WORKSHOP REPORT

UNDER THE U.S.-JAPAN COOPERATIVE CANCER RESEARCH PROGRAM

AREA	1. Basic Science 2. Clinical Science 3. Epidemiology & Behavioral Science

Date: April 24, 2009

1. Title of Workshop:

Immunotherapy Markers in Oncology: Progress and Challenges

2. Period of Workshop: from March 23 to March 24, 2009 2 days

3. Place of Seminar: Waikoloa, Hawaii island / Hawaii

4. Total Budget

a. Financial Support by JSPS: Total amount: 9,000 thousand yen

b. Financial Support by NCI : Total amount: U.S. dollar

c. Other Financial Support : Total amount:

5. Organizers

a. Japanese Organizer	
Name	Hideaki Tahara, MD, PhD
Institution / Department	Department of Surgery and Bioengineering Advanced Clinical Research Center Institute of Medical Science The University of Tokyo
Position	Professor
b. U.S. Organizer	
Name	Francesco Marincola, M.D.
Institution / Department	Department of Transfusion Medicine Clinical Center, National Institutes of Health
Position	Chief Infectious Disease and Immunogenetics Section

6. Participants

Number of Participants: Japanese: 19 U.S.: 19 Others: 0

a. List of Japanese-side Participants（Except for Organizer）

Name	Institution/Department	Position
Marimo Sato	Department of Bioengineering, the Advanced Clinical Research Center, the Institute of Medical Science, The University of Tokyo	Assistant professor
Yasunori Akutsu	Department of Frontier Surgery, Graduate School of Medicine, Chiba University	Lecturer
Yuichiro Doki	Department of Surgery, Graduate School of Medicine	Professor
Yoshihiko Hirohashi	First Department of Pathology, Sapporo Medical University, School of Medicine	Assistant professor
Kohzoh Imai	Sapporo Medical University, School of Medicine	
Masahisa Jinushi	Department of Bioengineering, the Advanced Clinical Research Center, the Institute of Medical Science, The University of Tokyo	Assistant professor
Akira Kanamoto	Department of Bioengineering, the Advanced Clinical Research Center, the Institute of Medical Science, The University of Tokyo	Assistant professor
Kazunori Kato	Department of Molecular Medicine, Sapporo Medical University, School of Medicine	Associate
Yutaka Kawakami	Division of Cellular Signaling, Institute for Advanced Medical Research, Keio University School of Medicine	Professor
Hisahiro Matsubara	Department of Frontier Surgery, Graduate School of Medicine, Chiba University	Professor
Kiminori Nakamura	Department of Molecular Medicine, Sapporo Medical University, School of Medicine	Assistant Professor
Hiroyoshi Nishikawa	Department of Cancer Vaccine, Department of Immuno-gene Therapy, Mie University Graduate School of Medicine	Lecturer
Noriyuki Sato	First Department of Pathology, Sapporo Medical University, School of Medicine	Professor
	Sapporo Medical University, School of Medicine	
Hiroshi Shiku	Department of Cancer Vaccine, Department of Immuno-gene Therapy, Mie University Graduate School of Medicine	Professor
Hiroya Takeuchi	Department of Surgery, Keio University School of Medicine	Assistant Professor
Minoru Toyota	Department of Biochemistry, Sapporo Medical University, School of Medicine	Professor
Hisashi Wada	Department of Surgery, Graduate School of Medicine	Research Associate
Tomonori Yaguchi	Division of Cellular Signaling, Institute for Advanced Medical Research, Keio University School of Medicine	Graduate student

b. List of US-side Participants（Except for Organizer）

Name	Institution/Department	Position
Magdalena Thurin	Cancer Diagnosis Program, National Cancer Institute (NCI), National Institutes of Health (NIH)	Program Director
Ena Wang	Infectious Disease and Immunogenetics Section (IDIS), Department of Transfusion Medicine, Clinical Center and Center for Human Immunology (CHI), NIH	Dir. Mol.Sci.
Lisa H Butterfield	Departments of Medicine, Surgery and Immunology, Division of Hematology Oncology, University of Pittsburgh Cancer Institute	Assist. Professor
Mary L Disis	Tumor Vaccine Group, Center for Translational Medicine in Women's Health, University of Washington	Investigator
Bernard A Fox	Earle A Chiles Research Institute, Providence Portland Medical Center, and Department of Molecular Biology, OHSU Cancer Institute, Oregon Health and Science University	Chief
Peter P Lee	Department of Medicine, Division of Hematology, Stanford University	Assoc. Professor
Jon M Wigginton	Discovery Medicine-Oncology, Bristol-Myers Squibb Inc.	Investigator
Stefan Ambs	Laboratory of Human Carcinogenesis, Center of Cancer Research, NCI, NIH	Investigator
Damien Chaussabel	Baylor Institute for Immunology Research and Baylor Research Institute	Associate Investigator
James Jacobson	Cancer Diagnosis Program, National Cancer Institute (NCI), National Institutes of Health (NIH)	
Mohammed Kashani-Sabet	Melanoma Clinic, University of California, San Francisco	Ass. Professor
John M Kirkwood	Departments of Medicine, Surgery and Immunology, Division of Hematology Oncology, University of Pittsburgh Cancer Institute	Professor
Michael T Lotze	Hillman Cancer Center, University of Pittsburgh, Pittsburgh	Professor
Raj Puri	Division of Cellular and Gene Therapies, Center for Biologics Evaluation and Research, Food and Drug Administration	Investigator
Antoni Ribas	Department of Medicine, Jonsson Comprehensive Cancer Center, UCLA, Los Angeles	Investigator
Howard Streicher	Cancer Therapy Evaluation Program, DCTD, NCI, NIH, Rockville	Senior Investigator
David F Stroncek	Cell Therapy Section, Department of Transfusion Medicine, Clinical Center, NIH, Bethesda	Investigator
Yingdong Zhao	Biometric Research Branch, NCI, NIH	Comput. Biologist

