay in OS curve separation. Arbitrarily assuming that treatment effect on OS was doubled, we determined how various delays in OS curve separation might impact sample sizes. **Methods:** We evaluated three dual-immunotherapy clinical trial scenarios in which the separation of OS curves either remained the same, decreased, or increased. Baseline OS estimates were from pivotal trial data for ipilimumab in melanoma (Robert C et al, *NEJM.* 2011). SAS PROC POWER with piecewise linear survival curve was then used to determine approximate corresponding sample sizes. **Results:** See Table. **Conclusions:** Demonstrating significance in order to gain regulatory approval is important for providing potentially life-saving medications. For trials evaluating dual-immunotherapies, despite doubling treatment effects, delays in OS curve separation of up to 6 months would require over 2700 subjects to reach statistical power. In an era where treatments are increasingly personalized, these numbers will be difficult to achieve, underscoring the importance of novel trial designs incorporating Bayesian hierarchical modeling, precompetitive collaborations, and/or the use of state-of-the-art predictive biomarkers.

		Ipilimumab + Dacarbazine	Ipilimumab + dacarbazine	Ipilimumab + dacarbazine + anti-PD-1 MAb	
			2-mo delay	4-mo delay	6-mo delay
Median OS (mos)	9.1	11.2	15	13.3	11.7
Hazard	0.076	0.076 (first 4 mos), then 0.054	0.076 (first 2 mos), then 0.042	0.076 (first 4 mos), then 0.042	0.076 (first 6 mos), then 0.042
Hazard ratio after delay*		0.71	0.55	0.55	0.55
Median OS after delay (mos)	9.1	12.9	16.6	16.6	16.6
Sample size			505	1061	2731

* Delay = delay in separation of OS curves.

3010

Poster Discussion Session (Board #2), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Use of tumor growth rate (TGR) to provide useful clinical information in phase I trials and reveal clear drug-specific profiles.**

Charles Ferte, Marianna Fernandez, Antoine Hollebecque, Serge Koscielny, Antonin Levy, Rastislav Bahleda, Christophe Massard, Jean-Charles Soria; Department of Medical Oncology, Institut Gustave Roussy, Villejuif, France; Institut Gustave Roussy, Villejuif, France

Background: The evaluation of treatment response by RECIST does not take into account the tumor growth kinetics along the treatment sequence and may induce misleading information regarding the evaluation of the drug activity. TGR incorporates the time between the tumor evaluations and allows for a dynamic and quantitative evaluation of the tumor kinetics. How TGR is modified along the introduction of experimental therapeutics and is associated with outcome in phase I patients remains unknown. **Methods:** Medical records from all patients (n=304) prospectively treated at Institut Gustave Roussy in 19 phase I trials between 2009 and 2011 were analyzed. TGR was computed both during the pre-treatment period (REFERENCE) and between baseline and first evaluation (EXPERIMENTAL). TGR is defined as: $\log_{10}(V_t/V_0)/dt$ and is represented as a percentage increase of tumor volume per month for clinical relevance. The associations between TGR, RECIST, the most commonly used prognostic scores (ECOG, RMH score, PFS ratio) and the outcome (OS, PFS) were computed (multivariate analysis). The effect of treatment, prognostic scores, histology, and the number of previous treatment lines on TGR were assessed. **Results:** Overall, we observed a reduction of TGR between the REFERENCE vs. EXPERIMENTAL periods (11.5 vs. 1.5, $P<.0001$). Although most of the patients were classified as stable disease (57%) or progressive disease (28%) by RECIST at the first evaluation, 88% and 69% of them exhibited a decrease in TGR, respectively. TGR reveals clear drug specific profiles along the 19 experimental regimens tested, allowing for an early evaluation of the drug activity. The TGR decrease was associated with both PFS ($P=.004$) and OS ($P=.001$) and remained significant even after adjustment to the other prognostic scores for phase I patients. **Conclusions:** Adding TGR assessment in phase I patients is simple and provides clinically relevant information: (i) It allows for an early and precise assessment of the tumor growth, (ii) It reveals drug-specific profiles, suggesting its potential use for the early assessment of drug activity, (iii) TGR is independently associated with prognosis in phase I patients.

3011

Poster Discussion Session (Board #3), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Mucin 1 (MUC1) expression in patients (pts) with early stage non-small cell lung cancer (NSCLC): Relationship between immunohistochemistry (IHC), tumor characteristics, and survival.**

Paul Leslie Mitchell, Shane Battye, Tom John, Carmel Murone, Simon Knight, Gerd Bode, Andreas Schroeder, Khashayar Asadi; Ludwig Medical Oncology Unit, Olivia Newton-John Cancer and Wellness Centre, Austin Health, Melbourne, Australia; Department of Anatomical Pathology, Austin Health, Melbourne, Australia; Ludwig Institute for Cancer Research/Austin Health, Melbourne, Australia; Ludwig Institute for Cancer Research, Heidelberg, Australia; Austin Hospital, Melbourne, Australia; Merck-Serono, Darmstadt, Germany; Department of Anatomical Pathology, Austin Health, Heidelberg, Australia

Background: MUC1, a glycoprotein highly expressed in many malignancies, is being explored as an antigen for immunotherapy. How best to measure MUC1 expression as well as its prognostic value in NSCLC are still under discussion. **Methods:** TMAs were constructed using triplicate 1mm cores of FFPE tumour and stained with 214D4 (recognises protein core) and MA695 (recognises carbohydrate epitope) anti-MUC1 antibodies (abs). TMAs were assessed for polarisation, both cyto and mem staining intensity (scored 0-3) and proportion cells +ve (0-100%; scored 0-5), averaged for multiple cores. A composite score (intensity x cells +ve) was derived, ranging from 0-15 (3+ in >75% cells). **Results:** TMAs from 521 pts were analysed: male 362 (69.5%); never smoking 35 (7%); adeno 259 (49.7%), squamous 180 (34.5%), large cell 39 (7.5%); nodal N0 340 (65.3%), N1 71 (13.6%), N2 107 (20.5%). Results of IHC staining intensity, proportion positive cells and depolarisation were very similar for the two abs. There was high concordance in the composite score for the abs ($R^2=0.71$, $p<0.0001$). Polarisation was discordant in 7.9% of cases. For 77 cases with paired primary/ N2 nodal tissue, mean 214D4 scores were 8.3 and 8.9 and MA695 10.6 and 9.9 respectively. Discordant staining in primary but not in node was seen in 5.2% and 10.4% with 214D4 and MA695 respectively. Increased expression as assessed by 214D4 (HR=1.26; 95% CI 1.014-1.565, $p=0.04$ log rank test) and MA695 (HR=1.20, 95% CI 1.042-1.605; $p=0.02$) was associated with improved survival. The prognostic value of depolarisation has yet to be analyzed. **Conclusions:** Over 93% of cases were MUC1 IHC positive. Composite scores for the 2 abs were highly correlated and depolarisation largely concordant. MUC1 expression was generally maintained in paired primary/nodal tumour. Increased expression of MUC1 was associated with improved survival however further investigation is needed to determine which abs best predict outcomes.

	214D4	MA695
No staining	3.5%	6.4%
Mean intensity		
Cyto	1.8	1.7
Mem	2.1	2.2
Mean cells +ve	3.9	3.6
Mean composite score	10.1	9.6
Adeno	13.0	12.0
Squamous	7.1	6.9
Large cell	7.1	7.8
Depolarization	66.3%	62.0%

3012

Poster Discussion Session (Board #4), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Effective incorporation of biospecimen collection for translational studies into a phase III trial: The NeoALTTO experience (BIG 01-06).**

Paolo Nuciforo, Jose Jimenez, Claudia Aura, Debora Fumagalli, Marion Maetens, Ludmila Prudkin, Françoise Rothe, Josep Vazquez, Sherene Loi, Holger Eidtmann, Christos Sotiriou, Jose Baselga; Molecular Pathology Laboratory, Vall d'Hebron Institute of Oncology, Barcelona, Spain; Vall D'Hebron Institute of Oncology, Barcelona, Spain; Breast Cancer Translational Research Laboratory - Jules Bordet Institute, Brussels, Belgium; Breast Cancer Translational Research Laboratory, Jules Bordet Institute, Brussels, Belgium; SOLTI, Barcelona, Spain; Translational Breast Cancer Genomics Lab, Melbourne, Australia; Universitätsklinikum Schleswig-Holstein - Klinik für Gynäkologie und Geburtshilfe, Kiel, Germany; Jules Bordet Institute, Brussels, Belgium; Memorial Sloan-Kettering Cancer Center, New York, NY

Background: Translational research studies in the context of clinical trials using targeted therapies are essential for the identification of biomarkers of response or resistance. However, many challenges (e.g. logistic, technical) can impede proper biological sample collection. The NEOALTTO study provided an excellent opportunity to address these issues. **Methods:** In this large neoadjuvant phase III multicenter, multinational clinical trial, serial samples (plasma, serum, frozen and FFPE specimens) from patients with breast cancer were collected in 86 sites (23 countries) at several time points (baseline, week 2 and surgery). Standardized operating procedures were made available to sites together with pre-assembled kits for sample collection, processing, storage, and shipment to reduce the variability and increase compliance with the study protocol. All biospecimens were centralized for long term storage. Baseline fresh/frozen samples were processed for downstream analyses according to a pre-defined workflow. Evaluable sample population (ESP) was determined based on the following criteria: tumor cellularity, RNA integrity (RIN), and yield. **Results:** A total of 12,193 serial samples from 449 of 455 randomized patients was collected. The overall sample missing rate was 9.5% (includes samples not collected by site, withdrawn patients and pCR). Missing rates were 9%, 8%, 13%, and 9% for plasma, serum, frozen and FFPE specimens, respectively. At least 1 frozen tumor tissue was available for 423 (94%), 431 (96%), and 334 (74%) patients at baseline, week 2, and surgery, respectively. After 2 extraction cycles, 629 and 516 baseline frozen samples were processed for RNA and DNA extraction, respectively. The ESP was 71% (58% when RIN considered) and 80% for downstream RNA- and DNA-based analyses, respectively. Incorrect material, inappropriate sample processing and low or absent tumor cellularity were some of the factors affecting the ESP. **Conclusions:** These results show that careful upfront logistic and technical planning in NEOALTTO had a significant impact on the compliance and the quality of collected material, which may ultimately result in valuable research data. Clinical trial information: NCT00553358.

3013

Poster Discussion Session (Board #5), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**EGF-based cancer vaccine: Optimizing predictive and surrogate biomarkers.**

Tania Crombet Ramos, Elia Neninger, Jorge Gonzalez, Pedro Camilo Rodriguez, Beatriz Garcia, Xitlally Popa, Zaima Mazorra, Carmen Viada, Patricia Lorenzo Luaces, Gisela Gonzalez, Agustin Lage; Center of Molecular Immunology, Havana, Cuba; Hermanos Ameijeiras Hospital, Havana, Cuba; National Institute of Oncology, Havana, Cuba

Background: EGFR is overexpressed in many epithelial tumors. EGF is one of the most important growth factors that stimulates EGFR, in a paracrine way. CIMAVax-EGF is a therapeutic cancer vaccine intended to induce antibodies against EGF. It is composed by recombinant EGF conjugated to P64 from *N.Meningitidis* as a carrier and Montanide, as adjuvant. **Methods:** Two controlled trials were done in advanced NSCLC and castration-resistant prostate cancer patients (CRPC). A multicentric, randomized Phase III trial was designed to assess the efficacy, immunogenicity, and safety of CIMAVax-EGF in advanced NSCLC patients. Patients with histology- or cytology-proven NSCLC at stage IIIB/ IV were enrolled. All subjects received 4 platinum-based cycles before entering the study. On the other hand, a multicentric, randomized Phase II trial was designed to assess the immunogenicity and safety of the vaccine in CRPC patients. The EGF cancer vaccine was administered in combination with mitoxantrone and prednisone. **Results:** 405 patients bearing NSCLC and 200 men with CRPC were enrolled in the 2 trials. In both studies the vaccine was immunogenic. Antibody titers against EGF increased with vaccination and EGF concentration in sera showed a fast reduction after immunization. The anti-EGF antibody response was directly correlated with overall survival. CIMAVax-EGF was safe and most prevalent adverse events were grade 1-2 injection site pain, fever, headache, nausea, vomiting, and chills. The vaccine significantly increased the survival of the NSCLC patients while it did not augment significantly the overall survival of the CRPC patients. In both studies, EGF concentration was measured at baseline and it was found to be much higher than in normal subjects. High EGF concentration predicted greater benefit after vaccination. On the contrary, NSCLC and prostate control patients with high levels of EGF had a poorer outcome. **Conclusions:** Antibody response against EGF is a surrogate marker of survival. High EGF concentration might be a predictive marker of vaccine efficacy and a poor prognostic biomarker for non-vaccinated NSCLC and CRPC patients. The predictive and prognostic value of the EGF concentration will be validated prospectively. Clinical trial information: IIC EC 081.

3014

Poster Discussion Session (Board #6), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Immune gene expression in primary melanomas to predict lower risk of recurrence and death.**

Shanthi Sivendran, Rui R. Chang, Sara Harcharik, Lawrence Hall, Sebastian Bernardo, Marina Moskalenko, Robert Phelps, Meera Sivendran, Ariella Cohain, Analisa DiFeo, Michael Parides, Mark Lebwohl, Philip Friedlander, Jacques Banchereau, Nina Bhardwaj, William K. Oh, Steven J. Burakoff, Karolina Palucka, Miriam Merad, Yvonne M. Saenger; Hematology/Oncology Medical Specialists, Lancaster General Health, Lancaster, PA; Mount Sinai School of Medicine, New York, NY; Department of Dermatology, Geisinger Health Systems, Danville, PA; Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH; Icahn School of Medicine, Mount Sinai Medical Center, New York, NY; Baylor Institute for Immunology Research, Dallas, TX; New York University, New York, NY; Division of Hematology and Medical Oncology, The Tisch Cancer Institute, Mount Sinai School of Medicine, New York, NY; The Tisch Cancer Institute, New York, NY

Background: Improved biomarkers are needed to define recurrence risk in patients with completely resected skin melanomas. Standard prognostic indicators used in staging, including depth, ulceration, and mitotic rate, while useful, often fail to accurately predict recurrence for individual patients. The immune system may prevent recurrence in this population, but no evidence-based immune biomarkers are in clinical use. Biomarker development has been hindered by clinical standards necessitating that the entire specimen be formalin fixed and paraffin embedded (FFPE) for morphology evaluation, a process damaging to RNA. **Methods:** To define a biomarker for melanoma recurrence, mRNA copy number of immune-related genes from FFPE melanoma was measured using NanoString, a hybridization assay suited for analysis of partially degraded RNA. Genes predictive of non-recurrence were defined using receiver operating characteristic (ROC) curves in a training cohort and then validated in an independent patient cohort. **Results:** A panel of 21 genes predictive of non-recurrence were defined using ROC curves in a training cohort (N=44). This result was validated in an independent patient cohort (N=37, AUC=0.794). Protein levels of the most differentially expressed gene, CD2, also associated with non-recurrence ($p<0.001$). The immune gene panel and CD2 staining associated with prolonged survival ($p<0.001$ and $p=0.019$, respectively). **Conclusions:** mRNA copy number of immune-related genes in primary FFPE melanomas predicts non-recurrence and prolonged survival. This data highlights the impact of immunosurveillance in primary human melanoma and the identified gene panel may be a useful tool for patient stratification for adjuvant immunotherapy studies.

3015

Poster Discussion Session (Board #7), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Targeting CD137 to enhance the antitumor efficacy of cetuximab by stimulation of innate and adaptive immunity.**

Holbrook Edwin Kohrt, Roch Houot, Kipp Weiskopf, Matthew Goldstein, Peder Lund, Ruth R Lira, Emily Troutner, Lori Richards, Amanda Rajapaksa, Anton Ostashko, Wen-Kai Weng, Lieping Chen, Debra Czerwinski, A. Dimitrios Colevas, John Sunwoo, Ronald Levy; Stanford Cancer Institute, Stanford, CA; Centre Hospitalier Universitaire Pontchaillou, Rennes, France; Stanford University, Stanford, CA; Yale University, New Haven, CT; Stanford University, School of Medicine, Stanford, CA

Background: Cetuximab therapy results in beneficial, yet limited, clinical improvement for patients with KRAS wildtype (WT) colorectal (CRC) and head and neck (HN) cancer. The efficacy of cetuximab, an IgG1 monoclonal antibody against EGFR, is due in part to antibody-dependent cell-mediated cytotoxicity (ADCC) by natural killer (NK) cells. CD137 is a costimulatory molecule expressed following activation on NK and memory, antigen-specific, CD8 T cells. **Methods:** We investigated the hypothesis that the combination of cetuximab with anti-CD137 mAb will enhance innate and adaptive immunity, thereby improving cetuximab's anti-tumor efficacy in preclinical models and a prospective trial, NCT01114256. **Results:** NK cells increased their expression of CD137 by a factor of 30-40 when exposed to cetuximab-coated, EGFR-expressing HN and CRC cell lines. An agonistic anti-CD137 mAb enhanced NK cell degranulation and cytotoxicity 2-fold (~45 to 90% tumor lysis assayed by chromium release). The combination of cetuximab and anti-CD137 mAbs was synergistic in a syngeneic, human-EGFR-transfected murine tumor leading to complete tumor resolution and prolonged survival. NK cell depletion, significantly, and CD8 T cell depletion, partly, abrogated the anti-tumor efficacy of this combination. A series of HN and both KRAS WT and mutant CRC xenotransplant models demonstrated synergy with cetuximab and anti-CD137 mAbs. In our clinical trial, 54 patients with HN cancer receiving cetuximab therapy, circulating and intratumoral NK cells upregulated CD137 with amplitude influenced by duration post-cetuximab and host FcγRIIIa polymorphism. Interestingly, in 10 HLA-A2⁺ patients, following cetuximab, an increase in EGFR-specific, CD137-expressing, CD8 T cells directly correlated with the percent increase in CD137-expressing NK cells. **Conclusions:** Our results demonstrate the synergy of combining an agonistic mAb, anti-CD137, augmenting ADCC and T cell memory following a tumor-targeting mAb, cetuximab, in HN and KRAS mutant and WT CRC cancer. These results support a novel, sequential antibody approach by targeting first the tumor and then the host innate and adaptive immune system. Clinical trial information: NCT01114256.

3016

Poster Discussion Session (Board #8), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Association of tumor PD-L1 expression and immune biomarkers with clinical activity in patients (pts) with advanced solid tumors treated with nivolumab (anti-PD-1; BMS-936558; ONO-4538).**

Joseph Grosso, Christine E. Horak, David Inzunza, Diana M. Cardona, Jason S. Simon, Ashok Kumar Gupta, Vindira Sankar, Jong-Soon Park, Georgia Kollia, Janis M. Taube, Robert Anders, Maria Jure-Kunkel, Jim Novotny, Jr., Clive R. Taylor, Xiaoling Zhang, Therese Phillips, Pauline Simmons, John Cogswell; Bristol-Myers Squibb, Princeton, NJ; Duke University Medical Center, Durham, NC; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD; Keck School of Medicine of the University of Southern California, Los Angeles, CA; Dako North America, Inc., Carpinteria, CA

Background: The immune checkpoint receptor programmed death-1 (PD-1) negatively regulates T-cell activation. In a phase I study, nivolumab, a PD-1 receptor blocking antibody, demonstrated durable clinical activity. Evaluation of the expression of the PD-1 ligand, PD-L1, by IHC with the 5H1 Ab suggested a correlation between pretreatment tumor PD-L1 expression and clinical response (Topalian et al, NEJM 2012). **Methods:** 304 pts with non-small cell lung cancer (NSCLC, n=127), melanoma (MEL, n=107), renal cell (RCC, n=34), colorectal (n=19) or prostate cancer (n=17) received nivolumab between 2008-2012 (0.1–10 mg/kg IV Q2W) during dose escalation and/or cohort expansion. Formalin-fixed paraffin-embedded tumor tissue and peripheral blood mononuclear cells (PBMC) were analyzed to explore potential pharmacodynamic/ predictive biomarkers associated with nivolumab therapy. Tumor surface PD-L1 expression was evaluated by IHC using an automated assay based on a sensitive and specific PD-L1 mAb (28-8) distinct from 5H1. PD-L1 positivity (PD-L1+) was defined as $\geq 5\%$ cell membrane staining of any intensity. Lymphocyte subsets in PBMC were measured using flow cytometry. **Results:** Tumor membrane PD-L1 was measured in 101 MEL and NSCLC pts. 17/38 (45%) of MEL and 31/63 (49%) of NSCLC biopsies were PD-L1+. A numerically higher objective response rate (ORR), longer progression-free survival (PFS), and overall survival (OS) was seen with PD-L1+ in MEL pts (Table). Analysis of the association of PD-L1 expression with ORR, PFS and OS in NSCLC is ongoing. A correlative analysis of pt response with pre-/post-dose levels of lymphocytes will be presented. **Conclusions:** These data, using a novel, automated PD-L1 IHC assay and mAb, support the hypothesis of tumor PDL1+ predicting activity of nivolumab in advanced cancer, which is being prospectively assessed in phase III trials. Clinical trial information: NCT00730639.

	Endpoint	PD-L1 status	N event/N subj (%)	Median, Mo (95% CI)
MEL	OS	+	8/16 (50%)	21.1 (9.4, -)
		-	10/18 (56%)	12.5 (8.2, -)
	PFS	+	9/16 (56%)	9.1 (1.8, -)
		-	12/18 (67%)	2.0 (1.8, 9.3)
	ORR	+	7/16 (44%)	
		-	3/18 (17%)	

3018

Poster Discussion Session (Board #10), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Ipilimumab in the real world: The U.K. expanded access programme (EAP) experience in advanced melanoma.**

Saif Ahmad, Wendi Qian, Sarah Gabrielle Ellis, Muhammad Adnan Khattak, Avinash Gupta, Kiruthikah Thillai, Ruth E. Board, Jenny Nobes, Angus Dalgleish, Simon Aird Grumett, Anthony Maraveyas, Sarah Danson, Toby Talbot, Maria Marples, Ruth Plummer, Satish Kumar, Mark R. Middleton, James M. G. Larkin, Christian Ottensmeier, Philippa Corrie; Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom; Cambridge Cancer Trials Centre, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom; University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom; The Royal Marsden NHS Foundation Trust, London, United Kingdom; Oxford University Hospitals NHS Trust, Oxford, United Kingdom; Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom; Oncology Department, Royal Preston Hospital, Preston, United Kingdom; Clinical Oncology, Norfolk & Norwich University Hospital, Norwich, United Kingdom; Department of Oncology, St George's Hospital Medical School, London, United Kingdom; Royal Wolverhampton Hospitals, Wolverhampton, United Kingdom; Castle Hill Hospital, Hull, United Kingdom; Cancer Research Centre, Weston Park Hospital, Sheffield, United Kingdom; Royal Cornwall Hospitals NHS Foundation Trust, Truro, United Kingdom; St. James's Institute of Oncology, Leeds, England; Northern Institute for Cancer Research, Newcastle University, Newcastle, United Kingdom; Velindre Cancer Centre, Cardiff, United Kingdom; The Royal Marsden Hospital NHS Foundation Trust, London, United Kingdom; Southampton University Hospitals NHS Foundation Trust, Southampton, United Kingdom; Oncology Centre, Cambridge University Hospitals NHS Foundation Trust (Addenbrooke's Hospital), Cambridge, United Kingdom

Background: Since publication of the registration trial in 2010 (Hodi et al, *NEJM* 2010;363:711-23), real world use of ipilimumab (Ipi) in previously treated advanced melanoma patients has extended beyond the specific trial entry criteria of ECOG PS 0-1. We undertook a review of UK patients (pts) treated in the international EAP prior to European licensing of Ipi in August 2011, to compare real world survival outcomes. **Methods:** UK clinicians registered in the EAP provided anonymised data using pre-specified variable fields for all pts. The EAP stipulated pts should have previously treated, unresectable stage III or IV metastatic melanoma and receive Ipi 3 mg/kg, 3 weekly IV, for up to 4 cycles. Response using RECIST criteria was assessed 12 weekly. Grade ≥ 3 adverse events (AEs) using CTCAE v3.0 were collected. **Results:** To date, information on 162 pts has been received from 16 UK sites. Primary sites were: 78% cutaneous, 4% ocular, 1% mucosal, 17% unknown. 78% pts had M1c disease, 14% had brain metastases. N° prior therapies ranged from 0-4, 72% pts received 1 prior therapy. Median age was 60 years, men>women (1.6:1). ECOG PS was: 38% 0, 47% 1, 14% 2, 1% 3. BRAF status was known in 38% cases and WT in 75% of these. 19% pts were on steroids at baseline. N° cycles delivered was 4 in 52%, 3 in 13%, 2 in 16%, 1 in 17% pts. Most frequent reason for stopping early was clinical evidence of disease progression (71%), death (16%) or unacceptable AE (12%). 32% pts experienced a grade ≥ 3 AE, the most common being diarrhoea (13%) and fatigue (8%). Complete and partial responses were reported in 1% and 21% of treated pts. At median follow-up of 17 months, median progression free survival and overall survival (OS) were 2.8 and 5.7 months, 1 year OS was 30%. Comparing outcomes of various pt subgroups, the strongest prognostic factor for OS was ECOG PS at the start of treatment ($p<0.0001$). For pts with PS 0 or 1, median OS was 8.8 months (compared with 10 months in the registration trial). More detailed safety and efficacy data on pt subgroups will be presented. **Conclusions:** This review, representing the largest Ipi EAP UK dataset, reports overall poorer survival outcomes than in the registration trial, but pts with similar characteristics to the trial population lived longer.

3019

Poster Discussion Session (Board #11), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Nonclinical evaluation of the combination of mouse IL-21 and anti- mouse CTLA-4 or PD-1 blocking antibodies in mouse tumor models.**

Maria Jure-Kunkel, Mark Selby, Katherine Lewis, Gregg Masters, Jose Valle, Joseph Grosso, Gennaro Dito, Wendy Curtis, Richard Garcia, Matthew Holdren, Alan J. Korman, Stacey Dillon; Bristol-Myers Squibb, Princeton, NJ; Bristol-Myers Squibb, Redwood City, CA; Bristol-Myers Squibb, Seattle, WA; Bristol-Myers Squibb, Mt. Vernon, IN

Background: Interleukin 21 (IL-21), a γ c chain family cytokine, is produced primarily by CD4+ T cells and has many effects on immune cells, including enhancing CD8+ T cell and NK cell proliferation and cytotoxicity. Recombinant IL-21 (rIL-21) therapy resulted in objective responses in ~20% of melanoma and renal cell carcinoma patients. In mouse models, monoclonal antibody (mAb) blockade of CTLA-4 prolongs antigen-specific T cell responses, while blockade of programmed death 1 (PD-1) reverses tumor induced T cell suppression. Ipilimumab, a CTLA-4 blocking mAb, significantly improved overall survival in patients with metastatic melanoma in 2 phase III trials, and in phase I studies a PD-1 blocking mAb (nivolumab) has antitumor activity in various cancers. Side effect profiles for each mAb have been related to their mechanism and are generally manageable. It was hypothesized that combination of IL-21 plus CTLA-4 or PD-1 blockade may enhance antitumor responses, potentially leading to improved clinical activity. **Methods:** Preclinical studies were conducted to test the antitumor activity of mouse IL-21 (mIL-21) in combination with an anti-mouse PD-1 (mPD-1) mAb (4H2-IgG1) or with an anti-mCTLA-4 blocking mAb (9D9-IgG2b) in syngeneic mouse tumor models, including MC38, CT-26, EMT-6, and B16F10. mIL-21 was tested at doses ranging from 50-200 μ g/dose, administered up to 3d/wk. mCTLA-4 mAb or mPD-1 mAb were administered 3-4x total at 200-300 μ g/dose. **Results:** Combination treatments produced enhanced antitumor activity vs. monotherapy. In the MC38 model, mIL-21 treatment led to 30% median tumor growth inhibition (TGI) by d29, while mPD-1 mAb produced 60% median TGI and 1/10 tumor-free mice. Combination of both agents led to synergistic antitumor activity, with complete regressions (CR) in 7/10 mice and 99.9% median TGI ($p=0.046$). CTLA-4 mAb + mIL-21 also produced synergistic activity in the MC38 model. By d21, mIL-21 monotherapy induced 34% TGI while CTLA-4 mAb resulted in 28% TGI, with no CR in either group. Combination resulted in 6/8 mice with CR and 86% TGI ($p<0.05$). **Conclusions:** These results support the use of rIL-21+nivolumab and rIL-21+ipilimumab in recently initiated clinical trials.

3020

Poster Discussion Session (Board #12), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Effect of anti-CTLA-4 antibody treatment on T-cell repertoire evolution in treated cancer patients.**

Edward Cha, Yafei Hou, Mark Klinger, Craig Cummings, Malek Faham, Antoni Ribas, Lawrence Fong; University of California, San Francisco, San Francisco, CA; Sequentia, Inc., South San Francisco, CA; Med-Hematology & Oncology, University of California, Los Angeles, Los Angeles, CA

Background: CTL-associated antigen 4 (CTLA-4) is an immune checkpoint expressed by T cells. While treatment with anti-CTLA-4 antibody can induce clinical responses in advanced cancer patients, its effects on the breadth of the T cell response is unknown. **Methods:** We used a sequencing-based method, LymphoSIGHT, to assess T cell repertoire diversity in 46 patients with metastatic castration resistant prostate cancer or metastatic melanoma. Peripheral blood mononuclear cells were obtained from patients prior to and during treatment with anti-CTLA-4 antibody. mRNA was amplified using locus-specific primer sets for T cell receptor (TCR) beta, and the amplified product was sequenced. Sequence reads were used to quantitate absolute TCR frequencies using standardized clonotype determination algorithms with normalization by spiked reference TCR sequences. Following clonotype quantitation, repertoire differences between serial samples were assessed by the Morisita index, a statistical measure of population dispersion. **Results:** 97 paired samples were assessed, of which 46 (47%) had increases and 22 (23%) had decreases in TCR diversity by more than 2-fold. By comparison, none of 9 untreated sample pairs underwent more than a 2-fold change in diversity ($P = 0.005$, Fisher's exact test, two tailed). TCR repertoire differences between monthly samples were markedly higher than for time-matched controls. After the first treatment, median Morisita index between samples was 0.197 for treated samples versus 0.039 for untreated ($P = 0.0005$, Mann-Whitney U test). The median number of clones that significantly changed in abundance was 421 for treated versus 45 for controls. In patients with multiple time points, this rapid clonotype evolution continued through treatment. Despite this global turnover in repertoire, a subset of high frequency clones, including CMV-specific T cells, remained relatively constant over the course of the study. **Conclusions:** CTLA-4 blockade increases the global rate of T cell clonotype turnover and influences TCR diversity. This evolution of the TCR repertoire may reflect a mechanism by which CTLA-4 blockade enhances tumor-specific T cells over time.

3021

Poster Discussion Session (Board #13), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Correlation of PDL1 tumor expression and outcomes in renal cell carcinoma (RCC) patients (pts) treated with pazopanib (paz).**

David J Figueroa, Yuan Liu, Robert C. Gagnon, Christopher Carpenter, Mohammed Dar, Arundathy N. Bartlett-Pandite; GlaxoSmithKline, Research Triangle Park, NC; GlaxoSmithKline, Collegeville, PA; GlaxoSmithKline Research and Development, Collegeville, PA

Background: The interaction between PDL1 (B7H1) and its receptor PD-1 on activated T cells plays an important role in the inhibition of T-cell responses and contributes to suppression of antitumor immune responses. Tumor PDL1 expression has been associated with poor outcomes in RCC. This study investigates the correlation between PDL1 tumor expression and outcomes in RCC pts treated with paz. **Methods:** Using IHC, we retrospectively analyzed baseline FFPE tumor samples for PDL1 from 2 paz RCC studies: a single arm phase II trial and randomized placebo (pbo)-controlled phase III study. PDL1 expression was analyzed by MedTox Laboratories using the anti-PDL1 MouseIgG1 clone 5H1 (Thompson) on the Leica automated IHC platform. Additional dual PDL1/CD68 staining was carried out to delineate tumor and macrophage PDL1 expression. Tumor PDL1 expression was quantified by H-Score (HS) and PDL1+ macrophages were assessed semi-quantitatively. Association between PDL1H scores and PFS was investigated by Kaplan-Meier analysis using optimal cutoff of PDL1tumor HS (minimum p value, log rank test). **Results:** The optimal cut-point of PD-L1 tumor HS, relative to PFS, was identified as HS > 3. In the phase II study (46 available samples out of 225), HS range was 0-150 and most samples had negative (HS = 0, n = 34, 74%) or low (HS 1-3, n=4, 9%) PDL1 expression. Pts with HS > 3 (n = 8, 17%) had significantly shorter PFS (2.6 mo) than those with HS ≤ 3 (12 mo; p = .0005). In the phase III study (N = 160 available samples: paz, 113 of 290; pbo, 47 of 145), HS range was 0-280. Most patients had negative (n = 122/160, 76%) or low (n = 9/160, 6%) PD-L1 expression, with 18% (29/160) having HS > 3. Pbo-arm pts with HS > 3 (n = 6/47, 13%) had shorter PFS (2.3 vs 5.5 mo p = .0207). Paz-arm pts with HS > 3 (n = 23/113, 20%) trended toward shorter PFS (7.3 vs 11 mo, p = .1405). **Conclusions:** PDL1 appears to be a prognostic marker with PDL1 HS > 3 associated with shorter PFS. Limitations of the study include the retrospective nature of the analysis with limited pt samples available, low or negative PDL1 expression in the vast majority of pts, and use of archival samples that may not accurately reflect PDL1 status at study entry. Additional results (tumor volume, OS) will be presented.

3022

Poster Discussion Session (Board #14), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**A phase I open-label study of Ad-RTS-hIL-12, an adenoviral vector engineered to express hIL-12 under the control of an oral activator ligand, in subjects with unresectable stage III/IV melanoma.**

Gerald P. Linette, Omid Hamid, Eric D. Whitman, John J. Nemunaitis, Jason Chesney, Sanjiv S. Agarwala, Alexander Starodub, John A Barrett, Andrew Marsh, Lori A. Martell, Angela Cho, Thomas D. Reed, Hagop Youssoufian, Andrea Vergara-Silva; Washington University in St. Louis, St Louis, MO; The Angeles Clinic and Research Institute, Los Angeles, CA; Atlantic Melanoma Center, Morristown, NJ; Mary Crowley Cancer Research Center, Dallas, TX; University of Louisville, Louisville, KY; St. Luke's Hospital and Health Network, Easton, PA; Indiana University Health Goshen Center for Cancer Care, Goshen, IN; ZIOPHARM Oncology, Inc., Boston, MA; Intrexon Corporation, Blacksburg, VA

Background: IL-12 is a pleiotropic cytokine with known antitumor activity; however, clinical use was limited by toxicity when delivered systemically. Ad-RTS-hIL-12 is an adenoviral vector engineered for controlled expression of IL-12 with the RheoSwitch Therapeutic System (RTS) and an oral activator, INXN-1001. **Methods:** In a phase I, 3+3 dose escalation study, subjects with unresectable stage III/IV melanoma were administered 1×10^{12} viral particles (Ad-RTS-hIL-12) intratumorally on the first day of up to six 21-day cycles, and INXN-1001 (5, 20, 100, and 160 mg) orally on days 1-7 of each cycle. **Results:** Dose escalation is complete with 14 subjects treated. Median prior therapeutic agents was 3 (range 1-4). Common related adverse events included chills (11, 78.6%), pyrexia (11, 78.6%), fatigue (10, 71.4%), and nausea (10, 71.4%). With a biologically effective dose of 160 mg, MTD for INXN-1001 was not reached. One death unrelated to study drug was secondary to septicemia. Clinical activity was observed in 5 of 7 subjects treated at doses of INXN-1001 ≥ 100 mg, but not at < 100 mg, and included prominent inflammatory responses in injected and non-injected lesions, decreases in size of injected and non-injected lesions, and reduction in tumor-associated pain. One subject at the 160-mg dose had stable disease for 20 weeks. Clinical activity in dose cohorts ≥ 100 mg coincided with a 4-fold median increase from baseline in peak serum levels of IL-12 and IFN- γ compared with lower dose cohorts. Flow cytometric analyses of PBMCs revealed 7-fold (≥ 100 mg dose cohorts) median increases from baseline in peak levels of absolute numbers of CD3+ and CD8+ T cells. ELISPOT and T-cell proliferation assays for antigen-specific responses are ongoing. **Conclusions:** Intratumoral delivery of IL-12 via an adenoviral vector with RheoSwitch enabled finely-controlled expression of IL-12 levels by an oral ligand. Ad-RTS-hIL-12 plus INXN-1001 (160 mg) was well tolerated and induced biological and clinical activity in subjects with advanced melanoma. Phase II studies are ongoing at the biologically effective dose. Clinical trial information: NCT01397708.

3023

Poster Discussion Session (Board #15), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Deletion and mutation of IL10RA gene on chromosome 11q23 to avert CpG-B oligodeoxynucleotides-induced apoptosis of human chronic lymphocytic leukemia B cells.***Xueqing Liang, Veronika Bachanova, Wei Chen; University of Minnesota, Minneapolis, MN*

Background: Targeting TLR9 expressed on human CLL cells with CpG-B oligodeoxynucleotides leads to IL-10-induced tyrosine phosphorylation of STATs and apoptosis of B-CLL cells. However, B-CLL cells from a small subset of patients were resistant to CpG-B ODN treatment. Here, we investigated the molecular mechanism by which B-CLL cells are sensitive or resistant to CpG-B ODN treatment. **Methods:** Purified CD19⁺CD23⁺CD5⁺ primary B-CLL cells were cultured for 5 days with/without CpG-B ODN (CpG 2006, CpG 685) or rh-IL-10. B-CLL cell apoptosis were determined by viable cell counts, Annexin V/PI and TMRE staining, Western blot and intracellular staining of the activation/cleavage of caspases, PARP, Bax translocation and cytochrome c release, IL-10R1 expression and IL-10 binding by flow cytometry, *IL10RA* gene deletion by FISH, IL10R1 S138G mutation by Bi-PASA, The tyrosine or serine phosphorylation of STATs by Western blot. **Results:** Fifteen CpG-sensitive and 11 CpG-resistant primary CLL samples were comparatively studied. B-CLL cells from 15/15 CpG-sensitive samples were induced into apoptosis by either CpG-B ODNs or IL-10 in a treatment time and dose-dependent manner. Both CpG-B ODNs and IL-10 significantly increased pTyr701-STAT1/pTyr705-STAT3 expression and induced apoptosis via the mitochondrial apoptotic pathway in 15 primary B-CLL cells. No *IL10RA* gene deletions were detected, 13/15 patients were IL10R1 S138G AA wildtypes and 2/15 patients were AG heterozygotes. In contrast, CpG-B ODNs or IL-10 failed to induce apoptosis in 11/11 CpG-resistant B-CLL cells. Interesting, 2/11 CLL samples had *IL10RA* genes deletion and 7/11 had IL10R1 S138G GG homozygote mutation. *IL10RA* gene deletion significantly decreased the IL10R1 expression; both *IL10RA* gene deletion and mutation significantly decreased the IL-10 binding, abolished or reduced CpG-B ODNs or IL10-induced pTyr701-STAT1/pTyr705-STAT3 expression in B-CLL cells. **Conclusion:** Deletion and mutation of *IL10RA* gene on chromosome 11q23 averts CpG-B ODN-induced apoptosis of human B-CLL cells, which may serve as biomarker to predict sensitivity or resistance of CLL to CpG-B ODN treatment.