c. List of Other Country Participants

Name	Institution/Department /Position	Country

7. Agenda and Topics of Workshop

This meeting will provide an overview of the most recent scientific achievements in the field of biomarkers for currently used and novel immunotherapy approaches for cancer. There are many examples of effective cancer immunotherapy including monoclonal antibodies, cytokines, immune-stimulatory and immune-inhibitory reagents and vaccines that can trigger powerful and durable cancer control even as is limited to small number of patients. The potential to eradicate cancer is remarkable even if the disease is wide spread but the definite targets and mechanism of action remain elusive, due to the limits of our knowledge. Therefore, the progress in this field would be greatly accelerated if novel hypothesis-generating strategies could be considered to improve patients' outcome. One of the approaches is to be able to identify the molecular/phonotypical features of patients that will respond to these therapies and those for whom other therapies could be applied or need to be developed. The clinical utility of these markers identified in pretreatment samples would predict the clinical course of disease since the improved outcome would be associated with effective treatment. In particular, the meeting will focus on: Markers predictive of treatment: 1) Predictors of survival/clinical benefit defined with a biomarker or as a set of biomarkers that could predict responsiveness to treatment at the time of patient's enrollment., 2) Another focus will be the emerging field of genetic variation of cancer risk and markers that can influence clinical outcome in response to immunotherapy treatment. 3) Predictors of toxicity defined as biomarkers that could predict the likelihood to suffer major toxicity or anaphylaxis at the time of patient's enrollment. 4) Surrogate (end-point) biomarkers defined as those biomarkers that could provide information about the likelihood of clinical benefit/survival at earlier stages compared to prolonged disease-free or overall survival analysis. 5) Mechanistic biomarkers defined as those that may explain or validate the mechanism(s) of action of a given treatment. 6) Identify bio-informatics strategies for the comparison of high throughput information among different institutions.

This meeting will also focus on novel cutting-edge strategies suitable for high-throughput screening of clinical samples for the identification (such biomarkers will be more likely identified by paired comparison of pre- and post-treatment samples) selection and validation of biomarkers relevant to disease outcome and/or serve as surrogate equivalents to clinical outcome.

*Agenda was attached separately

8. Scientific Achievements

D Chaussabel - *A bottom up view of immunology*- Profiling genome activity from PBMCs looking at DNA, RNA and protein trying to define a modular analysis framework: driving principle. Interpretation: reduce dimensions and increase visualization. Enhance robustness (ménage noise and improve validity across platforms). Translation: develop metric that will be used at the bedside. The idea is to have frameworks that apply to various diseases. Discuss paper and modular frame works. Fingerprinting human disease compared with normal. Looking for similar finger prints among diseases; but what about the tissue rather than the circulating lymphocytes? Is there more convergence? Is there more restriction or expansion of modules compared with PBMCs. Patients with B cell deficiencies have various patterns ., the finger prints are different because they have different disease over their deficiencies that may explain the differences. Time to apply to prediction of response in immune therapy. Now they are building the second generation of modules (based on Illumina platform) High risk of transplant rejection looking (260 modules): liver transplant; good prediction. Same with acute HIV infection compared with healthy also based on geographic origin of HIV infected patients ethnic background). Melanoma vaccine trial with Palucka: healthy controls and then patients at base line as well as patients that are different at base line. Also changes are seen in time such as IFN modules increased during IFN therapy. Expanding range of applications .: exposing fibroblasts in immune deficient patients: exposure to TNF, IL1b and Poly IC .. Lack of NEMO etc..MyD88 deficiency, IRAK4 -/- Irak 4+/- beautiful paper and figures. Ag specific immune responses in patients with melanoma and could identify genes specific for T reg peptides while it is different with TH1 peptides. Important points about the peer review process the GEO access and the recognition for data accrual and not only for the mechanistic interpretation. These data are strongly reminiscent of others observations in patients with chronic HCV; these patients; patients with decreased response to ex vivo challenge of their PBMCs with IFN-α appear to bear a decreased likely hood to respond to therapy with IFN-a and Rabavarin. These differences were considered to be potentially related to genetic background as it was observed that PBMCs from patients with of African American (AA) origin were least likely to respond to IFN-α stimulation ex vivo and at the same time to recover from hepatitis is in response to treatment compared with patients with a European American (EA) background.