3024

Poster Discussion Session (Board #16), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Anti-CD20-interferon-alpha fusion protein has superior in vivo activity against human B-cell lymphomas compared to rituximab and enhanced complement-dependent cytotoxicity in vitro.**

Reiko E Yamada, Kristopher K Steward, Gatara Ngarmchamnanrith, Ryan K Trinh, Sanjay Khare, Raj Sachdev, Iqbal S Grewal, Sherie L Morrison, John Timmerman; University of California, Los Angeles, Los Angeles, CA; ImmunGene and Valor Biotherapeutics, Thousand Oaks, CA

Background: We previously reported an anti-CD20-interferon (IFN)-alpha fusion protein able to induce apoptosis and promote in vivo eradication of a human CD20-expressing mouse B cell lymphoma (Xuan et al, *Blood* 2010). We now report the activity of a recombinant anti-CD20-human IFN α fusion protein against human non-Hodgkin B cell lymphomas (NHL). **Methods:** Anti-CD20-hIFN α was evaluated against a panel of human Burkitt, diffuse large B cell (DLBCL), and mantle cell lymphoma cell lines. Proliferation was measured by [3 H]-thymidine, complement-dependent cytotoxicity (CDC) by PI flow cytometry, and antibody-dependent cellular cytotoxicity (ADCC) by LDH release using PBMC effectors. NHL xenografts were grown in SCID mice. **Results:** Anti-CD20-hIFN α induced stronger growth inhibition than rituximab, particularly against Burkitt and germinal center-type DLBCL NHLs. Tumor growth inhibition by anti-CD20-hIFN α was associated with substantial apoptosis in some cell lines. Anti-CD20-hIFN α exhibited potent ADCC activity against Daudi, Ramos, and Raji cells, identical to rituximab. Surprisingly, anti-CD20-hIFN α exhibited superior CDC compared to rituximab against Daudi, Ramos, and Raji cells, that was dependent upon linkage of IFN α to the anti-CD20 antibody, and correlated with improved complement fixation. Importantly, anti-CD20-hIFN α achieved superior efficacy compared to rituximab and control fusion protein against multiple NHL xenografts in SCID mice (Raji: $p=0.002$ and OCI-Ly19: $p<0.0001$). At antibody doses at which Raji xenografts progressed through rituximab, anti-CD20-hIFN α eradicated 50-88% of established tumors. Non-targeted control fusion protein had only minor effects on tumor growth. **Conclusions:** Anti-CD20-hIFN α has stronger direct anti-proliferative and CDC activities than rituximab against human NHL while retaining potent ADCC activity, and also has the ability to eradicate established NHL xenografts in vivo. These results support the further development of anti-CD20-hIFN α for the treatment of B cell NHL, and a phase I first-in-human clinical trial is currently being planned.

3025

Poster Discussion Session (Board #17), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Phase I study of the safety, pharmacokinetics (PK), and pharmacodynamics (PD) of the oral inhibitor of indoleamine 2,3-dioxygenase (IDO1) INCB024360 in patients (pts) with advanced malignancies.**

Gregory Lawrence Beatty, Peter J. O'Dwyer, Jason Clark, Jack G Shi, Robert Charles Newton, Richard Schaub, Janet Maleski, Lance Leopold, Thomas Gajewski; Abramson Cancer Center of the University of Pennsylvania, Philadelphia, PA; Incyte Corporation, Wilmington, DE; The University of Chicago, Chicago, IL

Background: INCB024360 is a potent, selective inhibitor of IDO1. As the catabolism of tryptophan (Trp) to kynurenine (Kyn) by IDO1 inhibits immune responses and IDO1 expression is elevated in many human cancers, IDO1 inhibition may potentiate effective antitumor immune responses. **Methods:** This dose-escalation study in adult pts with advanced malignancies used a 3+3 design to determine MTD, toxicity, PK, PD, and tumor response rate. Daily doses of INCB024360 were evaluated in 28-day cycles in 8 cohorts (50 mg once daily; 50 mg, 100 mg, 300 mg, 400 mg, 500 mg, 600 mg, or 700 mg BID). Treatment continued until disease progression or unacceptable toxicity. PK and PD samples were drawn on days 1 and 15. **Results:** 52 pts have been treated. Tumor types included colorectal (56%), melanoma (12%), and other (33%). The most common adverse events ($\geq 20\%$) were fatigue, nausea, decreased appetite, vomiting, constipation, abdominal pain, diarrhea, dyspnea, back pain, and cough. The most common grade 3 or 4 adverse events were abdominal pain, hypokalemia, and fatigue (9.6% each). One DLT each was observed at 300 mg BID (grade 3 radiation pneumonitis) and 400 mg BID (grade 3 fatigue); no DLTs were observed in the 18 pts treated with 600 mg or 700 mg BID. There were no objective responses. At 56 days, stable disease was seen in 15 patients and lasted ≥ 112 days in 7 patients. Significant dose-dependent reductions in plasma Kyn/Trp ratios and Kyn levels were detected at all doses and in all pts. Maximal effects were observed at doses ≥ 300 mg BID. With repeat dosing, 700 mg BID provided an average plasma concentration ~ 5 -fold the projected IC_{90} . Overall, doses ≥ 300 mg BID achieved greater than 90% inhibition of IDO1 throughout the dosing period. **Conclusions:** INCB024360 was generally well tolerated at doses of up to 700 mg BID and there appears to be no correlation of dose with toxicity. Doses ≥ 300 mg BID were capable of $>90\%$ inhibition of IDO1 activity and found to effectively normalize plasma Kyn levels. The recommended dose as monotherapy is 600 mg BID. Clinical trial information: NCT01195311.

3026

Poster Discussion Session (Board #18), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**A phase I study of indoximod in combination with docetaxel in metastatic solid tumors.**

Erica Jackson, Elizabeth Claire Dees, John S. Kauh, R Donald Harvey, Anthony Neuger, Richard Lush, Scott J. Antonia, Susan E. Minton, Roohi Ismail-Khan, Hyo S. Han, Nicholas N. Vahanian, William Jay Ramsey, Charles J. Link, Howard Streicher, Daniel Sullivan, Hatem Hussein Soliman; University of South Florida, Morsani College of Medicine, Tampa, FL; UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC; Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA; The Winship Cancer Institute of Emory University, Atlanta, GA; H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; Moffitt Cancer Center and Research Institute, Tampa, FL; NewLink Genetics, Ames, IA; Division of Cancer Treatment and Diagnosis, National Cancer Institute, Bethesda, MD

Background: The indoleamine-2, 3-dioxygenase (IDO) pathway catabolizes tryptophan to create a state of immunosuppression. Indoximod (1-methyl-(D)-tryptophan, D-1MT) is an IDO pathway modulator. Pre-clinical studies in MMTV-neu mouse models have shown indoximod combined with chemotherapy was more effective in causing tumor regression than either agent alone. Based on this data, a phase IB trial was designed to study the safety of the combination of docetaxel (Doc) and indoximod. The primary goal of this trial was to determine the MTD for the combination of Doc and oral indoximod. Secondary endpoints were PK data and efficacy for indoximod/Doc. **Methods:** This phase IB study consisted of 5 dose levels (DL). Doc was dosed IV q 3 wks at 60 mg/m² in DL 1-4 and 75 mg/m² at DL 5. Indoximod was dosed at 300, 600, 1,000, 2,000, and 1,200 mg PO BID continuously in DL 1-5 respectively. MTD was determined using a 3+3 design. The DLT rule was 1st cycle ≥G3 non-heme AEs or ≥G4 heme AEs despite supportive care or that delay therapy >14d. The PK of indoximod and Doc was analyzed using a HPLC assay. PK was measured on C1D1 and 8. Standard eligibility/exclusion criteria applied along with exclusion of patients previously treated with ipilimumab. Treatment was continued until disease progression, intolerance, or unacceptable side effects. **Results:** Total # of patients treated at DL1-5 were 7, 6, 6, 2, and 6 respectively, with 22 total patients evaluable for response. DLTs included: G3 dehydration (at 300 mg), G5 neutropenic colitis (at 600 mg), G3 hypotension (at 2,000 mg) and G3 mucositis (at 2,000 mg). DL 5 was well tolerated and is the recommended phase II dose. The most frequent adverse events were fatigue (58.6%), anemia (51.7%), hyperglycemia (48.3%), infection (44.8%), and nausea (41.4%). There were 4 PRs (2 breast, 1 NSCLC, 1 thymic), 9 SD, and 9 PD. There were no drug-drug interactions, and PK was similar to Doc and indoximod single-agent studies. **Conclusions:** The Doc+ indoximod combination was well tolerated with no increase in expected toxicities or unexpected PK interactions. It was active in a pretreated population of patients with metastatic solid tumors. The RP2D is 75 mg/m² of Doc with 1,200 mg of indoximod BID for the current phase II metastatic breast cancer trial. NCT01191216 Clinical trial information: NCT01191216.

3027

Poster Discussion Session (Board #19), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Recombinant adenoviral human p53 gene infusion in treatment of malignant pleural effusions and malignant ascites.***Kaiweng Hu; East Hospital of Beijing University of Traditional Chinese Medicine, Beijing, China*

Background: Malignant pleural or peritoneal effusions are frequently developed in the late-stage malignant tumors in chest or abdominal cavity. Various drainage methods and intra-cavity chemotherapy have transient and limited benefits. Here we evaluated the role of recombinant adenoviral human p53 gene (rAd-p53) infusion for patients with malignant pleural or peritoneal effusions. **Methods:** Thirty-two patients with historically diagnosed malignant pleural (18 cases) or peritoneal (14 cases) effusions, 19 males and 13 females with an average age of 61 years old (37–80 years), were included this study. The malignant pleural effusions were caused by primary lung cancers (8 cases), lung metastatic tumors (4 cases), breast cancers (3 cases), pleural mesothelioma (2 cases), lymphoma (1 cases), and the peritoneal effusions were caused by ovarian cancers (5 cases), primary liver cancers (4 cases), liver metastatic tumors (2 cases), colon cancer (1 case), gastric cancer (1 case), and prostate cancer (1 case). After draining most of the fluids, 4×10^{12} viral particles (VP) of rAd-p53 diluted into 1,000 ml of saline solution for intra-abdominal cavity infusion and 2×10^{12} VP of rAd-p53 diluted into 500 ml of saline solution for intra-chest cavity infusion, were given weekly for 4 weeks. The response rate was evaluated. The complete response is defined as the complete disappearance of pleural or peritoneal fluid and negative cytologic findings for >4 weeks, and the partial response is defined as a decrease over 50% of the fluid without the need of drainage and negative cytologic findings for >4 weeks. **Results:** The pleural effusion showed a complete response in 6 patients (33.3%) and a partial response in 6 patients (33.3%). The peritoneal effusion had a complete response in 3 patients (21.4%) and a partial response in 7 patients (50.0%). The overall response rate was 68.8% (22/32). The symptoms associated with the malignant effusion relieved in 27 patients (84.4%). There were no serious side effects observed except for self-limited fever found in all the cases. **Conclusions:** Intra-abdominal or chest cavity infusion of rAd-p53 is a safe and effective treatment for some malignant pleural or peritoneal effusions.

3028

Poster Discussion Session (Board #20), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Adoptive T-cell therapy (ACT) with TILs for metastatic melanoma: Clinical responses and durable persistence of anticancer responses in peripheral blood.**

Marco Donia, Rikke Andersen, Eva Ellebaek, Trine Zeeberg Iversen, Mads Hald Andersen, Per thor Straten, Inge Marie Svane; Center for Cancer Immune Therapy/Department of Oncology, Copenhagen University Hospital Herlev, Herlev, Denmark; Center for Cancer Immune Therapy, Copenhagen, Denmark; Department of Oncology and Center for Cancer Immune Therapy, Department of Hematology, Copenhagen University Hospital Herlev, Herlev, Denmark; Center for Cancer Immune Therapy/Department of Haematology, Copenhagen University Hospital, Herlev, Denmark; Center for Cancer Immune Therapy/Department of Haematology & Oncology, Copenhagen University Hospital, Herlev, Denmark

Background: TIL treatment holds the promise to introduce a new treatment paradigm into oncology practice. To demonstrate the logistical feasibility of this complex approach in Europe, at Herlev Hospital, Denmark, we have initiated a trial for patients with metastatic melanoma and evaluated the melanoma-specific immunity in the peripheral blood. **Methods:** We present the updated results of trial NCT00937625. The study takes place in a medium-size academic center (30 in-patient oncology beds) equipped with a 36 square meters cGMP cell production lab integrated in a hematopoietic stem cell transplantation unit. Patients were treated with autologous TILs preceded by standard lymphodepleting chemotherapy but followed by attenuated regimens of IL-2 (n=6 low-dose s.c. for 14 days; n=9 i.v. intermediate decrescendo dose). Melanoma-specific responses were assessed with intracellular cytokine staining. **Results:** We have generated TILs from 28/31 patients, with 15 patients treated so far and many TILs cryopreserved for future use. Patients were treated with an average of $>50 \times 10^9$ CD4⁺, CD8⁺ and a small but consistent fraction of $\gamma\delta$ TILs. The lower doses of IL-2 have significantly decreased the classical toxicity of the treatment associated with more harsh IL-2 regimens, and response evaluation showed the achievement of three CR lasting > 1 year and four PR. Clinical responses were associated with high numbers of tumour reactive T-cells infused. Importantly, in most responding patients we observed induction and durable persistence (up to 1 year) of anti-melanoma T-cell responses in the peripheral blood. **Conclusions:** A high response rate including durable complete responses can be induced after treatment with TILs followed by an attenuated regimen of IL-2, which significantly reduced the occurrence of severe side effects. Effective TIL treatment is associated with induction and long-term persistence in the blood of T cells producing in vitro anticancer responses. By showing that TIL-based ACT is logistically feasible and accessible to medium-size academic centers, we open the possibility for testing this treatment in a large randomized setting in Europe. Clinical trial information: NCT00937625.

3029

Poster Discussion Session (Board #21), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Allogeneic dendritic cell (DC) vaccination as an “off the shelf” treatment to prevent or delay relapse in elderly acute myeloid leukemia patients: Results of phase I study.**

Arjan A van den Loosdrecht, Saskia Santegoets, Sandra van Wetering, Satwinder Kaugh Singh, Malika Koppes, Anthony Hall, Tanja D. de Gruijl, Gert J. Ossenkoppele, Ada M Kruisbeek; Department of Hematology, Cancer Center Amsterdam, Amsterdam, Netherlands; Department of Medical Oncology, VU University Medical Center, Amsterdam, Netherlands; DCPrime BV, Leiden, Netherlands

Background: Vaccines against tumor-associated antigens represent an appealing strategy for preventing tumor recurrence. A novel immunotherapy platform is represented by the DCOne cell line, which originates from a human myeloid leukemia cell line, endogenously expresses a range of tumor associated antigens and can be differentiated into mature dendritic cells. **Methods:** A phase I study enrolled 12 AML patients (age range 58-71) who were either in CR1/CR2 (n=5) or had smouldering disease (n=7), at high risk of relapse and ineligible for available post-remission therapies, in a 3+3 design, starting with 4 bi-weekly intradermal DCOne DC vaccinations of 10E6 cells (n=3), 25E6 (n=3) or 50E6 (n=6). Patients were monitored for clinical and immunological responses for 126 days and surviving patients were followed up after study completion. **Results:** Treatment was well tolerated in all patients, with expected toxicities of injection site reactions (< grade 2). During the study 3 patients died: 2 from infections and 1 from leukemia. Patients who survived more than 6 months post-vaccination showed remarkable survival (22 mo after the first patient was recruited, 3 patients have been alive for 22, 20 and 16 months respectively). One patient with smouldering AML at entry achieved CR2 after vaccination; one patient who was in CR2 at entry, relapsed 8 mo after vaccination and entered CR3 following a single low dose of 5-AZA. Remarkably, 1/5 patients that were evaluable by IFN γ ELIPOTs showed vaccination-induced specific T cell responses to WT-1 and PRAME, antigens present in DCOne DC. In addition, increased post vaccination delayed type hypersensitivity reactions were observed in all cohorts. Furthermore, induction of systemic immune responses, with increases in CD4+ and CD8+ T cell proliferative responses and/or seroconversion to DCOne DC and/or AML blasts was seen in 5 out of 9 patients. **Conclusions:** Vaccination with DCOne derived DC is feasible in AML, generated cellular and humoral immune responses, and interesting clinical responses. The hypothesis that immune responses correlate with clinical benefit will be investigated in a randomised phase II trial. Clinical trial information: NCT01373515.

3030

Poster Discussion Session (Board #22), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Effect of oral cyclophosphamide on the immunogenicity of DPX-Survivac in ovarian cancer patients: Results of a phase I study.**

Neil Lorne Berinstein, Amit M. Oza, Kunle Odunsi, Mohan Karkada, Jeannine A Vilella, John J. Nemunaitis, Michael Morse, Tanja Pejovic, James Bentley, Rita Nigam, Genevieve Weir, Lisa MacDonald, Marianne Stanford, Tomasz Burzykowski, Marc Mansour; Sunnybrook Health Sciences Centre, Toronto, ON, Canada; Princess Margaret Cancer Center, University Health Network, Toronto, ON, Canada; Roswell Park Cancer Institute, Buffalo, NY; Immunovaccine, Inc., Halifax, NS, Canada; Winthrop-University Hospital, Mineola, NY; Mary Crowley Cancer Research Center, Dallas, TX; Duke University Medical Center, Durham, NC; Oregon Health & Science University, Portland, OR; QEII Health Sciences Center, Halifax, NS, Canada; International Drug Development Institute, Louvain la Neuve, Belgium

Background: Survivin, a protein involved in regulation of apoptosis, is highly expressed in many tumor types and has reported prognostic value. DPX-Survivac is a cocktail of survivin HLA class I peptides (A1, A2, A3, A24 and B7) formulated in the novel adjuvanting vaccine platform DepoVax. A phase I study examined the safety and immune potency of DPX-Survivac in combination with cyclophosphamide in ovarian cancer patients. **Methods:** 18 of 19 advanced ovarian cancer patients treated with platinum chemotherapy and showing no disease progression completed their vaccine therapy. Cohort A (6 pts) received three 0.5 mL vaccine injections 3 weeks apart; cohorts B and C (6 pts each) received three 0.1 mL or 0.5 mL vaccine injections in combination with metronomic low dose oral cyclophosphamide. Adverse events were assessed using CTCAE v4.0. Blood was collected to study immune function (MDSCs, T regs and B cells) and vaccine-induced T cell immunity (ELISpot, tetramer analysis and multi-parametric intracellular cytokine staining). Repeated measures of immunity at baseline and after 1, 2 and 3 injections were analyzed using a general linear model. **Results:** DPX-Survivac was well tolerated with no significant systemic AEs. Local injection AEs occurred in all patients. Grade 3 local reactions occurred in 3 patients (1 pt in B and 2 pts in C). 11 of 12 patients receiving the combination therapy produced immune responses by at least 2 assays, generally established with one or two vaccinations and increased or maintained with boosters. A dose response was observed, with cohort C patients producing significantly higher magnitude responses (C vs B, $P=.013$). Low dose cyclophosphamide significantly enhanced the 0.5 ml dose (C vs A, $P=.015$). Notably, antigen specific CD8 T cells were detected ex vivo in PBL's using tetramers and further characterized as polyfunctional by multi-parametric ICS, suggesting a robust immune response. **Conclusions:** DPX-Survivac is well tolerated and immunogenic. Immune modulation with low dose oral cyclophosphamide can dramatically enhance the immunogenicity of this vaccine. The efficacy of the proposed DPX-Survivac vaccine therapy needs to be tested in a randomized phase II study. Clinical trial information: NCT01416038.

3031

Poster Discussion Session (Board #23), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Risk of HCC recurrence with cellular immunotherapy following radiofrequency ablation.**

Jiuwei Cui, Wei Li, Nanya Wang, Hengjun Zhao, Haofan Jin, Chao Niu, Guanjun Wang; First Hospital of Jilin University, Changchun, China; Cancer Center, First Hospital of Jilin University, Changchun, China

Background: Although radiofrequency ablation (RFA) has brought promising therapeutic outcome in hepatocellular carcinoma (HCC) treatment, the curative rate was compromised by high recurrence and metastasis after RFA. Immunosuppression in patients with HCC is an important factor leading to its recurrence and metastasis. This study was designed to observe the efficiency and safety of application of cellular immunotherapy (CIT) after RFA for HCC patients. **Methods:** Sixty-two patients with HCC who were treated with radical RFA were divided into two groups: RFA alone (32 patients) and RFA/CIT (30 patients). Autologous mononuclear cells were collected from the peripheral blood and separated by apheresis, and then induced into natural killer cells, $\gamma\delta$ T cells, cytokine-induced killer cells. These cells were identified by flow cytometry with their specific antibodies and then were infused intravenously to RFA/CIT patients for 3 or 6 courses. The tumor recurrent status of these patients was evaluated with computed tomography (CT) every 3 months after RFA. Progression-free survival (PFS), liver function, viral load, and adverse effects were examined. **Results:** The median PFS in RFA group was 12.0 (9.1-14.8) months while median PFS in RFA/CIT group has not yet been reached. It indicated that sequential RFA/CIT significantly reduced the risk of HCC recurrence [hazard ratio (HR) = 0.136]. In RFA/CIT group, six courses had better survival prognosis than three courses ($P < 0.05$). Viral load of hepatitis C decreased in two of three patients without antiviral therapy in RFA/CIT group, but was increased in RFA alone group. The RFA/CIT group maintained the hepatic function at the level before CIT. The hepatic function in 28.1% (9/32 cases) patients in RFA group was deteriorated. Only one developed fever (38.5 °C) after one infusion and recovered 2 hours later. Otherwise, there was no toxic effect in the RFA/CIT group. **Conclusions:** These preliminary results suggested that combination of sequential CIT with RFA for HCC patients was efficient and safe, and may be helpful in the prevention of the recurrence for the patients with HCC after RFA. It also suggested that the CIT may reduce the risk of recurrence by dual effects of anti-tumor and anti-virus.

3032

Poster Discussion Session (Board #24), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Outcome with stereotactic radiosurgery (SRS) and ipilimumab (Ipi) for malignant melanoma brain metastases.**

Sana Shoukat, David Mitchell Marcus, Monica Rizzo, David H. Lawson, Yuan Liu, Mohammad Khurram Khan; Internal Medicine, Emory University School of Medicine, Atlanta, GA; Department of Radiation Oncology, Winship Cancer Institute of Emory University, Atlanta, GA; Department of Surgery, Emory University, Atlanta, GA; Emory University School of Medicine, Atlanta, GA; Biostatistics and Bioinformatics Shared Resource at Winship Cancer Institute, Atlanta, GA; Department of Radiation Oncology, Winship Cancer Institute, Emory University School of Medicine, Atlanta, GA

Background: SRS with Ipi for brain metastases from malignant melanoma has been explored for overall survival (OS) (Knisely JP, Yu JB, Flanigan J, et al. Radiosurgery for melanoma brain metastases in the ipilimumab era and the possibility of longer survival. J Neurosurg. 2012;117:227-33). We present the first retrospective analysis to determine if this combination is safe and improves OS, while accounting for lactate dehydrogenase (LDH). **Methods:** Patients with melanoma brain metastases who underwent SRS between 1998-2010 (n=124) were compared with those who additionally received Ipi (n=11). The primary endpoint was median OS from time of SRS, calculated using Kaplan-Meier method. Cox proportional hazard model was carried out for univariate and multivariable survival analysis. The secondary endpoints were local control at initial site of SRS, anywhere intra-cranial failure, need for repeat SRS, and toxicity. **Results:** Median OS for the entire cohort was 6.9 months. Patients in the Ipi group had an improved median OS of 28.3 months vs. 6.8 months in the non-Ipi group (p = 0.013). No difference was noted in local control, anywhere intracranial failures, toxicity (radionecrosis, hemorrhage, patient reported memory deficits), or need for repeated SRS. MVA (Table) showed that Ipi independently predicted for improved OS even when taking into account LDH and ECOG performance status. The only confounding factor within the Ipi group was younger age of the Ipi cohort (43 vs. 55 yrs, p = 0.006). **Conclusions:** Use of SRS with Ipi appears to be safe and associated with an impressive increase in median OS in patients with brain metastases from malignant melanoma; this combination should be further investigated.

Univariate and multivariable analysis.

Variables		Univariate analysis		Multivariable analysis	
		HR (95%CI)	P value	HR (95%CI)	P value
KPS	<= 60	2.11 (1.16 -3.84)	0.011	0.47(0.28-0.80)	0.005
	<=80, > 60	1.60 (1.09 -2.35)			
	> 80				
ECOG	0	0.46 (0.29-0.72)	<.001		
DS-GPA	1+				
	1	2.64 (1.48 - 4.72)			
	2	1.92 (1.18 - 3.14)			
Number of brain mets	3	1.09 (0.69-1.73)	0.004		
	4				
	1	0.50 (0.32 - 0.78)			
	2	0.47 (0.26 -0.83)			
Ipi	Not Given	2.56 (1.18 - 5.51)	0.013	2.30 (1.05-5.04)	0.037
LDH	Given		0.001	0.47 (0.29-0.78)	0.004
	<= 250	0.45 (0.27 - 0.73)			
	> 250				

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Poster Discussion Session (Board #25), Sat, 1:15 PM-5:15 PM and
4:45 PM-5:45 PM**Intraperitoneal radioimmunotherapy (RIT) for desmoplastic small round cell tumor (DSRCT): Initial results from a phase I trial.**

Shakeel Modak, Michael P. La Quaglia, Jorge A. Carrasquillo, Pat Zanzonico, Catherine Enero, Neeta Pandit-Taskar, Hye Jin Kang, Nai-Kong V. Cheung; Memorial Sloan-Kettering Cancer Center, New York, NY

Background: DSRCT, a rare sarcoma of adolescents and young adults usually arising from the peritoneum, is lethal in >80% of patients despite aggressive multimodality therapy. Recurrences often present as multifocal peritoneal implants, making it uniquely suited for intraperitoneal (IP) targeting. We hypothesized that targeted radiotherapy may improve local control and reduce relapses. IP RIT, by virtue of prolonged residence time and slow transfer to the circulation, may selectively target IP DSRCT while minimizing organ toxicity. The anti-4Ig-B7H3 murine monoclonal antibody 8H9 binds to 96% of primary DSRCT (*Med Pediatr Oncol* 39:547). ^{131}I -8H9 injected intra-Ommaya is safe (*J Neurooncol* 97:409). **Methods:** We initiated a phase I study to test the safety of IP RIT with ^{131}I -8H9. Cohorts of 3-6 patients were treated with ^{131}I -8H9 at escalated doses from 30mCi/m²-60mCi/m² as a single IP injection. A tracer dose of 2mCi ^{124}I -8H9 was given IP before ^{131}I -8H9 to acquire PET images and biodistribution data. Pharmacokinetics (PK) was studied using serial blood draws. **Results:** 15 heavily prior-treated patients: 13 with DSRCT, 2 with rhabdomyosarcoma received 30, 40, 50mCi/m² ^{131}I -8H9 (3 at each dose level) or 60mCi/m² (n=6). Dose-limiting toxicity was not seen. Three patients (n=1 each) had transient, self-limiting, possibly therapy-related grade 3 toxicities: neutropenia, hepatic transaminase elevation and thrombocytopenia. No patient required hematopoietic stem cell rescue. Blood half life was 32.5±11.5h (n=12) and mean peritoneal residence time was 14.6h (n=3). Mean absorbed dose to blood based on blood sampling was 0.56±0.21 rad/mCi (n=14). Mean absorbed doses (rad/mCi) to kidney, liver, lung and spleen were 1.72, 1.92, 0.64 and 1.03 respectively (n=3). Dehalogenation was insignificant: >80% iodine remained protein-bound in blood (n=10). 6/7 DSRCT patients treated without evaluable disease remain in remission at a median of 11.1 months post ^{131}I -8H9. **Conclusions:** IP ^{131}I -8H9 was safe and ^{124}I -8H9 provided valuable PK and dosimetry data. Since maximum tolerated dose was not reached we have expanded patient accrual to a planned dose of 90mCi/m². Clinical trial information: NCT01099644.

3034

General Poster Session (Board #13A), Mon, 8:00 AM-11:45 AM

HER2 evaluation process for neoadjuvant targeted therapies in breast cancer.

Yoshio Mizuno, Naoko Takeda, Junichi Yamada, Hiroaki Abe, Yuko Inoue, Hiroshi Seto, Kazuhiko Sato, Tokyo-West Tokushukai Hospital; Breast Oncology Center, Tokyo-West Tokushukai Hospital, Tokyo, Japan; Department of Clinical Pathology, Tokyo-West Tokushukai Hospital, Tokyo, Japan; Inoue Ladies Clinic, Tokyo, Japan; Seto Hospital, Saitama, Japan; Tokyo-West Tokushukai Hospital, Tokyo, Japan

Background: Treatment with a combination of HER2-targeted therapies has emerged as an addition of trastuzumab to neoadjuvant chemotherapy regimens in breast cancer patients and it goes on increasing. For clinical HER2 determination, immunohistochemical (IHC) analysis is an attractive method and all IHC 2+ cases are categorized as equivocal and should be reflexed to fluorescence in situ hybridization (FISH) testing. However, research in recent years with respect to false-negative cases for HER2 testing have been reported, and it comes to the question of what considering the indication for trastuzumab in the neoadjuvant chemotherapy. To clarify these controversial points in applying the results of HER2 testing in the clinical setting, we performed a retrospective analysis of core needle biopsy (CNB) and surgical specimen results. **Methods:** 422 patients underwent primary operations for early breast cancer at Tokyo-West Tokushukai Hospital (Tokyo, Japan) from October 2008 to December 2012. Among these patients, 262 patients who received CNB prior to operation were enrolled. Those patients who received preoperative chemotherapy or had DCIS were excluded. With regard to diagnostic criteria, HER2 positivity was defined as either 3+ by IHC or FISH analysis amplification ratio of ≥ 2.2 . In addition, if in any cases which CNB samples or surgical specimens showed HER2-positive had defined true HER2-positive cases, we assessed the false-negative results of the HER2 test via IHC using CNB samples and surgical specimens. **Results:** In a matched cohort of 262 patients, 59 cases showed HER2-positive (five cases were CNBs negative to surgical specimens positive, 14 cases were CNBs positive to surgical specimens negative, and 40 cases were both positive). If we decide for selection of trastuzumab target cases by CNBs only, we make mistakes in indications of trastuzumab for five (8.5%) of 59 HER2-positive cases who were CNBs negative to surgical specimens positive. **Conclusions:** There are quite a few cases show false negatives for HER2 testing in CNB samples. In cases of considering the indication for trastuzumab in the neoadjuvant chemotherapy, even if the CNB samples resulted in negative, we consider that retesting with FISH analysis should be carried out.

3035

General Poster Session (Board #13B), Mon, 8:00 AM-11:45 AM

Correlative biomarker analysis of sequential tumor biopsies in a ph I mode of action (MoA) study in neoadjuvant head and neck squamous cell carcinoma (HNSCC) patients (pts) treated with RG7160 (GA201), a novel dual-acting, monoclonal antibody (mAb) designed to enhance antibody-dependent cellular cytotoxicity (ADCC), with cetuximab (C) as reference.

Christoph Mancao, Lilla Di Scala, Paul Delmar, Stéphane Temam, Jean-Charles Soria, James F. Spicer, Mark McGurk, Jean-Pierre Delord, Jerome Sarini, Niels Halama, Stefan Evers, Alexandre Passioukov; F. Hoffmann-La Roche Ltd, Basel, Switzerland; Institut Gustave Roussy, Villejuif, France; King's College London, Guy's Hospital, London, United Kingdom; King's College London, Guy's Hospital, London, United Kingdom; Institut Claudius Regaud, Toulouse, France; Institute Claudius Regaud, Toulouse, France; National Center for Tumor Diseases, University of Heidelberg, Heidelberg, Germany; Roche Glycart AG, Schlieren, Switzerland

Background: GA201 is a novel humanized anti-epidermal growth factor receptor (EGFR) mAb with a dual MoA: glycoengineered to enhance ADCC on top of inhibition of EGFR signaling. In an open-label, multi-center trial of pts with HNSCC, an exploratory biomarker analysis of sequential tumor biopsies was performed to investigate the single/duplex marker correlation structure. **Methods:** Pts received 2 doses of 700 or 1,400 mg GA201 or C (day 1, 8). Tumor biopsies were taken at baseline (BL) and pre-surgery (day 15). Immunohistochemistry immune-cell counts (single/duplex markers), EGFR-pathway markers and intra-tumoral cytokines (LUMINEX) were assessed. Advanced exploratory statistical methods were used to analyse inter-relationship between BL and on treatment markers, and with response (as determined by FDG-PET). **Results:** All immune markers (single and duplex) presented highly heterogeneous median values at BL, but cluster analysis emphasized their strong inter-correlation. These markers were unrelated to the BL tumor EGFR and pERK expression. Strongest bivariate correlation was seen between (CD16, CD68), (CD3, 4, 8) and (CD4, NKp46). GA201 treatment induced positively correlated dynamic changes (chg) between (CD8, CD68), while C did so for (CD4, CD16) and (CD16, CD68). Strong and negative correlation between (CD56chg, PETchg) was seen only in pts treated with 1,400 mg GA201. Intra-tumoral cytokines like CXCL12 showed good correlation with BL CD3, 16 and 68 infiltration. Principle component analysis also confirmed a good association between most BL immune markers and was able to differentiate strongest PET responders in the 700 mg GA201 cohort. **Conclusions:** Multivariate statistical methods were used to demonstrate strong interdependencies between immune-effector markers and to highlight their promising associations with response to GA201 treatment, likely due to ADCC processes in the tumors. Clinical trial information: NCT01046266.

Serum lactate dehydrogenase (LDH) as a prognostic selection criterion for ipilimumab treatment in metastatic melanoma.

Christian U. Blank, Sander Kelderman, Harm van Tinteren, Bianca Heemskerk, Rob van den Brom, Geke A. Hospers, Alfons JM van den Eertwegh, Ellen Kapiteijn, Jan Willem De Groot, Rob L. Jansen, W. Edward Fiets, Marcin Krzystanek, Zoltan Szallasi, Andrew James Scott Furness, Alex Renn, Martin Eric Gore, Paul Lorigan, Ton Schumacher, John B. A. G. Haanen, James M. G. Larkin; The Netherlands Cancer Institute-Antoni Van Leeuwenhoek Hospital, Amsterdam, Netherlands; The Netherlands Cancer Institute, Amsterdam, Netherlands; Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands; University Medical Center Groningen, Groningen, Netherlands; Department of Medical Oncology, University Medical Center Groningen/University of Groningen, Groningen, Netherlands; VU University Medical Center, Amsterdam, Netherlands; Leiden University Medical Center, Leiden, Netherlands; Isale Clinic, Zwolle, Netherlands; Department of Medical Oncology, Maastricht University Medical Center, Maastricht, Netherlands; Medical Center Leeuwarden, Leeuwarden, Netherlands; Technical University of Denmark, Lyngby, Denmark; The Royal Marsden Hospital NHS Foundation Trust, London, United Kingdom; The Royal Marsden NHS Foundation Trust, London, United Kingdom; The Christie Hospital NHS Foundation Trust, Manchester, United Kingdom; The Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Netherlands

Background: Ipilimumab, a CTLA4 blocking antibody, has been shown to improve survival in metastatic melanoma patients in phase III trials. However, only a subset of patients experiences long-term survival benefit. Therefore, we analysed two independent retrospective sets of patients treated in 'real world' settings to identify prognostic factors that correlate with better outcome after ipilimumab therapy. **Methods:** Advanced melanoma patients, eligible for ipilimumab therapy, were treated in the Dutch and UK Expanded Access Programs (EAP) and after European licensing on the 3mg/kg schedule. Baseline characteristics and peripheral blood parameters were assessed and patients were monitored for the occurrence of adverse events and responses. **Results:** A total of 166 patients were enrolled in the Dutch EAP. Best overall response rate and disease control rate were (DCR) 17% and 35%. Median follow-up was 17.9 months, with a median progression free survival of 2.9 months. Median overall survival (OS) was 7.5 months, and OS at 1 year 37.8% and at 2 years 22.9%. Univariate analysis revealed that gender, age, Breslow thickness, baseline and week 6 lymphocyte count (ALC), and prior treatment had no influence on OS, while mWHO, M stage, baseline LDH, S100, erythrocyte sedimentation rate (ESR), and the slope of ALC did. In a multivariate model only baseline LDH and ESR remained as significant independent prognostic factors. The relevance of LDH was validated in an independent cohort of 68 patients from the UK. Stratifying the patients in the NL cohort according to LDH levels (\leq upper limit normal (ULN), 54.4% of patients versus $>2\times$ ULN, 16.9% of patients) separated into groups of patients observing DCR of 48% versus 14%, and median OS of 13.7 versus 3.1 months, while toxicity was similar in both groups (48% and 41%). None of the patients was alive at 15 months after treatment if baseline LDH was $>2\times$ ULN in both the NL and UK cohorts. **Conclusions:** Overall survival upon ipilimumab treatment is lower in an expanded access population as compared to phase III trials. LDH $>2\times$ ULN at baseline is a potential prognosticator in patients receiving ipilimumab and should be considered in guiding ipilimumab treatment initiation.

3037

General Poster Session (Board #13D), Mon, 8:00 AM-11:45 AM

A novel method to identify and monitor endogenous tumor-reactive T cells by high expression of CD11a (LFA-1) and PD-1 (CD279) as immunologic readout for evaluating the efficacy of PD-1 blockade.