This observation raises the question of whether patients with melanoma or HCV that have better changes to respond to therapy are characterized by a different genetic background compared to those likely to do poorly. A recent analysis performed in our laboratories [Pos et al. in preparation] in which PBMCs from 48 consecutive AA normal donors were compared to PBMC from 48 consecutive EA normal donors matched for age and sex failed to demonstrated dramatic differences between the response of the two ethnic groups to IFN-a measured as activation of various STAT-proteins as well as at the global transcriptional analysis level. Thus, this analysis suggests that alterations in IFN signaling are likely to represent a secondary effect of the presence of cancer cells or viral particles that in turn may interfere with the innate immune response of the host. This being the case, it will be likely in the future that more insights about the mechanisms leading to altered IFN signaling in cancer patients will be gathered by a more in depth analysis of cancer biology and the products released by cancer cells that may affect immune cells activity locally and at the systemic level. Indeed melanoma has been shown in by various investigators to bear strong differences in the expression of ISGs and that ISG expression in melanoma is coordinately associated with a immune phenotype (at least at the transcriptional level) in which several other transcript are over expressed such as several cytokines, chemokines, pro-angiogenic factors etc. This immune signatures have been associated with better prognosis of melanoma patients in the past.

M Kashani-Sabet - *New diagnostic and prognostic markers in melanoma* - Identification of check points in the progression of melanoma. B-RAf mutations are early but they are very early and do not describe progression. Arrays differentiating radial to progression there is only loss of gene expression. Also there are two subtypes of melanoma that cannot separated by BRaf mutation but the modifiers that are associated with the vertical growth phase loss. Also nevis compared with melanomas also identify several immune regulatory genes of the IFI16, CCL2 and 3, CXCL1, 9 and 10 etc, they tend to be unregulated in primary melanoma compared with nevi but become down-regulated in the metastatic phase. A new paper in press in PNAS coming out next week by Kashani is the development of a multi-marker diagnostic assay for melanoma. A training set of 534 nevi and melanoma and 4 validation sets and found ARPC2, FN1, RGS1, SSP1 and WNT2 to be over-expressed in melanoma compared with nevi and they are. In melanoma there is a uniform top to bottom expression. Specificity of 94% with ROC of 911 by intensity, top bottom high sensitivity, combined specificity of 95 and sensitivity of 91 % and high ROC. Useful for Spitz nevi differentiation and dysplastic nevi (95% accuracy). In misdiagnosed cases with reclassified correctly. The markers were also evaluated on independent cohorts including the German Cancer Registry (Heidelberg/Kiel cohort). A multi-marker approach was used and several stages are evaluated and demonstrated that it could predict sentinel node status and disease specific survival ($p<0.001$) changing from 96 to 69% survival. Multi-marker score is an independent predictive of sentinel node positivity higher than depth and ulceration. IN the German cohort the Multi marker score the best predictor of DSS. A molecular map of melanoma progression is being built from melanocyte to various growth phases and metastatization. They will not be evaluated in the ECOG data set with the help of John Kirkwood.

Y Kawakami - *Molecular mechanisms for cancer cell induced immune-suppression and resistance. Potential biomarkers associated with response to immunotherapy* - Three are project identification of shared tumor antigens, methods to induce effective antigen spreading and understanding mechanism inducing immune suppression and resistance. Particular interest is why cancer cells stimulate regulatory mechanisms focusing on MAPK WNT and Braf mutations. BRaf and NRas mutations are early in melanoma. Depletion of BRaf with shRNA decreases melanoma invasion. Also there are IgGs in patients with melanoma that recognize the mutation. Others found epitopes associated with HLA-A*0201. However, inhibition of BRaf or STAT-3 depleted the expression of several cytokine including IL-6, IL-8 etc. Also a MEK inhibitor inhibits the expression of IL-10. Also VEGF expression is inhibited by shRNA for ERK1/2. Inhibition of ERK also induces the enhancement of T cell responses and protection of mice from cancer. 888 mel very sensitive to MEK inhibitor. Also production of IL-10 by melanoma cells with accumulated b-catenin, thus b catenin is associated with IL-10 production in very high expression. Transfection of b-catenin induced production of IL-10, culture of DC with supernatant of melanoma cells with high b catenin induce IL-10 producing DC but this is decreased by shRNA for β-catenin and in general more tolerogenic DCs. Functionally, T cells make less TNF-α when stimulated with DC cultured with supernatant from β-catenin positive melanomas and FOXP3 is higher. In vivo, b-catenin in mouse does not promote IL-10. In a xenogenic model human melanoma cells (397) negative for b-catenin and transfected produce IL-10 and then T cell activation tranfected in the nude mouse produce less IFN-γ and have lowered lytic activity. However, IL-10 blocking antibodies do not affect the phenomenon suggesting that something else is responsible. Screening 800 kinase siRNA to identify which are involved in immune suppression STKX kinase inhibits IL-10 and TGF-β production. Cancer stem cells; epithelial-mesenchymal transition (EMT) is induced by SNAIL transfection inducing e-cadherin also

increase in IL-10 and TGF-β. Co-culture with human PBMCs induces FOXP3 which is blocked by anti TSP1 antibodies. Thus co-culture of PBMCs with melanoma cells transfected with SNAIL will increase FOXP3 cells that can be reduced by anti-TSP treatment. Blocking SNAIL intra-tumorally with siRNA induces increase in CD4 and CD8 T cells, thus in vivo SNAIL may be involved in immune suppression. Similar results can be obtained by anti-TSP1 (downstream of SNAIL) can induce better T cell infiltrates. SNAIL trasfected melanoma is resistant to immunotherapy in mouse models and may be a biomarker for tumor responsiveness in humans to immune therapy.

S Ambs - *Differences in tumor immune biology by race/ethnicity* - Laboratory of human carcinogenesis, Center for cancer Research –Gene expression profiling comparing two technique groups prostate cancer: AA have higher death rates from all cancer sites combined, national center for health statistics. Biology is contributing factor. 8q24 cancer susceptibility locus of AA. AMBS compared 33 AA and 36 EA microdissected tumors and analysis at gene and pathway level. About 162 were different several metastases related. Also a gene least from Rhodes at al Cancer res 2002 62 4427; compared this list with the ethnicity; there was no difference between AA and EA thus the tumors develop similarly. The big differences instead where related to immune responses (10 to the minus 30). Also these genes are mainly associated to the development of SLE and other inflammatory diseases but not with other cancers.

All her associated with IFN such as IFNG, STAT1, CXCL9-11 CCL5 CCL4 CCR7, IL15 and 16, USG15, etc, Mx1, IRF-1 and 8, 2 AND OAS2. Tap 1 and 2. These are over expressed in AA. Hypothesis, are some cancers in AA infected by viruses?

Similar results were obtained studying breast tumors and comparing stroma with cancer tissue by micro-dissection and validated by immune-histochemistry by a validation set. Increased macrophage infiltration in AA tumors (CD68); e4531 and higher micro vessel density CD31. Gene enrichment analysis (how does it relate to other published gene lists?). Most were related to other data bases associated with studies in which IFN effects were studied in cell lines, or studies in SLE. No Treg differences were noted between AA and EA but there was a general correlation with ER status and overall outcome where it was easier in basal type breast CA. Very important conclusive FIGURE on IFNs and their role in cancer. Also the DARC (Duffy antigen receptor) gene binds cytokines and interacts with CD82 that interacts with metastasis. IRF-5 variants in AA are associated with SLE in AA. HCV genotype 1.

J Kirkwood - *Melanoma as immunotherapy model* - The importance of phase III and phase II large studies are critical to learn and have sufficient definitive information. IL-2 no even phase III trial has even been done Patel at al ESMO 2008. Meta analysis of all phase II trials published a year ago from John, and various outcomes markers where identified but what is the best predictor and in general the most patients are treated less differences are noted between therapies. Multiple pathways are targeted but no agent as been shown to affect survival. Also constitutive activation of STAT-3 has been suggested as a prognostic factor in general (what about therapy?). Also relevance of Thw (Tatsumi and storkcus JEM 2002 195 and the same Cancer res 2003, 63). Other therapies are of course IFN, GM-CSF IL-2 vaccine and anti-CTL4. Extensive experience with IFN. There is evidence of improved survival and though is minor but there is a significant difference. Also Gogas paper is important showing that autoimmunity is predictive of adjuvant therapy benefit NEJM paper). The study was repeated already and was indeed the same result. These however are markers but not predictors. Any baseline predictor for IFN therapy, IL1 IL-6 and TNF alpha are predictors. What about shorter therapy, one month of therapy more tolerable (**1,000 patients on this trial**); results are pending. Also Moschos and Kirkwood et al observed the CD3 positive T cell and CD11c DC went up after IFN for 1 month. There was a decrease in Stat3 decrease both prot and phosphor protein. Vaccines and IFN and GMCSF. HLA A2 treated and also non A2 are controls: results pending. Many vaccines like also GM2 and the morton cancer vax did worse with the vaccine. Results with ACTA4 are controversial and discussed also by Toni Ribas. Elevated LDH is in favor of anti-CTLA4 though per se is in general a poor prognostic factors (this was however an exclusion factor for the therapy);

H Shiku - *Cancer Vaccine Development in Japan* – Cholesterol-based nano-particles mixed with protein (HER2, NY-ESO-1 and MAGE); they demonstrated HLA I and II associated presentation as well as high expression of Ag in DC in lymph nodes). TCR gene transfer– Anima model with CMS5 sarcoma in DUC18 transgenic mice recognizing mERK2 in collaboration with Paul Allen and Bob Schriber (context $H2K^d$). Adoptive T cell transfer is given day 2, or 4 or 7, only at day two they work. Cytokine staining demonstrated that the more cytokines where produced the better the outcome (multi-functionality); beautiful slide about the expression of various markers decreasing with time. Tumor challenge did not rescue the multi-functionality. (remember SAR CD27 and telomeres) only cells derived from day two adoptive transfer can reject the tumor. In addition ablation of T regs (stimulation of GITR with

stimulating antibody or depletion with anti T reg antibody) induces the multifunctionality. Also peptide vaccination rescues multifunctional T cells in vivo. In conclusion, T regs decrease the function of T cells and vaccination blocks this effect. Also MAGEA4 TCR transgenic transfer was tested in NOD mice (gamma-chain KO mice). The addition of antigen vaccination increased the effectiveness and the multi-functionality of these T cells. To improve the function of the T cells they used a Codon optimized TCR by silencing the endogenous TCR and transfecting the ectopic expression of the transgenic gene. This increases the expression (what about NK cells?).