Haidong Dong, Svetomir Markovic, Christopher J Krco, Eugene D. Kwon; College of Medicine, Mayo Clinic, Rochester, MN; Mayo Clinic, Rochester, MN

Background: Tumor immunotherapies directed towards enhancing natural or endogenous anti-tumor T-cell immunity in patients with advanced malignancies are currently being implemented in clinic with promising results. In order to optimize therapeutic protocols and monitor the effectiveness of such therapies, a reliable T-cell marker is needed. **Methods:** We utilized CD11a (LFA-1, lymphocyte functional-associated antigen 1), an integrin up-regulated on effector and memory CD8 T-cells, and PD-1 (programmed death-1), an immunoregulatory receptor expressed by activated T cells, as biomarkers to identify, quantitate and monitor endogenous tumor-reactive cytotoxic lymphocytes (CTLs) in two mouse tumor models and the peripheral blood (PB) of 12 patients with stage IV melanoma. **Results:** High expression of CD11a and PD-1 was identified among CD8 T-cells residing within primary and metastatic murine tumor sites, as well as in spontaneous murine breast cancer tissues. In the PB of melanoma patients, tumor antigen-specific CD8 T cells were associated with a population of CD11a high CD8 T-cells which co-expressed high levels of PD-1, as opposed to eleven healthy donors who had a much lower frequency of PD-1+ CD11a high CD8 T-cells ($p < 0.0001$). Phenotypic analysis showed that CD11a high CD8 T-cells are proliferating (Ki67 positive) activated (CD62L low, CD69 high) T-cells. Increased CD11a high CD8 T-cells and delayed tumor growth were observed in PD-1 deficient mice, suggesting that the antitumor effector function of CD8 T cells is compromised by co-expression of elevated levels of PD-1. **Conclusions:** CD11a high CD8 T-cell population expresses high levels of PD-1 and is likely the cellular target of PD-1 blockade therapy. High expression of CD11a (LFA-1) and PD-1 (CD279) by CD8 T-cells may represent a novel biomarker to identify and monitor endogenous tumor-reactive CTLs. This may not only provide an immunological readout for evaluating the efficacy of therapy, but also contribute to the selection of patients with solid malignancies likely to benefit from anti-PD-1 therapy.

3038

General Poster Session (Board #13E), Mon, 8:00 AM-11:45 AM

Relationships of peripheral blood lymphocyte counts (PBLC) with antitumor activity of NGR-hTNF given in combination with chemotherapy (CT).

Alessandra Bulotta, Vanesa Gregorc, Gilda Rossoni, Gabriele Todisco, Maria Grazia Viganò, Cristina Ammannati, Giulia Mazzola, Antonio Lambiase, Claudio Bordignon; Department of Oncology, Istituto Scientifico Ospedale San Raffaele, Milan, Italy; Department of Oncology, Istituto Scientifico San Raffaele, Milan, Italy; MolMed, Milan, Italy

Background: Antitumor effects of NGR-hTNF (N), a tumor-targeted antivascular agent, are driven at low dose by an early vessel stabilization that greatly enhances both intratumoral CT uptake and T-cell infiltration. Synergism with CT was shown in immunocompetent mice, but not in nude mice lacking functional T cells. **Methods:** By an individual patient pooled analysis of 396 patients (pts) from 7 ph II trials in 6 tumor types, we estimated the effects of baseline PBLC on the antitumor activity of N (with or without CT) and CT alone. Low dose N ($0.8 \mu\text{g}/\text{m}^2$) was given in combination with CT in 171 pts. Control groups of 140 and 85 pts receiving N and CT alone, respectively, were also analyzed. CT was doxorubicin or a platinum-based regimen. In all trials, response to therapy was assessed every 6 weeks by RECIST. Endpoints of interest were response rate (RR, complete plus partial response), disease control rate (DCR, RR plus stable disease), duration of response (DOR) and progression-free survival (PFS). In logistic and Cox regression analyses, PBLC data were dichotomized in high or low levels by the median cutpoint (1.5/mL; 95% CI, 1.4-1.6). Multivariate models included age, sex, PS and tumor type as covariates. **Results:** In both N-alone and CT-alone groups, there was no statistically significant difference in treatment effect according to baseline PBLC. Conversely, high PBLC were related to better treatment outcome in the N plus CT group, compared to low PBLC. In this N plus CT group, high PBLC (vs low) were associated with higher RR (OR=2.8; 95% CI, 1.2-6.3; $p=.01$) and DCR (OR=2.6; 1.3-4.9; $p=.004$), and with longer DOR (HR=0.39; 0.16-0.96; $p=.04$) and PFS (HR=0.60; 0.43-0.85; $p=.004$). For high vs low PBLC, RR was 29% vs 14%, DCR 76% vs 57%, median DOR 8.2 vs 6.3 months, and median PFS 5.0 vs 3.0 months, respectively. On multivariate analyses, high PBLC remained an independent predictor of increased RR (OR=2.7; $p=.01$) and DCR (OR=2.6; $p=.005$), and improved DOR (HR=0.36; $p=.04$) and PFS (HR=0.60; $p=.005$). **Conclusions:** Consistently with preclinical data, these results highlight the potential value of PBLC in predicting tumor response to NGR-hTNF in combination with CT, which merits further clinical validation

Clinical trial information: NCT00994097-NCT00484432-NCT00484276-NCT00484211-NCT00483509-NCT00483080-NCT01358071.

3039

General Poster Session (Board #13F), Mon, 8:00 AM-11:45 AM

Phase I study of weekly treatment with paclitaxel injection concentrate for nanodispersion (PICN), a novel solvent and protein-free formulation of paclitaxel.

Minish Mahendra Jain, Chetan Dilip Deshmukh, Shailesh Arjun Bondarde, Niraj Bhatt, Vijay Shinde, Shravanti Bhowmik, Ganesh Divekar; KEM Hospital and Research Center, Pune, India; Deenanath Mangeshkar Hospital and Research Center, Pune, India; Shatabdi Super Specialty Hospital, Nashik, India; Kailash Cancer Hospital and Research Centre, Goraj, India; Sun Pharma Advanced Research Co., Ltd., Mumbai, India; Sun Pharma Advanced Research Co., Ltd., Cranbury, NJ

Background: PICN is a novel solvent and protein-free 100-110 nm particle formulation of paclitaxel stabilized with polymer and lipid using Nanotecton Technology. Paclitaxel has shown superior safety and efficacy profile when administered on a weekly schedule. We studied safety, tolerability, and pharmacokinetics (PK) of PICN using a weekly schedule in patients with advanced solid malignancies. **Methods:** Patients aged 18-65 years with advanced solid malignancies, ECOG performance status ≤ 2 , estimated survival ≥ 12 weeks, and adequate organ function were enrolled. A standard phase I, 3+3 dose escalation design to determine the maximum tolerated dose (MTD) of PICN administered on a weekly schedule (three consecutive weeks, one week recovery) was employed. PICN dose was escalated at pre-determined fixed dose levels of 80, 100, 125, 150, 175, and 200 mg/m². PICN was administered as a 30 min infusion without any premedication for hypersensitivity. **Results:** Twenty-one patients treated with PICN had a mean age of 52.1 yrs (range 35-67); 20 were female and entered with metastatic breast cancer (MBC; n=15), cervical cancer (n=3), skin cancer (n=2). One male had oral cancer. Doses studied were 80 (n=3), 100 (n=3), 125 (n=3), 150 (n=3), 175 (n=6), and 200 (n=3) mg/m². Despite the lack of dexamethasone premedication, no patient receiving PICN reported hypersensitivity reaction. Two DLTs (neutropenia and febrile neutropenia; both grade 3) were reported at PICN 200 mg/m². PICN PK (AUC₀₋₂₄, AUC_{0-∞}, and C_{max}) increased in a dose proportionate manner from 80 to 200 mg/m². Grade 3 or worse related AEs were: neutropenia, leucopenia, peripheral neuropathy, febrile neutropenia, anemia, thrombocytopenia, fatigue, syncope, hypotension and maculopapular rash. Partial responses were observed in MBC (100, 125, 150, 175, and 200 mg/m²) and cervical cancer (80 mg/m²). **Conclusions:** PICN on thrice monthly schedule was tolerable in the dose range evaluated. Two DTLs were reported: neutropenia and febrile neutropenia (both grade 3). Anti-tumor activity was observed in MBC and cervical cancer. Final trial data for PICN PK, safety, and efficacy will be presented at the conference. Clinical trial information: CTRI/2011/11/002124.

3040

General Poster Session (Board #13G), Mon, 8:00 AM-11:45 AM

Effect of vaccination with autologous tumor-loaded dendritic cells on intratumoral regulatory T cells in metastatic melanoma patients.

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Background: Vaccination with dendritic cells (DC) is still a valid experimental option for metastatic melanoma (MM). However, only a few patients experience long-lasting objective responses and the majority of clinical responders afterwards relapse and die. Which mechanisms are actually responsible for this “secondary resistance” to whole tumor antigens-loaded DC vaccines is largely unknown. It has been hypothesized that suppressive immune cell subpopulations, regulatory T cells in particular, may progressively accumulate in tumor tissues thus hampering therapy-induced antitumor immune responses along time. To elucidate this issue we evaluated changes induced by immunologically effective DC vaccination in the composition of tumor-associated T cell subpopulations. **Methods:** 12 patients with MM previously enrolled in a phase I/II DC vaccine trial and for which tumor tissue taken before and after at least 4 induction vaccine doses were available, were included in the study. Intratumoral lymphocytes were evaluated by CD3, CD4, CD8, FoxP3 and GrB immunostainings, and quantified by a computer-assisted method. A nonparametric two-tailed Wilcoxon signed-rank test was utilized for evaluating differences in the distribution of the number of cell positive for each marker on the total cell counts in pre- and post-vaccine biopsies. **Results:** Our data showed a considerable and statistically significant decrease of intratumoral FoxP3⁺ regulatory T cells in melanoma tissues after DC vaccination. In addition, the concurrent increase of intratumoral activated cytotoxic T lymphocytes, as shown by CD8 and Granzyme B stainings, indicated that this decrease has likely a functional relevance. **Conclusions:** Our findings that vaccination with DC loaded with autologous tumor lysate strongly reduces the intratumoral content of regulatory T cells add strength to the rationale for the development of potentially more effective combination schedules where whole tumor antigen-loaded DC vaccine prime and partially activate tumor-specific low-affinity T cells in a first tumor antigen-focusing step, followed by boosting with non-maximal doses of anti-CTLA-4 antibodies.

3041

General Poster Session (Board #13H), Mon, 8:00 AM-11:45 AM

T-regulatory cell function analysis in locally/regionally advanced melanoma patients treated with ipilimumab.

Robert Alan VanderWeele, Lisa H. Butterfield, Hui-Min Lin, Diana E. Cunningham, Yan Lin, John M. Kirkwood, Ahmad A. Tarhini; University of Pittsburgh Cancer Institute, Pittsburgh, PA; Department of Biostatistics, University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh, Pittsburgh, PA; University of Pittsburgh Medical Center, Pittsburgh, PA

Background: We conducted an immunogenicity and biomarker analysis in stage IIIB-C melanoma patients treated with neoadjuvant ipilimumab (ipi) and reported a significant increase in the frequency of circulating T-regulatory cells (T-reg) (CD4+CD25hi+ Foxp3+; $p=0.02$ CD4+CD25hi+CD39+; $p=0.001$) from pre-ipi (baseline) to post-ipi (6 weeks) in treated patients (Tarhini et al., ASCO 2012). Also, increases in T-reg were associated with improved PFS ($p=0.034$; HR=0.57). We hypothesized that ipi induces its clinical activity in part through its effect on T-reg, and despite the observed increase in circulating T-reg frequency; their suppressor function is reduced. **Methods:** Patients were treated with ipi (10 mg/kg IV q3weeks x 2doses) bracketing definitive surgery. PBMC were collected at baseline and at 6 weeks. T-reg were isolated from pre-ipi (baseline) and post-ipi (6 weeks) PBMC samples utilizing Miltenyi Biotec T regulatory isolation kit (CD4+CD25+CD127^{dim/-}). Isolated T-reg were incubated with OKT3/IL-2/CD28-stimulated and CFSE-labeled CD4+CD25- responder T-cells from the same patient time point at 1:1, 1:2 and 1:5 ratios. Flow cytometry was used to evaluate the number of cell divisions of CFSE labeled responder cells and, therefore, the degree of T-reg proliferation suppression. **Results:** Thirty-five patients were enrolled in the study; of those, 18 patients had adequate PBMC samples with sufficient T-reg isolated for T-reg functional analysis. Preliminary analysis of the first 13 patients shows a trend toward decreased suppressive function of T-reg after treatment with ipi. Expressed as “percent of maximum inhibition”, pre- vs. post-ipi T-reg showed 61% vs. 47% suppressive activity ($p=0.15$) at the 1:1 ratio. **Conclusions:** There was a trend toward decreased suppressive function of T-reg after treatment with ipi, supporting the function modulation hypothesis. T-reg functional analysis for the remaining 5 patients is ongoing and we will perform comparative studies of changes in T-reg function with changes in T-reg frequency and clinical outcomes in the entire cohort.

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General Poster Session (Board #14A), Mon, 8:00 AM-11:45 AM

Circulating endothelial microparticles (EMP) modifications as predictive biomarkers of resistance to chemotherapy for breast cancer (BC).

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Background: Growing evidence indicates that EMP may both modulate angiogenesis and endothelial injury in cancer and cardiovascular disease. However, it has not been shown whether in vivo release of EMP might reflect the tumor response or the antiangiogenic effects of chemotherapy (CT). The aim of this work was to evaluate the relationship between the levels of small-size EMP (sEMP) and resistance to CT in locally advanced and metastatic BC. **Methods:** Citrated platelet-free plasma was obtained from BC patients before and after 3-4 cycles of chemotherapy. Small-size CD144+ sEMP (0.1-0.5 μ m diameter) were prospectively quantified using a high resolution Apogee A50 flow cytometer. Association of EMP with clinical variables, response to CT and survival was analyzed. Response (partial or complete) was defined by RECIST criteria. **Results:** 45 BC patients were included (20, metastatic; 25, locally advanced). Treatment included anthracyclines in 66.7% of patients and taxanes in 15.5%. Small-size EMP baseline counts were higher in premenopausal women ($p=0.008$), but no association with other clinical or pathological characteristic was found. A significant decrease of circulating sEMP was observed after treatment with CT in the whole group of patients with paired samples available ($n=33$): pre-CT: 416.2 ± 365 vs. post-CT: 340.7 ± 458 ($p=0.005$). Lower chemotherapy-induced sEMP decrease was associated to treatment resistance: ROC analysis demonstrated a 66.7% sensitivity and 72.2% specificity of lower sEMP decrease for lack of response to CT using a decline cut-point of -40 sEMP (close to median decrease: -47). With the same cut-point of low sEMP decrease, odds ratio for treatment resistance was 5.2 (95% confidence interval, 1.17-23.04; $p=0.03$). No clear association of the degree of sEMP decrease was found for disease or progression free survival in the neoadjuvant or in the metastatic setting. **Conclusions:** This study suggests that circulating sEMP decrease after CT and are tightly associated with treatment resistance in BC patients. These findings indicate pathophysiological roles for sEMP in BC and support their potential role as treatment resistance biomarkers.

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General Poster Session (Board #14B), Mon, 8:00 AM-11:45 AM

Investigating genes and micro-RNAs that may predict clinical benefits of anti-CTLA-4 therapy.

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Background: Blockade of T cell co-inhibitory receptor CTLA-4 with a monoclonal antibody, ipilimumab, has led to augmented anti-tumor immune responses, clinical benefit, and FDA approval of ipilimumab for the treatment of metastatic melanoma. Only a subset of patients benefit from anti-CTLA-4 therapy. In order to identify genes, microRNAs, and signaling pathways that are modulated by anti-CTLA-4, which may be used for potential correlation with clinical outcomes or provide additional targets for therapy, we purified and analyzed CD4⁺T cells from patients treated with anti-CTLA-4 for changes in gene and microRNA expression profiles. **Methods:** On an IRB-approved phase Ia presurgical clinical trial, 6 patients with localized bladder cancer were treated with two doses of ipilimumab at 10 mg/kg at weeks 1 and 4. Pre-therapy and post-therapy blood samples were collected for CD4⁺ T cell enrichment by using the T cell isolation kit from Miltenyi Biotec (Auburn, CA). RNA and microRNA were isolated from purified CD4⁺T cells using Qiagen RNA isolation kits for Affymetrix microarray and microRNA array analyses. Microarray data were then analyzed using Ingenuity iReport (Redwood City, CA). RT-PCR and Western blot were used to confirm significant changes in genes or pathways identified in microarray analyses. **Results:** Anti-CTLA-4 treatment resulted in modulation of differentially expressed genes (DEGs). After two doses of treatment, anti-CTLA-4 significantly changed expression of a total of 289 DEGs. Further pathway analyses indicated that anti-CTLA-4 induced a variety of pathways involved in cell proliferation and immune modulation, including PI3K/AKT, MAP/ERK, and IFN/JAK-STAT pathways. We have also identified 9 microRNAs that potentially regulate the expression of genes changed by anti-CTLA-4 therapy. **Conclusions:** Anti-CTLA-4 treatment results in modulation of multiple genes, microRNAs, and pathways, which likely play important roles in anti-tumor immune responses. We are currently testing a number of these identified genes and microRNAs as potential predictive biomarkers for anti-CTLA-4 therapy in a small cohort of patients who had complete response vs. progression of disease after anti-CTLA-4 therapy.

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General Poster Session (Board #14C), Mon, 8:00 AM-11:45 AM

Clinical and pharmacodynamic (PD) results of a phase I trial with AMP-224 (B7-DC Fc) that binds to the PD-1 receptor.

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Background: PD-1/B7-H1 (PD-L1) axis blockade can reinvigorate T cells, and overcome tumor immune evasion of multiple tumor types. AMP-224 is the first recombinant B7-DC-Fc fusion protein tested in patients that binds to and modulates the PD-1 axis through a unique MOA. The MoA hypothesis for AMP-224 is depletion of PD-1^{high} expressing T-cells representing exhausted effector cells. Subsequent replenishment of the T-cell pool with functional T-cells may restore immune function. **Methods:** Patients with advanced solid tumors received low dose CTX on Day 0, followed by AMP-224 (IV infusion) on Days 1 and 15 of each 28-day cycle in doses ranging from 0.3 to 30 mg/kg. Blood samples were assessed serially for changes in lymphocyte subsets, PD-1^{HI} T cells and T cell effector function. IHC staining of paired biopsies for B7-H1, CD8, PD-1, CD4 and FoxP3 was performed to assess immunological status of the tumor at baseline and following treatment and then relative to peripheral readouts. **Results:** 42 patients (83% melanoma) were treated with varying doses of AMP-224 [0.3 mg/kg (*n* = 6); 1 mg/kg (*n*=4); 3 mg/kg (*n* = 4); 10 mg/kg (*n* = 22); 30 mg/kg (*n* = 6)]. Infusion reactions were common (69% across dose cohorts) and occurred mostly at higher doses (86% at the 10 mg/kg dose). No drug-related inflammatory adverse events were identified contrary to PD-1 blocking antibodies. Fresh pre-treatment biopsies were collected from 33/42 (78.5%) patients and paired biopsies have been collected thus far from 19/36 (52.7%) patients on study. 31% of baseline tumors were B7-H1+. Several PD readouts in the periphery showed reductions in PD-1^{HI} cells and emergence of a functional T cell response (increases in IFN γ +, TNF α +, IL-2+ CD4 and CD8 T cells) in individual patients where partial response, stable disease, and mixed responses were seen. **Conclusions:** Data from peripheral readouts is consistent with hypothesized AMP-224 MoA. B7-H1+ was not always predictive of functional response to AMP-224 immunotherapy. Comprehensive PD readouts and evaluation of PK/PD relationships will be presented and may ultimately predict restoration of immune competence even in the presence of initial disease progression. Clinical trial information: NCT01352884.

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General Poster Session (Board #14D), Mon, 8:00 AM-11:45 AM

A trial of autologous ex vivo expanded NK cell-enriched lymphocytes with docetaxel in patients with advanced NSCLC as second- or third-line treatment: Phase IIa study.*Suk-Young Park; The Catholic University of Korea, Daejeon St. Mary's Hospital, Daejeon, South Korea*

Background: Cancer immunotherapy has been attractive for a long time with diverse clinical attempts and results. In particular, NK cells have received considerable attention because of their potential role in immune surveillance in vivo. MICA/B on tumor cells, known as the representative ligand for NKG2D receptor on NK cell, has been reported to be modulated by a variety of stresses including some chemotherapeutic agents and it is anticipated that enhancing MICA/B expression is contributory to anti-cancer treatment. With recent development of expanding autologous ex-vivo NK cell enriched lymphocytes (NKL), we designed a trial to augment the anti-cancer effect by co-administering NKL and docetaxel (D), one of the second-line agents in patients with advanced NSCLC. **Methods:** We first identified some chemotherapeutic agents, such as cisplatin and D, that induce peak MICA/B expression on HeLa cell during the 24-36 hours and designed a trial of combination of NKL with D administered within the same day. Eligible patients were 20-75 years old, ECOG PS 0-2, previously received one chemotherapy, and had stage IIIB/IV histologically proven NSCLC with measurable lesions. NKL were prepared and provided from NKBIO CO. Feasibility, adverse effect, and PFS were evaluated and compared with historical control of weekly D. **Results:** 19 patients were enrolled before early closure. NKL production and administration were feasible in all cases even with disseminated disease. No additional AE was observed in addition to that reported in D alone. PFS 3M and RR 10.5% with 2 PR were observed and similar to historical control (PFS 2.9M, RR 8.8%). **Conclusions:** To our knowledge, it seems to be the first report on the combination of NKL with D in patients with advanced NSCLC. Autologous NKL production and co-administration with D were feasible without further toxicity or complication. Benefit in PFS and RR, as compared with historical control, was not detected in this study population with advanced NSCLC, but further study to see whether the combination of NKL and chemotherapy has anti-cancer effect is desirable to be performed in low tumor burden state, such as less advanced or remission induced state.

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General Poster Session (Board #14E), Mon, 8:00 AM-11:45 AM

Up-regulation of stromal cell-derived factor by il-17 and il-18 via PI3K dependent pathway.

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Background: Stromal cell-derived factor-1 (SDF-1) is a versatile chemotactic cytokine and a sole ligand of CXCR4. SDF-1-CXCR4 axis is the most commonly expressed pathway in cancer cells, and is also responsible for metastasis, homing of stem cell, HIV infection, and autoimmune diseases. Interleukin (IL)-17 plays a key role in the pathogenesis of inflammation by inducing SDF-1 to propagate inflammation and vascular endothelial growth factor to provoke angiogenesis. Recent studies revealed that IL-17 pathway is interwoven with multiple cytokines and downstream pathways. Members of the IL-1 β family, including IL-18, have recently gained attention. By measuring SDF-1 production regulated by individual and simultaneous cytokine stimulations, two cytokines were explored to define their effects, their downstream signal transduction pathways, and the impact of their antibodies. **Methods:** Synovial tissue was obtained from patients with rheumatoid arthritis and their age- and sex-matched controls with osteoarthritis. Fibroblast-like synoviocytes (FLS) were isolated and stimulated with a combination of IL-17 and IL-18 and quantified SDF-1 production with ELISA and their transcripts with RT-PCR. Another subset of FLS were preconditioned with PI3K inhibitor, 100 nM wortmannin, and stimulated with either 5 ng/mL of IL-17, 10 ng/mL of IL-18, or combination of both cytokines. Mann-Whitney test was adopted for statistical analysis. **Results:** Both IL-17 and IL-18 increased SDF-1 level and its mRNA transcript, not only individually but also synergistically ($p < 0.05$). In the group where PI3K inhibitor was applied, the individual and synergistic promotion of SDF-1 production by IL-17 and IL-18 were inhibited ($p < 0.05$). Concomitant application of anti-IL-17 and anti-IL-18 led to further decline of SDF-1 production ($p < 0.01$). **Conclusions:** This is the first report of PI3K-dependent synergism between IL-18 and IL-17, and adds a novel perspective of the role of IL-18 in immune regulation. The individual effects of these two cytokines, and their interaction through PI3K, suggest an interrelationship between the IL-1 family and IL-17.

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General Poster Session (Board #14F), Mon, 8:00 AM-11:45 AM

Therapeutic superiority of a TCR-like antibody to an intracellular WT1 oncogene peptide compared with the tyrosine kinase inhibitor imatinib in a mouse model of Philadelphia chromosome positive (Ph+) ALL.

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Background: Acute and chronic leukemias demonstrate significantly increased expression of the Wilms tumor gene 1 (WT1) product, including CD34⁺ CML stem cells, making WT1 an attractive therapeutic target. However, WT1 protein is intracellular and currently un-druggable. ESKM is a fully human IgG1 antibody that targets a 9 amino acid sequence (RMF) of the protein WT1 in the context of HLA-A0201. **Methods:** BV173 is an HLA-A0201 positive Ph+ ALL cell line. It over-expresses WT1 and binds strongly to ESKM. We evaluated the in vitro and in vivo efficacy of ESKM in combination with TKIs. Antibody-dependent cell-mediated cytotoxicity (ADCC) was evaluated in vitro by chromium release assay, utilizing human PBMC effectors. In vivo tumor growth was assessed in NSG mice bearing disseminated luciferase tagged BV173 with bioluminescence imaging and flow cytometry of the bone marrow after sacrifice. Imatinib was used at maximum tolerated doses for these mice as determined in pilot studies. **Results:** The addition of imatinib in vitro did not affect the ability of ESKM to perform ADCC. The BV173 engrafted NSG mice treated with ESKM with and without TKIs showed tumor regression one week after beginning therapy, clearing leukemia from the liver and spleen. Mice relapsed primarily in the bone marrow, with increasing luciferase signal after two weeks of therapy. Compared to untreated control animals, after 5 weeks of therapy, imatinib alone only reduced tumor growth by 45%; ESKM alone reduced growth by 81%, and the combination of ESKM and imatinib reduced growth by more than 95%. Flow cytometry of cells remaining after treatment showed binding of ESKM, suggesting escape was not due to down regulation of the epitope. **Conclusions:** In this mouse model of Ph+ ALL, ESKM antibody therapy is superior to imatinib and the combination of both modalities is additive. This antibody is efficacious in vitro and in vivo against WT1 overexpressing leukemias, in context of HLA-A0201. This combination holds promise as a therapy for leukemias in patients who are HLA-A0201 positive, with the potential of improved cytoreduction in patients with Ph+ leukemias.

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General Poster Session (Board #14G), Mon, 8:00 AM-11:45 AM

Pharmacokinetics (PK) of blinatumomab and its clinical implications.

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Background: Blinatumomab is an investigational, bispecific, single-chain T cell engaging (BiTE) antibody of 55 kD that targets CD19 on B cells and CD3 on T cells. Blinatumomab induces polyclonal T cell activation and proliferation, resulting in redirected lysis of CD19⁺ target cells. Comprehensive analysis of its PK and the clinical implications is presented. **Methods:** PK data from 131 patients enrolled in 3 phase 1 or 2 studies of non-Hodgkin lymphoma (NHL; N=76), relapsed/refractory acute lymphoblastic leukemia (r/r ALL; N=36), and ALL with minimal residual disease (MRD; N=19) were analyzed. Blinatumomab was given by constant IV (cIV) infusion at 0.5, 1.5, 3, 5, 10, 15, 30, 60 and 90 $\mu\text{g}/\text{m}^2/\text{d}$ over 4 weeks/cycle. Serum blinatumomab concentrations were assessed using a validated bioassay. PK parameters, including volume of distribution (V), half-life ($t_{1/2}$) and clearance (CL), were analyzed with noncompartmental methods. CL was derived by dividing infusion rate by blinatumomab concentration at steady state (C_{ss}). Mean CL from each patient was used to determine disease heterogeneity and for covariate analyses. Effective dose was assessed with PK, *in vitro* and clinical B cell data. **Results:** At the doses tested, blinatumomab had linear PK that was stable over time. V (~5 L) approximated that of monoclonal antibodies. Blinatumomab had a $t_{1/2}$ of ~2 h, with systemic CL of ~2 L/h. Minute blinatumomab concentrations were detected in the urine of 3 (of 13) NHL patients at a dose of 60 $\mu\text{g}/\text{m}^2/\text{d}$. There was no trend of NHL, r/r ALL or ALL with MRD; CrCL, age, gender, weight, or body surface area (BSA) influencing CL. Preliminary analysis showed comparable mean CL values in patients with CrCL ≥ 30 mL/min, ranging from 1.9 to 2.6 L/h. To achieve C_{ss} above the *in vitro* EC₉₀ value for leukemia cell lines (470 pg/mL) and complete B cell suppression in patients, 15 $\mu\text{g}/\text{m}^2/\text{d}$ of blinatumomab given as cIV over 4 weeks/cycle is desired for ALL treatment. **Conclusions:** Blinatumomab PK was linear, stable, and independent of NHL, r/r ALL or ALL with MRD. Kidney involvement in its excretion was limited at the investigated doses. cIV administration is required due to the short $t_{1/2}$. Flat or BSA-based dosing can be used in adults. A cIV dose of ≥ 15 $\mu\text{g}/\text{m}^2/\text{d}$ provides adequate exposure for ALL treatment. Clinical trial information: NCT00274742, NCT01209826, NCT00560794.

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General Poster Session (Board #14H), Mon, 8:00 AM-11:45 AM

Phase II study of dendritic cell vaccination combined with recombinant adenovirus-p53 in treatment for patients with advanced pancreatic carcinoma.

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Background: There are few choices of treatments for advanced pancreatic carcinoma (PC) due to the resistances to chemo- or radio-therapy. Immunotherapy based on dendritic cell (DC) vaccines and p53-based gene therapy are two promising therapeutic modalities. They also demonstrated favorable safety profiles. In this study, we compared the immunological and clinical response between DC vaccine therapy and DC vaccine combined with recombinant adenovirus-p53 (rAd-p53) gene therapy. **Methods:** Thirty-six patients with a stage IV pancreatic cancer, 21 men and 15 women with an average age of 56.2 years old, were included in this study and randomly assigned to two groups: 16 patients in DC group (DCG) and 20 in DC plus rAd-p53 group (DPG). The DCG patients received autologous antigen-loaded DC (antigen from isolated pancreatic cancer cells) and the DPG patients received both DC and rAd-p53. DC vaccines were injected intra-dermally once every week for 4 injections and rAd-p53 were given by intravenous injections once per 3 days for 5 times at a dose of 3×10^{12} viral particles. The response, safety and peripheral blood lymphocyte subsets were investigated. **Results:** The post-treatment CD3+, CD3+CD4+, CD4+/CD8+ ratio of patients' peripheral blood in both groups were increased. But the percent of CD4+CD25+ regulatory T cells were significantly decreases. In DPG, 5 patients had a partial response (PR) and 4 patients had stable disease (SD) according to the RECIST standard. The 3 and 3 DCG patients achieved a PR and SD, respectively. The disease control rates (PR+SD) were 45.0% and 37.5% for DPG and DCG, respectively. The 6-month overall survival rates were 50.0% and 43.8% for DPG and DCG, respectively. The median survival times were 6.8 and 5.5 months for DPG and DCG, respectively. Mild to medium grade fever was observed in most of the patients in the two groups. No serious adverse events were found. **Conclusions:** DC-based immunotherapy and p53 gene therapy are safe and appropriate treatments for patients with advanced pancreatic carcinoma. The combined treatments showed more beneficial results than the DC immunotherapy alone.

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General Poster Session (Board #15A), Mon, 8:00 AM-11:45 AM

Clinical guideline on the prophylactic use of G-CSF on neutropenia by chemotherapy.

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Background: Febrile neutropenia (FN) is common in patients with chemotherapy. It requires conventional treatment, however, many studies have reported that G-CSF reduces the incidence of FN; the results were not clear and the physicians use it at their discretion. In this guideline we evaluated the efficacy and safety of the prophylactic use of G-CSF. **Methods:** We analyzed controlled trials (G-CSF, pegylated form or placebo) given to adult or pediatric patients with chemotherapy for leukemia (LEU), lymphoma and solid tumors (L&ST) or stem cell transplant (SCT), without infections and large radiation ports. Two independent reviewers applied CONSORT to determine the methodological quality; for ranking the evidence we used GRADE and the recommendations were developed by Delphi method. We developed subgroups according to age and type of intervention to analyze the outcomes (risk, duration, severity of FN and adverse events). We performed random-effects or fixed-effects meta-analysis methods according to their heterogeneity. **Results:** Of 1,776 studies, 112 were included. For the risk of FN between G-CSF or pegylated form vs placebo found that G-CSF reduces the risk in adults with LEU (RR 0.89, 95% CI 0.81-0.98; $p=0.024$), L&ST (RR 0.758, 95% CI 0.68-0.84; $p=0.000$) and SCT (RR 0.85, 95% CI 0.74–0.97, $p=0.017$). The risk of developing severe neutropenia reduces in the adults with L&ST with the factor (RR 0.79, 95% CI 0.71-0.88; $p=0.000$) and pediatric patients with LEU (RR 0.789, 95% CI 0.71-0.88; $p=0.000$). While the duration of neutropenia in children with L&ST the time reduces with the factor (SMD -0.559; 95% CI -0.841 to -0.28; $p=0.000$). The G-CSF vs pegylated form, the evidence was inconclusive. **Conclusions:** When the risk and duration of neutropenia is present we suggest the use of G-CSF in adult and pediatric patients. For adults, we suggest the use of pegylated form, but for pediatric patients we do not have a specific suggestion because the evidence is nonexistent, so it is necessary to carry out clinical trials to obtain evidence.

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General Poster Session (Board #15B), Mon, 8:00 AM-11:45 AM

Blinatumomab exposure and pharmacodynamic response in patients with non-Hodgkin lymphoma (NHL).

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Background: Blinatumomab (AMG 103) is an investigational, bispecific, T cell engaging (BiTE) antibody targeting CD19-expressing B cells. We describe the exposure-pharmacodynamic (PD) response of blinatumomab in patients with NHL, using a quantitative pharmacology approach. **Methods:** In a phase 1 study, 76 patients with NHL received blinatumomab by continuous intravenous infusion (cIV) at doses of 0.5 to 90 $\mu\text{g}/\text{m}^2/\text{d}$ in 4- or 8-week cycles. Pharmacokinetics (PK) was determined. PD responses evaluated included lymphocytes and cytokines measured during treatment, and sum of the products of the greatest diameters of tumor size in lymph nodes (SPD) at the end of treatment. Blinatumomab concentration at steady state (C_{ss}) and the cumulative area under the concentration (AUC_{cum})–time curve over the period before the evaluation of SPD were used to evaluate the exposure-SPD relationship. **Results:** Blinatumomab showed linear PK. Early PD responses were characterized by B cell depletion, T cell redistribution, and transient cytokine release. Following cIV at doses from 0.5 to 90 $\mu\text{g}/\text{m}^2/\text{d}$, B cells declined at a first-order rate with a dose-dependent rate constant, ranging from 0.16 to 1.0 h^{-1} . Complete B cell depletion was achieved within 48 hours at doses $\geq 5 \mu\text{g}/\text{m}^2/\text{d}$. A dose-independent decrease in T cell counts was observed within 24 hours after dosing, and T cells returned to baseline within 2 weeks of treatment. Cytokine elevation occurred in some patients and was dose-dependent. Blinatumomab exposure-SPD relationship was best described by an inhibitory E_{max} model ($E = E_0 - (I_{max} * C) / (IC_{50} + C)$). According to the model estimation, a 50% reduction in SPD would be achieved when C_{ss} is 2141 pg/mL and AUC_{cum} is 1381 $\text{h} * \mu\text{g}/\text{L}$, equivalent to a blinatumomab dose of 54 $\mu\text{g}/\text{m}^2/\text{d}$ given over 27 days. **Conclusions:** B lymphocytes were completely depleted from the circulation at blinatumomab doses $\geq 5 \mu\text{g}/\text{m}^2/\text{d}$. Depletion was faster at higher doses. Higher blinatumomab C_{ss} and AUC_{cum} were associated with better tumor reduction. Tissue accessibility may explain the higher dose requirement for SPD reduction versus peripheral B cell depletion. The PK/PD model has utility for the design of future studies of blinatumomab in NHL. Clinical trial information: NCT00274742.

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General Poster Session (Board #15C), Mon, 8:00 AM-11:45 AM

Double-loaded mature dendritic cell (DC) therapy for non-HLA-restricted patients with advanced ovarian cancer: Final results of a clinical phase I study.

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Background: Prognosis of ovarian cancer remains poor after initial responsiveness to surgery and chemotherapy followed by high recurrence and mortality rates and new experimental approaches are warranted. Our goal was to evaluate a novel DC-based vaccine, which exploits a unique dual loading strategy to amplify specific anti-tumor short- and long-term immune responses to delay or even prevent recurrent and metastatic disease. **Methods:** Monocytes were collected via apheresis, matured into DCs and pulsed with two universal tumor associated antigens (uTAA) in our GMP facility. DCs were loaded with TERT and Survivin via two different pathways (mRNA and peptide) to elicit CD8⁺ and CD4⁺T cells directly. Endpoints of the study were tolerability and safety, immunological and clinical responses. T cell responses against the IMP and loaded antigens were evaluated by cytokine bead array (CBA) and intracellular staining assays. **Results:** 15 non HLA-restricted patients with advanced ovarian cancer were enrolled 8 weeks after standard treatment (surgery and chemotherapy). Each patient was vaccinated intradermally on a weekly or fortnightly basis with a maximum of 8 doses of 13×10^6 double loaded DCs. The majority of treatment related side effects were grade 1 fever and erythema. Overall the therapy was well tolerated. Immune response data is available for 14/15 patients, 1 was withdrawn after the first administration. The IMP leads to strong immune responses with high frequency (>90%), which is proven for both uTAAs in CD8⁺ as well as CD4⁺ T cells. A clear positive trend in progression free survival is demonstrated compared to matched historical control. **Conclusions:** Therapy with our unique double loaded DC vaccine was feasible, safe and well-tolerated by patients. The vaccine was highly immune stimulatory and elicited both, long-term and short-term anti-tumor immune responses, establishing a promising platform for immune therapy for ovarian cancer and all solid tumors in general. The first two authors contributed equally. Clinical trial information: NCT01456065.

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General Poster Session (Board #15D), Mon, 8:00 AM-11:45 AM

Awareness and understanding of cancer immunotherapy in Europe.

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Background: Use of immunotherapies in the treatment of cancer is growing and a range of new immunotherapeutic strategies are being evaluated. It is important that healthcare providers (HCP) understand these treatments and how they compare with and may complement established therapies. As part of the activities of the POINT expert group, we commissioned a survey of current awareness, attitudes and perceptions of cancer immunotherapy in Europe. **Methods:** From August-September 2011, 426 healthcare professionals (HCPs: oncologists, surgeons and oncology nurses) from France, Germany, Italy, Spain and the UK (~85 respondents/country) completed online interviews. Representatives of patient advocacy groups (PAGs) in each country were surveyed by telephone interview. **Results:** Nearly all (98%) HCPs were aware of cancer immunotherapy. While 68% of HCPs indicated a high level of interest, knowledge levels were lower. Only 24% of the HCPs had direct experience with cancer immunotherapies, while others reported they knew a lot (12%), a reasonable amount (28%) or little (34%) about immunotherapy strategies but had not used them (76%). Overall perceptions of cancer immunotherapy among HCPs were largely positive (60%) and rarely negative (3%). The key advantages of cancer immunotherapy were perceived to be good safety and tolerability (75%), a targeted mechanism of action (61%) and good efficacy (48%). The leading barriers to use of immunotherapies were costs of treatment (58%), past clinical trial failures (45%) and access/formulary restrictions (44%). Most (75%) HCPs had already discussed cancer immunotherapy with their patients and 70% of patients were reported as being receptive to the concept. The majority of PAGs indicated they were currently unable to advise patients about cancer immunotherapy due to a lack of or confusing information and poor understanding. **Conclusions:** Awareness of cancer immunotherapy in Europe is high and it is generally perceived as a positive addition to established treatment options. However, the understanding varies and the direct experience is limited. There is a clear need for further educational activities concerning cancer immunotherapy, as well as further clinical data on long-term efficacy and safety.

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General Poster Session (Board #15E), Mon, 8:00 AM-11:45 AM

An Fc-optimized NKG2D-Ig fusion protein for induction of NK cell reactivity against breast cancer.

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Background: The anti-tumor activity and clinical success of the monoclonal antibody trastuzumab, approved for treatment of HER2/neu-overexpressing breast cancer, is at least partially mediated by induction of antibody dependent cellular cytotoxicity (ADCC). However, only about 20% of patients show HER2/neu overexpression, and trastuzumab treatment is associated with side effects. The ligands of the activating immunoreceptor NKG2D (NKG2DL) are widely expressed on malignant cells, but generally absent on healthy tissue. We aimed to take advantage of this tumor-restricted expression by using NKG2DL as target-antigens on breast cancer cells. To this end we generated NKG2D-Ig fusion proteins with modified Fc moieties and studied their ability to induce NK cell anti-tumor reactivity. **Methods:** The Fc parts within the constructs were modified by amino acid exchange as previously described (Lazar 2006; Armour 1999). Direct effects on tumor cell viability as well as induction of NK cell activation, degranulation, cytotoxicity and IFN- γ release in cultures with breast cancer cell lines expressing different HER2/neu levels were determined. **Results:** Compared to NKG2D-Fc containing a wildtype Fc part (NKG2D-Fc-WT) or trastuzumab, our mutants (S239D/I332E and E233P/L234V/L235A/ Δ G236/A327G/A330S) displayed highly enhanced (NKG2D-Fc-ADCC) and abrogated (NKG2D-Fc-KO) affinity to the NK cell Fc receptor, respectively. In contrast to trastuzumab, no direct effect of the constructs on tumor cell viability was observed. In cultures of NK cells and breast cancer cells, NKG2D-Fc-KO significantly reduced NK reactivity due to blocking immunostimulatory NKG2D-NKG2DL interaction. NKG2D-Fc-WT substantially enhanced NK reactivity by induction of ADCC, while the effects of NKG2D-Fc-ADCC by far exceeded that of NKG2D-Fc-WT and, in case of HER2/neu-low targets also that of Herceptin. **Conclusions:** Fc-engineered NKG2D-Ig fusion protein effectively target breast cancer cells for NK anti-tumor reactivity. Due to the tumor-restricted expression of NKG2DL, NKG2D-Fc-ADCC may constitute an attractive means for immunotherapy especially of HER2/neu-low or -negative breast cancer.

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General Poster Session (Board #15F), Mon, 8:00 AM-11:45 AM

Rituximab pharmacokinetics in children and adolescents with de novo intermediate and advanced mature B-cell lymphoma/leukemia: A Children's Oncology Group (COG) report.

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Background: The COG trial ANHL01P1 was undertaken to determine pharmacokinetics (PK) and safety following the addition of rituximab (R) to FAB/LMB 96 chemotherapy in children and adolescents with stage III/IV mature B-NHL and B-ALL±CNS disease. **Methods:** Patients received R (375mg/m²) on day -2 and 0 of two induction cycles and day 0 of two consolidation cycles. R levels were measured prior to any antibody infusion, during induction cycles (1 hour prior and 30-60 minutes after each R dose) and following consolidation cycles (1, 3, 6 and 9 months after last R dose). R was measured by ELISA with goat anti-rituximab antibody as the capture reagent and goat anti-mouse IgG-conjugated to horseradish peroxidase as the detection reagent. R terminal half-life (t_{1/2}) was calculated if at least 3 time points after the last dose were measurable in an individual subject. **Results:** Serum R levels are reported in the Table. Highest peak levels were achieved following the second dose of each induction cycle with sustained troughs and a t_{1/2} of 26-29 days. Group C patients tended to have lower R levels than Group B. Patients with LDH ≥2xULN were noted to have lower R levels during induction 1 compared to those with LDH <2xULN. Children (<13 years) exhibited higher peak concentrations, similar trough levels and a shorter t_{1/2} than adolescents (≥13 years). None of these differences reached significance after adjusting for multiple comparisons. **Conclusions:** R can be safely added to FAB chemotherapy with high early R peak/trough levels and a long terminal half-life. The efficacy of R combined with FAB chemotherapy is currently being investigated in an ongoing international intergroup trial. Clinical trial information: NCT00057811.

	Group B		Group C	
	N	Mean±SEM (µg/mL)	N	Mean±SEM (µg/mL)
Trough1	21	0±0	15	0±0
Peak1	17	208.3±8.3	14	182.6±8.9
Trough2	20	147.1±15.3	14	119.1±21.3
Peak2	18	299.2±18.7	11	245.2±31.1
Trough3	16	106.6±32.1	9	54.8±8.4
Peak3	15	267.5±15.0	9	252.8±10.5
Trough4	17	190.3±11.3	9	174.6±14.0
Peak4	15	383.5±24.5	8	321.1±32.4
1mo	20	72.7±8.3	10	56.3±6.2
3mo	16	15.5±2.7	11	16.9±3.1
6mo	18	2.9±0.6	7	1.3±0.5
9mo	14	1.3±0.4	0	-

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General Poster Session (Board #15G), Mon, 8:00 AM-11:45 AM

Generation of tumor-specific cytotoxic T-lymphocytes from peripheral blood of colorectal cancer patients for adoptive immunotherapy.

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Background: Adoptive T-cell transfer (ACT) using autologous TIL, grown ex vivo and then infused into the cancer patient after lymphoablative chemotherapy, has emerged as an effective treatment for patients with metastatic melanoma. However, this approach has been hampered by the difficulty of isolating TILs from tumors other than melanoma, and of amplifying a sufficient quantity of autologous tumor-reactive T cells. So we decided to adopt a recently described procedure for generating in vitro large numbers of anti-tumor HLA-restricted CTLs, by stimulating patient's CD8-enriched peripheral blood mononuclear cells (PBMCs) with DCs pulsed with apoptotic solid tumor cells (TCs) as a source of tumor antigens. **Methods:** 61 patients affected by CRC were enrolled. Tumor biopsies were obtained at surgery, together with 100 ml of heparinized peripheral blood. Tumors were dissociated to a single-cell suspension and cultured in order to obtain tumor cell line from each patient. Dendritic cells (DCs) were generated from previously separated PBMCs, using a magnetic positive selection of CD14+ monocytes, cultured in presence of Interleukin-4 and recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF). Anti-tumor cytotoxic T lymphocytes (CTLs) were elicited using DCs as antigen-presenting cells, autologous apoptotic tumor cells as source of antigens and T CD-8 lymphocytes enriched effectors, with weekly stimulation. To evaluate the cytotoxic activity of CTLs, interferon- γ (IFN- γ) secretion was assessed by ELISPOT. **Results:** Tumor cell lines and DCs were obtained from 19 out of 61 patients. ELISPOT was performed so far for 6 patients: strong IFN- γ secretion was detected at the third, fourth and fifth stimulations for one patient and at the second for another patient, whereas for three patients a weak secretion was detected during the second and the third stimulations. CTLs from one patient did not react to the stimulations. **Conclusions:** Generation of CTLs suitable for ACT immunotherapy is feasible from peripheral blood in patients with CRC.

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General Poster Session (Board #15H), Mon, 8:00 AM-11:45 AM

A phase Ib dose-escalation study of TRC105 (anti-endoglin antibody) in combination with capecitabine for advanced solid tumors (including patients with progressive or recurrent HER2-negative metastatic breast cancer).

Ellis Glenn Levine, Andres Forero, Tracy O'Connor, Ben K. Seon, Manoj A Jivani, Bonne J. Adams, Charles P. Theuer; Roswell Park Cancer Institute, Buffalo, NY; University of Alabama at Birmingham, Birmingham, AL; TRACON Pharmaceuticals, Inc., San Diego, CA

Background: CD105 (endoglin) is an endothelial cell membrane receptor highly expressed on angiogenic tumor vessels that is essential for angiogenesis and upregulated by hypoxia and VEGF inhibition. TRC105 is an anti-CD105 monoclonal antibody being studied in phase II trials that potentiates chemotherapy in preclinical models. **Methods:** Pts with advanced solid tumors (for purposes of dose escalation) or pts with metastatic HER2-negative breast cancer (following completion of dose escalation), ECOG PS 0-1, and normal organ function were treated with intravenously administered TRC105 wkly plus capecitabine at 1,000 mg/m² BID for 14 of every 21 days, and assessed for safety, PK, and response. **Results:** Fourteen patients (median age = 52; M:F 4:10; median of 3 prior regimens; 10 with breast and 4 with colorectal cancer) were enrolled. Dose escalation proceeded from 7.5 mg/kg TRC105 to the recommended single agent phase II dose of 10 mg/kg of TRC105 in combination with capecitabine, without development of dose limiting toxicity. Fourteen pts were treated at 7.5 mg/kg (n=4) or 10 mg/kg (n=10) TRC105 wkly + 1,000 mg/m² BID/14d capecitabine of repeating 21 day cycles. Patients experienced expected TRC105 related adverse events of grade 1 or grade 2 infusion reaction, epistaxis, gingival bleeding, telangiectasia, headache, rash, and fatigue. Grade 3 headache and grade 3 vomiting were each seen in one patient. Adverse events characteristic of each individual drug were not increased in frequency or severity when the two drugs were administered together. Mucocutaneous telangiectasia, a marker of TRC105 target modulation, was observed at both dose levels. A heavily pretreated male breast cancer patient remained on study for 9 months with a RECIST-defined partial response. Stable disease beyond 9 weeks was observed in three patients. **Conclusions:** The recommended single agent phase II dose of 10 mg/kg TRC105 wkly was well tolerated in combination with capecitabine. The combination treatment could be advanced in HER2-negative breast or colorectal cancer. Clinical trial information: NCT01326481.

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General Poster Session (Board #16A), Mon, 8:00 AM-11:45 AM

Immunomodulatory effects of a dipeptidyl peptidase inhibitor, ARI-4175, and NK cell activation in a colon cancer model.

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Background: A possible mechanism of the antitumor effect of therapeutic antibodies is antibody-dependent cellular cytotoxicity (ADCC). We reported that an inhibitor of dipeptidyl peptidase (DPP)4-like serine proteases, Ari-4175, significantly slowed the growth of *KRAS*-mutated HCT-116 xenografts in nude mice, either as a single agent or with cetuximab (Ctx). Ari-4175 is not cytotoxic in vitro, and since it was able to overcome the resistance to Ctx due to the *KRAS* mutation in the HCT-116 line, we hypothesized that the antitumor activity involves the activation of NK cells and enhancement of ADCC. **Methods:** Ari-4175 was administered orally to nude mice at 200 μ g q.d. x5 days/week. Peripheral blood or spleens were assayed for immune parameters, ex vivo, at various time points. Expression of surface markers on myeloid and NK cells were monitored by flow. Natural cytotoxicity and ADCC were assayed using HCT116 cells and Ctx using a flow based assay. Cytokines were measured using Luminex assays. **Results:** Ari-4175 induced upregulation of CD69 on NK cells on day 2, followed by upregulation of CD16 on day 7. Coordinate with these changes in peripheral blood, splenocytes from treated mice exhibited significantly increased in vitro cytotoxicity toward the HCT116 cells. Increased serum levels of inflammatory cytokines, including IL-1b, IL-6, MCP-1, GCSF, and IL-2 were seen. A significant expansion of a distinct CD45⁺CD14⁺Gr-1⁺MHC-II⁺CD11b⁺CD11c^{variable} myeloid cell population in both peripheral blood and spleens of mice was also seen after one week of treatment. **Conclusions:** Ari-4175 showed dramatic anti-tumor effect in *KRAS*-mutant CRC xenografts when given alone or in combination with Ctx. In vitro data suggest that the therapeutic effect of 4175 might partially be due to the augmentation of ADCC through elevated expression of CD16 on NK cells. In addition, Ari-4175 appears, in vivo, to expand a unique myeloid cell population, which may be responsible for an inflammatory cytokine response and subsequent activation of NK cells. Our study provides a mechanistic rationale for testing Ari-4175 in a clinical trial and possible biomarker endpoints for evaluation in peripheral blood samples.

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General Poster Session (Board #16B), Mon, 8:00 AM-11:45 AM

A phase Ib dose-escalation study of TRC105 (anti-endoglin antibody) in combination with bevacizumab (BEV) for advanced solid tumors.

Lee S. Rosen, Francisco Robert, Daniela Matei, Jonathan Wade Goldman, David S. Mendelson, E. Gabriela Chiorean, Robert Matthew Strother, Ben K. Seon, Delia Alvarez, Bonne J. Adams, Charles P. Theuer, Michael S. Gordon; UCLA Santa Monica Hematology-Oncology, Santa Monica, CA; University of Alabama at Birmingham, Birmingham, AL; Indiana University School of Medicine, Indianapolis, IN; The David Geffen School of Medicine at University of California, Los Angeles, Santa Monica, CA; Pinnacle Oncology Hematology, Scottsdale, AZ; University of Washington, Indianapolis, IN; Roswell Park Cancer Institute, Buffalo, NY; TRACON Pharmaceuticals, Inc., San Diego, CA

Background: CD105 (endoglin) is an endothelial cell membrane receptor highly expressed on angiogenic tumor vessels that is essential for angiogenesis and upregulated by hypoxia and VEGF inhibition. TRC105 is an anti-CD105 monoclonal antibody that potentiates VEGF inhibitors in preclinical models. This study assessed safety, PK and preliminary efficacy of TRC105 in combination with BEV. **Methods:** Pts with advanced solid tumors, ECOG PS 0-1, and normal organ function were treated with escalating doses of IV TRC105 (3, 6, 8 or 10 mg/kg/wk) plus bevacizumab (BEV) at 15 mg/kg q3wk or 10 mg/kg q2wk. **Results:** Thirty one pts (median age = 62; M:F 14:17; median 4 prior regimens; primarily metastatic colorectal or ovarian cancer) were treated with TRC105 wkly + BEV. TRC105 3 mg/kg wkly + 15 mg/kg q3wk BEV was well tolerated. Concurrent administration of TRC105 6 mg/kg wkly + 15 mg/kg BEV q3wk resulted in headaches in 4 of 5 pts on cycle 1 day 1 (two grade 3). Dose escalation to the recommended single-agent phase II dose of 10 mg/kg TRC105 weekly + BEV (10 mg/kg q2wk) was tolerated when the initial TRC105 dose was introduced one week after BEV dosing and administered over 2 days. Headache was the only serious adverse drug reaction observed. Adverse events characteristic of each individual drug were not increased in frequency or severity. Target TRC105 serum concentrations were achieved at 6 mg/kg. Mucocutaneous telangiectasia, a marker of TRC105 target modulation, was observed beginning at 6 mg/kg and was dose proportional. Five of 19 heavily pretreated, BEV or VEGF receptor tyrosine kinase inhibitor (TKI) refractory pts with colorectal and ovarian cancer, each with marked tumor burden, experienced radiographic reductions in tumor volume (10-17%). Three of these patients remained on study longer than the prior VEGF inhibitor treatment and two are ongoing. Seven ongoing patients have been treated for 2-8 months. **Conclusions:** TRC105 10 mg/kg wkly was well tolerated with BEV 10 mg/kg q2wk. The combination demonstrated activity in BEV and VEGF TKI refractory pts. Randomized phase II trials of BEV +/- TRC105 have commenced in renal cell cancer and glioblastoma. Clinical trial information: NCT01332721.

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General Poster Session (Board #16C), Mon, 8:00 AM-11:45 AM

An engineered immunotherapy (NKTR-214) with altered selectivity toward the IL2 receptor: Efficacy and tolerability in a murine tumor model.

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Background: Cytokine-based immunotherapy has been successful for the treatment of cancer, with potential for durable responses in multiple indications. One approach towards stimulating the immune system is to target the heterotrimeric interleukin2 receptor, IL2R. NKTR-214 uses polymer technology to alter receptor subunit selectivity to favor expansion of CD8+ memory effector T cells (CD8T) in the tumor over CD4+ regulatory T cells (Treg). In addition, polymer conjugation is designed to improve exposure and enhance tumor localization, significantly improving efficacy, modulating vascular leak syndrome (VLS) and allowing flexible dosing regimens. **Methods:** C57BL/6 mice bearing established subcutaneous B16F10 melanoma were treated with NKTR-214 at a variety of doses (0.25-4.0 mg/kg) and schedules (q5dx3 to q14dx2). Mice treated with clinically validated IL2 were administered 3mg/kg, bidx5 for 2 cycles. Efficacy was measured by monitoring tumor volumes. Tolerability was evaluated by survival. VLS was measured by injection of Evans Blue dye followed by colorimetry in lungs. Tumor immunotyping, by flow cytometry. **Results:** Tumors from mice receiving NKTR-214 had a CD8/Treg ratio of over 1,000 versus 14 for IL2. NKTR-214 administered at 2 mg/kg, q9dx3 was identified as the optimal regimen and showed tumor growth delay of 26 days compared to 9 days for optimally dosed IL2. 90% of NKTR-214 treated mice tolerated treatment compared to 67% for IL2. VLS was completely resolved prior to administration of the next dose of NKTR-214, unlike IL2. NKTR-214 was well tolerated in rats at two schedules, at MTD. **Conclusions:** NKTR-214 is a highly differentiated cytokine with a new mechanism of action that may provide options for cancer immunotherapy. Polymer conjugation of a clinically validated cytokine alters the IL2R selectivity to favor expansion of tumor killing immune cells (CD8T) over regulatory immune cells (Treg) in the tumor. The conjugate is also designed to improve exposure and enhance tumor localization, offering more options of dose and schedule. The optimal dose and schedule is cytokine-sparing, provides substantial tumor growth delay, and reduces toxicity.

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General Poster Session (Board #16D), Mon, 8:00 AM-11:45 AM

Antitumor activity of concurrent blockade of immune checkpoint molecules CTLA-4 and PD-1 in preclinical models.

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Background: Interaction of immune checkpoint molecules PD-1 and CTLA-4 and their respective ligands attenuates antitumor T cell responses. In clinical studies, PD-1 blocking antibody (Ab) nivolumab (BMS-936558) or the CTLA-4 blocking Ab ipilimumab result in durable responses in multiple human malignancies. We describe the evaluation of concurrent treatment with anti-PD-1 and anti-CTLA-4 mAbs in preclinical models. **Methods:** Antitumor activity of treatment with murine homologs of anti-PD-1 (4H2-mIgG1) and anti-CTLA-4 (9D9-mIgG2b) was evaluated in MC38, a murine colon adenocarcinoma model. The effects of concurrent treatment on T cell infiltration of tumors, tumoral expression of PD-L1 and cytokine levels were explored. The preclinical safety profile of concurrent nivolumab + ipilimumab was assessed in a cynomolgus macaque model. **Results:** Concurrent treatment of MC38 tumors with 4H2-mIgG1 + 9D9-mIgG2b (10 mg/kg Q3d x 3) results in synergistic antitumor activity whereas efficacy with sequential dosing was similar to either agent alone. With concurrent treatment, dose reductions of one Ab relative to a fixed dose of the other resulted in retention of some antitumor activity. Anti-PD-1 enhanced CD8+ T cell infiltration of MC38 tumors and increased tumor PD-L1 expression. Anti-CTLA-4 treatment increased intratumoral CD8+ T cells and reduced intratumoral T regulatory cells. While concurrent treatment did not result in further increases in T cell infiltration, it increased expression of intratumoral cytokines. Anti-PD-1 resulted in down regulation of cell surface and intracellular levels of PD-1 in CD8+ T cells. In cynomolgus macaques, concurrent nivolumab + ipilimumab resulted in dose-dependent gastrointestinal toxicities (diarrhea; body weight loss) not observed in earlier cynomolgus studies with nivolumab and rarely with ipilimumab. These preclinical observations provided the rationale for a dose escalation trial (NCT01024231) of combined nivolumab + ipilimumab in advanced melanoma. **Conclusions:** Concurrent treatment with anti-PD-1/anti-CTLA-4 resulted in synergistic antitumor activity in preclinical models and supports the evaluation of the combination in clinical studies.

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General Poster Session (Board #16E), Mon, 8:00 AM-11:45 AM

Phase I clinical trial of a genetically modified and oncolytic vaccinia virus GL-ONC1 with green fluorescent protein imaging (NCT009794131).

Khurum Hayat Khan, Anna-Mary Young, Joaquin Mateo, Nina Tunariu, Timothy Anthony Yap, David Shao Peng Tan, David Mansfield, Mabel Wong, Ruth Riisnaes, Kevin J. Harrington, Johann Sebastian De Bono; The Institute of Cancer Research, The Royal Marsden NHS Foundation Trust, Sutton, United Kingdom; The Royal Marsden Hospital NHS Foundation Trust, London, United Kingdom; Institute of Cancer Research and Royal Marsden Hospital, London, United Kingdom; The Royal Marsden NHS Foundation Trust, Sutton, United Kingdom

Background: GL-ONC is a genetically engineered virus attenuated by insertion of the *ruc-gfp* (Renilla luciferase and Aequorea green fluorescent protein fusion gene), *beta-galactosidase* (*lacZ*) and *beta-glucuronidase* (*gusA*) reporter genes into the FL14.5L, J2R (thymidine kinase) and A56R (hemagglutinin) loci, respectively. A phase I trial of intravenous (i.v) GL-ONC1 was pursued to evaluate safety, tolerability, tumour delivery, neutralising antibody development and antitumor activity. **Methods:** GL-ONC1 was administered at escalating doses (1×10^5 , 1×10^6 , 1×10^7 , 1×10^8 , 1×10^9 , 3×10^9 plaque forming units (pfu) on day 1; 1.667×10^7 and 1.667×10^8 , 1.667×10^9 pfu on days 1-3) utilizing a 28-day cycle and a 3+3 dose escalation design. Paired biopsies before treatment and on day 8 for pharmacodynamic and viral delivery evaluation were obtained. Green fluorescent protein (GFP) imaging was performed on skin rash and mucosal tumour lesions at baseline and after each cycle. **Results:** To date, 33 patients (pts) across 8 cohorts have been treated with 1 dose limiting toxicity reported of grade 3 transaminitis after a single infusion at 1×10^9 pfu. Other reported adverse events (n) included pyrexia (26), musculoskeletal pain (10), fatigue (8), nausea and vomiting (4). 2 pts had transient transaminitis; both had liver metastases, which may have contributed to this. 2 pts developed minimally symptomatic poxvirus skin pustules, which appeared green by GFP and were positive to viral plaque assay (VPA). Overall, stable disease (SD) by RECIST was seen at >24 weeks (n=6) and 8-12 weeks (n=5). 2 out of 4 pts in cohort 8 (one with cholangiocarcinoma and another with non-small cell lung cancer) achieved SD for median 5.5 months, with a drop in tumour markers at the time of infusions. **Conclusions:** GL-ONC1 is well tolerated; more frequent delivery of the virus (2 weekly, at the same dose) is planned in an attempt to increase agent exposure. Clinical trial information: NCT009794131.

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General Poster Session (Board #16F), Mon, 8:00 AM-11:45 AM

Circulating myeloid-derived suppressor cells (MDSC) and correlation to poor prognosis, Th2-polarization, inflammation, and nutritional damages in patients with gastric cancer.

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Background: Recent studies have shown that myeloid-derived suppressor cells (MDSC), which have been identified in most patients, are potent suppressors of T cell activation. MDSC have been reported to be correlated with tumorigenesis and the progression of tumors. We have previously reported significant correlations of MDSC with nutritional damage and inflammation in many types of cancer. In this study the quantitative detection of MDSC in peripheral blood was analyzed in the prognosis in patients with gastric cancer. **Methods:** We tested PBMCs from 29 preoperative patients with gastric cancer and 18 healthy volunteers using flow cytometric analysis (CD14⁺CD11b⁺CD33⁺). Markers for nutritional status (serum levels of total protein), inflammation (NLR: neutrophil/lymphocyte ratio, serum levels of vascular endothelial growth factor: VEGF) and Th2-polarization (PBMC's production of IL-6 and IL-10) were measured in this study. The prognosis of the patients was assessed by Kaplan-Meier tests for correlation with levels of MDSC. **Results:** MDSC levels in 29 preoperative patients with gastric cancer were significantly higher than in normal volunteers. These levels were not associated with pathological characteristics. However they were significantly correlated with the production of IL-6 and IL-10, VEGF levels and NLR. A significant inverse correlation with levels of total protein was obtained. MDSCs were increased in stage IV patients compared with healthy volunteer and 2-year survival rate of the patients with higher levels of MDSC (> 1.104%PBMC) was significantly poorer (median OS, 498 vs 473 days; $p = 0.048$), while there was no significant difference in patients with stages I, II, and III. **Conclusions:** These data suggest that increased MDSC is an effective biomarker for immunosuppression due to Th2-polarization, nutritional impairment, systemic inflammation and poor prognosis in advanced patients with gastric cancer.

Biologic meshes in cancer patients: A double-edged sword—Differences in production of IL6 and IL12 caused by acellular dermal matrices in human immune cells.

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Background: Acellular dermal matrices (ADM) have been used in different fields of surgery for almost 20 years. In 2005 Breuing et al first described its use in breast cancer patients. It is assumed that it is safe to use in an oncologic setting, but data from controlled studies are still missing. Because of its lack of cells ADM are considered not to cause an immune reaction. With increasing knowledge about the importance of immunology in breast cancer more information about ADM on different immune cell populations is needed. IL6 and IL12 are two central cytokines and key regulators of immune suppression and activation. **Methods:** Stratattice (ST; LifeCell) CollaMend (CM; Bard Davol), Biodesign (BD; Cook Biotech) as well as TiLoop a synthetic mesh (TL; pfm medical) were used in this study. We isolated myeloid dendritic cells (MDC), untouched plasmacytoid dendritic cells (PDC), naïve B-cells and CD8+ T-cells using the MACS System and co-cultured them with the biologic meshes or TL. For positive controls, we used CpG ODN 2216 3 µg/ml and LPS in a concentration of 100 ng/ml. Cytokine concentration of IL12p70 and IL6 were determined after seven days by using sandwich Elisa sets. Statistical significance was determined by the nonparametric Friedman-Test. The single hypothesis was calculated with a paired Wilcoxon Test. **Results:** There was a highly significant difference between the different ADM and TL in the immunologic response. The statistical difference for IL 6 was $p = 0.0006131$ for B cells and $p = 0.00418$ for T cells between TL and ADM. ST also caused significantly more IL6 than CM and BD. We found similar differences in IL 12 with $p = 0.00194$ for B cells and $p = 0.003636$ in T cells in regard to the difference between TL and ADM. For IL 12 there was no statistical difference between the ADM. We didn't see any significant differences in the cytokine profile between the various ADM/TL in the MDC and PDC subpopulations. **Conclusions:** Despite the assumed lack of immune answer to ADM, immune cells reacted in our study with significantly different cytokine profiles. These findings can have implications regarding the activation or suppression of effector cells in a cancer patient.

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General Poster Session (Board #16H), Mon, 8:00 AM-11:45 AM

Immunotherapy with CpG-ODN in neoplastic meningitis: A phase I trial.

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Background: TLR9 agonists, such as phosphorothioate oligodeoxynucleotides containing CpG motifs (CpG-ODNs), are immunostimulating agents with antitumor effects in animal models. Neoplastic meningitis is a devastating disease, with no efficient therapeutic available. A phase I trial was conducted in patients with neoplastic meningitis to define the safety profile of subcutaneous injections, combined or not with intra-CSF administration of a CpG ODN. **Methods:** The diagnosis of neoplastic meningitis was based on a positive CSF cytology or on the association of clinical symptoms with meningeal enhancement on MRI. Cohorts of 3-6 patients were treated for 5 weeks with escalating doses of CpG-28 (level 1 and 2: 0.1 and 0.3 mg/kg/week subcutaneously (sc); level 3 to 6: 0.3mg/kg sc associated with 3/7/12/18 mg intrathecally every other week). Concomitant treatments with radiotherapy or chemotherapy were allowed. The primary endpoint was tolerance. Secondary endpoints were time until neurological progression and survival. **Results:** Twenty-nine (29) patients were included. Primary cancer was malignant glioma (n=15), lung carcinoma (n=7), breast cancer (n=3), melanoma or melanocytoma (n=2), ependymoma (n=1), and colorectal cancer (n=1). At diagnosis median age was 56 years and median KPS was 70%. The treatment was well tolerated. Adverse effects possibly or probably related to the studied drug consisted in grade 2 lymphopenia, anemia and neutropenia (n=19), local erythema at injection sites (n=14), fever (n=10) and seizure (n=11). Two serious adverse events were considered as possibly or probably related to the protocol: one local infection and one intra-cerebral haemorrhage. Interestingly, median survival was higher in patients (n=8) who were concomitantly treated with bevacizumab at time of protocol (19 weeks vs 15 weeks, p=0.11). **Conclusions:** CpG-28 was well tolerated at doses up to 0.3mg/kg subcutaneously and 18 mg intrathecally. Main side effects were limited to local erythema, lymphopenia and fever. Combination with bevacizumab warrants further clinical investigations. Clinical trial information: P060301.

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General Poster Session (Board #17A), Mon, 8:00 AM-11:45 AM

Use of an antibody-drug conjugate targeting tissue factor to induce complete tumor regression in xenograft models with heterogeneous target expression.

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Background: Tissue factor (TF) is the main initiator of coagulation, that starts when circulating factor VII(a) (FVII(a)) binds membrane bound TF. In addition, the TF:FVIIa complex can initiate a pro-angiogenic signaling pathway by activation of PAR-2. TF is aberrantly expressed in many solid tumors, and expression has been associated with poor prognosis. TF-011-vcMMAE, an antibody-drug conjugate (ADC) under development for the treatment of solid tumors, is composed of a human TF specific antibody (TF-011), a protease-cleavable valine-citrulline (vc) linker and the microtubule disrupting agent monomethyl auristatin E (MMAE). **Methods:** TF-011 and TF-011-vcMMAE were functionally characterized using in vitro assays. In vivo anti-tumor activity of TF-011-vcMMAE was assessed in human biopsy derived xenograft models, which genetically and histologically resemble human tumors. TF expression in xenografts was assessed using immunohistochemistry. **Results:** TF-011 inhibited TF:FVIIa induced intracellular signaling and efficiently killed tumor cells by antibody dependent cell-mediated cytotoxicity in vitro, but showed only minor inhibition of TF procoagulant activity. TF-011 was rapidly internalized and targeted to the lysosomes, a prerequisite for intracellular MMAE release and subsequent tumor cell killing by the ADC. Indeed, TF-011-vcMMAE efficiently and specifically killed TF-positive tumors in vitro and in vivo. Importantly, TF-011-vcMMAE showed excellent anti-tumor activity in human biopsy-derived xenograft models derived from bladder, lung, pancreas, prostate, ovarian and cervical cancer (n=7). TF expression in these models was heterogeneous, ranging from 25-100% of tumor cells. Complete tumor regression was observed in all models, including cervical and ovarian cancer xenografts that showed only 25-50% TF positive tumor cells. **Conclusions:** TF-011-vcMMAE is a promising new ADC with potent anti-tumor activity in xenograft models that represent the heterogeneity of human tumors, including heterogeneous TF expression. The functional characteristics of TF-011-vcMMAE allow efficient tumor targeting, with minimal impact on coagulation.

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General Poster Session (Board #17B), Mon, 8:00 AM-11:45 AM

Novel heteroclitic XBP1 peptides evoking antigen-specific cytotoxic T lymphocytes targeting various solid tumors.

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Background: Activation of the unfolded protein response (UPR) allows for tumor cells to survive prolonged endoplasmic reticulum stress and hypoxic conditions. XBP1 is an upstream element and a critical transcriptional activator of the UPR, and its up-regulation in a variety of human solid tumor cancers makes it as a promising immunotherapeutic target. The purpose of these studies was to evaluate immunogenic HLA-A2 XBP1-specific peptides for their ability to elicit cytotoxic T lymphocytes (CTL) against a variety of solid tumor cell lines. **Methods:** Upon the validation of XBP1 peptides for their strong HLA-A2 bindings and stabilities, peptide-specific CTL were generated ex vivo by repeated stimulation of CD3⁺ T lymphocytes obtained from HLA-A2⁺ normal donors with XBP1 peptides-pulsed antigen-presenting cells, either dendritic cells or T2 cells. **Results:** A cocktail of heteroclitic XBP1 **US₁₈₄₋₁₉₂** (YISPWILAV) and heteroclitic XBP1 **SP₃₆₇₋₃₇₅** (YLFPQLISV) peptides with significantly improved HLA-A2 affinity and stability from their native counterparts were used to evoke XBP1 antigen-specific CTL. The CTL were predominantly CD3⁺CD8⁺ T cells (>80%) containing a high percentage of Effector Memory (EM; CCR7⁻CD45RO⁺) cells, which were distinctively evoked by repeated stimulation with the XBP1 peptides. In addition, the XBP1-CTL displayed a high level of cellular activation (CD69⁺/CD3⁺CD8⁺). The XBP1-CTL demonstrated effective anti-tumor responses including cell proliferation and IFN- γ production, as well as degranulation (cytotoxic activity) against HLA-A2⁺ breast cancer (MB231, MCF7), colon cancer (LS180, SW480) and pancreatic cancer (8902, Panc1, PL45) cell lines, which over-express both unspliced and spliced XBP1 antigens. Importantly, the specific anti-tumor activities were detected primarily in the EM CTL subset. **Conclusions:** These results suggest the immunotherapeutic potential of a cocktail of heteroclitic XBP1 **US₁₈₄₋₁₉₂** and XBP1 **SP₃₆₇₋₃₇₅** peptides to elicit effective anti-tumor responses against various solid tumors, and provide the framework for clinical development of vaccine trials to improve patient outcome.

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General Poster Session (Board #17C), Mon, 8:00 AM-11:45 AM

AFM11, a CD19/CD3 bispecific tandab, to facilitate T-cell-mediated killing of CD19+ cells.

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Background: CD19, due to broader expression on B cell subtypes, is an attractive alternative to CD20 as a target for treatment of B cell malignancies. T cells are potent tumor-killing effectors that cannot be recruited by full length antibodies, however TandAb technology harnesses their cytotoxic nature for oncology indications. The CD3 RECRUIT TandAb AFM11 enables T cells to potently and specifically kill CD19+ tumors and possesses advantageous PK properties enabling intravenous dosing. **Methods:** We constructed AFM11, a human bispecific tetravalent antibody with two binding sites for both CD3 and CD19. In vitro efficacy and safety were evaluated on CD19+ cell lines and primary tumors. In vivo efficacy was evaluated in a murine NOD/scid xenograft model reconstituted with human PBMC. **Results:** In vitro assays demonstrate higher potency and efficacy of target cell lysis by AFM11 relative to a bispecific tandem scFv. CD8+ T cells dominate early cytotoxicity (4 hrs) while after 24 hrs both CD4+ and CD8+ T cells equally contribute to tumor lysis with **EC₅₀** of 0.5 – 5 pM; cytotoxicity is independent of cell CD19 density. AFM11 exhibits similar cytotoxicity at Effector:Target ratios from 5:1 to 1:5 and facilitates T cell serial killing of its targets. AFM11 activates T cells only in the presence of CD19+ cells. In PBMC cultures AFM11 induces CD69 and CD25 expression, T cell proliferation, and production of IFN- γ , TNF- α , IL-2, IL-6, and IL-10. Depletion of CD19+ cells from PBMC abrogates these effects, and indicates strict CD19+ target-dependent T cell activation. Thus, AFM11 should not elicit the devastating cytokine release observed when full length antibodies bind CD3. Cell lysis by AFM11 is restricted to CD19+ targets as CD19- bystanders are not lysed in co-culture assays. Up to one week co-incubation with AFM11 does not inhibit T cell cytotoxicity and thus it does not induce anergy. In vivo AFM11 exhibits a dose-dependent growth inhibition of Raji tumors; a single dose of AFM11 exhibits similar efficacy as 5 daily injections. **Conclusions:** AFM11 is a highly efficacious novel drug candidate for the treatment of CD19+ malignancies with an advantageous safety profile and anticipated dosing regimen.

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General Poster Session (Board #17D), Mon, 8:00 AM-11:45 AM

A phase I study of ad.p53 DC vaccine in combination with indoximod in metastatic solid tumors.

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Background: Indoleamine 2,3 dioxygenase (IDO) is an inducible tryptophan-catabolizing enzyme that downregulates the immune system. Many tumor cell types overexpress IDO to avoid elimination by infiltrating cytotoxic T cells. 1-methyl-D-tryptophan (1-MT, indoximod) is an IDO pathway modulator. Published preclinical data suggested that blockade of IDO with indoximod enhances the immunologic response to dendritic cell (DC) vaccines. Ad.p53 is an adenovirus used for generating DC vaccines directed against p53 epitopes. Ad.p53 when given to previously treated SCLC patients significantly increased their response rate to subsequent chemotherapy. The primary goal in phase I is the MTD of indoximod + Ad.p53. **Methods:** This phase I study used a 3+3 design with 7 indoximod dose levels (DL) (100 mg, 200 mg, 400 mg, 800 mg QD then 800 mg, 1,200 mg, and 1,600 mg PO BID)+up to 6 fixed dose Ad.p53 DC vaccinations q2wks. Standard eligibility/exclusion criteria applied along with exclusion of patients previously treated with ipilimumab. Treatment will continue until disease progression or unacceptable toxicity. DLT rules are 1st cycle \geq G3 AE related to treatment. **Results:** Total patients accrued to phase I=32. Types treated were 22 breast, 3 colon, 2 gastric, 1 ovarian, 1 NSCLC, 1 oropharynx, 1 sarcoma. No. of patients treated at DL 1-7 were 3, 3, 4, 6, 6, 3, 6, respectively. Toxicities possibly related to therapy were G1-2 fatigue, nausea, constipation, diarrhea, photosensitivity, hyponatremia, hyperglycemia, AST elevation. The MAD was 1,600 mg BID of indoximod with no DLTs noted. All discontinuations were due to progression of disease. No objective responses to study therapy were noted, but 6/10 breast patients treated with carboplatin/gemcitabine or gemcitabine alone responded to salvage therapy. **Conclusions:** The combination of indoximod+Ad.p53DC vaccine was well tolerated. The RP2D of indoximod is 1,600 mg BID + Ad.p53DC vaccine. There may be a chemosensitizing effect of Ad.p53DC+indoximod with chemo. The phase II trial in metastatic breast is looking at the response rate of indoximod+Ad.p53DC vaccine followed by salvage carboplatin/gemcitabine in previously treated patients. NCT01042535 Clinical trial information: NCT01042535.