N Sato - *Clinical trials, markers of immune response against tumor antigens expressed by human cancer initiating cells* (stem cells with cancer initiating ability)-. Does the fundamental tumor Ag exist? W31 (highly tumorigenic initiating clone) and Brash Cell as cancer initiating cell counterparts (transformed with H-ras); CD34+/CD38-, CD138-, CD20+ CD133+ etc.). Novel isolation technique: Expression of ABC transported accounting for resistance to chemotherapy (verapamil can interfere with the function of ABC (i.e ABCG2). By giving verapamil the stem cells can be characterized as a side population that looses expression when verapamil is added. Cells sorted this way have higher colony formation in vivo and higher cancer initiating ability in SKID mice. Array analysis demonstrated that two proteins are more expressed in the stem cells: SMCP (sperm mitochondrial cystein rich protein and it is a typical cancer testis antigen). Over expression increases the tumorigenic expression in mice which is reduced by siRNA. The other is SOX-2 (sex determining region Y box-2) expressed in brain, pancreas and intestine by PCR but not by immune histochemistry. IN cancer only few cells are positive in primary lung and breast cancer. These cells have a basaloid phenotype. Several cancers were tested comparing 13 basaloyd cancer compared with 12 non-basaloyd and the two proteins were expressed only in the basaloyd. The expression correlated with prognosis and increased resistance to CPT. Susceptibility to CTLs is similarly susceptible to lysis compared with decidual cancer cells. An epitope of SOX-2 *109 KYRP… epitope associated with HLA-A*2402 has been identified). Other proteins have been identified.

Y Doki - *Study of NY-ESO-1 data from patients with NY-ESO-1* -. A CT antigen with strong antibody responses as well as CTL responses. The use a Cholteseryl Pullulan nano-particles (see first talk)that absorb the protein and express in the APCs. Several aspects of monitoring were discussed including ELISA, IHC, Ag spreading, and Epitope analysis. Loss of NIH was seen with vaccination, some cases of antigen-spreading were observed and a specific region of the NY-ESO-1 protein was found to be most immunogenic. In the future only this region will be used for immunization.

M Jinushi - *MFG-E8: a negative regulator and potential biomarker to predict clinical activities of GVAX* -. GM-CSF gene transferred tumor cell vaccines (GVAX) was tested. The clinical trial induces strong anti-tumor responses. DFCI cancer Vaccine center. Phase III against hormone refractive Prostate cancer no significant different and increase mortality due to disease progression, thus termination of the clinical protocol. GM-CSF deficient mice develop autoimmune manifestations including pulmonary alveolar proteinosis, SLE and insulitis and diabetes. GM-CSF regulates the phagocytosis of apoptotic cells by APCs. Anzler at all JEM and Lauber Immunity 2004 analysis of phagocyte receptors MFG-8 is down-regulated of apoptotic cell phagocytosis. Defects are responsible for impaired apoptotic cell phagocytosis in GM-CSF -/- mice. Its transduction normalizes cytokine productin in reponsoe to apoptotic cells (TGFb, IL1b IL-4 IL12p70 and IL23p19 that regulated T helper cell differentiation. GM-CSF regulates the differentiation of TH cell differentiation by MFG-E8. TLR stimulation suppresses MFG-E8 production by APCs resulting in increased allo-MLR in apoptotic cell loader macrophages driven splenocyte proliferation. Jinushi M et al JCI 2007 Dual roles of GM-CSF in pro- and atni-inflammatory reactions (great picture!). Blockade of MFG-E8 in tumor cells potentiates GVAX therapeutic immunity (B16 model). GVAX/RGE (inhibitor of MFG-E8) vaccines decrease Tregs and decreases tumor specific CD8+ T cell effectors with decrease of FOxP3 and increase in CD69 expressing CD8 T cells. MFG-E8 expressin melanonoma patients with advanced stage Is high and not detected in non advanced stage melanoma and nevi. Conclusion: MFG is a negative regulator of GVAX induced immunity by regulating Treg/Teff balance. It is a prognostic factor and may predict response to GVAX therapy. H Stricher pointed out should be used as partial surrogate to look at prediction or to see the prognosis after vaccine therapy?? It should be used for the interpretation of clinical data. Lotze: the most effective was TLR4 but not TLR9 (weak effect, why?); in the tumor microenvironment MFG ??? What are the endogenous factors that may bind to TLR4 that may affect the production of down-regulation? We do not know.