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General Poster Session (Board #17E), Mon, 8:00 AM-11:45 AM

A phase II study of NPC-1C: A novel therapeutic monoclonal antibody (mab) to treat pancreatic (P) and colorectal (CR) cancers.

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Background: NPC-1C is a chimeric mab developed to treat P and CR cancers. This mab was selected from a panel of hybridomas derived from biologically screened, pooled human allogeneic colon cancer tissues. The NPC-1C target appears to be a variant of MUC5AC expressed specifically by human P and CR tumors with minimal cross-reactivity to normal GI mucosa. **Methods:** A phase IIa study with NPC-1C is enrolling patients (pts) with advanced P and CR cancer refractory to standard therapy. Primary objectives are to measure efficacy by analysis of CT scans pre- and post-therapy, clinical lab tests, and physical examinations. Secondary objectives are to determine safety, pharmacokinetics (PK), and select immune responses to the mab. Analyses of pts peripheral blood mononuclear cells (PBMCs) for antibody-dependent cell-mediated cytotoxicity (ADCC) and immune cytokine profiling utilizing the Milliplex MAP Human Cytokine/Chemokine Panel are planned to assess for immunologic outcome and correlation with clinical benefit. **Results:** Pts received 1.5 mg/kg IV of NPC-1C every two weeks. 10 subjects (8 colorectal, 2 pancreatic) have enrolled on study (2/10 non-evaluable). Treatment was well tolerated with grade 1 and 2 constitutional symptoms noted. Coombs positivity was reported in 4/8 pts shortly after the infusions, but only grade 1 hemolysis was reported (in 2/8 patients). There was 1 episode of each of the following grade 3 AE's: weakness, increased bilirubin, and abdominal pain, possibly related to NPC-1C. 5 of 19 pts treated in this and the phase Ia study demonstrated stable disease (SD) on scans after completing the first course of treatment (4 doses). **Conclusions:** Preliminary results with NPC-1C show signs of clinical activity based on SD in heavily pretreated pts with P and CR cancer. Safety has been established at the 1.5 mg/kg dose. A new lot of NPC-1C, produced with improved sterility and purification procedures and demonstrating no red cell agglutination, has been manufactured under GMP conditions. We plan to introduce this new lot at the current 1.5 mg/kg dose level. If there are no dose limiting toxicities, we plan to dose escalate to a higher MTD at which we will re-evaluate clinical efficacy. Clinical trial information: NCT01040000.

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General Poster Session (Board #17F), Mon, 8:00 AM-11:45 AM

Anti-60S ribosomal protein L29 antibody: New anticancer agent discovered from human sera.

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Background: Incidence of hepatocellular carcinoma (HCC) is lower in autoimmune hepatitis (AIH) than chronic viral hepatitis. In AIH, serum immunoglobulin G (IgG) levels are associated with the clinical features. In this study, we searched IgG showing anti-tumor effect in sera of AIH patients. **Methods:** Total IgG was extracted from sera of AIH patients by using protein G. Anti-tumor effects of the total IgG were evaluated by MTT assay using human HCC Huh7 cells and PLC/PRF/5 cells. Autoantigens in membrane proteins of Huh7 cells were screened by immunoprecipitation followed by liquid chromatography-mass spectrometry (LC-MS) shotgun analysis. **Results:** In one AIH patient without any cancers, addition of total IgG extracted from her serum to the culture inhibited the proliferation of Huh7 cells and PLC/PRF/5 cells, and decreased intracellular levels of β -Catenin and Cyclin-D1. In this patient, autoantigens in membrane proteins were screened, and 60S ribosomal protein L29 (RPL29) was identified from the MS/MS spectra and the SwissProt database using the Mascot Search engine. RPL29 expression in human HCC cell lines including Huh7, PLC/PRF/5, Hep3B, HepG2, HLE, HLF and SK-Hep-1 were identified by Western blot. Next, in the other 25 AIH patients, we investigated the correlation between serum anti-RPL29 levels by indirect ELISA using recombinant RPL29 and anti-tumor effects of total IgG extracted from their sera. Serum anti-RPL29 levels were significantly correlated with anti-tumor effects of total IgG extracted from their sera ($P < 0.0001$). On the other hand, addition of recombinant RPL29 to the culture canceled anti-tumor effects of total IgG extracted from sera of AIH patients showing higher serum anti-RPL29 levels. Additionally, these anti-tumor effects of total IgG extracted from sera of AIH patients were shown by MTT assay using human pancreatic cancer AsPC-1 cells and Panc-1 cells. **Conclusions:** Anti-RPL29 antibodies showing anti-tumor effect were discovered from human sera. Anti-RPL29 inhibits cancer cell proliferation via down-regulation of Wnt/ β -Catenin signaling pathway. Serum anti-RPL29 may be one of immune systems by which the human does not develop cancers.

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General Poster Session (Board #17G), Mon, 8:00 AM-11:45 AM

Multicenter trial for assessing tolerability of combination therapy with cisplatin, irinotecan, and PSK in extensive-stage small-cell lung cancer: RNCLC-01 study.

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Background: Polysaccharide-K (PSK, Krestin) is a protein-bound polysaccharide, extracted from cultured mycelium of *Coriplus versicolor*, and is associated with immunostimulatory activity. Although combination use of PSK with chemotherapy has been shown to prolong the response period in patients with small-cell lung cancer (SCLC), the efficacy of PSK in combination chemotherapy including cisplatin has been less examined. We examined safety and efficacy of combination therapy with cisplatin, irinotecan, and PSK in extensive-stage (ED) SCLC. **Methods:** Eligible pts included: histologically confirmed ED-SCLC without prior chemotherapy, age under 75 years, ECOG performance status ≤ 1 , with evaluable disease and adequate organ function. Treatment: irinotecan 60 mg/m² on days 1, 8, and 15 and cisplatin 60 mg/m² on day 1 every 4 weeks for 4 to 6 courses, and oral PSK 3,000 mg/body daily. The treatment with PSK was continued after the end of cisplatin and irinotecan administrations, and was combined as a part of second-line therapy after exacerbations. **Results:** Between January 2008 and July 2010, 17 patients were enrolled, and the efficacy and prognosis were evaluated in 15 of 17 patients. Response rate was 66.6%, one-year survival rate was 66.6%, and two-year survival rate was 33.2%. The major toxicities were diarrhea and myelosuppression, and the common toxicity of PSK-containing combination chemotherapy was not observed. **Conclusions:** The combination chemotherapy with cisplatin, irinotecan, and PSK was effective and well tolerated in ED-SCLC. Clinical trial information: NCT00546130.

A randomized phase II/III study of naptumomab estafenatox plus IFN- α versus IFN- α in advanced renal cell carcinoma.

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Background: Naptumomab estafenatox/ANYARA (Nap) is a fusion protein of an antibody (5T4) and a superantigen (SEA/E-120). After phase I studies (Borghaei. J Clin Oncol. 2009, 27:4116) a prospective, randomized phase II/III trial of Nap + IFN- α (A) vs IFN- α (I) was conducted. **Methods:** Patients (pts) with RCC were randomized in an open label study to receive A or I. The primary endpoint was OS. Secondary endpoints were PFS, response rate and safety. Baseline (bl) plasma IL-6 was predictive of pazopanib (Tran. Lancet Oncol. 2012, 13:827) and MVA-5T4 vaccine (Harrop. Cancer Immunol Immunother. 2012, 61:2283) benefit in RCC pts. IL-6 and anti-SEA/E-120 antibodies (a-S) were analyzed. A subgroup SG1 had bl levels below median for IL-6 (<7 pg/ml) and a-S. Another subgroup SG2 had IL-6 below 13 pg/ml (Tran. Lancet Oncol. 2012, 13:827) and excluding upper quartile of a-S according to phase 1 levels (Borghaei. J Clin Oncol. 2009, 27:4116). **Results:** From 5/2007 to 10/2010 513 pts were treated (ITT) with a median follow-up time for censored pts of 43 months. Unexpectedly, pts in certain territories had increased bl a-S (median of 61 pmol/ml in Russia vs 34 in UK). The table summarizes efficacy results. The primary endpoint was not met. Multivariate analysis adjusted for risk scores and subsequent TKI usage verified Nap benefit in pts with low IL-6 and normal a-S. Nap was well tolerated. Pyrexia (A:46%/I:18%), nausea (21%/11%), back pain (18%/6%), vomiting (16%/7%) and chills (12%/4%) were more common after Nap. **Conclusions:** The study did not meet primary endpoint. In pts with low IL-6 and normal levels of a-S, addition of Nap to IFN- α improves OS and PFS. The results warrant further studies with Nap in sequence or combo with e.g. TKIs in this subgroup. More generally, as bl IL-6 appears to be prognostic and predictive of outcome on treatment with TKIs and immunotherapies this may be a stratification factor for RCC studies. Clinical trial information: NCT00420888.

Population	Treatment	N	Response CR+PR	PFS median months	HR (95% CI)	OS Median months	HR (95% CI)
ITT	A	253	35 (14%)	5.8	0.92 (0.77, 1.11)	17.1	1.08 (0.88, 1.33)
	I	260	40 (15%)	5.8		17.5	
SG1	A	67	19 (28%)	13.7 (p=0.016)	0.62 (0.42, 0.92)	63.3 (p=0.020)	0.59 (0.37, 0.95)
	I	63	10 (16%)	5.8		31.1	
SG2	A	68	13 (19%)	8.5 (p=0.162)	0.75 (0.51, 1.10)	30.4 (p=0.036)	0.65 (0.42, 0.99)
	I	63	11 (17%)	5.8		21.7	

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General Poster Session (Board #18A), Mon, 8:00 AM-11:45 AM

A phase II study of Recchia's immunomodulatory schedule (RIS) as a maintenance therapy in high-risk tumors after a response.

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Background: F. Recchia et al. (Interleukin-2 and 13-cis retinoic acid in the treatment of minimal residual disease: A phase II study. *Int J Oncol.* 20: 1275-1282, 2002) previously defined the possibility of achieving a chronic immune stimulation through a prolonged low dose sc interleukin 2 (IL2)-based schedule, leading to a prolongation of PFS in advanced cancer patients (pts). A confirmatory phase II study was started. **Methods:** Pts diagnosed of high risk solid tumors in response to chemotherapy were included. RIS consisted in low dose IL2 (1.8 MU sc per day, 5 days per week, 3 weeks per month) plus oral isotretinoin (CRA, 0.5 mg/kg) the same days of IL2 and/or sc PEG-interferon (50 µg per week of treatment). No Chx was allowed with RIS, but concurrent maintenance with TKI or Hx was permitted. **Results:** Since 08/2007 to 01/2013, 69 pts (39 M/30 F, median age 60 y, range 31-78) were included. 51 pts were treated with stage IV disease. 36 pts entered in complete response (CR), 29 in partial response (PR), 1 with progressive disease, 3 adjuvant. 4/69 grade 3/4 toxicity, (6%): 1 fatal poliserositis resistant to cortisone after 20 months of RIS, 1 stopped RIS for reaction requiring steroids, 1 for multiinfarction dementia, 1 for grade 3 liver toxicity. 3 pts presented other alterations managed along the course of the RIS (1 PVT, 1 ITP, 1 angina resolved after stent placement). Grade 1-2 fever, fatigue and joint pain were commonly observed but usually controlled with NSAIDs. Among the 68 pts evaluable for response, one pt progressed, 26 presented stable disease (median TTP 3 mo, range 2-28), 3 presented PR (1 melanoma, 1 prostatic ca, 1 ovarian ca; 9, 11 and 28 mo respectively), and 38 maintained or presented CR (median TTP 19 mo, range 2-62+). Three pts in PR were converted to a CR during RIS (1 pancreatic ca, 1 ovarian ca, 1 larynx ca). **Conclusions:** In this study we confirm the previous results reported by F. Recchia et al. RIS represents a good alternative for high risk pts with solid tumors after systemic chemotherapy induced response with acceptable tolerability and offers in selected pts a possibility of obtaining a CR after a PR. Further validation and randomized studies are warranted.

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General Poster Session (Board #18B), Mon, 8:00 AM-11:45 AM

Physicochemical, functional, and pharmacologic comparability between the proposed biosimilar rituximab GP2013 and originator rituximab as the foundation for biosimilarity.

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Background: Development of a biosimilar involves extensive characterization of the originator product and a target-directed iterative development process ensuring comparability to the originator with similar clinical efficacy, safety and quality. Here we report the physicochemical, functional and pre-clinical pharmacological characterization of a proposed rituximab biosimilar (GP2013). **Methods:** A variety of physicochemical methods were used to analyze primary and higher order structure, post-translational modifications and size heterogeneity. Functional characterization included a series of bioassays (in vitro target binding, ADCC, CDC and apoptosis) and SPR-based Fc receptor binding assays. Comparative PK and PD were assessed in cynomolgus monkeys, the pharmacologically most relevant species. **Results:** GP2013 has the same primary amino acid sequence and higher order structure as the originator rituximab and both were comparable with regard to charge variants, specific amino acid modifications, glycan pattern and size heterogeneity (low- and high-molecular weight variants & particles). Functionally GP2013 could not be distinguished from originator rituximab preclinically. In primates, PK analysis confirmed bioequivalence between GP2013 and originator rituximab with nearly identical AUC values and 90% CIs entirely within the standard acceptance range of 0.8-1.25. Bioequivalence of PD response was also shown, with 95% CIs of areas under the effect-time curves (AUEC) ratios for relative change from baseline in B-cell populations within the 0.8-1.25 acceptance range. **Conclusions:** Using a broad panel of analytical methods it was shown that GP2013 is highly similar to originator rituximab at the physicochemical level. In addition, the preclinical comparability exercise confirmed that GP2013 and originator rituximab are pharmacologically similar with regard to FcR and CD20 binding, ADCC, CDC and apoptosis potency, PK exposure and B-cell depletion. As such, we anticipate that the ongoing clinical trials will help provide confirmatory evidence of similar efficacy and safety to the originator product.

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General Poster Session (Board #18C), Mon, 8:00 AM-11:45 AM

High hydrostatic pressure to induce immunogenic cell death in human tumor cells.

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Background: Recent studies have identified molecular events characteristic of immunogenic cell death. These include surface exposure of calreticulin, HSP70 and HSP90, release of intranuclear HMGB1 and secretion of ATP from dying cells. Several chemotherapeutic agents, including anthracyclins, oxaliplatin and bortezomib, and hypericin-based photodynamic therapy have been described to induce the immunogenic cell death in human tumor cells. We investigated the potential of high hydrostatic pressure (HHP) to induce immunogenic cell death in human tumor cells. **Methods:** Prostate and ovarian cancer cell lines and primary tumor cells were treated by HHP and we analyzed the kinetics of the expression of immunogenic cell death markers. HHP killed tumor cells expressing immunogenic cell death markers were tested for their ability to activate dendritic cells (DCs), to induce tumor specific T cells and regulatory T cells. **Results:** HHP induced rapid expression of HSP70, HSP90 and calreticulin on the cell surface of all tested cell lines and primary tumor cells. HHP also induced release of HMGB1 and ATP from treated cells. The kinetics of expression was similar to doxorubicin, HHP, however, induced 1.5-2 fold higher expression of HSP70, HSP90 and calreticulin. The interaction of DCs with HHP-treated tumor cells led to the faster rate of phagocytosis, significant upregulation of CD83, CD86 and HLA-DR and release of IL-6, IL-12p70 and TNF α . The ability of HHP-killed tumor cells to promote DCs maturation was cell contact dependent. DCs pulsed with tumor cells killed by HHP induced high numbers of tumor-specific CD4⁺ and CD8⁺IFN-g-producing T cells even in the absence of additional maturation stimulus. DCs pulsed with HHP treated tumor cells also induced the lowest number of regulatory T cells among the tested conditions. Cells treated by HHP can be cryopreserved in liquid nitrogen and retain their immunogenic properties upon thawing thus allowing for their convenient use in the manufacturing of cancer immunotherapy products. **Conclusions:** High hydrostatic pressure is a reliable and very potent inducer of immunogenic cell death in the wide range of human tumor cell lines and primary tumor cells.

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General Poster Session (Board #18D), Mon, 8:00 AM-11:45 AM

VTX-2337, a TLR8 agonist, plus chemotherapy in recurrent ovarian cancer: Preclinical and phase I data by the Gynecologic Oncology Group.

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Background: Given the absence of clear molecular drivers in high-grade serous ovarian cancer, targeting the tumor micro-environment with immunotherapy is an emerging approach. VTX-2337 is a potent, small molecule agonist of TLR8 which stimulates the innate immune response, and was previously evaluated as a single agent in cancer patients. We report data combining VTX-2337 with chemotherapy in recurrent ovarian cancer. **Methods:** VTX-2337 was tested in an ovarian cancer mouse model with an intact human immune system. Additionally, an open-label phase I study of VTX-2337 + pegylated liposomal doxorubicin (PLD) in recurrent ovarian cancer (NCT01294293, N=13) was performed. PLD (40 mg/m²) was given on day 1 of a 28-day cycle. Three dose levels of VTX-2337 (2.5, 3.0, 3.5 mg/m², N=13) were serially tested and given by SC injection on days 3, 10, and 17. VTX-2337 (3.0 mg/m²) was also tested with paclitaxel (80 mg/m²; N=7) given on days 1, 8, and 15 of a 28 day cycle. Responses were evaluated using RECIST1.1. PK and serum immune cytokines were measured. Patients remained on therapy until toxicity or progression. **Results:** In the mouse model, clinical responses to PLD were increased. Innate immunity along with CD8+ T cell responses were also induced. In humans, treatment with PLD + VTX-2337 increased various cytokines and chemokines (G-CSF, MCP-1, MIP-1 β , TNF- α). The PK of PLD was not affected by VTX-2337. The combination was well tolerated with no DLTs. AEs consisted of those seen with single-agent PLD (Gr 3/4 toxicities, N=6) or VTX-2337 (Gr 1/2 injection site reaction, transient fever, flu-like symptoms). There was 1 partial response (13%) and 63% had stable disease. Paclitaxel + VTX-2337 was also well tolerated. **Conclusions:** VTX-2337 enhances the effect of PLD in a preclinical model of ovarian cancer, and the combination is well-tolerated in patients. Clinical data and biomarkers consistent with immunostimulation, provide rationale for the on-going randomized, placebo-controlled, phase II trial comparing PLD vs PLD + VTX-2337 (GOG-3003, NCT01666444). Clinical trial information: NCT01666444.

3078

General Poster Session (Board #18E), Mon, 8:00 AM-11:45 AM

Prognostic factors for the efficacy of catumaxomab in patients (pts) with malignant ascites (MA): Meta-analysis from two phase III studies.

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Background: Data from two phase III studies (IP-REM-AC-01 and CASIMAS) were used for a meta-analysis to identify factors with a potential impact on the efficacy of catumaxomab (CATU) in pts with MA. **Methods:** 389 pts treated with CATU plus paracentesis and 88 control pts (paracentesis only) were included. Efficacy parameters were overall survival (OS), puncture-free survival (PuFS) and time to first puncture (TTPu). The impact of the prognostic factors Karnofsky Index (KI), total serum protein (protein) and presence/absence of distant metastases (metastases) at inclusion was analysed for these parameters. **Results:** Pts treated with CATU showed a significant prolongation of PuFS (44 vs 11 d, HR 0.34, $p<0.0001$), TTPu (88 vs 13 d, HR 0.26, $p<0.0001$) and OS (88 vs 68 d, HR 0.62, $p=0.007$) compared to control pts. Pts treated with CATU and a KI of 80–100 at inclusion had significantly better OS compared to those with a KI of 60–79 (120 vs 57 d, HR 0.55, $p<0.001$) and improved PuFS (55 vs 27 d, HR 0.61, $p<0.001$) but not improved TTPu (88 vs 96 d, HR 0.80, $p=0.186$). Linear Cox regression of KI as a co-variable showed a significantly positive impact of high KI on catumaxomab efficacy for OS (HR 0.97, $p<0.001$) and PuFS (HR 0.98, $p<0.001$) but not TTPu (HR 0.99, $p=0.054$). Cox regression analysis of the presence of distant metastases and low ($<$ normal) total serum protein on CATU efficacy showed a negative impact on OS (metastases: HR 1.43; $p<0.001$; protein: HR 1.39, $p=0.002$), PuFS (metastases: HR 1.37, $p=0.003$; protein: HR 1.45, $p<0.001$) and TTPu (metastases: HR 1.28, $p=0.069$; protein: HR 1.41, $p=0.011$). Treatment effects on PuFS and TTPu were observed in all subgroups for all factors but the effect on OS compared to controls was only found in pts with KI 80–100 and in those with no distant metastases and with normal/high total serum protein. **Conclusions:** Karnofsky Index, distant metastases and total serum protein at screening were identified as factors having a significant impact on OS in CATU-treated pts and better prognostic pts compared to controls. In contrast, the effect of CATU on ascites control (PuFS, TTPu) was observed in all subgroups irrespective of whether the pts were in the good or poor prognostic group.

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General Poster Session (Board #18F), Mon, 8:00 AM-11:45 AM

Targeting myeloid-derived suppressor cells and the PD-1/PD-L1 axis to enhance immunotherapy with anti-CEA designer T cells for the treatment of colorectal liver metastases.

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Background: Immunotherapy for colorectal cancer liver metastases (CRCLM) is limited by the intrahepatic immunosuppressive environment mediated in part by myeloid derived suppressor cells (MDSC), which expand in response to tumor. T cell suppression can be mediated by programmed death ligand-1 (PD-L1, CD274) on MDSC binding to programmed death-1 (PD-1, CD279) on T cells. We hypothesize blocking PD-L1 will improve adoptive cellular therapy efficacy for CRCLM through inhibition of MDSC-mediated T cell suppression. **Methods:** “Designer” T cells (dTc) were produced from activated murine splenocytes transduced with chimeric antigen receptor (CAR) specific for CEA. C57BL/6 mice were injected with CEA⁺ MC38 tumor cells via spleen, and liver MDSC (CD11b⁺Gr1⁺) were purified with immunomagnetic beads after two weeks. MDSC were co-cultured with stimulated dTc with or without in vitro PD-L1 blockade. **Results:** MDSC expanded 2.4-fold in response to CRCLM, and expressed high levels of PD-L1 (63.8% PD-L1⁺). PD-L1 was equally expressed on both monocytic (CD11b⁺Ly6G⁻Ly6C⁺) and granulocytic (CD11b⁺Ly6G⁺) MDSC subsets (43.6% PD-L1⁺ and 27.9% PD-L1⁺, respectively). Expression of related ligand, PD-L2 was found to be negligible in both subsets. The cognate inhibitory receptor, PD-1, was expressed on dTc (23.8% PD-1⁺) and native T cells (37.3% PD-1⁺). Increasing endogenous T cell expression of PD-1 significantly correlated with MDSC expansion ($r=0.9774$, $p<0.0001$) in response to CRCLM. Co-culture of dTc with MDSC demonstrated the suppressive effect of MDSC on dTc proliferation which was abrogated with in vitro targeting of PD-L1. The percentage of dTc proliferating in the presence of CEA⁺ tumor decreased from 72.2% to 29.3% ($p<0.001$) with the addition of MDSC, and immunosuppression was reversed with blockade of PD-L1, which resulted in a 1.6-fold increase in dTc proliferation ($p=0.01$). **Conclusions:** Liver MDSC expand in the presence of CRCLM and mediate suppression of anti-CEA dTc via PD-L1. Our results indicate that blockade of PD-L1:PD-1 engagement is a viable strategy for enhancing the efficacy of adoptive cell therapy for liver metastases.

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General Poster Session (Board #18G), Mon, 8:00 AM-11:45 AM

Effect of therapeutic targeting of EMP2 on breast and endometrial cancer stem cells.*Madhuri Wadehra, Meagan Kiyohara, Negin Ashki, Ann Chan; DGSOM at UCLA, Los Angeles, CA*

Background: There is increasing evidence that tumor-initiating cancer stem cells (CSCs) contribute to tumor metastasis and therapeutic resistance. In breast and endometrial cancers, metastatic CSCs are defined as CD44+/CD24- and ALDH+, and in this study, we define another marker epithelial membrane protein-2 (EMP2) as a novel target for this population of cells. EMP2 is an oncogene whose expression has been shown to correlate with tumor progression and survival in a number of human cancers including triple-negative breast, ovarian, and endometrial tumors. **Methods:** A number of cancer cell lines were utilized both in vitro and in vivo to determine if EMP2 expression levels alter the number and expression of CSC markers. To translate this work, EMP2 and ALDH expression were correlated in primary human endometrial and breast cancers. To determine the therapeutic efficacy of our fully-human EMP2 IgG1 antibody, xenografts from breast or uterine cancer were treated, removed, and then reinjected into secondary mice. **Results:** In this study, we show new evidence that EMP2 is highly expressed in CSCs. High levels of EMP2 increase the tumor forming potential of both endometrial and breast cancer lines. In tumors created from these cells, high levels of ALDH+ expression were also observed. In contrast, reduction in EMP2 decreased CD44 expression and ALDH activity both in vitro and in vivo, with the net consequence of poorly vascularized and slow growing tumors. We have recently developed a novel monoclonal antibody to EMP2 and have started testing its therapeutic efficacy. In vitro treatment with EMP2 IgG1 reduced HIF-1a, CD44, and ALDH levels, ultimately leading to caspase mediated apoptosis. Treatment of breast cancer cells with EMP2 IgG1 reduced tumor load in both subcutaneous and metastatic models of breast and endometrial cancer resulting in a significant improvement in survival. Reinjection of these cells post treatment into secondary mice failed to efficiently form, and histological examination of the tumors revealed significant necrosis and elimination of ALDH+ CSCs. **Conclusions:** These results suggest that targeting EMP2 may reduce CSCs and represent an attractive target for further therapeutic development.

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General Poster Session (Board #18H), Mon, 8:00 AM-11:45 AM

Novel murine anti-HER2 monoclonal antibodies to induce apoptosis and regulate miR-21 in breast cancer cell.

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Background: MicroRNAs play critical roles in biological and pathological processes. Of these, miR-21 is frequently observed to be overexpressed in various cancers. However, treatment of breast cancer cells with trastuzumab enhances the level of miR-21 by a relative fold of 2.26 (Ichikawa, PLoS ONE, 2012, p.5). In this study, we demonstrate that two murine anti-HER2 mAbs, 10H8 and 8H11, while inhibiting cell proliferation and inducing apoptosis, do not perturb the level of miR-21 in the same way as trastuzumab. To understand the differential effects of trastuzumab and the two mAbs, epitope mapping studies were also conducted. **Methods:** SKBR3 cells were used to confirm HER2 binding and determine the antibodies' impact on proliferation, apoptosis, and miRNA expression. Cell proliferation upon a 3-day antibody treatment (10 to 15 μ g/ml) was measured using MTS and Crystal Violet Assays. Apoptotic cells were identified using Annexin V-FITC flow cytometry. To determine their epitope mapping, cells were grown on coverslips overnight, fixed, blocked, and incubated in AF488-mAb or AF555-mAb, and images were captured with IX81. QRT-PCR was used to measure the relative levels of miR-21, using miR-16 as the endogenous control. **Results:** Cell proliferation assays showed that the treatment of 10H8 resulted in a better growth inhibition in SKBr3 cells than that of trastuzumab (ex: 80.2% vs 84%, respectively). Combining 10H8 and 8H11 produced less viable cells and more early- and post-apoptotic cells than those treated with trastuzumab alone. In addition, whereas trastuzumab increased the relative quantity of the known oncogene, both 10H8 and 8H11 maintained a steady level of miR-21 (0.99 and 1.2, respectively). **Conclusions:** Initial results indicate that 10H8 and 8H11 inhibit cell proliferation and induce apoptosis in HER2-overexpressing cells. However, unlike trastuzumab, 10H8 and 8H11 do not involve miR-21 up-regulation. Moreover, the three mAbs do not have overlapping epitopes on HER2. Further investigations of the antibodies' impact on intracellular signaling pathways and the structural determination of the HER2 binding sites would provide further understanding of the therapeutic potentials of these two mAbs.

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General Poster Session (Board #19A), Mon, 8:00 AM-11:45 AM

Phase I clinical study of NK105, paclitaxel-encapsulating micelles, on a weekly schedule, in patients with malignant tumors (dose-escalation phase).

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Background: NK105 is the formulation of a novel drug delivery system that encapsulates paclitaxel (PTX) in polymeric micelles and possibly resolves the safety issues associated with the additives contained in the conventional PTX formulation. In the phase II study of NK105 administered to gastric cancer patients on a 150Emg/m² triweekly schedule, peripheral sensory neuropathy was mitigated compared to prior data for conventional PTX, without any reduction in efficacy. Based on the dose-density theory, we conducted a phase I study of NK105 on a weekly schedule to determine the dose-limiting toxicity (DLT) and recommended dosage (RD), and to evaluate the pharmacokinetic profile. **Methods:** Patients with advanced solid tumors refractory to standard therapy received NK105 at dosage levels of 50-100 mg/m² as a 30-min infusion without premedication, once a week for three weeks, followed by one week of rest. Pharmacokinetic analysis was conducted in cycles 1 and 2. **Results:** Sixteen patients were enrolled in the study. In the 100Emg/m² cohort (n=7), one patient experienced DLT (grade 4 neutropenia lasting 5 days), and dose reduction or delay was necessary in 4 of the 7 patients during the first course due to neutropenia. It was decided that 100 mg/m² was the maximum tolerated dosage, and the RD was set at 80 mg/m². Grade 3 or more severe adverse drug reactions reported for the 80Emg/m² cohort were neutropenia, anemia, fatigue, hearing impaired and ataxia. Comparison of the pharmacokinetic parameters of NK105 with those of PTX at the same dosage (100 mg/m²) showed that the **AUC_{0-inf}** and Vdss of NK105 were approximately 50-fold and 1/15 of the reported PTX values, respectively. A refractory gastric cancer patient and an esophageal cancer patient each showed a partial response, at dosages of 80 mg/m² and 100 mg/m², respectively. **Conclusions:** 80Emg/m² weekly administration of NK105 was well tolerated and showed anti-tumor activity, including partial responses and several occurrences of stable disease. The expansion phase of the study is ongoing at the RD of 80 mg/m². Clinical trial information: JapicCTI-101233.

3083

General Poster Session (Board #19B), Mon, 8:00 AM-11:45 AM

Superparamagnetic nanoparticles targeting cancer cells invading the extracellular matrix.

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Background: Metastatic cancer cells secrete matrix metalloproteinases (MMP), which allow cancer cells to burrow their way to the nearby vasculature. Therefore, MMPs are potential targets for anticancer treatment. Nanoparticles (NPs) can be crafted to adhere to the ECM and subsequently be released in response to advance of invasive cells. The objective of the study is to elucidate the interaction of cancer cells in a three-dimensional culture, with functionalized SPIONS targeted to the extracellular matrix around them. **Methods:** SPIONS, 15-20 nm in size were functionalized using different coatings: ficoll 400, sucrose, lysine-arginine, dextran50,000. Cellular uptake studies using cervical adenocarcinoma HeLa cell lines were performed to determine the optimum formulation taken up by the cells, using electron microscopy and Prussian blue staining for optical microscopy. Two types of extracellular matrices were used: Rat-tail Collagen type I and ECM gel from Engelbreth-Holm-Swarm murine sarcoma with NPs embedded in them. To assess the capability of cells to invade the matrix, cells were grown on the surface of the matrices for 1 week To evaluate the ability to grow, expand and migrate inside the matrix, cells were embedded within the matrix and left for 14 days. Comparison was made in the presence or absence of SPIONS. **Results:** Sucrose-coated SPIONS were taken up the best by HeLa cell lines as evaluated by MRI. MMP-1 Secretion allowed HeLa cell invasion of collagen type-1 matrix unidirectionally. Cells could adhere, proliferate, differentiate and migrate in the absence of SPIONS. Cells positive for MMP-9 invaded ECM gel from Engelbreth-Hol-Swarm murine sarcoma matrix also only in the absence of SPIONS. Cells that were found engulfing SPIONS showed morphological features of apoptosis as nuclear pyknosis and karyorrhexis. **Conclusions:** Targeting NPs to the ECM surrounding cancer cells that have developed a metastatic potential represents an attractive platform for cancer therapeutics. The findings show a great promise for development of new theranostic agents, that can be directed to the tumor environment using external magnetic fields, with subsequent suppression of invasion and even destroying malignant cells.

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General Poster Session (Board #19C), Mon, 8:00 AM-11:45 AM

The effect of copper nanoparticles on the progression of tumor in vivo.

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Background: The study of the anticancer activity of copper nanoparticles (CuNP) measuring 70-75 nm in rats transplantable tumors. **Methods:** Experiments were carried out on white outbreed male rats with transplanted sarcoma 45 (9.10-dimethyl-1.2-benzanthracene-induced fusiform cell fibrosarcoma, S-45) and Pliss's lymphosarcoma (Pliss). 0.5 ml of tumor tissue suspension (1×10^6 cells) in saline was inoculated subcutaneously in rat dorsal region for induction of sarcoma 45 or Pliss's lymphosarcoma. 7-8 days after tumors transplantation the animals were divided into three groups. Group 1: control (rats received 0.3 ml of saline only). Group 2: CuNP in 0.9% w/v of NaCl were injected intratumorally (1.25 mg/kg bodyweight). Group 3: The same amount of CuNP was injected intraperitoneally. Animals received nanoparticles four times a week for one week, then after a week's break, the procedure was repeated. The associated nanoparticles are spherical in shape and have an oxide film on its surface. CuNP effect on tumor growth was determined by the mass (M) and size (V) of tumors. **Results:** The analysis of the received data showed that administration of CuNP to rats with S-45 caused reduced growth rates or cases with complete regression (8 of 17 cases) in 67.0% of experimental animals independently of injection way ($V = 0.81 \pm 0.3 \text{ cm}^3$, $M = 0.91 \pm 0.3 \text{ g}$). The other 33% of rats with S-45 showed tumor growth ($V = 7.58 \pm 1.3 \text{ cm}^3$, $M = 9.2 \pm 1.6 \text{ g}$). Overall, for the entire group of animals with S-45, nanoparticles inhibited tumor growth of 45%. In the control group there was an increase of tumor growth ($V = 9.0 \pm 1.8 \text{ cm}^3$, $M = 10.4 \pm 2.5 \text{ g}$). CuNP introduction to animals with Pliss caused a delay in tumor growth and partial or complete regression (12 of 28 cases) in 40.0-48.0% of the rats in the route of Cu injection ($V = 1.4 \pm 0.8 \text{ cm}^3$, $M = 10.4 \pm 2.5 \text{ g}$). Observation of the remaining animals revealed tumor growth ($V = 60.6 \pm 5.93 \text{ cm}^3$, $M = 67.13 \pm 8.7 \text{ g}$). In the whole group tumor growth inhibition was 50.0%. In the control group we observed increase of tumor growth ($V = 62.1 \pm 5.21 \text{ cm}^3$, $M = 75.9 \pm 8.2 \text{ g}$). **Conclusions:** Thus the study showed that the copper nanoparticles possess antiproliferative activity, can inhibit the growth of transplanted tumors in rats and may be potentially used in anticancer medical therapy.

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General Poster Session (Board #19D), Mon, 8:00 AM-11:45 AM

Preclinical testing using novel CT20p peptide-nanoparticle combination in breast cancer.

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Background: Patients with metastatic breast cancer may be initially responsive to treatment, but a significant number develop refractory disease. There is a critical unmet need to develop effective therapeutic approaches given the unique metabolism of tumor cells. **Methods:** We examined the cytotoxic properties of a novel peptide, CT20p, derived from the C-terminus of Bax. For delivery to cells, the amphipathic nature of CT20p allowed it to be encapsulated in polymeric nanoparticles (NPs). NPs were made using aliphatic hyperbranched polyester (HBPE) that incorporated surface carboxylic groups and interior hydrophobic cavities for encapsulation of CT20p. To examine the cytotoxic potential and targeting capacity of CT20p-NP-HBPE, we treated MDA-MB-231 and MCF-10A breast cancer cell lines with the combination and measured changes in mitochondrial function, cell metabolism and induction of cell death. The ability of CT20p-NP-HBPE to cause tumor regression was examined by subcutaneously implanting MDA-MB-231 cells in nude mice. **Results:** Initial results showed that CT20p caused the release of calcein from mitochondrial-like lipid vesicles, without disrupting vesicle integrity, and, when expressed as a fusion protein in cells, localized to mitochondria. While the peptide alone had little effect upon intact cells, when encapsulated and delivered by nanoparticles, CT20p-HBPE-NPs proved an effective killer of breast cancer cells. CT20p-NP-HBPE initiated non-apoptotic cell death within 3 hours of treatment by targeting mitochondria and deregulating cellular metabolism. Nanoparticles alone or nanoparticles encapsulating a control peptide had minimal effects. The cytotoxicity of CT20p-NP-HBPE was most pronounced in breast cancer cells, sparing normal, epithelial cells. In implanted breast tumors, CT20p-NP-HBPE accumulated in tumors within 24 hours and reduced tumor burden by 50-80%. **Conclusions:** These results reveal the innovative features of CT20p that allow nanoparticle-mediated delivery to tumors and the potential application in combination therapies that target the unique metabolism of cancer cells.

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General Poster Session (Board #19E), Mon, 8:00 AM-11:45 AM

Active immunotherapy in patients with progressive disease (PD) after first-line therapy: Racotumomab experience.

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Background: Racotumomab is a therapeutic vaccine that induces a cellular and humoral immune response against NeuGc-containing gangliosides expressed in several tumors but not in normal human tissues. A previous randomized, double blinded, placebo-controlled trial has demonstrated low toxicity of racotumomab and a statistically significant benefit in overall survival (OS) in patients with advanced non-small-cell lung cancer (NSCLC) who had achieved partial or complete response or disease stabilization after first line therapy. **Methods:** An open, non-randomized study was performed to evaluate if racotumomab could also be beneficial in patients with progressive disease. Patients with recurrent and advanced stages (IIIB/IV) of NSCLC, in progression after completion of first-line onco-specific treatment as per the NCCN Oncology Therapeutic Guidelines (surgery, chemotherapy and/or radiotherapy) were included in the study. Most of them had received 4 to 6 cycles of cisplatin/vinblastin. Vaccination consisted of 5 intradermic doses of racotumomab (1 every 14 days), followed by 1 dose every 28 days until patient refusal or worsening of ECOG status. The patients did not receive second-line therapy. **Results:** 180 patients were included in an intent to treat (ITT) survival analysis (Kaplan Meier estimate), after at least 10 months of follow-up. Median survival was 8.06 months. OS rate (%) at 24 months was 21%. A control group of 85 consecutive patients treated at the same institution by the same investigators, who did not receive second-line therapy or racotumomab showed a median survival of 6.26 months (log rank test $p = 0.011$). OS rate (%) at 24 months was only 7%. A per protocol survival analysis including only the 124 patients (68.8%) who received ≥ 5 doses of racotumomab showed a median survival of 12 months. OS rate (%) at 24 months was 30%. **Conclusions:** Patients with PD after first-line treatment show favorable results in survival when vaccinated with racotumomab. This result is similar to previous clinical trials where racotumomab was administered to patients with objective response (partial or complete) or stable disease after first line therapy.

3087

General Poster Session (Board #19F), Mon, 8:00 AM-11:45 AM

Effect of an adjuvant breast cancer vaccine on disease-specific survival of breast cancer patients with depressed lymphocyte immunity.

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Background: Breast cancer patients were vaccinated in the adjuvant setting with an autologous, allogeneic whole cell vaccine to evaluate the effect on host lymphocyte immunity and disease-specific survival. **Methods:** We began preparing whole cell preparations for a vaccine study in 1995. Stage I and II breast cancer patients had host lymphocyte immunity against tumors associated antigens evaluated before and after vaccination. Those patients with depressed immunity, determined by a lymphocyte blastogenesis assay (LBA), were offered the whole cell vaccine. Patients were given six intradermal injections (three weekly followed by three monthly). Ten weeks after the last injection the LBA was repeated. Thirty-seven patients were vaccinated in the adjuvant setting with the whole cell autologous, allogeneic vaccine. **Results:** The vaccine was well tolerated with no severe toxicities. Some patients experienced slight pain and swelling at the injection site and slight chills and fever. The vaccinated patients had a mean follow-up of 12.7 years with mean follow-up of 8.9 and 9.2 years for the patients with normal and depressed immunity, respectively, in the historic control. The 10-year survival was 95% (20 of 21 patients) in the normal immunity historic control, 59% (33 of 56 patients) in the depressed immunity historic control and 89% (33 of 37 patients) in the patients with depressed immunity that were vaccinated in the present clinical trial. **Conclusions:** The disease-specific survival of the vaccinated patients with depressed immunity in this trial is significantly greater than that of the historic controls of unvaccinated patients with depressed immunity to their tumor associated antigens. This study confirms the importance of maintaining good host lymphocyte immunity after completion of standard therapy and validates the value of cancer immunotherapy in the adjuvant setting.

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General Poster Session (Board #19G), Mon, 8:00 AM-11:45 AM

A phase II study of intratumoral administration of the autologous immature dendritic cell (DC) vaccine used after lesion photodynamic therapy (PhDT) and immunomodulation with cyclophosphamide (Cy) in pretreated patients with disseminated malignant melanoma.

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Background: Immature DC can be loaded in vivo with tumor-specific antigens after tissue destruction and induce specific immune response even in pretreated patients with unfavorable prognosis. We assess clinical efficacy of this approach in melanoma patients after at least 1 line of drug therapy in metastatic setting. **Methods:** Totally, 14 pts were enrolled: 11 men, 3 women. Stage M1a in 4 (28%) pts, M1b in 1 (7%) and M1c was in 9 (64%) pts. Patient received Cy 300 mg IM at D0, photoditasine (PH) at D3, PhDT therapy on injectable lesion 2 hrs after PH (662 nm, 300 J). The immature DC derived from peripheral blood stem-cells were injected in irradiated lesion 6 hrs after photodynamic therapy at D3 and daily until D7 of the 21-day cycle. Vaccine dose was $60-100 \times 10^6$ cells. Toxicity was measured by CTC AE v4, efficacy by RECIST 1.0. Primary endpoint was clinical benefit rate (CBR), secondary – overall survival (OS), response rate (RR), time to progression (TTP) and toxicity. **Results:** 14 pts were evaluable for toxicity, 13 for response. 37 cycles of therapy were performed. 2 patients achieved PR lasting 393 and 218+ days. 5 patients were stable for 33+, 108, 113, 168 and 239 days. RR was 15 (95% CI 2-39)%, CBR – 54 (95% CI 27-81)%. Median OS was 334 days, TTP – 113 days. RR was well correlated with OS ($p=0,014$). No SAE or AE of 4th degree occurred. The only grade 3 toxicity was fever. All grade 1-2 AEs were immune-related: fever (the most frequent AE), pain in the lesions, flu-like reactions, myalgia as well as high serum ALT, AST and bilirubin in patients with liver metastases. **Conclusions:** Thus, using the described approach for immunotherapy of melanoma has clinical efficacy and worth further developing.

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General Poster Session (Board #19H), Mon, 8:00 AM-11:45 AM

A phase I study of an MVA vaccine targeting p53 in cancer.

Vincent M. Chung, Nicola Hardwick, Joshua D.I. Ellenhorn, Jonathan R. Espenschied, Dean Lim, Peiguo Chu, Dajun Qian, Joseph Kim, Joseph Chao, Marwan Fakih, Yun Yen, Don J. Diamond; City of Hope, Duarte, CA; Cedars-Sinai Medical Center, Los Angeles, CA; City of Hope Cancer Center/Beckman Research Institute, Duarte, CA; City of Hope National Medical Center, Duarte, CA

Background: Despite chemotherapy, survival for most patients with metastatic gastrointestinal cancer is <2 years. These dismal statistics reflect lack of effective therapy. We are conducting a first-in-human trial of modified vaccinia Ankara (MVA), an attenuated viral vaccine that targets wild-type (wt) p53. This approach applies to the majority of p53-involved tumors, since the bulk of the p53 sequence is identical, except for individual point mutations. Our phase I study evaluates the safety and tolerability of the vaccine. **Methods:** Patients with metastatic colon, gastric and pancreas cancer that failed standard treatment were eligible for vaccination if >10% of the cells stained strongly positive for p53. A standard 3+3 design was employed and patients were accrued to either the 10^8 pfu or 5.6×10^8 pfu dose levels. A total of 3 injections were given, each spaced apart by 3 weeks and patients were evaluated for dose limiting toxicities. **Results:** Three patients were accrued to the 10^8 pfu dose level; 2 patients with colon cancer and 1 patient with pancreatic cancer. There were no dose limiting toxicities and the injection was well tolerated and only local irritation at the injection site was seen. The dose was escalated to 5.6×10^8 pfu; 3 patients were accrued, 1 with colon cancer and 2 with pancreatic cancer. There were no dose limiting toxicities observed in the 3 patients. Grade 2 toxicities that were at least possibly related to the vaccine include injection site reaction and fatigue seen in one patient each. One patient with pancreatic cancer had stable disease on CT after completion of the 3 injections. This dose level is being expanded to accrue an additional 6 patients to evaluate safety and immunogenicity using cytokine flow cytometry to characterize p53-specific CD4⁺ and CD8⁺ T cell response. Of the cell-surface markers employed, PD1 showed extraordinary strong expression prior to vaccine injection. All patients had a humoral response to the MVA backbone. **Conclusions:** Our MVA p53 vaccine is well tolerated with minimal grade 1-2 toxicities. The highest dose tested is 5.6×10^8 pfu and additional patients are being accrued to evaluate immunogenicity. Since many cancers have mutant p53, this is an attractive target and may potentially be used with many other cancers. Clinical trial information: NCT01191684.

3090

General Poster Session (Board #20A), Mon, 8:00 AM-11:45 AM

VXM01, an oral T-cell vaccine targeting the tumor vasculature: Results from a randomized, controlled, first-in-man study in pancreatic cancer patients.

Friedrich Hubertus Schmitz-Winnenthal, Lars Grenacher, Tobias Friedrich, Heinz Lubenau, Marco Springer, Klaus M. Breiner, Nicolas Hohmann, Walter E. Haefeli, Gerd Mikus, Juergen Weitz, Alexis Ulrich, Markus W. Büchler, Philip Knebel, Thomas Schmidt, Yingzi Ge, Andreas G. Niethammer, Philipp Beckhove; Clinic for General, Visceral and Transplantation Surgery, University of Heidelberg, Heidelberg, Germany; Diagnostic and Interventional Radiology University Heidelberg, Heidelberg, Germany; Vaximm GmbH, Mannheim, Germany; Vaximm AG, Basel, Switzerland; Clinical Pharmacology and Pharmacoepidemiology University of Heidelberg, Heidelberg, Germany; Translational Immunology Division, German Cancer Research Center, Heidelberg, Germany

Background: VXM01 is an orally available, bacterially transmitted DNA vaccine targeting VEGFR-2. Pre-clinically, VXM01 showed anti-tumor activity in multiple tumor types. This first-in-human study was designed to evaluate the safety and tolerability of VXM01. Secondary endpoints included VEGFR-2 specific T-cell responses, tumor perfusion changes, and related biomarkers. **Methods:** A randomized, double-blinded, placebo-controlled, dose-escalation study was conducted in 45 patients with advanced pancreatic cancer. VXM01 or placebo was given on days 1, 3, 5, and 7. Doses were escalated from 10(6) CFU to 10(10) CFU over 5 dose groups, each including 6 VXM01 and 3 placebo patients. VEGFR-2 specific T-cell activity was monitored by ELISpot and T(reg) specificity assays before, during and after the vaccination course. Tumor perfusion was assessed by DCE-MRI on days 0 and 38. Biomarkers included CA19-9, VEGF-A and collagen IV. **Results:** Patients were enrolled from 12/2011 to 10/2012. Most commonly observed AEs were leukopenia, abdominal pain, and diarrhea, which were all equally distributed between treatment and placebo group. While a mild elevation in average blood pressure was observed in the VXM01 group over the placebo group, the hypertension adverse event rate did not differ between both groups. No DLTs were observed. VEGFR-2 specific effector T-cell response was increased in 57% of evaluable VXM01 treated patients, during and after the vaccination course. In 25% of the VXM01 group, the T-cell response score post-vaccination was higher than maximum placebo levels. In contrast, VEGFR-2 specific T(reg) responses were overall reduced in vaccinated patients. DCE-MRI data indicated a >33% drop in K(trans)/tumor perfusion in 35% of evaluable VXM01 treated patients vs. 10% in the placebo group. Mean changes were -4% (VXM01) and +15% (placebo). Reduced tumor perfusion correlated with VEGFR-2 specific T-cell responses and biomarker responses. **Conclusions:** VXM01 appeared safe and was well tolerated without DLTs across 5 tested dose levels. The data suggest further that VXM01 induces and enhances a VEGFR-2 specific T-cell response and impacts tumor perfusion. Clinical trial information: ISRCTN68809279.

3091

General Poster Session (Board #20B), Mon, 8:00 AM-11:45 AM

A phase I/II randomized trial using GM-CSF-producing and CD40L-expressing bystander cell line (GM.CD40L) vaccine in combination with CCL21 in stage IV lung adenocarcinoma: Preliminary results.

Jhanelle Elaine Gray, Alberto Chiappori, Charles C. Williams, Mary Colleen Pinder, Eric B. Haura, Jongphil Kim, Germaine Gonzalez, David Noyes, Scott J. Antonia; H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; Moffitt Cancer Center and Research Institute, Tampa, FL

Background: Our GM.CD40L vaccine (an allogeneic tumor cell-based vaccine generated from human bystander cell line) recruits and activates dendritic cells, which then migrate to regional lymph nodes, where T cell activation occurs, leading to systemic tumor cell killing. The CCL21 chemokine helps to recruit T cells and leads to enhanced T cell responses. The GM.CD40L.CCL21 combination has demonstrated additive effects in NSCLC mouse models. **Methods:** We initiated a phase I/II randomized study to evaluate GM.CD40L (Arm A) vs. GM.CD40L.CCL21 (Arm B) in patients with lung adenocarcinoma who had failed first-line therapy. Primary endpoints were safety and tolerability of Arm B in phase I and progression-free survival (PFS) in phase II; secondary endpoints included anti-tumor immune responses/T-cell responses by ELISpot assay on PBMC. Immune-related response criteria as determined by the investigator served to determine discontinuation from study treatment. Intradermal vaccines were administered every 14 days for 3 doses and then monthly X3. A two-stage minimax design was used. **Results:** In phase I, 3 patients received GM.CD40L.CCL21; no dose-limiting toxicities occurred. Between 4/2012 and 12/2012, Arm A enrolled 11 and Arm B enrolled 16 patients, including those in phase I (median age: 70/67.5 years, females: 45.5%/37.5%, PS1: 54.5%/75%, median prior regimens: 3/5 for Arm A vs. Arm B, respectively). Most common toxicities for Arm A vs. Arm B were injection site reaction (45.5%/43.8%), fatigue (9.1%/37.5%), anorexia (0%/12.5%), and pain in extremity (0%/12.5%). Median PFS for Arm A vs. B was 4.4 vs. 4.4 months ($p=0.37$). Of the 6 patients who remained on study post RECIST v1.1 progression, all demonstrated further progression on subsequent scans and were removed from the study. Of patients evaluable for efficacy, stable disease was 3/7 and progressive disease was 6/7 for Arm A vs. Arm B, respectively. Analyses of ELISpot assay on the PBMC are underway. **Conclusions:** GM.CD40L.CCL21 vaccine is well tolerated; thus far, median PFS results are similar to GM.CD40L vaccine. Updated results of the phase II trial will be presented. Clinical trial information: NCT01433172.

3092

General Poster Session (Board #20C), Mon, 8:00 AM-11:45 AM

Systematic approach for development of new immunotherapeutics for lung cancers through cancer genomics analysis.

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Background: Oncoantigens are defined to be proteins that are very specifically expressed in cancer cells and that have the oncogenic activity and high immunogenicity, and are considered to be promising targets for immunotherapy such as therapeutic cancer vaccines. **Methods:** We have established a strategy as follows to identify new oncoantigens; i) screening of highly transactivated genes in the majority of 120 lung cancers using cDNA microarray representing 27,648 genes coupled with enrichment of tumor cells by laser microdissection, ii) verification of no expression of each candidate gene in normal tissues by northern-blot analysis, iii) validation of the clinicopathological significance of its high level of expression with tissue microarray containing 300 lung cancers, iv) verification of a critical role of each gene in the growth or invasiveness of cancer cells by RNAi and cell growth/invasion assays, v) screening of the epitope peptides recognized by HLA-A*0201- or A*2402-restricted cytotoxic T lymphocyte (CTL). We conducted phase I clinical trials of these therapeutic peptide vaccines for lung cancer patients. **Results:** We identified 35 oncoantigens and screened dozens of 10-amino-acid peptides, each of which corresponded to a part of TTK, LY6K, IMP-3, CDCA1, KIF20A, CDC45L, and FOXM1, and was a candidate to be presented on the surface of HLA-A*0201 or HLA-A*2402 that induced in vitro CTL response. Phase I clinical studies indicated that five epitope peptides could strongly induce the CTL activity in cancer patients. For example, we conducted a phase I study for HLA-A*2402-positive, advanced non-small cell lung cancer patients who failed to standard therapy, using the combination of 1, 2 or 3 mg/body of each peptides from LY6K, CDCA1, and KIF20A mixed with adjuvant once a week. This cancer vaccine therapy demonstrated tolerability and had very high immunogenicity of even 1 mg/body dose to induce antigen-specific CTLs in cancer patients. **Conclusions:** Through systematic genomics-based approach and clinical study, we have identified five epitope peptides, which could induce CTLs very effectively in cancer patients, and therefore it warrants further clinical studies. Clinical trial information: NCT01069575.

3093^A

General Poster Session (Board #20D), Mon, 8:00 AM-11:45 AM

Evaluation of cyclophosphamide as an immune enhancer for the NY-ESO-1/ISCOMATRIX vaccine in patients with metastatic melanoma.

Oliver Klein, Ian D. Davis, Grant A. McArthur, Andrew Mark Haydon, Phillip Parente, Nektaria Dimopoulos, Heather Jackson, Eugene Maraskovsky, Wendie Hopkins, Rodica Stan, Weisan Chen, Jonathan S. Cebon; Ludwig Institute for Cancer Research, Melbourne, Australia; Department of Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Australia; Alfred Hospital, Melbourne, Australia; Eastern Health Clinical School, Box Hill Hospital, Monash University, Melbourne, Australia; Ludwig Institute for Cancer Research, Heidelberg, Australia; CSL Limited, Parkville, Australia; Ludwig Institute for Cancer Research, Heidelberg, Australia

Background: We have previously demonstrated potent immunogenicity of the NY-ESO-1/ISCOMATRIX vaccine in patients with resected melanoma; however the same vaccine induced only a few vaccine antigen specific immune responses in patients with advanced disease. Therefore, we have enrolled a second cohort of patients with advanced melanoma in the clinical trial LUD2002-013 to investigate whether pre-treatment with the immune-modulator cyclophosphamide could improve the immunogenicity of the NY-ESO-1/ISCOMATRIX vaccine. **Methods:** LUD2002-013 was an open-label phase II study intended to evaluate the safety and immunogenicity of the NY-ESO-1/ISCOMATRIX vaccine in patients with advanced melanoma. The first cohort of patients received vaccine alone; a second cohort with 19 patients was added after evaluation of responses in Cohort 1 and received vaccine in combination with low-dose cyclophosphamide. Patients received 3 injections of NY-ESO-1 ISCOMATRIX preceded, in Cohort 2, by cyclophosphamide at a dose of 300 mg/m² every four weeks. Assessment of clinical and immunological responses was undertaken at week 11. **Results:** Fifteen patients of Cohort 2 completed at least one cycle of vaccination. No objective responses were observed with three patients having stable disease for more than three months. The inclusion of cyclophosphamide into the vaccination protocol did not lead to any significant toxicity. Seven of fourteen patients in Cohort 2 developed a vaccine induced NY-ESO-1 specific CD4 T cell response, a significant increase compared to cohort 1 ($p=0.019$). No differences were observed in the frequency of vaccine induced antibody or CD8 T cell responses. No change in the frequency of peripheral blood regulatory T cells or myeloid derived suppressor cells was detected. **Conclusions:** The administration of low dose cyclophosphamide has significantly increased the NY-ESO-1 specific CD4 T cell response of the NY-ESO-1/ISCOMATRIX vaccine in patients with metastatic melanoma. Given the emerging importance of CD4 T cells in tumour regression, the present findings warrant further clinical exploration of combining cyclophosphamide with vaccines and other immune-modulatory agents. Clinical trial information: NCT00518206.

3094

General Poster Session (Board #20E), Mon, 8:00 AM-11:45 AM

NfP2X₇, a novel target for immune therapeutic approaches in cancer treatment.

Jan Nesselhut, Julian Barden, Dagmar Marx, Nicole Cillien, Wiebke Goebel, Michael Herrmann, Raymond Y. Chang, Thomas Nesselhut; Praxisgemeinschaft fuer Zelltherapie, Duderstadt, Germany; Biosceptre International, Sydney, Australia; Meridian Medical Group, New York, NY

Background: P2X₇ receptor, a ligand-gated channel, is activated by extracellular ATP and mediates ATP induced apoptosis. Several polymorphisms leading to loss of function of P2X₇ have been described. Overexpression of non-functioning P2X₇ receptor (nfP2X₇), able to form a Ca²⁺ channel but not an apoptotic pore, is found on every type of cancer cell but no normal cells, making it a perfectly selective target for immune therapy approaches. **Methods:** Samples from various tumor types were analyzed for the expression of nfP2X₇ using immunohistochemistry. MoDC from n=6 patients with advanced metastatic sarcoma (2 osteosarcoma, 4 soft tissue sarcoma) with disease progression after failure of extensive standard therapies were primed against the nfP2X₇ using a specific peptide. MoDC were harvested, analyzed by flow cytometry and applied to the patient intradermally. Specific T-cell response was analyzed by IFN-gamma Elispot. **Results:** nfP2X₇ is overexpressed in essentially all analyzed tumor samples from all cell types: epithelial, mesenchymal, neural, germinal examined in large numbers (n = 5 to >1,000). The uniquely displayed epitope associated with the ATP binding site exposed on nfP2X₇ but hidden in P2X₇ was found on the cancer cell surface but not on the surface of any normal cells. MoDC primed with a specific peptide against nfP2X₇ show a distinct DC morphology and an up-regulation of the maturation marker CD83. Patients are 6-9 months (onset June 2012) under treatment at time of abstract submission. 2 patients have a disease stabilization. One further patient with a metastatic, rapidly growing osteosarcoma showed a mixed response. IFN-gamma Elispot, which was exemplarily performed, shows a nfP2X₇-specific T-cell response. **Conclusions:** Efficient inductions of an antitumor response by dendritic cell therapy require the definition and choice of an optimal target, being expressed in almost all kinds of tumors. nfP2X₇, a set of non-functioning forms of the P2X₇ receptor unable to initiate apoptosis but maintaining channel activity, could therefore be an optimal universal candidate. The efficacy of dendritic-cell based therapy may be improved by priming the MoDC against nfP2X₇ and thus may improve the clinical outcome.

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General Poster Session (Board #20F), Mon, 8:00 AM-11:45 AM

Booster inoculations of the AE37 peptide vaccine enhance immunological responses in a phase II study.

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Background: We are conducting a multicenter randomized phase II trial of AE37, the Ii-Key hybrid peptide of HER2 776-790 (AE36). The purpose of the study is to determine if the AE37 vaccine can prevent recurrence in disease-free conventionally treated node-positive (NP) and high-risk node-negative (NN) breast cancer patients at significant risk for recurrence. Since clinical efficacy is anticipated to occur as the result of long lasting memory immune responses induced by vaccination, repeated booster inoculations were scheduled as part of the trial. Here we present data on immune responses in patients who received boosters up to 24 months after completion of the primary vaccination series (PVS). **Methods:** The trial is enrolling NP or high-risk NN patients with any degree of HER2 expression (IHC 1-3+ or FISH > 1.2) rendered disease-free following standard of care therapy. The vaccine group (VG) received AE37+GM-CSF and control group (CG) GM-CSF alone in 6 monthly i.d. inoculations followed by boosters administered every 6 months x 4. Immunologic responses were assessed in vivo by dermal reactions at the inoculation site, and in vitro, against the AE36 peptide, with proliferation and IFN- γ ELISPOT assays. **Results:** 25 patients in the VG and 23 in the CG have completed their boosters. After the last booster (BRC24), 100%, 54% and 54% in the VG (vs. 9%, 18% and 27% in the CG) responded by dermal reaction, proliferation and IFN- γ ELISPOT, respectively. Mean dermal reactions (orthogonal mean in mm) in vaccinated patients was 25.9 ± 3.13 at completion of the PVS (R6) and increased to 35.47 ± 4.35 at BRC24 ($p=0.01$). VG patients increased their proliferation response (stimulation index, SI) to AE36 from 0.97 ± 0.046 at baseline (R0) before vaccination to 2.27 ± 0.57 at R6 ($p=0.0003$) which was maintained until BRC24 (SI 2.21 ± 0.33 , $p<0.0001$). The number of IFN- γ specific spots/ 10^6 PBMC increased from 26.88 ± 12.36 at R0 to 40.35 ± 17.02 ($p=0.07$) at R6, up to 62 ± 16.82 ($p=0.0076$) at BRC24. **Conclusions:** Our data demonstrate that AE37 vaccine boosters enhance the immune responses against HER elicited during the PVS, thus sustaining long lasting immunity, a prerequisite for possible clinical efficacy which is currently being evaluated. Clinical trial information: NCT00524277.

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General Poster Session (Board #20G), Mon, 8:00 AM-11:45 AM

Safety and efficacy of the HER2-derived GP2 peptide vaccine in combination with trastuzumab for breast cancer patients in the adjuvant setting.

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Background: GP2 is a 9 amino acid HLA-A2/A3 restricted HER2-derived peptide. GP2 + GM-CSF has been shown to be safe and effective in eliciting anti-HER2 immune response in breast cancer patients. Preclinical data has demonstrated that pretreatment of cells with trastuzumab (Tz) enhances susceptibility to lysis by GP2-specific cytotoxic T lymphocytes (CTLs). We conducted a phase Ib study to evaluate the combination of the GP2 vaccine and Tz. **Methods:** HLA-A2/A3 + patients with HER2 overexpressing breast cancer receiving Tz as standard therapy were enrolled. The study was designed as a 3+3 dose escalation trial with an expansion cohort evaluating 4 dose levels of the vaccine administered as 6 inoculations given every 3 weeks in combination with Tz (6mg/kg). Toxicity was graded 48-72 hr post vaccination using NCI Toxicity Criteria. Ejection fraction (EF) was monitored every 3 mo. Immunologic response was assessed in vivo by injection site local reaction (LR) and in vitro by quantifying the number of GP2-specific CTLs by HLA-A2: IgG dimer assays and their functional activity by ELISPOT. **Results:** 19 patients enrolled (median age 47 yr, mean tumor size 3.4 cm, 74% were grade 3, 53% ER/PR+, 63% node positive, 74% received anthracycline based therapy). Maximum local toxicities were grade 1 (77% of patients) and grade 2 (6%), and maximum systemic toxicities were grade 1 (24%) and grade 2 (5%). There were no grade 3 or 4 local or systemic toxicities. There was no significant change in EF at 3 mo ($57 \pm 1\%$, $p=0.23$) or 6 mo ($59 \pm 1\%$, $p=0.8$) compared to baseline ($58 \pm 0.9\%$). Mean post-vaccine series LR was significantly larger than initial vaccination LR (68.2 ± 8.6 mm vs 28.0 ± 10.3 mm, $p=0.0004$). In vitro assays demonstrated an increase in the maximal number of post- versus pre-vaccination GP2-specific CTLs by dimer assay (1.45 ± 0.19 vs $0.96 \pm 0.19\%$, $p=0.06$) and increased ELISPOT activity [median 86 range (3-194) vs 34 (range 0-295) spots/ 10^6 cells]. **Conclusions:** GP2 vaccine in combination with Tz is both safe and immunogenic in HER2-overexpressing breast cancer patients in the adjuvant setting. Toxicity was limited to mild local and systemic reactions. There were no dose limiting toxicities or cardiac events.

3097

General Poster Session (Board #20H), Mon, 8:00 AM-11:45 AM

Risk factors for development of delayed urticarial reactions in the phase II trial of HER2 peptide vaccines plus GM-CSF versus GM-CSF alone in high-risk breast cancer patients to prevent recurrence.

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Background: We are monitoring the incidence of delayed urticarial reactions (DURs) in our phase II trial evaluating adjuvant HER2-specific vaccines (AE37 and GP2) for the prevention of breast cancer recurrence. Here, we characterize DURs and analyze risk factors for their development. **Methods:** After completion of standard of care therapy, disease-free node-positive or high-risk node-negative patients (pts) were randomized to receive either a peptide+GM-CSF (VG) or GM-CSF (CG). Pts receive 6 monthly intradermal inoculations during the primary vaccine series (PVS) then four boosters (B) every 6 mos. Immune response is measured by delayed type hypersensitivity (DTH) pre- (R0) and post-PVS (R6) and local reaction (LR) at R1 – R6. **Results:** Twenty-four (6.1%) of 393 initiated patients report a DUR; 13 VG (vDUR), and 11 CG (cDUR); vDUR - 9 AE37, 4 GP2. Time to onset of symptoms is 9 ± 5 days (d) and is similar in vDUR/cDUR ($p = 0.27$). DURs manifest as hives/pruritis in all patients. Average duration of symptoms is $32.6 \text{ d} \pm 8.8 \text{ d}$ (no difference in vDUR/cDUR [$p = 0.23$]). Episodes have resolved with antihistamines or IV/oral steroids. Ten (4 cDUR, 6 vDUR) patients have had recurrent episodes that have resolved similarly. 75% of first episodes occur between R6-B3. For DUR patients v. those who have not had a DUR (noDUR), there are no differences in demographics. DTH response is similar in vDUR pts v. noDUR VG pts (R0- $p = 0.34$; R6- $p=0.40$). cDUR pts had a greater DTH response v. CG noDUR pts at R6 ($13.2 \text{ v } 4.7 \text{ mm}$, $p=0.01$). LRs are greater in DUR pts compared to noDUR pts after the second vaccination (R2 – $66.2 \text{ v } 48.2 \text{ mm}$, $p=0.02$). LR for DUR pts decrease and are less than noDUR at R6 ($45.4 \text{ v } 57.4 \text{ mm}$, $p=0.09$). Relative risk for developing DUR for LR $> 100 \text{ mm}$ at R2 is 3.49 (1.58-7.68, 95% CI [$p=0.004$]). At 29.9 months median follow-up, there have been no recurrences in VG and CG DUR v. 75.9% DFS for noDUR ($p=0.05$). **Conclusions:** DURs occur infrequently and without long-term sequelae. Pts at risk for developing DUR are identified early in the vaccine series using LR. Robust immune response in DUR may explain the survival benefit demonstrated here. Clinical trial information: NCT00524277.

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General Poster Session (Board #21A), Mon, 8:00 AM-11:45 AM

Phase I/II clinical trial of a genetically modified and oncolytic vaccinia virus GL-ONC1 in patients with unresectable, chemotherapy-resistant peritoneal carcinomatosis.

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Background: For therapy-resistant peritoneal carcinomatosis (PC) viruses exhibiting oncolytic properties open up new perspectives. Our phase I/II study in patients with refractory PC (NCT01443260) is designed to assess the safety, MTD, and anti-tumor activity of GL-ONC1, a recombinant vaccinia virus (VACV) genetically engineered to selectively replicate in and destroy cancer cells. **Methods:** GL-ONC1 was administered intraperitoneally up to 4 times every 28 days under a standard 3+3 dose escalation design. Safety was assessed using CTCAEv4.0. Anti-tumor activity was determined by “fluid biopsies” obtained via repetitive paracenteses and by serial PET-CT scans. Patient samples were collected for pharmacokinetics, pharmacodynamics and viral shedding analysis. **Results:** Up to now, 4 patients have received 10 doses of GL-ONC1 ranging from 10^7 to 10^8 infectious viral particles per application. Adverse events have generally been limited to grade 1/2, being mostly transient flu-like symptoms as well as increased abdominal pain resulting from treatment-induced peritonitis. No DLT was reported. No viral shedding was observed. In one gastric cancer patient, effective intraperitoneal replication of GL-ONC1 was demonstrated for more than 3 weeks. Using either anti-EpCAM or anti-VACV specific antibodies, around 5% of all ascitic cells were found to be EpCAM-positive 3 days after treatment in this patient, and only around 5-10% of these cancer cells were VACV positive at the same time point. In contrast, 4 days later (i.e. 7 days after virotherapeutic treatment), less than 2% of all ascitic cells were still EpCAM-positive, and more than 90% of these cancer cells were VACV positive. Of note, VACV-positive cancer cells morphologically showed significant degenerative changes. **Conclusions:** Preliminary data demonstrate that GL-ONC1 is well tolerated when infused intraperitoneally. Importantly, a single intraperitoneal delivery of GL-ONC1 was found to be sufficient to cause a dramatic decline in the number of malignant cells in the ascitic fluid, suggesting that GL-ONC1 effectively removes tumor cells in the ascites of patients with PC. Clinical trial information: NCT01443260.

3099

General Poster Session (Board #21B), Mon, 8:00 AM-11:45 AM

A phase I trial of intratumoral administration of HF10 in patients with refractory superficial cancer: Immune correlates of virus injection.

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Background: HF10 is a spontaneously occurring, oncolytic, mutant Herpes Simplex Virus type 1 (HSV-1). Several deletions/insertions in its genome render it nonpathogenic. HF10 has been tested in solid tumors accessible for injection. **Methods:** We report correlative studies from an open label, non-randomized, multicenter, single dose escalation phase I study in patients with refractory superficial cancer. The study was a “3 + 3” design with 4-dose cohorts at escalating doses of HF10 (1×10^5 TCID₅₀/dose with incremental dose escalations up to 1×10^7 TCID₅₀/dose), which has been completed. Body fluids (qPCR), peripheral blood (flow cytometry) and serum (30-plex cytokine assay) were examined for viral levels, quantitative immune cell variation, and cytokines, respectively. **Results:** Seventeen patients were enrolled and 15 treated (9 H/N; 4 melanoma; 1 colon; 1 sarcoma). Best response was stable disease in six patients and progressive disease in nine patients. Three of the 15 patients had an adverse event possibly related to the study therapy. These AEs were grade 1 hypotension (1) and flu-like symptoms (2): typical of treatment with oncolytic viruses. qPCR analysis transiently revealed virus in the saliva of two patients (day 2 and day 22); viral clearance was achieved after 1 and 7 days respectively. Comparing the two highest and two lowest dose HF10 cohorts, CD8+PD1+ cells were decreased with increasing HF10 dose ($p=0.023$). Increased monocyte population (CD14+CD11c+) appeared to correlate with increased HF10 dose ($p=0.063$). IL-8 increased in all samples ($p=0.0078$ Wilcoxon Signed rank test) post injection. **Conclusions:** Single dose intratumoral injection was well tolerated with mild-drug related AEs and rapid viral clearance. Six patients achieved stable disease during the study period. There appears to be a generalized IL-8 related inflammatory response coincident with increased peripheral blood monocytes after HF10 administration. Decreased CD8+PD1+ cells may indicate a shift towards a non-exhausted, functional CTL phenotype. These results justify the currently accruing study of multiple administrations of HF10 at the highest administered dose. Clinical trial information: NCT01017185.

3100

General Poster Session (Board #21C), Mon, 8:00 AM-11:45 AM

Role of mesenchymal stem cells in delivering Newcastle disease virus to glioma cells and glioma stem cells and enhancing the oncolytic effect of the virus by secreting TRAIL.

Shimon Slavin, Gila Kazimirsky, Amotz Ziv-Av, Chaya Brodie; International Center for Cell Therapy and Cancer Immunotherapy, Tel Aviv, Israel; Mina & Everard Goodman Faculty of Life-Sciences, Bar-Ilan University, Ramat-Gan, Israel, Ramat-Gan, Israel; Bar Ilan University, Ramat-Gan, Israel; Henry Ford Hospital, Detroit, MI

Background: Newcastle disease virus (NDV), an avian paramyxovirus, is tumor selective and oncolytic by induction of apoptosis. Preclinical and clinical studies in patients with glioblastoma (GBM) using NDV demonstrated occasional clinical benefits with no major side effects. Limitations to the use of NDV as virotherapy of GBM is the inefficient delivery into cancer cells in the brain. **Methods:** Mesenchymal stromal cells (MSCs) can migrate towards cancer cells. We examined potential delivery of oncolytic effect of NDV (MTH-68/H) against glioma cell lines and glioma stem cells (GSCs) and the ability of MSCs to deliver NDV to glioma cells and GSCs in culture. **Results:** NDV induced a dose-dependent cell death in the glioma cells U87, A172 and U251 with maximal effects at 10 MOI. In contrast, we found only small level of apoptosis or changes in self-renewal in three GSCs infected with NDV. We found that MSCs derived from bone marrow, adipose tissue and cord were successfully infected by NDV and were able to deliver the virus to co-cultured glioma cells and GSCs. In addition, treatment of glioma cells and GSCs with culture supernatant of infected MSCs increase apoptosis of glioma cells as compared to the effect of direct infection of glioma cells. Moreover, the culture supernatants of the infected MSCs induced cell death in GSCs that were resistant to the oncolytic effect of NDV, suggesting that factor(s) secreted by the infected MSCs sensitized the glioma cells and GSCs to the cytotoxic effects of NDV. Using antibody array and ELISA we identified TRAIL as the factor secreted from infected MSCs. Indeed, treatment of infected glioma cells with TRAIL increased the cytotoxic effect of NDV and sensitized GSCs to the oncolytic effects of NDV. **Conclusions:** MSCs can be employed to deliver NDV to GBM. In addition, MSCs can also sensitize glioma cells and GSCs to oncolysis by NDV. Considering the resistance of GSCs to chemotherapy and radiation therapy, treatment of GBM with MSC-mediated targeted oncolytic NDV may provide a new clinical tool for treatment of GBM and eradication of GSCs.

3101

General Poster Session (Board #21D), Mon, 8:00 AM-11:45 AM

Activity of PU-H71, a novel HSP90 inhibitor, and bortezomib in Ewing sarcoma preclinical models.

Srikanth R. Ambati, Eloisi Caldas Lopes, Kohji Kosugi, Ullas Mony, Ahmet Zehir, Andre L. Moreira, Paul A. Meyers, Gabriela Chiosis, Malcolm A.S. Moore; Memorial Sloan-Kettering Cancer Center, New York, NY; Program in Molecular Pharmacology and Chemistry, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY

Background: Heat shock protein (HSP) 90 regulates the disposition and activity of a large number of deregulated proteins in Ewing sarcoma. We have shown pre-clinical efficacy of PU-H71, a novel HSP90 inhibitor developed at MSKCC, in Ewing sarcoma. Unfolded proteins as a result of HSP90 inhibition are degraded via the ubiquitin-proteasome pathway or the autophagy pathway. We investigated the effects of combined inhibition of HSP90 and the proteasome pathway. **Methods:** We studied the effects of PU-H71 and bortezomib alone and in combination on cell proliferation and viability in multiple Ewing cell lines, benign stromal cells and hematopoietic stem cells. We performed cell cycle analysis, clonogenic assay, immunoblot analysis, reverse phase protein array and in vivo experiments in NOD/SCID IL2R gamma null (NSG) mice using the A673 cell line transduced with GFP luciferase. Using A673 metastatic model in NSG mice, we investigated the disease burden when treated with PU-H71, bortezomib and in combination. **Results:** In vitro, PU-H71 and bortezomib treatment resulted in caspase mediated cell death in a dose-dependent and time-dependent manner. Using PU-H71 beads we showed that some of the critical proteins, including IGF1R, hTERT and EWS-FLI1, are stabilized by HSP90. Exposure to PU-H71 resulted in depletion of AKT, ERK, MYC, C-kit, IGF1R and EWS-FLI1. Combination index (CI)-Fa plots and normalized isobolograms indicated synergism between PU-H71 and bortezomib. We noted increased expression of cleaved PARP and cleaved caspase 3 when Ewing cell lines were exposed to a combination of PU-H71 and bortezomib. Based on PK studies we treated mice (4 groups) using vehicle, PU-H71 75mg/kg i.p three times/wk, bortezomib 0.8mg/kg i.v twice/wk or a combination of PU-H71 and bortezomib for 4 wk. Ewing xenografts were significantly inhibited when treated with combination of PU-H71 and bortezomib compared to vehicle or either drug alone. **Conclusions:** Both PU-H71 and bortezomib have single agent activity against Ewing sarcoma. However they exhibit synergism when combined in in vitro and in vivo models providing a strong rationale for clinical evaluation in Ewing sarcoma.

3102

General Poster Session (Board #21E), Mon, 8:00 AM-11:45 AM

Do ascorbic acid and viscum album improve PFS in NSCLC?