H Takeuchi - *EpCAM, a tumor associated antigen to isolate circulating tumor cells in gastrointestinal cancers* - use of anti-Epcam may affect tumor stage and progression, characterizes the investigate the prognostic impact of Epcam in 130 patients with GI cancers, 35 non-metastatic …., Use a system to isolate cells from circulation that express EpCam using magnetic beads. Intact CTCs are checked. Big

differences with healthy donors and increase with stage. Also CTC decrease with chemotherapy is they have stable disease but increase if the patient has progression of disease. Also EpCAM expression was studied by RT-PCR in esophageal cancer and found to be not different in various stages but survival demonstrated big differences in the survival. In cell lines of esoph cancer a proliferation assay was performed showed that introduction of EpCAM increase the expression of Cyclins (G2 and A) by microarray validated by PCR (also Cyclin D, E and c-myc) thus EpCAM expression accelerates cell cycle. Thus EpCAM targeted immune therapy; anti-EpCAM antibodies decrease tumor growth in animal models but more recently there are clinical trials.

L Michael - *You eat what you are: autophagy and immunity* - Hybrid metabolism: mytochondria and glyucolysis**.** Phase II trial: UPCI 06-035 in pancreatic cancer. Biomarkers in pancreatic cancer HJ Zeh Winicoff S,…Marrangoni AAM Cancer Biomark 2005 1(6): 259-69 to distinguish pancreatic cancer compared to pancreatitis. Signal 0 is initiated by PAMP or DAMPs: DAMPs are several intracellular **.** Cancer is the result of a chronic release of DAMPS. **HMGB-1** second most abundant protein after β-actin. HMGB-1 is associated with necrosis (type III death) but not during apoptosis. HMGB1 suppresses apoptosis. Thus, in type I death is sequestered in the nucleus, in type II death autophagy something different happens. During stress autophagy and apoptosis are increased. Those patients that do not respond to therapy have higher autophagy, Starvation promotes autophagy as does Rapamycin in the presence of HMGB1 there is autophagy but if KO cells for HMGB1 there is apoptotic cell death by Caspase 3 assay. Exogenous HrHMGB2 increasesw autophagy in cancer cell lines. Loss of oxidative phophsorylation in HMGB1-/- negative cells.Authophagy measures: HMGB1, p62, Beclin 1, LC3 spots or LC3-II, DAMP miRs, Mitochondrial markers (p75, NDUSF1- Dermott select study 120 pts with RCC looking for CAIX as a prognostic marker).

T Ribas - *Invasive and non invasive monitoring of cancer therapy* - BFF = best friends of Franco best friends forever. Tremelimumab over 650 patients treated both negative endpoint analysis both confirm a low but reproducible response rate (10%). Most responses however are long term, 20 to 30% have autoimmune toxicities there is a critical need to understand the mechanism of action. Study Peripheral circulation “how you define an immune response with those assays?”. Fixed limits_ take a negative and define a confidence interval> Components of assay variability 1) technical (different protocols) Analytical (Same SOP, variations in replicates) Physiological (Same person, different results over time). Definitions of a positive immune response: Mean+3SD Fixed value cutoff, useful for populations with similar baseline set values. RCV (reference change value: allows. No expansion was observed during CTLA4 no decrease in T regs. Post treatment gene expression profiling from ENA shows activation of T cells (get Ena info). Phosphoflow techniques suggest that T cells from Gary Nolan lab and studied pZAP70, pLCK, p38 pErk pAKT and pSTAT1. Cellular barcoding (Pacific Ortange) to run cells together to minimize variation and then compare expression for various phosphoproteins in different cell types and observed that tremelimimab induces changes in pLck (decrease in CD4 cells increases in p38 in DI14 cells increases in pSTAT1 in CD4 and CD14 cells pSTAT3 increase and pSTAT5 decrease. There was a decrease in pErk in both CD4 and CD14. The surprising result was the impact on monocytes that could not be expected by the anti-CTLA4 therapy but data shows that monocytes to express CTLA4. Also there is increase in Th17 cells. What about intra-tumoral data? This shows a clear difference from the peripheral circulation. We should look at the whole kinetics in vivo using PET-based molecular imaging .PET reporter gene ^{18}F-FHBG in HSV1-sr39tk. Engineering antibodies for control of PK and targeting. A phase 2 open label single arm clinical trial to study the mechanism of action of CP-675, 206 in patients with…. . Tumors do not change early in the response against ACTL4 the size of tumor early in not a predictor of response which may come later (inflammation and necrosis increases size). FDG and FLT was however useful in showing the early response. In the spleen also there were changes visualizing tremelimumba induced release of CTLA4 cell cycle checkpoint; finally nanotechnology with James Heath at Cal Tech trying to miniaturize tetramer technology for use in FNA. DNA encoded antibody libraries (DEAL), can they be used to select cells based on tetramers. Third generation solid state sorting on MHC array multiplexing and having high capture efficiency therefore allowing testing of tumors with minimally invasive techniques. This allows the study of many cells at one time by solid phase immobilization (Kwon G, Radu C and Ribas A).