Fatima Zehra Raza, Smita Ranjan, Goetz H. Kloecker; University of Louisville, Louisville, KY; Brown Cancer Center, Louisville, KY; James Graham Brown Cancer Center, University of Louisville, Louisville, KY

Background: Intravenous vitamin C achieves 70 times higher plasma concentrations of ascorbate as a similar oral dose. Such pharmacologic concentrations (0.3-20mM) kill cancer cells but not normal cells, through extracellular H_2O_2 generation. Ascorbate treatment depleted ATP and induced autophagy in prostate cancer cells; gemcitabine with ascorbate resulted in 50% inhibition of growth in pancreatic tumor xenografts in mice models. A phase I/II study for pancreatic cancer patients is underway. Viscum album (mistletoe) is widely used in Europe as complementary therapy. Its cytotoxic and immunomodulatory effects are poorly understood. Additional treatment with subcutaneous mistletoe injections improved quality of life in gastric cancer patients in a study. **Methods:** We report the case of a 54 year old male smoker with adenocarcinoma of the lung (pT4N2M1a), EGFR/RAS/ALK negative. After 5 cycles of chemotherapy (carboplatin/taxol x 3, then carboplatin/pemetrexed x 2) scans showed continued progression. The patient made an informed decision to stop further chemotherapy and initiated complementary medical means, receiving high dose IV vitamin C (75 g, 2 x week) and subcutaneous mistletoe injections every other day, for the last seven months. **Results:** Six months into the non-standard therapy, a PET/CT shows a PR by RECIST and decreased FDG activity of the biopsy proven primary and metastatic site. The patient feels well, a year now after being diagnosed with advanced NSCLC. **Conclusions:** Whether the PR and the surprisingly long PFS is due to IV ascorbic acid and/or mistletoe is unknown. A review of the literature reveals reports of similar astonishing clinical results after treatment with either agent. A registry and biorepository of cases treated with alternative modalities may discover markers and biological mechanisms to explain these surprising reports.

3103

General Poster Session (Board #21F), Mon, 8:00 AM-11:45 AM

Economic analysis of pharmacokinetic (PK) studies in phase I clinical trials.

Badi Edmond El Osta, Gerald Steven Falchook, Diane Schaub, Harihara Subramanian, Maryam Daneshmand, Dina Harleaux, Daniel D. Karp; Department of Investigational Cancer Therapeutics, Phase I Clinical Trials Program, The University of Texas MD Anderson Cancer Center, Houston, TX; Office of Performance Improvement, The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX; Clinical & Translational Research Center (CTRC), The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Although PK evaluation is a prevalent and critical component of cancer drug development, optimization of the cost-value relationship of PK studies has not been evaluated. **Methods:** We reviewed 26 phase I protocols that were activated and performed in the Clinical & Translational Research Center at U.T. MD Anderson Cancer Center from 2010 to 2012. 23 protocols met the residual analysis criteria of a normal and independent distribution. We collected protocol-specific data including medication, route, frequency, mechanism of action, number of enrolled patients, number of PK time points, and PK charges. **Results:** All 23 protocols were funded by industry sponsors. During 2010-2012: the total number of patients was 560; the median number of patients enrolled per protocol at our site was 18 (range 3-84); the median number of total PK studies per patient performed on each protocol was 19.46 (4.11-36.92); the median charge of PK time points was \$42.33 (35.02-80.00); the median charge of all PK time points per patient on a protocol was \$813.00 (329.00-1676.00); the median number of total PK studies per patient on a protocol during cycle 1 was 15.20 (2.40-27.47); the median charge of PK studies during cycle 1 was \$43.24 (36.93-80). The number of PK time points and the amount of PK charges did not significantly correlate with monotherapy vs. combination regimens, oral vs. parenteral route, or small molecules vs. other (cytotoxic, vaccine, monoclonal antibodies). However, protocols (all cycles combined) with small molecules had a trend of lower PK time point median charges (41.97 vs. 52.67; $p=0.09$). Analysis of PKs in cycle 1 demonstrated that protocols with small molecules had a trend of higher PK time point median charges (61.33 vs. 42.38; $p=0.08$). **Conclusions:** The number and charges of PK studies was independent of the study drug or route. Future studies comparing budgeted vs. performed PKs and industry vs. investigator-initiated trials are warranted.

Systematic analysis of potential targets for immunotherapy in acute myeloid leukemia.

Christopher Simon Hourigan, Meghali Goswami, Nawal Alkharouf, Medha Bhagwat, Heidi May Sardon, Ann L Williams, J. Phillip McCoy, Sawa Ito, Stephen Anthony Strickland, Bipin N. Savani, James W Fraser, Hossein Sadrzadeh, Amir Tahmasb Fathi, Lu Qin, Allan Hess, B Douglas Smith, Judith E. Karp, A. John Barrett; Myeloid Malignancies Section, Hematology Branch, National Heart, Lung and Blood Institute, Bethesda, MD; Bioinformatics Program, National Institutes of Health Library, Bethesda, MD; Flow Cytometry Core Facility, National Heart, Lung and Blood Institute, Bethesda, MD; Stem Cell Allogeneic Transplantation Section, Hematology Branch, National Heart, Lung and Blood Institute, Bethesda, MD; Vanderbilt University Medical Center, Nashville, TN; Massachusetts General Hospital, Boston, MA; Center for Leukemia and the Bone Marrow Transplant Unit, Division of Hematology/Oncology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD

Background: The ability to target myeloid malignancies using immunotherapy, without allogeneic transplantation, depends on the capability to target leukemic clones while sparing normal tissues. A variety of putative leukemia associated antigens (LAA) have been identified but an evidence-based list of targets for acute myeloid leukemia (AML) has not yet been established. **Methods:** De-identified, clinically annotated, samples of peripheral blood and/or bone marrow aspirate from untreated AML patients were collected under IRB-approved protocols from three NCCN cancer centers. Samples were analyzed for commonly observed somatic mutations in ASXL1, DNMT3A, FLT3, IDH1/2, KIT, NPM1, NRAS, RUNX1, TET2, and WT1. Gene expression of 75 consensus LAAs were determined using a custom-designed RT-PCR array. 12 samples underwent extended LAA analysis by flow sorting into “bulk leukemia” and “stem cell enriched” populations. LAA expression was normalized using the geometric mean of three control genes. **Results:** Samples from 48 AML patients (30 blood, 22 marrow) were suitable for analysis. Average age of patients was 53 (24-86), 50% were female. Cytogenetics were favorable (17%), intermediate (65%) or adverse (19%); 29% presented with a white blood cell count >50,000. Over 10,000 individual data-points were collected. Five distinct patterns of LAA expression were observed in blood from AML patients compared to healthy donors. **Conclusions:** Understanding the heterogeneity and patterns of AML LAA expression between individuals allows the rational prioritization of potential targets for immunotherapy. Based on our data we predict that targeting any single LAA will likely often be ineffective but that it may be possible to create an inclusive panel of multiple targets with coverage of most AMLs, eliminating the need for individualized personalization of therapy. Such AML antigen signatures may also have utility for minimal residual disease monitoring.

1) Common, often highly overexpressed	Cyclin A1, WT1
2) Common, rarely highly overexpressed	BAALC, PR3
3) Rare, but highly overexpressed	PRAME, MSLN, MECOM
4) Rare, and rarely highly overexpressed	Survivin, RHAMM
5) Not overexpressed	AURKA, EXOSC5, MAGE A1/A3/C1, NUDCD1, RPSA, SSX2IP

TPS3105[^]

General Poster Session (Board #21H), Mon, 8:00 AM-11:45 AM

Clinical and preclinical activity of SL-401, a targeted therapy directed to the interleukin-3 receptor on cancer stem cells and tumor bulk of hematologic malignancies, against blastic plasmacytoid dendritic cell neoplasm (BPDCN).

Arthur E. Frankel, Jung Hee Woo, Jeremy Preston Mauldin, Hetty Eileen Carraway, Olga Frankfurt, Stephen J. Forman, Christopher Brooks, Ivan Bergstein, Thomas Cirrito, Eric K. Rowinsky; University of Texas Southwestern, Dallas, TX; Scott & White Cancer Research Institute, Temple, TX; Johns Hopkins School of Medicine, Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD; Northwestern University Department of Medicine Division of Hematology-Oncology, Chicago, IL; City of Hope, Duarte, CA; Stemline Therapeutics, Inc., New York, NY

Background: Blastic Plasmacytoid Dendritic Cell Neoplasm (BPDCN), a rare and aggressive dendritic cell-derived hematologic malignancy that typically involves the skin and invariably progresses to a leukemic phase, has a dismal prognosis with a median survival of approximately 14 months. Since BPDCN cells express high levels of the interleukin-3 receptor (IL-3R), SL-401, a novel targeted therapy directed to IL-3R, is being developed to treat BPDCN and other IL-3R-expressing hematologic malignancies. SL-401 is a recombinant biologic comprised of IL-3 conjugated to a truncated diphtheria toxin, a potent inhibitor of protein synthesis (Frankel et al, *Prot Eng* 13, 575, 2000). SL-401 is cytotoxic in vitro to IL-3R-expressing leukemia blasts (Frankel et al, *Leukemia* 14, 576, 2000) and inhibits tumor growth in vivo (Black et al, *Leukemia* 17, 155, 2003). Recently, SL-401 demonstrated ultra-high anti-tumor potency against BPDCN cells in the femtomolar (10^{-15} M) range (Angelot-Delettre et al, *Blood* 118 Suppl 2588, 2011). **Methods:** In a Phase I/II trial of SL-401 in patients with IL-3R-expressing advanced hematologic malignancies, 4 patients with heavily pretreated BPDCN received a single cycle of SL-401 as a 15-minute infusion daily for 5 days. Results: All patients had CD4+/CD56+/CD123+ (IL-3Ralpha) expressing blasts and had failed previous combination chemotherapy regimens and allogeneic bone marrow transplantation. There were no serious adverse events. Three patients treated with SL-401 at 12.5 μ g/kg/day (the planned pivotal Phase IIb trial dose) experienced complete responses (CRs). The CRs included disappearance of BPDCN in the skin, bone marrow, peripheral blood, spleen and lymph nodes. CR durations are 5, 3+, and 1+ months to date. Conclusions: Given these robust clinical responses, as well as the mechanistic rationale for SL-401 in BPDCN, additional BPDCN patients are being evaluated in the study and a pivotal Phase IIb multi-cycle trial in this ultra-orphan indication is being planned. Clinical trial information: NCT00397579.

TPS3106

General Poster Session (Board #22A), Mon, 8:00 AM-11:45 AM

A phase I study of lirilumab (BMS-986015), an anti-KIR monoclonal antibody, administered in combination with ipilimumab, an anti-CTLA4 monoclonal antibody, in patients (Pts) with select advanced solid tumors.

Naiyer A. Rizvi, Jeffrey R. Infante, Geoffrey Thomas Gibney, Erin Marie Bertino, Sarah A. Cooley, Kiki Lekatis, Jon M. Wigginton, Andres A. Gutierrez, Ashok Kumar Gupta, Su Young Kim, F. Stephen Hodi; Memorial Sloan-Kettering Cancer Center, New York, NY; SCRI/Tennessee Oncology, PLLC, Nashville, TN; Moffitt Cancer Center, Tampa, FL; The Ohio State University Wexner Medical Center, Columbus, OH; University of Minnesota, Minneapolis, MN; Bristol-Myers Squibb, Princeton, NJ; Dana-Farber Cancer Institute, Boston, MA

Background: Immune checkpoint blockade represents a novel form of cancer immunotherapy. Killer cell immunoglobulin-like receptors (KIR) and cytotoxic T lymphocyte antigen-4 (CTLA-4) are immune receptors that down-regulate NK and T cell activity, respectively. The anti-KIR antibody, lirilumab (BMS-986015), potentiates innate immunity by blocking signaling through inhibitory KIRs and has demonstrated modest side effects in a Phase I trial. The anti-CTLA-4 antibody, ipilimumab, potentiates adaptive immunity and has demonstrated improved overall survival in pts with advanced melanoma and preliminary evidence of clinical activity in Phase I and II trials. We hypothesized that coordinate modulation of innate and adaptive immunity by combining anti-KIR and anti-CTLA4 antibodies could achieve enhanced biologic and clinical activity compared to either agent alone. Here, we describe a Phase I study of lirilumab plus ipilimumab in pts with selected advanced solid tumors. **Methods:** This study will be performed in two parts and enroll approximately 150 pts. During dose escalation, pts with advanced melanoma, non-small cell lung cancer and castrate resistant prostate cancer, will be enrolled. During cohort expansion, 20 pts with each tumor type will be enrolled at the maximum tolerated dose (MTD), or the maximum administered dose, if no MTD is defined. The primary study objectives are to delineate the safety and tolerability, dose limiting toxicities, and MTD of this combination. Secondary objectives are to assess preliminary anti-tumor activity, pharmacokinetics, and immunogenicity of this combination in all pts, and the pharmacodynamic effects on tumor infiltrating lymphocytes in a cohort of melanoma pts. Exploratory objectives include a thorough assessment of the modulation of innate and adaptive immunity by this combination in peripheral blood and/or tumor specimens, and preliminary evaluation of the association of these changes with clinical outcome. Clinical trial registration number: NCT01750580 Clinical trial information: NCT01750580.

TPS3107

General Poster Session (Board #22B), Mon, 8:00 AM-11:45 AM

A phase I study of the safety, tolerability, pharmacokinetics, and immunoregulatory activity of urelumab (BMS-663513) in subjects with advanced and/or metastatic solid tumors and relapsed/refractory B-cell non-Hodgkin's lymphoma (B-NHL).

Ignacio Melero, Tara C. Gangadhar, Holbrook Edwin Kohrt, Neil Howard Segal, Theodore Logan, Walter John Urbani, F. Stephen Hodi, Patrick Alexander Ott, Jose Luis Perez-Gracia, Jedd D. Wolchok, Aadhar Shah, John F. Kurland, Lewis J. Cohen, Ronald Levy, Jon M. Wigginton, Stacie M. Goldberg; Clinica Universidad de Navarra and CIBEREHD, Pamplona, Spain; University of Pennsylvania, Philadelphia, PA; Stanford Cancer Institute, Stanford, CA; Memorial Sloan-Kettering Cancer Center, New York, NY; Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; Earle A. Chiles Research Institute-Providence Cancer Center, Portland, OR; Dana-Farber Cancer Institute, Boston, MA; Department of Medical Oncology, Clínica Universidad de Navarra, Pamplona, Spain; Bristol-Myers Squibb, Princeton, NJ; Stanford University, School of Medicine, Stanford, CA

Background: CD137 (4-1BB) is a costimulatory molecule that belongs to the TNF superfamily. It is upregulated on activated lymphocytes, NK cells and dendritic cells and plays an important role in the potentiation of antigen-specific immune responses in T-cell directed therapy as well as in antibody-dependent cell-mediated cytotoxicity. Urelumab is an agonistic antibody targeting CD137 which has demonstrated antitumor activity against a variety of cancers in pre-clinical and clinical studies. We describe a phase I study to investigate the clinical and biologic effects of treatment with urelumab in patients with advanced solid tumors and B-cell non-Hodgkin's lymphoma (B-NHL). **Methods:** This phase I study (n=70) will include dose escalation (Part 1) using a 6+9 design, cohort expansion (Part 2), and tumor-specific cohort expansion (Part 3). In Part 1, successive cohorts of pts with advanced solid tumors will be treated as follows: Cohort 1 (0.1 mg/kg q3weeks) and Cohort 2 (0.3 mg/kg q3weeks). In Part 2, both cohorts (1 +2) will expand to 20 patients with advanced solid tumors. In Part 3, additional tumor-specific cohorts with B-NHL, colorectal cancer, and head and neck cancer (10 subjects each) will be enrolled at the highest tolerated dose. The primary objective of this study is to evaluate the safety and to define the MTD of the respective doses of 0.1 and 0.3 mg/kg administered every 3 weeks with special attention to hepatic toxicity. Secondary objectives include assessment of the preliminary antitumor activity, pharmacokinetics, and immunogenicity. Exploratory objectives include investigation of the immunoregulatory activity in peripheral blood and paired tumor biopsy specimens and associations with clinical outcome. Part 1 (dose escalation) has been completed without any DLTs. Clinical trial information: NCT01471210.

TPS3108

General Poster Session (Board #22C), Mon, 8:00 AM-11:45 AM

A phase Ib, open-label, multicenter study of urelumab (BMS-663513) in combination with rituximab in subjects with relapsed/refractory B-cell malignancies.

Holbrook Edwin Kohrt, John E. Godwin, Izidore S. Lossos, Michael E. Williams, John Timmerman, Brian K. Link, Stacie M. Goldberg, Analia McGirr, John F. Kurland, Jon M. Wigginton, Lewis J. Cohen, Ronald Levy; Stanford Cancer Institute, Stanford, CA; Earle A. Chiles Research Institute, Portland, OR; Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL; University of Virginia Medical Center, Charlottesville, VA; University of California, Los Angeles, Los Angeles, CA; University of Iowa Hospitals and Clinics, Iowa City, IA; Bristol-Myers Squibb, Princeton, NJ; Stanford University, School of Medicine, Stanford, CA

Background: CD137 (4-1BB) is a costimulatory molecule that belongs to the TNF superfamily. It is upregulated on activated lymphocytes, NK cells and dendritic cells and plays an important role in the potentiation of antigen-specific immune responses as well as in antibody-dependent cell-mediated cytotoxicity (ADCC). Urelumab is an agonistic antibody targeting the CD137 receptor. Preclinical evidence has shown that there is modulation of CD137 expression on NK cells after exposure to rituximab. Anti-CD137 agonist monoclonal antibody has been shown to have single-agent anti-lymphoma activity and to potentiate the anti-lymphoma activity of rituximab through enhancing ADCC. We hypothesized that upregulation of CD137 on NK cells by rituximab followed by urelumab could afford a mechanism-based approach to achieve enhanced biologic and/or clinical activity compared to either single agent alone. Here we describe a phase Ib study to investigate the clinical and biologic effects of combined treatment with urelumab and rituximab in patients with relapsed/refractory B-cell malignancies. **Methods:** This phase I study (n=100) will include dose escalation (Part 1) using a 3+3+3 design and cohort expansion (Part 2). In Part 1, successive cohorts of patients with relapsed/refractory B-NHL will be treated as follows: Cohort 1 (0.1 mg/kg q3weeks) and Cohort 2 (0.3 mg/kg q3weeks) with both cohorts in combination with rituximab 375 mg/m² given weekly for the first 4 weeks of each 12 week cycle. In Part 2, cohorts of CLL (n=30), follicular lymphoma (FL) (n=30), and diffuse large B-cell lymphoma (DLBCL) (n=20) will be treated at the dose level found to be safe for the urelumab/rituximab combination. The primary objective of the study is to evaluate the safety and define a safe and effective dose of the urelumab/rituximab combination. Secondary objectives include assessment of the antitumor activity, pharmacokinetics, and immunogenicity. Exploratory objectives include investigation of the immunoregulatory activity of this combination in peripheral blood and paired tumor biopsy specimens and the association of these effects with clinical response/toxicity. Clinical trial information: NCT01775631.

TPS3109

General Poster Session (Board #22D), Mon, 8:00 AM-11:45 AM

Phase I dose escalation study of recombinant interleukin-21 (rIL-21, BMS-982470) in combination with ipilimumab (Ipi) in patients (pts) with advanced or metastatic melanoma (MM).

Shailender Bhatia, Brendan D. Curti, Michael S. Gordon, Jason Chesney, Theodore Logan, John A. Thompson, Nels Royer, Rachel Bittner, David Fontana, Joseph Grosso, Pamela L. Clemens, Lewis J. Cohen, Christoph Matthias Ahlers, Jon M. Wigginton, Patrick Hwu; University of Washington Medical Center/Fred Hutchinson Cancer Research Center/Seattle Cancer Care Alliance, Seattle, WA; Earle A. Chiles Research Institute, Portland, OR; Pinnacle Oncology Hematology, Scottsdale, AZ; University of Louisville, Louisville, KY; Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; Seattle Cancer Care Alliance, Seattle, WA; Bristol-Myers Squibb, Seattle, WA; Bristol-Myers Squibb, Princeton, NJ; The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Ipilimumab is a monoclonal anti-CTLA-4 antibody that promotes T-cell activation and has been approved for the treatment of pts with advanced melanoma. The cytokine rIL-21 is a T-cell and NK-cell growth factor that has also demonstrated antitumor activity in selected solid tumors including MM. We hypothesize that coordinated stimulation of T and/or NK cell function with rIL-21 in conjunction with T-cell checkpoint inhibitor blockade with Ipi will achieve enhanced biologic and clinical activity compared to either agent alone. Here we describe an ongoing phase Ib study to investigate the clinical and biologic effects of combined treatment with rIL-21 and Ipi in pts with MM. **Methods:** The phase I study includes dose escalation (Part 1) using a 6 + 6 design and cohort expansion (Part 2). In Part 1 (n=48), successive cohorts of pts with melanoma will be treated with rIL-21 in combination with Ipi as follows: Arm A, rIL-21 (10, 30, or 50 µg/kg daily x 5) + Ipi (3 or 10 mg/kg Q3W) in a 3 week cycle; or Arm B, rIL-21 (30, 100, or 150 µg/kg weekly) + Ipi (3 or 10 mg/kg Q3W) in a 3 week cycle. In Part 1, all subjects will receive an initial cycle with rIL-21 monotherapy (lead-in) for biomarker and PK assessment that will be the same as the dose of rIL-21 specified for the cohort. Four cycles of combination treatment will follow the lead-in with restaging evaluation after 4 combination cycles. Subjects with initial benefit are eligible for retreatment at progression. In Part 2, pts (n=25/arm) will be randomly assigned to one of 3 cohorts: Ipi monotherapy at 3 mg/kg Q3W or Ipi + weekly rIL-21 or Ipi + daily rIL-21 at the MTD determined for each schedule in Part 1. The primary objectives of this study are to evaluate the safety of rIL-21 + Ipi and to define the MTD of the respective rIL-21 + Ipi regimens. Secondary objectives include assessment of the preliminary antitumor activity, pharmacokinetics, and immunogenicity. Exploratory objectives include investigation of the immunologic effects of this combination in peripheral blood and paired tumor biopsy specimens, and the association of these effects with clinical outcome. Clinical trial information: NCT01489059.

TPS3110

General Poster Session (Board #22E), Mon, 8:00 AM-11:45 AM

A phase I dose-escalation and cohort expansion study of lirilumab (anti-KIR; BMS-986015) administered in combination with nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients (Pts) with advanced refractory solid tumors.

Rachel E. Sanborn, William Howard Sharfman, Neil Howard Segal, F. Stephen Hodi, Jedd D. Wolchok, Walter John Urba, Bernard A. Fox, Suzanne Louise Topalian, Drew M. Pardoll, Kelly L. Covello, Dan McDonald, Su Young Kim, Ashok Kumar Gupta, Jon M. Wigginton, Thomas Gajewski; Earle A. Chiles Research Institute and Providence Cancer Center, Portland, OR; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD; Memorial Sloan-Kettering Cancer Center, New York, NY; Dana-Farber Cancer Institute, Boston, MA; Bristol-Myers Squibb, Princeton, NJ; The University of Chicago Comprehensive Cancer Center, Chicago, IL

Background: Immune checkpoint blockade represents a novel form of cancer immunotherapy. Killer cell immunoglobulin-like receptor (KIR) and programmed death-1 (PD-1) are immune receptors that down-regulate NK and T cell activity, respectively. Lirilumab, an anti-KIR antibody that potentiates innate immunity, has demonstrated modest side effects in a phase I monotherapy trial. Nivolumab, a PD-1 receptor blocking antibody that potentiates adaptive immunity, has shown clinical activity with various solid tumors in phase I and II trials. We hypothesized that coordinate modulation of innate and adaptive immunity with anti-KIR and anti-PD-1 antibodies could achieve more favorable biologic and clinical activity than either agent alone. Here, we describe a phase I study of lirilumab plus nivolumab in pts with advanced solid tumors, the first collaborative clinical trial being conducted by the International Immuno-Oncology Network (II-ON). **Methods:** This study will be performed in two parts and enroll approximately 150 pts. During dose escalation, pts with any solid tumor, except primary central nervous system tumors, will be enrolled. During cohort expansion, pts (N=16/cohort) with non-small cell lung carcinoma – squamous and non-squamous histology, renal cell carcinoma, melanoma, colorectal carcinoma, or ovarian carcinoma will be enrolled at the maximum tolerated dose (MTD), or the maximum administered dose, if no MTD is defined. The primary study objectives are to delineate the safety and tolerability, dose limiting toxicities, and MTD of this combination. Secondary objectives are to assess preliminary anti-tumor activity, pharmacokinetics, and immunogenicity in all pts, and pharmacodynamic effect on tumor infiltrating lymphocyte subsets from melanoma pts. Exploratory objectives include a thorough assessment of innate and adaptive immunity modulation by this combination in peripheral blood and/or tumor specimens, as well as preliminary associations with clinical outcome. As of Feb 1, 2013, three pts have started therapy. Clinical trial registration number: NCT01714739. Clinical trial information: NCT01714739.

TPS3111

General Poster Session (Board #22F), Mon, 8:00 AM-11:45 AM

Phase I dose escalation study of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) in patients (pts) with advanced hepatocellular carcinoma (HCC) with or without chronic viral hepatitis.

Bruno Sangro, Todd S. Crocenzi, Theodore Hobart Welling, Mercedes Iñarrairaegui, Jesús Prieto, Carmen Fuertes, Laurie Delanty, William Feely, Jeffrey Anderson, Dennis M. Grasela, Jon M. Wigginton, Ashok Kumar Gupta, Ignacio Melero; Clinica Universidad de Navarra and CIBEREHD, Pamplona, Spain; Providence Cancer Center, Portland, OR; University of Michigan, Ann Arbor, MI; Bristol-Myers Squibb, Princeton, NJ

Background: Pts with advanced HCC have limited treatment options. Sorafenib, the current standard of care, achieves only modest overall survival improvements. There is a clear etiologic association between HCC and prior/concurrent hepatitis B (HBV) or C (HCV) infection. Programmed death-1 (PD-1) is an immune checkpoint receptor that inhibits T-cell activation when bound by ligands including PD-L1/L2. PD-L1 overexpression has been noted on HCC tumors, and PD-1/PD-L1 interaction may contribute to viral hepatitis induced T-cell exhaustion. Nivolumab, a PD-1 receptor blocking antibody, has shown efficacy against various solid tumor types in Ph 1 trials. We hypothesized that blockade of PD-1/PD-L1 interaction could enhance T-cell activation and mediate antitumor and/or antiviral activity in HCC pts. We describe a phase I, dose-escalation study of nivolumab in advanced HCC pts. **Methods:** Successive pt cohorts with histologically confirmed advanced HCC with/without HBV or HCV infection (N=72 max) will be treated on 3 distinct arms with IV nivolumab at 0.3, 1 and 3.0 mg/kg (uninfected or HCV-infected pts) or 0.1, 0.3, 1 and 3.0 mg/kg (HBV-infected pts) every 2 weeks using a 3+3 escalation scheme. Pts must have progressive disease or intolerance after ≥ 1 line of therapy or have refused sorafenib treatment, and a Child-Pugh class A. HBV-infected pts must be receiving antiviral therapy (viral DNA < 100 IU/mL) for ≥ 3 months. Pts with brain metastasis, encephalopathy, prior/current ascites requiring paracentesis, history of recent variceal bleeding, active coinfection with HIV, or both HBV and HCV, or concurrent hepatitis D and HBV infection will be excluded. Primary endpoints include characterization of safety, tolerability, dose-limiting toxicities and maximum tolerated dose of nivolumab. Secondary endpoints include assessment of the preliminary antitumor activity (per modified RECIST for HCC), PK and immunogenicity. Exploratory endpoints include evaluation of the relationship between tumor PD-L1 expression and clinical outcome, and nivolumab's antiviral and immunoregulatory activity in peripheral blood and/or tumor specimens. Clinical trial information: NCT01658878.

TPS3112

General Poster Session (Board #22G), Mon, 8:00 AM-11:45 AM

Phase I dose escalation study of recombinant interleukin-21 (rIL-21; BMS-982470) in combination with nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients (pts) with advanced or metastatic solid tumors.

Laura Quan Man Chow, Michael S. Gordon, Theodore F. Logan, Scott J. Antonia, Shailender Bhatia, John A. Thompson, Julie R. Brahmer, Gretchen Solberg, Rachel Bittner, David Fontana, Joseph Grosso, Lewis J. Cohen, Christoph Matthias Ahlers, Jon M. Wigginton, Charles G. Drake; University of Washington/Fred Hutchinson Cancer Research Center, Seattle, WA; Pinnacle Oncology Hematology, Scottsdale, AZ; Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD; Bristol-Myers Squibb, Princeton, NJ; The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD

Background: Programmed death-1 (PD-1) is an immune checkpoint receptor that attenuates T-cell activation by binding to its ligands, PD-L1 and PD-L2. Nivolumab, a PD-1 receptor blocking antibody, has shown durable antitumor activity in pts with various solid tumors in two phase I clinical trials. The cytokine rIL-21 has also shown antitumor activity in selected solid tumors. We hypothesized that combining rIL-21-induced stimulation of T-cell and NK-cell function in conjunction with T-cell checkpoint blockade using nivolumab could enhance biologic activity resulting in improved clinical outcomes, as compared with either agent alone. We describe a novel phase I study investigating the biologic activity and clinical outcomes of the combination in pts with advanced solid tumors. **Methods:** This ongoing study (N=160) includes a dose escalation phase (Part 1) using a 3 + 3 design followed by an expansion phase (Part 2). In Part 1 (N=60), successive pt cohorts with advanced solid tumors are being treated with escalating doses of rIL-21 (10, 30, 50, 75, or 100 μ g/kg IV) on two distinct schedules (Arms A and B) in combination with fixed-dose nivolumab (3 mg/kg q 2 weeks) in 6 week cycles. Arm A administers rIL-21 on a weekly schedule, given on day 1 in weeks 1–4 of the 6 week cycle. Arm B administers rIL-21 at 3x per week during weeks 1 and 3 of the 6 week cycle. In Part 2, pts with renal cell carcinoma (N=50) or non-small cell lung carcinoma (N=50) will each be randomized to treatment at the maximum tolerated dose (MTD) or maximum administered dose, if no MTD is determined, for Arm A or Arm B. Therapy for pts who are stable or responding in Parts 1 and 2 may be continued for up to 2 years or until treatment discontinuation criteria are met. Primary objectives are to evaluate the safety of rIL-21 + nivolumab, and to define the MTD of the 2 schedules. Secondary and exploratory objectives include a preliminary assessment of the antitumor activity, pharmacokinetics, immunoregulatory activity (peripheral blood, tumor) and immunogenicity of this combination. Clinical trial information: NCT01629758.

TPS3113

General Poster Session (Board #22H), Mon, 8:00 AM-11:45 AM

A phase I dose-escalation study evaluating the effects of nivolumab (anti-PD-1; BMS-936558; ONO-4538) in patients (pts) with select relapsed or refractory hematologic malignancies.

Alexander M. Lesokhin, Martin Gutierrez, Ahmad Sami Halwani, Stephen Maxted Ansell, Philippe Armand, Ivan Borrello, Zdenka E Segota, Adam D. Cohen, Moshe Talpaz, Deepika Cattray, Tracy Turner, Maria Mezes, Christina Hartman, Ashok Kumar Gupta, Su Young Kim, Jon M. Wigginton, John Timmerman; Memorial Sloan-Kettering Cancer Center and Weill Medical College of Cornell University, New York, NY; Hackensack University Medical Center, Hackensack, NJ; Huntsman Cancer Institute, Salt Lake City, UT; Mayo Clinic, Rochester, MN; Dana-Farber Cancer Institute, Boston, MA; Johns Hopkins University, Baltimore, MD; Holy Cross Medical Group, Fort Lauderdale, FL; Fox Chase Cancer Center, Philadelphia, PA; University of Michigan Comprehensive Cancer Center, Ann Arbor, MI; Bristol-Myers Squibb, Princeton, NJ; University of California, Los Angeles, Los Angeles, CA

Background: Programmed death-1 (PD-1) is an immune checkpoint receptor that inhibits T-cell activation upon interaction with its ligands PD-L1 or PD-L2. Increased PD-L1 expression has been reported with various hematologic malignancies and may prevent the host immune response from exerting an antitumor effect on the malignant cells. Nivolumab, a fully human IgG4 monoclonal PD-1 receptor blocking antibody, has demonstrated antitumor activity in pts with solid tumors including melanoma, renal cell carcinoma, and non-small cell lung carcinoma. We hypothesized that nivolumab could also mediate antitumor activity in pts with hematologic malignancies, a significant area of unmet medical need. We describe a phase I study to evaluate the effects of nivolumab in pts with select hematologic malignancies. **Methods:** This open-label, two-part study will enroll approximately 100 pts. During dose escalation, successive cohorts of pts with relapsed or refractory hematologic malignancies will be treated using a 6+3 escalation design. Pts will receive 1 or 3 mg/kg nivolumab IV every 2 weeks (wks) (the first dose will be followed by a 3-wk evaluation period), for 2 years, with the potential for an additional year of therapy for pts who progress during the follow-up period. Subsequently, 5 tumor-specific cohorts of 16 pts will be enrolled at the maximum tolerated dose (MTD) for multiple myeloma, B-cell lymphoma, T-cell lymphoma, Hodgkin lymphoma/primary mediastinal B-cell lymphoma, and chronic myelogenous leukemia. Response will be assessed at wks 4, 8, 16, 24, and every 16 wks thereafter. The primary study objective is to establish dose limiting toxicities, the MTD, and the recommended phase II nivolumab dose. Secondary objectives are to characterize nivolumab pharmacokinetics, immunogenicity, preliminary antitumor activity, and the potential association between PD-L1 expression on tumor cells and clinical efficacy. Exploratory objectives include investigation of the immunoregulatory effects of nivolumab in peripheral blood, bone marrow, and/or tumor. Pts are currently being enrolled at 3 mg/kg. Clinical trial information: NCT01592370.

TPS3114

General Poster Session (Board #23A), Mon, 8:00 AM-11:45 AM

An exploratory study of the biologic effects of nivolumab (Anti-PD-1; BMS-936558; ONO-4538) treatment in patients (pts) with advanced (unresectable or metastatic) melanoma (MEL).

Jeffrey Alan Sosman, Salvador Martin-Algarra, Jedd D. Wolchok, William Howard Sharfman, Shailender Bhatia, F. Stephen Hodi, Wen-Jen Hwu, Thomas Gajewski, Craig L. Slingluff, Howard Kaufman, Manish Gupta, Analia McGirr, Christine E. Horak, Christoph Matthias Ahlers, Jon M. Wigginton, Walter John Urba; Vanderbilt-Ingram Cancer Center, Nashville, TN; University of Navarra, Pamplona, Spain; Memorial Sloan-Kettering Cancer Center, New York, NY; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University, Baltimore, MD; University of Washington, Seattle, WA; Dana-Farber Cancer Institute/Harvard Cancer Center, Boston, MA; The University of Texas MD Anderson Cancer Center, Houston, TX; The University of Chicago, Chicago, IL; University of Virginia School of Medicine, Charlottesville, VA; Rush University Medical Center, Chicago, IL; Bristol-Myers Squibb, Princeton, NJ; Earle A. Chiles Research Institute and Providence Cancer Center, Portland, OR

Background: Programmed death-1 (PD-1) is an immune checkpoint receptor expressed by T cells that negatively regulates T-cell activity and may promote tumor immune evasion by binding to its ligands (PD-L1/L2) on tumor cells and/or antigen-presenting cells. Nivolumab is a fully human IgG4 PD-1 receptor blocking monoclonal antibody that has shown antitumor activity in phase I trials in pts with solid tumors, including advanced MEL. Objective responses were observed in pts whose diseases were refractory to multiple prior therapies. We describe an ongoing exploratory, open-label, multicenter translational study designed to further investigate the immunoregulatory activity and mechanisms of action of nivolumab in advanced MEL, including ipilimumab (anti-CTLA-4) naïve and refractory pts. **Methods:** This study is enrolling advanced MEL pts who are either ipilimumab naïve (n=40) or refractory (n=40). Eligible pts must not have received >3 prior therapies for metastatic disease and must consent to pre-and on-treatment biopsy (after 2 doses of nivolumab [Day 28]) of an accessible tumor. Nivolumab will be administered IV every 2 weeks (wks) at a dose of 3mg/kg and may continue for up to 13 cycles (104 wks) until clinically significant progression or treatment discontinuation criteria are met. Tumor responses will be assessed every 8 wks using RECIST 1.1 criteria. The primary objective is to investigate the pharmacodynamic (immunoregulatory) activity of nivolumab in the peripheral blood (PB), tumor, and tumor microenvironment. Secondary objectives include evaluation of safety and tolerability, preliminary antitumor activity, and immunogenicity of nivolumab, as well as the association between tumor PD-L1 expression and clinical efficacy. Exploratory objectives include characterization of pharmacokinetics (PK) and evaluation of the potential association between selected PB and/or tumor biomarkers and PK, clinical safety, and efficacy (eg, progression-free and overall survival). Clinical trial information: NCT01621490.

TPS3115

General Poster Session (Board #23B), Mon, 8:00 AM-11:45 AM

Chimeric antigen receptor (CAR⁺) modified T cells targeting prostate specific membrane antigen (PSMA) in patients (pts) with castrate metastatic prostate cancer (CMPC).

Susan F. Slovin, Xiuyan Wang, Melanie Hullings, Gabrielle Arauz, Shirley Bartido, Jason Stuart Lewis, Heiko Schöder, Pat Zanzonico, Howard I. Scher, Isabelle Riviere; Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins University; Memorial Sloan-Kettering Cancer Center, New York, NY; Memorial Sloan-Kettering Cancer Center, New York, NY

Background: A phase I dose-escalating study to assess safety, dose and targeting efficiency of genetically modified autologous human T cells targeted to PSMA was initiated. Preclinical models demonstrated anti-tumor activity and accumulation, migration, and persistence of these cells to tumor. The autologous PSMA-targeted T cells utilizes the P28z second generation chimeric antigen receptor following iv cyclophosphamide (Cy). For safety, the herpes simplex virus-1 thymidine kinase (hsvtk) gene is co-expressed with the P28z receptor, rendering T cells sensitive to ganciclovir for immediate T cell elimination. The expression of hsvtk enables PET imaging using radiolabeled FIAU to localize these T cells. **Methods:** Autologous T cells are activated from a leukapheresis product using anti-CD3/CD28 Dynabeads. Release criteria include mean vector copy number by Q-PCR and vector identity by Southern blot, absence of Replication Competent Retrovirus and residual Dynabeads. Pts were dosed from 10^7 to 3×10^7 CAR⁺ T cells/kg. All 7 pts received 300mg/m² of Cy one day before infusion. Baseline and post treatment imaging included FDG, FDHT and ¹⁸F-FIAU PET scans. Results: Three pts in cohort 1 received 1×10^7 CAR⁺ T cells/kg safely. A fourth pt received the same dose with a modified vector with higher copy number. One pt had stable disease for > 6 months; a second pt has stable scans for > 20 months; the third and fourth patients progressed. Of 3 pts in cohort 2, one received 1.5×10^7 CAR⁺ T cells/kg and 2 received 3×10^7 CAR⁺ cell/kg. All 3 had intermittent fever spikes up to 39°C associated with increased levels of IL-4, IL-8, IP-10, sIL-2ra and IL-6 suggesting T cell activation. CAR⁺ cells persisted in the circulation for up to 2 weeks. Scans with ¹⁸F-FIAU labeling suggests that imaging may be cell dose dependent. Conclusions: We have shown that pts can be safely treated with an ex vivo transduction, expansion and therapeutic protocol for the generation of PSMA targeted T cells. Cytokine production suggests in vivo activation and persistence of T cells in blood for up to 2 weeks. Ongoing imaging with ¹⁸F may be suboptimal; a second cohort of pts will be studied with ¹²⁴I-FIAU. Clinical trial information: NCT01140373.