K Kato - *Interleukin-13 receptor alpha 2 as a target for cancer therapy* - Identification of cell surface molecules candidates for cancer therapy. CAR- and integrin dependent adenovirus type 5 infection in not tumor specific. The classic process in not tumor specific. Thus, what makes it tumor specific? To overcome the problem a modified vector contains a Z33 motif. This increases the infection because it binds to IgG and allows binding to Fc in human Ad5. This may allow high efficiency of transduction in a tissue specific way. Increase transgene expression was observed in OV-3 cancer cells by anti-ERb-2 mab

constructs. Development of hybridoma libraries to screen for cancer surface markers using a bgal reporter system. The antigen is then identified by immune precipitation and TOF. 1B7 antibody could bind to prostate, pancreas and breast cancer but no normal cells. The antigen recognized turn out to be EpCAM. Also a novel target was identified by using the same method S11 and T13 clones which increased adenovirus infection. This antibody can bind pancreatic prostate and ovarian cancer and the molecular is PAP type 2A as an antigen. Several antigens have then identified among them CD213a2 (IL-13 receptor alpha 2). The heterodimer IL4 Ra and IL13 Rea1 is in the immune cells but in cancer the heterodimer is the combination of IL4Ra and IL-13a2 which is higher affinity than the Ra1 (known to be expressed in glioma and ovarian cancer. Also 3/13 melanoma cell lines were positive. Another antibody was developed and more staining was observed of melanoma cell lines with no differences between primaries and metastatic lesion. The expression appears to be regulated by methylation as hypo-methylation agents increase the expression. Toxin conjugates demonstrated antitumor effects in vitro. In the FDA has already started a trials to test the effectiveness but perhaps DAC should be added.

X Wu - *Systematic evaluation of genetic variants in inflammation pathway as predictors for cancer risk and clinical outcome* - Epidemiologic research evolution from traditional to molecular to integrative (questionnaire to serum DNA to more integrative techniques including tissue arrays etc). Most importantly different pathways are not mutually exclusive. Create a model based on all various informations looking for germ line as well as somatic variants. The leading hypothesis is that the inflammatory response may play a role in carcinogenesis. 59 SNPs in 36 genes were analyzed. SNPs were selected with those promoter UTR or coding region considering genes previously suggested by the literature to be relevant. Several cytokines etc were selected and were studied in 1500 lung cancer cases and 1700 matched controls. Very comprehensive epidemiologic information was obtained and 7 SNPs were found to be relevant. Among them there were variants in IL1a and IL1b with IL1b being a stronger factor for predisposition to lung cancer in heavy smokers. In bladder cancer 5 SNps were associated with increase MCP1 and IFNAR2 and two with decreased risk COX2 and IL4r (The COX-2allele was observed to be associated with reduced mRNA expression). Thus COX 2 UTR protects against bladder cancer risk. Potential of pharmacogenetics: BCG treatment in lung cancer; the response depends on high TH1 cellular immune response level. A study of more than 400 cases about half experienced recurrence; among them the same genes that were associated with risk of developing bladder cancer were also predictor of response; a survival tree analysis could be constructed to see that the presence of several SNPs can have a worst recurrence rate if the combination of poor predictors is present. ROC including IFNl+EP+Clin reached a .94 level compared with slightly above .5 based on the Clinical information alone. Recent study is being completed with 10,000 SNPs of which 400 belong to inflammation pathway. Many SNPs were identified to predict pneumonitis.

M Toyota - *The role of epigenetic changes in cancer immunotherapy* (Sapporo Medical University) -
Integration of IFN signaling to p53 responses. Identification of FGFR1 as a target of IFN-α, It is upregulated by IFN and also is expressed by liver cancer cells but not hepatocytes. Anti FGFR1 antibodies suppress growth of xenografts; the mechanism by altering the transmembrane receptor activity and in particular activation of IFN-α and IFN-b related genes. The mechanism is mediated to a combination of JAK IRF signaling and the MAPK pathway (FGFR1) By suppressing of reduction of the MAPK pathway and consequent over activation of the JAK/STAT/IRF pathway.
The role of DNA methylation: WNT signaling Growth factor stress and inflammation Nature Genetic 2004. Epigenetic inactivation of CHFR and mitotic checkpoint which is sensitive to microtubule inhibition restoration and results in cancer cell apoptosis: genetic and epigenetic alterations in colorectal cancer and comparison to age related-methylation. Also methylation of CIITA is associated with down regulation of MHC class II and lack of induction with IFN-γ. COBRA method was used to analyze the methylation patterns of the gene CIITA.

H Streicher - *A survival guide to correlating clinical results with immune responses and biomarkers* - CTEP sponsors about 200 INDs and 60 to 80 clinical trials; prioritize trials design by using biomarkers. Predictive biomarkers (GSK gene signature, predictive classifiers as Rich Simons); prognostic biomarkers also prior to treatment (Korn melanoma, Halabi Score, Gajeski Gene signature in melanoma, Tissues infiltrating lymphocytes, MDSC); mechanistic biomarkers (after treatment) and measures of treatment effect (surrogate biomarkers) immune responses, autoimmune responses, activated T cells tumor tissue Th1, cytokine, necrosis and vasculitis. incomplete understating of biology, translation requires identifying targets, predictive classifiers of response, etc.
Why do we need biomarkers? They could be used to stratify patients; example is CTLA4; the biology is too complicated to identify the factors see Jim Allison from Peggs KS and Quarada SA and Korman AJ Curr Opin Immunol 2006; 18, 206. When the biology is complicated the number of variables to evaluate