TPS3116

General Poster Session (Board #23C), Mon, 8:00 AM-11:45 AM

A phase I/II study of multiple peptides cocktail vaccine for advanced/recurrent ovarian cancer.

Satoshi Takeuchi, Tadahiro Shoji, Masahiro Kagabu, Tatsuya Honda, Fumiharu Miura, Hideo Omi, Anna Takada, Toru Sugiyama; Iwate Medical University School of Medicine, Morioka, Japan

Background: Despite improvement in chemotherapy including the molecular targeting agents, advanced or recurrent ovarian cancer (A/ROC) are still incurable. Some tumor associated proteins, hypoxia-inducible protein 2 (HIG2), and tumor related vascular endothelial growth factor receptor 1,2 (VEGFR1,VEGFR2) were found to be candidates as new targets, and their epitope peptides have been shown to have the ability to induce specific cytotoxic T lymphocyte (CTL) responses. We conducted a phase I/II study of these peptides cocktail vaccine (PCV) in patients with A/ROC in order to evaluate toxicity, immunological response, and tumor response. **Methods:** 23 patients positive for human leukocyte antigen (HLA)-A2402 (A24) and 15 patients with HLA-A0201(A02) were enrolled in this study. Enrollment is still on going up to 30 evaluable patients in each group. PCV for A24 comprises FOXM1(Forkhead Box M1), MELK(maternal embryonic leucine zipper kinase), HJURP(Holliday junction recognition protein), VEGFR1 and VEGFR2. As for A02- PCV comprises HIG2, VEGFR1and VEGFR2. Cocktails were made at a dose of 1mg of each peptide with GMP grade-adjuvant, MONTANIDE ISA51. Vaccination schedule included weekly subcutaneous administration for first 12 weeks, then bi-weekly administration for next 16 weeks, then further vaccination was done monthly for 8 times and 3-6 months as a maintenance step according to patient's need. Pre- and post-vaccination blood samples were obtained from the patients for toxicity assessment and immunological evaluation. Clinical responses were evaluated every three months by RECIST v 1.1. Results: PCV were generally well tolerated with no major adverse events, and most of the patients developed specific CTL responses. One patient showed complete response, two showed partial response and 10 showed stable disease in 22 evaluable patients. At the time of the analysis, median overall survival was 5 months (from 30 to 623 days) and 9 months(from 54 to 921 days),in A24 and A02, respectively. 11 patients remained alive with median follow up of 9 months. Conclusions: These findings suggest these peptides cocktail vaccines are safe and applicable for advanced/recurrent ovarian cancer. Clinical trial information: UMIN000003862, UMIN000003860.

TPS3117^

General Poster Session (Board #23D), Mon, 8:00 AM-11:45 AM

Intergroup ALFA/GOELAMS randomized phase II trial of lirilumab anti-KIR monoclonal antibody (IPH2102/BMS986015) as maintenance treatment in elderly patients with acute myeloid leukemia (EFFIKIR trial).

Norbert Vey, Hervé Dombret, Norbert Ifrah, Arnaud Pigneux, Claude Gardin, Marc E. Buyse, Pascale Andre, Anne Tirouvanziam-Martin, Robert Albert Zerbib, Renaud Buffet, Marcel Rozencweig; Institut Paoli Calmettes, Marseilles, France; Hematologie Adultes, Hôpital Saint-Louis, Paris, France; Centre Hospitalier Universitaire, Angers, France, Angers, France; Hopital Haut-Leveque, Pessac, France; Hematology, Hopital Avicenne APHP, University of Paris 13, Bobigny, France; International Drug Development Institute, Louvain la Neuve, Belgium; Innate-Pharma, Marseilles, France

Background: Inhibitory killer immunoglobulin-like receptors (KIR) negatively regulate natural killer (NK) cell-mediated killing of HLA class I-expressing tumors. Lack of KIR-HLA class I interactions has been associated with potent NK cell-mediated antitumor efficacy and increased survival in patients with acute myeloid leukemia (AML) upon haploidentical stem cell transplantation from KIR-mismatched donors (Ruggeri, *Blood* 2007). Anti-KIR antibody treatment resulted in long-term survival in SCID mice inoculated with lethal autologous AML cells (Romagne, *Blood* 2009). Lirilumab is a second generation fully human monoclonal antibody targeting the major inhibitory KIR on NK cells. The objectives of this study are to determine if maintenance therapy with lirilumab can improve leukemia-free survival (LFS) of elderly patients in first complete remission (CR1) of AML and to assess two dose schedules leading to either intermittent or continuous KIR occupancy. **Methods:** EFFIKIR is a randomized double-blind 3-arm placebo controlled trial of lirilumab in elderly patients in CR1 of AML. Patients aged 60 to 80 in CR1 of AML following standard induction and consolidation programs are randomly allocated to receive placebo or lirilumab given at either 0.1 mg/kg q 12 weeks or 1 mg/kg q 4 weeks according to a minimization algorithm adjusting for center, primary vs. secondary AML, no. of consolidation cycles (1 vs. 2) and cytogenetics (intermediate vs. high risk). Patients are to receive up to 2 yrs of therapy. ECOG performance status of 0-1, adequate hematologic, liver and renal function, and recovery from toxicities of prior chemotherapies are required. Patients are excluded if they are eligible for bone marrow transplantation and if the time interval since last consolidation exceeds 3 mos. The primary endpoint is LFS based on independent central review. The trial will accrue 50 patients in each arm and is powered (80%) to detect an improvement in LFS with a hazard ratio of 0.60 and a one-sided alpha of 0.05. Each dose schedule will be compared to placebo using a Hochberg procedure. The first patient was randomized on 12/11/2012. Clinical trial information: NCT01687387.

TPS3118[^]

General Poster Session (Board #23E), Mon, 8:00 AM-11:45 AM

Evaluation of the safety and immunogenicity of intratumoral injection of interferon gamma (IFN γ) during vaccination in patients with subcutaneous or cutaneous metastases of melanoma (Mel51; NCT00977145).

Craig L. Slingluff, Gina R. Petroni, Lynn Dengel, David W. Mullins, William W. Grosh, Geoffrey R. Weiss, Robert M. Strieter, Patrice Neese, Carmel Nail, James W. Patterson, Walter C. Olson, Kimberly A. Chianese-Bullock; University of Virginia School of Medicine, Charlottesville, VA; University of Virginia Health System, Charlottesville, VA; Memorial Sloan-Kettering Cancer Center, New York, NY; Dartmouth School of Medicine, Lebanon, NH; University of Virginia, Charlottesville, VA; Novartis, Cambridge, MA

Background: One mechanism to improve immunologic outcomes of vaccine therapy, and other immune therapies, is to optimize T cell trafficking to sites of tumor. CXCR3 is expressed by Th1 and Tc1 T cells and directs them to sites of inflammation by following the chemokine gradient. The ligands for CXCR3 (CXCL9 (MIG), CXCL10 (IP-10) and CXCL11 (I-TAC)) are induced by interferon gamma (IFN γ). This protocol tests whether administering peptide vaccine activates circulating tumor antigen-specific CD8⁺ CXCR3⁺ T cells, followed by intratumoral IFN γ to increase CXCR3 ligands (CXCL9-11) at the tumor site and thus to recruit the CXCR3⁺ T cells. **Methods:** This pilot clinical trial is enrolling patients (n=14) with subcutaneous or cutaneous metastases of melanoma (stage IIIB-IV), who have adequate accessible tumor in 1-4 lesions to provide 100-300 mm³ tumor on each of the three biopsy days, and with at least one lesion amenable to intratumoral IFN γ injection. Patients must also express HLA-A1, A2, A3, or A11. Patients undergo tumor biopsy d1, then are vaccinated days 1, 8, and 15 with a multi-peptide vaccine. A biopsy day 22 provides information on the effect of vaccination alone on T cell infiltration into tumor. IFN γ (up to 2 million units) is injected into at least 1 metastasis, which is biopsied day 24. Additional vaccines are given days 24, 43, and 64. Primary goals are to determine the safety of intratumoral interferon gamma (IFN γ) plus a multi-peptide melanoma vaccine, and to evaluate the biological effects of vaccine plus IFN-g at the tumor site, to include expression of CXCR3 ligands (CXCL9, CXCL10 & CXCL11) and the magnitude of infiltration of CD8⁺ CXCR3⁺ T cells and vaccine-specific T cells. Secondary goals include evaluating effects of vaccine on CXCR3 expression by circulating antigen-experienced CD4 and CD8 T cells, estimating the effects of vaccine plus IFN γ on changes in the percentage of FoxP3⁺ CD25^{hi} CD4⁺ (putative regulatory T cells, Tregs) among tumor infiltrating T cells and to obtain preliminary data on the clinical response of cutaneous or subcutaneous metastases of melanoma to the proposed combination regimen. Clinical trial information: NCT00977145.

TPS3119[^]

General Poster Session (Board #23F), Mon, 8:00 AM-11:45 AM

Results of the CARMA study to investigate catumaxomab therapy for ascites related to peritoneal carcinomatosis in clinical practice.

Christian M. Kurbacher, Jalid Sehouli, Manfred Welslau, G.-F. Tempelhoff, J. Kufahl, Christian Marth, Helmut Oettle, Barbara Schmalfeldt, Elisabeth Urban, Volker Kunzmann; Medical Centre Bonn Friedensplatz, Bonn, Germany; Department of Gynecology, Campus Virchow Clinic, Charité Medical University, Berlin, Germany; Praxis Aschaffenburg, Aschaffenburg, Germany; Klinikum Aschaffenburg, Frauenklinik, Aschaffenburg, Germany; Klinikum Deggendorf, Medizinische Klinik II, Deggendorf, Germany; Department of Obstetrics and Gynecology, Medical University of Innsbruck, Innsbruck, Austria; Onkologische Schwerpunktpraxis, Friedrichshafen, Germany; Technische Universität München, Frauenheilkunde, München, Germany; Fresenius Biotech GmbH, Munich, Germany; Medizinische Klinik und Poliklinik II, University of Würzburg, Würzburg, Germany

Background: The trifunctional antibody CATU (catumaxomab) is approved in the EU for intraperitoneal (IP) treatment of malignant ascites (MA) in patients (pts) with EpCAM-positive carcinomas. Clinical data for CATU are based on 2 phase III and several phase I/II trials. However, the routine use of CATU has not been evaluated systematically. Therefore, a prospective observational study (CARMA) was started in 2010 investigating the administration of CATU in a total of 160 pts with MA under routine conditions. Participating centers were hospitals and oncologic practices in Germany and Austria. Hereby, we report on the results of the 2nd interim CARMA analysis. **Methods:** This analysis included 103 pts with MA due to EpCAM-positive carcinomas: ovarian, n=37; gastric, n=13; pancreatic, n=10; colorectal, n=6, miscellaneous, n=37. Pts were treated with CATU at a routine setting at 4 increasing dosages over a 2-week interval. The primary endpoint was puncture-free interval (PFI), secondary endpoints included safety and overall survival (OS). **Results:** The study population mainly comprised pts with advanced-stage disease. In 65% distant metastases were present. Therapy was given in 24 hospitals (73 %) and 9 outpatient facilities (27 %). Pts suffered from typical MA related symptoms such as abdominal swelling (77%), pain (56%), dyspnea (27%), anorexia (31%), constipation (13%). In 67 pts (65%), CATU was given as planned, 36 pts (35%) received <4 infusions. Most frequent adverse events (AE) were fever (20%), nausea (14%) and diarrhea (6%). The median PFI was 57 days (d), the median OS was 100 d. For the subgroups ovar/non-ovar, a median PFI of 93/41 d and a median OS of 115/72 d was observed. **Conclusion:** CARMA represents the first systematic evaluation of CATU therapy given for MA under routine conditions. In accordance to previous prospective trials, the presented 2nd interim-analysis was able to demonstrate a clinically meaningful benefit of CATU, particularly impressive in ovarian cancer pts. CATU showed an acceptable safety profile, thus allowing for treatment at an outpatient setting in an adequately selected group of pts. The final CARMA analysis after including 160 pts is thus eagerly awaited. Clinical trial information: DRKS00000458.

TPS3120

General Poster Session (Board #23G), Mon, 8:00 AM-11:45 AM

A pilot study of preoperative, single-dose ipilimumab (Ipi) and/or cryoablation (Cryo) in women (pts) with early stage/resectable breast cancer (ESBC).

Adi Diab, Stephen Barnett Solomon, Virgilio Sacchini, Christopher Comstock, Majid Maybody, Jeremy C. Durack, Janice S. Sung, Brian Blum, Deirdre A. Neville, Alan Kotin, Jianda Yuan, Sujata Patil, Elizabeth Ann Morris, Edi Brogi, Monica Morrow, Jedd D. Wolchok, Clifford Hudis, James Allison, Larry Norton, Heather L. McArthur; Memorial Sloan-Kettering Cancer Center, New York, NY; Interventional Radiology and Image Guided Therapies, Memorial Sloan Kettering Cancer Center, New York, NY, New York, NY; The University of Texas MD Anderson Cancer Center, Houston, TX

Background: Intratumoral cryo combined with immune modulation generates a potent systemic anti-tumor immune response that might improve recurrence-free survival in ESBC. Cryo-mediated tumor destruction results in necrosis and immunogenic cell death which exposes dendritic cells (DC) to sufficient quantities of tumor antigens and inflammatory cytokines to induce their maturation and activation and elicit tumor specific T cell responses. To further amplify this immune response we use ipi, a human monoclonal antibody that blocks cytotoxic T lymphocyte antigen-4 (CTLA4). In preclinical murine models, the combination of cryo with CTLA4 blockade successfully mediates rejection of metastatic prostate cancer lesions and prevents growth of secondary tumors. We therefore hypothesize that this strategy could confer long-term immunity for pts with ESBC. In this study, we evaluate the safety of pre-op cryo and/or immune modulation with single dose ipi (at 10 mg/kg) in pts with ESBC. **Methods:** Pts are sequentially assigned to receive pre-op: cryo alone (Group A), ipi alone (B), or ipi with cryo (C). Cryo is administered 7-10 d prior to surgery. Ipi is administered 8-15 d prior to surgery (1-5 d prior to cryo). If at least 5/6 pts in each group proceed with surgery without delay, the regimen will be considered safe/tolerable. Primary aim: To evaluate the safety of pre-op cryo and/or ipi (10mg/kg) in pts with ESBC. Secondary aims: To characterize pre- and post-intervention radiographic and immunological (peripheral blood and tumor tissue) correlates. Eligibility: Pts ≥ 18 y of age with operable ≥ 1.5 cm invasive ESBC, no history of autoimmune disease and planned mastectomy. Study status: As of January 25, 2013 7/7 pts were enrolled to Group A (expanded after 1 pt had suspected incomplete cryo) and 5/6 pts were enrolled to Group B. Enrollment to Group C will open when the pts in Group B meet the safety endpoint 30 d (+/-10 d) after surgery. Toxicity evaluation continues for 12 wks after ipi administration for Groups B and C. Clinical trial information: NCT01502592.

TPS3121

General Poster Session (Board #23H), Mon, 8:00 AM-11:45 AM

A phase II study of live-attenuated *Listeria monocytogenes* immunotherapy (ADXS11-001) in the treatment of persistent or recurrent cancer of the cervix (GOG-0265).

Warner King Huh, William E. Brady, Kathleen N. Moore, Heather A. Lankes, Bradley J. Monk, Carol Aghajanian, Don S. Dizon, Paula M. Fracasso; University of Alabama at Birmingham, Birmingham, AL; GOG Statistical Data Center, Buffalo, NY; University of Oklahoma Health Sciences Center, Oklahoma City, OK; Gynecologic Oncology Group, Buffalo, NY; Creighton University School of Medicine at St. Joseph's Hospital and Medical Center, Phoenix, AZ; Memorial Sloan-Kettering Cancer Center, New York, NY; Women and Infants Hospital of Rhode Island, Providence, RI; University of Virginia, Charlottesville, VA

Background: This is a GOG/NCI-sponsored phase II study (NCT01266460, GOG 0265) of ADXS11-001 in patients with persistent or recurrent cancer of the cervix. ADXS11-001 is a live attenuated *Listeria monocytogenes* (*Lm*) immunotherapy bioengineered to secrete a HPV-E7 fusion protein targeting HPV-E7 transformed cells. A previous phase I dose escalation study determined the safety of ADXS11-001 in patients with late stage cervical cancer (Maciag PA. *Vaccine*. 2009 Jun 18;27(30):3975-83). The primary objectives of this study are to evaluate the tolerability and safety of ADXS11-001, and to assess the activity of ADXS11-001 in patients with persistent or recurrent cancer of the cervix. Secondary objectives are progression-free survival, overall survival and objective tumor response. **Methods:** Patient eligibility criteria: Females age ≥ 18 years with persistent or recurrent squamous or non-squamous cell carcinoma, adenosquamous carcinoma, or adenocarcinoma of the cervix with documented disease progression (disease not amenable to curative therapy). Patients must have measurable disease as defined by RECIST 1.1; at least one "target lesion" as defined by RECIST 1.1; have had one prior systemic chemotherapeutic regimen for management of their disease; have adequate organ function and must be free of active infection and not on antibiotics. This protocol is a 2-stage design with 12-month survival as the primary endpoint and with a planned sample size of up to 67 patients. Patients will receive ADXS11-001 at a dose of 1×10^9 CFU on Day 1 and repeat every 28 days for 3 total doses in the absence of disease progression or unacceptable toxicity, with each dose followed at 72 hours by a 7 day course of ampicillin, 500 mg QID. Tumor tissue and serum samples may be collected periodically for translational research. After completion of study treatment, patients are followed every 3 months for 2 years and then every 6 months for 3 years. As of January 31, 2013, enrollment has been completed in the 6-patient safety lead-in portion of the study. Clinical trial information: NCT01266460.

TPS3122

General Poster Session (Board #24A), Mon, 8:00 AM-11:45 AM

Novel combination of toll-like receptor (TLR)-7 agonist imiquimod and local radiotherapy in the treatment of breast cancer chest wall recurrences or skin metastases.

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Background: To assess the local and systemic effects of the novel combination of local radiotherapy (RT) with imiquimod (IMQ) applied topically to breast cancer metastatic to skin, and measure immunologic correlates (clinicaltrials.gov NCT01421017). Breast cancer is the 2nd most common tumor to metastasize to the skin. Current therapies for unresectable skin lesions are rarely curative. Patients ultimately die of visceral metastases, necessitating more effective therapies. IMQ, a synthetic TLR-7 agonist has profound effects on the tumor immune microenvironment and can lead to regression of cutaneous breast cancer metastases (Adams, *Clin Ca Res*, 2012). The trial was designed based on accumulated data supporting the synergy of combined RT/immunotherapy (Formenti, *JNCI*, 2013), and pre-clinical data demonstrating the synergy of topical IMQ and local RT in a mouse model of mammary adenocarcinoma which ulcerates through the skin, and mimics a chest wall recurrence. In the mouse model, the combination was superior with complete regressions of the treated tumors, responses at untreated sites and improved survival (Dewan, *Clin Ca Res*, 2012). **Methods:** Eligibility: patients with biopsy-confirmed breast cancer, measurable disease and skin metastases, ECOG PS 0-2 and adequate organ/marrow function. RT is delivered to 1 area of skin metastases in 5 fractions of 6 Gy (days 1, 3, 5, 8, 10). IMQ cream is applied topically 5 nights/week for 8 weeks, beginning on day 1. Following a brief phase I portion to allow dose optimization in the event of unanticipated adverse events (3-3 design), the phase II study evaluates efficacy with 25 additional patients planned. Primary endpoint is the response rate in untreated distant metastases, assessed by immune-related response criteria. The local tumor responses and safety of the combination will also be determined; tumor biopsies will be studied for immune-mediated rejection signatures and peripheral lymphocytes for antigen-specific T and B cell responses. To date, 10 patients have been enrolled. The phase I portion has been successfully completed with 6 patients without DLT. Phase II enrollment has begun. Clinical trial information: NCT01421017.

TPS3123

General Poster Session (Board #24B), Mon, 8:00 AM-11:45 AM

Trial proactive: A prospective, randomized, multicenter, open label phase III study of active specific immunotherapy with racotumomab plus best support treatment versus best support treatment in patients with advanced non-small cell lung cancer.

Roberto E Gomez, Amparo Macias, Tania Crombet, Ana Maria Vazquez, Rolando Perez, Maria Laura Ardigo, Agustin Lage; Laboratorio ELEA, Buenos Aires, Argentina; Center of Molecular Immunology, La Habana, Cuba; Center of Molecular Immunology, La Habana, Cuba; Recombio, Madrid, Spain; Center of Molecular Immunology, Havana, Cuba

Background: Gangliosides, especially NeuGc-GM3, are attractive targets for cancer immunotherapy. They are not expressed in normal human cells but are overexpressed and involved in tumor growth in several tumors (melanoma, neuroectodermal pediatric tumors and breast cancer). More than 70% of non-small cell lung cancers (NSCLC) express NeuGc-GM3. Racotumomab is an anti-idiotypic monoclonal antibody that mimics NeuGc gangliosides. Administered as a therapeutic vaccine it acts as an antigen, inducing a cellular and humoral immune response against NeuGc-GM3 and other gangliosides. Previous trials have shown its low toxicity and high immunogenicity. Recently, a multicenter, randomized, placebo controlled, double-blind trial in 176 NSCLC (IIIB and IV) patients with at least stable disease after first line therapy and progression free at inclusion showed a statistical significant survival benefit in favor of racotumomab.

Methods: This phase III, multinational, randomized (1:1), open label trial will evaluate efficacy and safety of racotumomab plus best supportive care (BSC) versus BSC. 1,082 patients with NSCLC (stages III or IV) showing response or stable disease after standard first-line therapy (platinum-based chemotherapy and radiotherapy) are eligible if they remain free of progression and have a PS \leq 1. Vaccination consists of an induction period (5 doses, 1 every 14 days) followed by a maintenance period (1 dose every 28 days, until worsening of the PS or unacceptable toxicity occur). Upon progression other onco-specific therapies may be used and vaccination can continue. Overall survival (OS) will be compared using a two-sided log rank test at a significance level (alpha) of 0.05 with 90% power to detect a hazard ratio of 0.79. There will be 3 interim analyses and a final analysis when 758 deaths occur. So far 190 patients have been randomized. An Independent Data Monitoring Committee oversees the study and has so far (last meeting September 2012) concluded that the trial can continue. The trial is registered in ClinicalTrials.gov under NCT01460472. Clinical trial information: NCT01460472.

TPS3124[^]

General Poster Session (Board #24C), Mon, 8:00 AM-11:45 AM

A randomized, double-blind, placebo-controlled, multicenter, multinational, phase II trial immunotherapy with L-BLP25 (tecemotide) in patients with colorectal carcinoma following R0/R1 hepatic metastasectomy.

Stefan Kasper, Friedrich Overkamp, Markus Hermann Moehler, Frank Kullmann, Hauke Lang, Michael Schoen, Victoria Smith-Machnow, Susanna Hegewisch-Becker, Daniel Seehofer, Wolf Bechstein, Michael Heike, Matthias Voehringer, Volker Heinemann, Richard Greil, Michael Geissler, Florian Lordick, Marc Peeters, Eric Van Cutsem, Peter Robert Galle, Carl Christoph Schimanski; Department of Medical Oncology, West German Cancer Center, University Hospital Essen, University Duisburg-Essen, Essen, Germany; Medical Practice for Oncology and Hematology, Recklinghausen, Germany; Department of Internal Medicine, Johannes Gutenberg Universität Mainz, Mainz, Germany; Klinikum Weiden, Medizinische Klinik I, Weiden, Germany; Universitätsmedizin Mainz, Mainz, Germany; Städtisches Klinikum Karlsruhe, Karlsruhe, Germany; iOMEDICO, Freiburg, Germany; Private Practice for Oncology, Hamburg, Germany; Department of General, Visceral, and Transplantation Surgery, Charité–Universitätsmedizin Berlin, Campus Virchow-Klinikum, Berlin, Germany; Goethe University Hospital, Frankfurt, Germany; Hospital Dortmund, Dortmund, Germany; Robert-Bosch-Krankenhaus, Stuttgart, Germany; Department of Hematology and Oncology, Klinikum Grosshadern and Comprehensive Cancer Center, LMU Munich, Munich, Germany; Universitätsklinikum der PMU, Salzburg, Austria; Department of Gastroenterology and Oncology, Klinikum Esslingen, Esslingen, Germany; University Cancer Center Leipzig, University Clinic Leipzig, Leipzig, Germany; Department of Oncology, Antwerp University Hospital, Edegem, Belgium; University Hospitals Leuven, Leuven, Belgium; Mainz University, Mainz, Germany; University Medical Center Mainz, 1st Department of Internal Medicine, Mainz, Germany

Background: 15-20% of all patients (pts) diagnosed with colorectal cancer (crc) develop metastases (mets) surgical resection remains the only potentially curative treatment available. Current 5-year survival rate following R0 resection of liver mets lies between 28-39%, recurrence occurs in up to 70% of pts. To date, adjuvant chemotherapy has not significantly improved clinical outcomes. The primary objective of the ongoing LICC trial (L-BLP25 In Colorectal Cancer) is to determine whether L-BLP25, an active MUC1-specific cancer immunotherapy, extends recurrence-free survival (RFS) time over placebo in crc pts following R0/R1 resection of liver mets known to highly express MUC1 glycoprotein. Phase III data from L-BLP25 in NSCLC will be reported at this meeting. **Methods:** This is a multinational, phase II, multicenter, randomized, double-blind, placebo-controlled trial with a sample size of 159 pts from 20 centers in 3 countries. Pts must have stage IV cr adenocarcinoma limited to liver mets. Following curative-intent complete resection of the primary tumor and of all synchronous/metachronous mets, eligible pts are randomized 2:1 to receive either L-BLP25 or placebo. L-BLP25 arm receives a single dose of 300 mg/m² cyclophosphamide (CPA) 3 d before 1st L-BLP25 dose, then primary treatment with sc L-BLP25 930 µg weekly for 8 weeks, followed by sc L-BLP25 930 µg maintenance doses at 6-week (year 1 and 2) and 12-week (year 3) intervals until recurrence. Control arm: CPA is replaced by saline solution and L-BLP25 by placebo. Primary endpoint (PE) is RFS time. Secondary endpoints: Overall survival (OS), safety, tolerance, RFS/OS in MUC-1 positive cancers. Exploratory immune response analyses are planned. Study start was in Q3 2011. 19 centers were initialized and 36 patients recruited, no SUSARs occurred. Study recruitment will end Q3 2013; follow-up until Q3 2017. PE assessment is in Q3 2016. Interim analyses are not planned. No major practical issues were identified during setup and early conduct of the study. Clinical trial information: 2011-000218-20.

TPS3125

General Poster Session (Board #24D), Mon, 8:00 AM-11:45 AM

A multi-peptide vaccine plus toll-like receptor agonists in melanoma patients, with evaluation of the vaccine site microenvironment and sentinel immunized node (Mel58; NCT01585350).

Craig L. Slingluff, Gina R. Petroni, Kimberly A. Chianese-Bullock, William W. Grosh, Geoffrey R. Weiss; University of Virginia School of Medicine, Charlottesville, VA; University of Virginia Health System, Charlottesville, VA; University of Virginia, Charlottesville, VA

Background: Recent data show clinical activity of cancer vaccines containing a defined cancer antigen, and a peptide vaccine for melanoma. However, immune responses to peptide vaccines are often transient and of low magnitude. The most common adjuvant for peptide vaccines for melanoma has been an incomplete Freund's adjuvant (IFA), which may have suboptimal adjuvant properties. Toll-like receptor (TLR) agonists offer the potential to improve the magnitude and persistence of antitumor T cell responses, either in combination with IFA or alone. CD40 ligation at the vaccine site microenvironment (VSME) may also improve adjuvant activity of TLR agonists and may be provided by CD4 T cell activation. We report a clinical trial of a multi-peptide vaccine using TLR agonists and IFA, with correlative studies in 3 immunologic compartments. **Methods:** This trial is enrolling patients with resected stage IIB-IV melanoma (n=48) and is designed to evaluate the safety and immunogenicity of vaccination with peptides and either of 2 toll-like receptor agonists (TLR3 agonist polyICLC; TLR4 agonist endotoxin), with or without IFA. Patients are vaccinated 6 times over 12 weeks with 12 Class I MHC-restricted nonamer peptides. An immunogenic tetanus helper peptide is included to activate CD4 T cells in the VSME and secondarily to ligate CD40. Goals include safety assessment, measures of CD8 T cell responses, and characterization of cellular and molecular events induced in the blood, VSME and vaccine-draining node (sentinel immunized node, SIN), as well as a preliminary assessment of whether vaccination with TLR agonists improves the persistence of CD8 and CD4 T cell responses to melanoma antigens compared to prior studies with IFA. This includes a first-in-humans evaluation of the safety and immunogenicity of LPS, a classic TLR4 agonist, as a vaccine adjuvant. Thus, there is a novel dose-escalation phase with this adjuvant, which has been safely administered in other settings by intravenous and inhalation routes. An aim of this study is to identify an improved adjuvant for use in future trials combining peptides with other immune therapies. Clinical trial information: NCT01585350.

TPS3126

General Poster Session (Board #24E), Mon, 8:00 AM-11:45 AM

Biomarker correlation to clinical response in phase I/II trials of the adjuvant breast cancer vaccine neuvax (nelipepimut-S or E75).

John S. Berry, Alfred F. Trappey, Alan K. Sears, Timothy J. Vreeland, Guy T. Clifton, Diane F. Hale, Ritesh Patil, Jarrod P. Holmes, Sathibalan Ponniah, Elizabeth Ann Mittendorf, George Earl Peoples, David C. Van Echo; Brooke Army Medical Center, San Antonio, TX; Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY; Naval Medical Center San Diego, San Diego, CA; Cancer Vaccine Development Program, United States Military Cancer Institute, USUHS, Bethesda, MD; The University of Texas MD Anderson Cancer Center, Houston, TX; Department of Surgery, Brooke Army Medical Center, Fort Sam Houston, TX; Walter Reed National Military Medical Center, Bethesda, MD

Background: We completed phase I/II clinical trials with NeuVax (nelipepimut-S), a HLA-A2/A3-restricted, HER2-derived peptide vaccine. The vaccine was administered in the adjuvant setting to prevent recurrence in breast cancer patients rendered disease-free with standard-of-care therapy. Here, we examine the relationship between in vitro immunologic response (IR) and clinical recurrence (CR) after 5-year follow-up. **Methods:** The phase I/II trials were performed as dose-escalation/schedule-optimization trials enrolling node positive and high-risk, node-negative patients (pts) with tumors expressing any level of HER2 (IHC 1+, 2+, or 3+). HLA-A2/A3+ pts were enrolled in the vaccine group (VG) while HLA-A2/A3-pts were followed prospectively as an untreated control group (CG). The VG was given 4-6 monthly intradermal inoculations of nelipepimut-S+GMCSF (immunoadjuvant) during the primary vaccine series (PVS). In vitro IR was assessed for E75-specific, cytotoxic T lymphocyte clonal expansion by HLA-A2:IgG dimer assay and expressed as mean dimer index (mdi) at baseline, after PVS (R6), and six months after the PVS. HER2 under-expression was defined as an IHC 1/2, and a FISH < 2.2. VG and CG pts were followed for CR over 60 months. P-values were calculated using the Fisher's exact test. Results: Of the 195 pts enrolled, 8 withdrew, leaving 187 evaluable pts; 108 in the VG and 79 in the CG. R6 dimer assays were available for 86 pts in the VG. The mean R6 dimer in the VG is 0.63 mdi+.08. Of the 30 pts with an R6 dimer above the mean, only one recurred, compared to eight of the 56 below the mean (p=.09). The difference between baseline and maximum mdi was available in 56 HER2 under-expressing VG pts. Of the 26 pts above the mean difference (1.08 mdi +.17), one recurred, compared to six CR in the 30 pts below the mean (p=.06). There were no CR in pts with HER2 under expression with a mean difference ranked in the top third. Conclusions: In prospective, completed phase I/II trials of NeuVax (nelipepimut-S), patients who exhibit robust in vitro IR have lower recurrence rates. This finding suggests that nelipepimut-specific CTL clonal expansion is a valid biomarker for CR in pts treated with NeuVax. Clinical trial information: NCT00841399.

TPS3127

General Poster Session (Board #24F), Mon, 8:00 AM-11:45 AM

A phase II study of a yeast-based therapeutic cancer vaccine, GI-6207, targeting CEA in patients with minimally symptomatic, metastatic medullary thyroid cancer.

Ravi Amrit Madan, Nishith K. Singh, Ann Wild Gramza, Antonio Tito Fojo, Christopher Ryan Heery, Joseph W. Kim, Sheri McMahon, Myrna Rauckhorst, Thomas H King, David Apelian, Jeffrey Schlom, James L. Gulley; Laboratory of Tumor Immunology and Biology, Medical Oncology Branch, National Cancer Institute, Bethesda, MD; National Cancer Institute, National Institutes of Health, Bethesda, MD; Medical Oncology Branch, National Cancer Institute, Bethesda, MD; GlobeImmune, Inc., Louisville, CO

Background: *Saccharomyces cerevisiae* has been genetically modified to express CEA protein and developed under a CRADA with GlobeImmune/NCI as a heat-killed immune-stimulating, therapeutic cancer vaccine (GI-6207). A phase I study with GI-6207 demonstrated safety, biomarker stabilization and enhanced immune response in some patients. CEA is over-expressed in multiple malignancies, including medullary thyroid cancer (MTC). Two therapies recently approved by the FDA for metastatic MTC (vandetanib, cabozantinib) come with toxicity and should be reserved for symptomatic/progressive disease. However, a large population of asymptomatic MTC patients has small tumor burden and/or disease that is more indolent. The standard management of these patients is observation. Preliminary data suggest that tumor growth measured by the rate of CEA and calcitonin increase can be quantified in a 3-6 months. Retrospective data from prostate cancer studies suggest vaccines can alter growth rates within 3-4 months. We hypothesize that GI-6207 can alter tumor growth rates in MTC and impact long-term outcome. **Methods:** A phase II study will evaluate the effect of GI-6207 on the rates of increase in calcitonin in metastatic MTC. 34 patients with minimally symptomatic, radiographically evaluable, metastatic MTC will be randomized 1:1. Arm A will receive vaccine for a year from the time of enrollment. Arm B will receive vaccine after 6 months of surveillance. GI-6207 will be administered subcutaneously at 4 sites (10 yeast units/site), every 2 weeks for 3 months, then monthly up to 1 year. The primary endpoint will compare the effect of GI-6207 on calcitonin kinetics between the vaccine and surveillance arms in the first 6 months. Secondary endpoints include immunologic responses (including antigen-specific T cell responses), objective responses, time to progression, and changes in CEA kinetics. If this trial can prospectively demonstrate that vaccines can alter tumor growth rates, and if such changes are associated with clinical outcomes, then changes in tumor growth rates may become a clinical metric to evaluate vaccine efficacy in MTC and other populations.

TPS3128

General Poster Session (Board #24G), Mon, 8:00 AM-11:45 AM

CALM study: A phase II study of intratumoral coxsackievirus A21 in patients with stage IIIC and stage IV malignant melanoma.

Robert Hans Ingemar Andtbacka, Howard Kaufman, Gregory A. Daniels, Lynn E. Spitler, Jose Lutzky, Sigrun Hallmeyer, Eric D. Whitman, John J. Nemunaitis, Karl Zhou, Roberta Karpathy, Jeffrey Ira Weisberg, Darren Shafren; Huntsman Cancer Institute, University of Utah, Salt Lake City, UT; Rush University Medical Center, Chicago, IL; UC San Diego Moores Cancer Center, La Jolla, CA; Northern California Melanoma Center, San Francisco, CA; Mount Sinai Comprehensive Cancer Center, Miami Beach, FL; Oncology Specialists, SC, Park Ridge, IL; Atlantic Melanoma Center, Morristown, NJ; Mary Crowley Cancer Research Center, Dallas, TX; I3 Research, Princeton, NJ; Viralytics, Ltd., Sydney, Australia

Background: Coxsackievirus A21 (CVA21; CAVATAK) is a naturally occurring "common cold" virus. CVA21 displays potent oncolytic activity against both in vitro cultures of cancer cells and against in vivo xenografts of human cancers in mouse models of melanoma, prostate cancer, breast cancer and multiple myeloma, all which exhibit high surface ICAM-1 expression, which is used for viral entry. In mouse human melanoma xenograft CVA21 challenge models, progeny virus released from infected cells is capable of targeting adjacent cells, entering the systemic circulation, and destroying micro-metastatic foci. In a phase 1 study, two intralesional injections of CVA21 were shown to reduce or stabilize the growth of injected melanoma lesions. **Methods:** The CALM study investigates the efficacy and safety of intratumoral CVA21 in approximately 63 pts with treated or untreated unresectable Stage IIIC-IVM1c melanoma. Pts are treated with up to 3×10^8 TCID₅₀ intratumorally on study days 1, 3, 5 and 8 and then every three weeks for a further 6 injections. Key eligibility criteria are ≥ 18 yrs old, ECOG 0-1, and at least 1 injectable cutaneous, sc, or nodal tumor >1.0 cm. The primary endpoint is irPFS at 6 months following tx; secondary endpoints include durable response rate and OS. A 2-stage Simon's minimax design will be employed. Based on data from previous trials and literature, a target overall irPFS at 6 months of 22.5% versus a projected rate of 10% in the target population is selected. With an alpha level of 5% and 80% of power, a total of 54 evaluable patients will be required to test the null hypothesis that the true irPFS rate is $<10\%$ versus the alternative hypothesis that the true irPFS rate is at least 22.5%. Thirty-five patients will be treated at Stage I. If 3 or more objective responses (CR or PR) are achieved by modified RECIST 1.1 criteria in the first 35 patients, then the study will complete enrollment. Results: Currently, 21 patients have been enrolled on the study and treated with CVA21 injections. The treatment has been well tolerated. The Stage I interim efficacy endpoint of > 3 objective responses has been achieved, and the second stage of the study is proceeding with a planned enrollment of a total of 63 patients. Clinical trial information: NCT01227551.