is too big to use all of them to follow the patients during clinical trials though "ACTL4 is a proof of principle drug". Measuring immune responses is not going to be enough other markers should be identified. Predictive markers are critical. Also surrogate biomarkers should be considered that could help the monitoring of patients. Clinical response rates are not necessary relevant be. Predictive biomarkers: K-ras mutation predict response to Cetuximab NEJM OCT 23 2008. Also Her 2 in breast cancer and EGFR mutation in NSCLC in the Japanese population. Adoptive therapy SAR group; strong patient selection bias with good results with total body radiation; talk about CD27 and telomere length (would total body radiation affect the biology of the tumors? Has anybody looked at what happens to the inflammatory response I the tumor? We need to change the way we do phase 1 and 3 (also consider John Kirkwood picture about number of patients and overall effects of therapy). When should we go to phase III? Gene expression based predictive classifiers) Oncotype DX breast.

Y Akutsu - *Immunologic and epigenetic approaches for the treatment of esophageal cancer - Combination of DC and radiation (radio-immunotherapy)*; Radiation kills tumor directly, increases antigenicity of tumors dead cells can be source of Ag and also elicits an abscopal effect by enhancing the immune response. In a mouse model the combination was much more effective than each individual approach. Latin from away from Scopus reported by Mole RH in 1953 Br J Radiol 26.

How does radiation affect the immune system? Increase in CD8 T cells in tumor draining lymphnodes and stimulates also HSP gp96 and stress; can this explain the effectiveness of TBR in the adoptive immune therapy trials? This talk is critical to test the hypothesis relevant to SAR work. This is key because if local radiation could do the same it may be unnecessary to perform TBI. Gp96 can indeed enhance immune responses. *Hystone deacetylase* inhibition for therapy; one of the epigenetic mechanisms Acetylation makes chromatic loose and transcription can occur. Obviously all of this needs to be understood. Analysis of genes that are affected by inhibition of acetylase in cancer. Very important to have this information together with the effects of demethylation. Effects on chemosensitization; also CAR (coxsakie and adenovirus receptor). No expression of CAR in tumors (Esoph cancer).

B Fox - *Monitoring of tumor specific immune responses to undefined antigens* - RLM vaccine protection model. All trials have cytoxan plus GVAX against prostate by three arms only vaccine the other only cytoxan and then pheresis and vaccine and looking at PSA doubling time was better in patients receiving cytoxan. However it is very difficult to understand the immune responses because there is a strong allo response and not clearly known antigens relevant to the autologous tumor. Potential solutions: screen pre and post sera looking for developing antibodyies (invitogen Protein array immune response biomarker arrays to test thousands of proteins). All patients make some kind of antibody responses some of them are in common but there are also gene specific. Correlation with PSA doubling time correlated with immune responses toward other prostate antigens; Leukapherses are used to prepare APCs and also tested for T cell responses (i.e. against galactin A). T cells are stimulated with anti-CD3 and anti-IL2 that expands cells keeping the same Ag-specific frequency. ELISA and ICS is used to test specificity but also the CD107 assay. Can we test against autologous and allo-tumor they make TNF, IFN and GMCSF producing cells that are also CD 107 positivity; with Ed Walker more characterization is performed looking at different other markers see they last papers also one in Trends in Immunology. Big differences in the production of various cytokines but is galectin A expressed by the patients' tumors; Cell Search TM system to isolate cancer stem cells. The number of CTS (stem cells) is a predictor of survival in various cancer. Now they are studying whether CTS express the galectin A.

J Wulfkuhle - *Novel proteomics approaches to immune monitoring - Reverse phase protein micro arrays for signal pathway profiling of tumors in high throughput fashion.* Whitelaw Emma **–** They are studying signaling following therapy with various singling pathways inhibitors/targeting agents based on selection of micro-dissected populations they are spotted in micro arrays. LCM is necessary because it is important to differentiate the microenvironment effects. Tissues are printed at various dilutions with samples from a trial on in the same array. The advantage is that it needs only one antibody simplifying the identification of good antibodies. Validated antibodies should present only one band and should be inhabited by specific phosphor peptides (they have a bank of 300 validated phopho-antibodies: work with Laura Esserman at UCSF looking at breast cancer; in some patients there is concordance between tissue lysate and LCM tissue but in the majority very little (demonstrating the use of LCM). Correlation with clinical data demonstrated a correlation between Her2 expression and phophorylation of Her 2; there was however a subset that had a lot of HerP but low level of amplification. This patients were excluded because they had low Her but they might have responded because they had a lot of phophoHer. Same study was done on the EGFR. *Tissue handling processing variable on the state of phosphoproteins - novel molecular fixatives* - The are so many variable that may affect; time taken to put material preserve material and concentration of endogenous enzymes, tissue thickness and penetration time, storage temperature,

staining and preparation that might affect phosphor. Changes in melanoma with time and phosphoprotein concentration. Incubation of tissue with phophatase or kinase inhibitors results in changes of phophorylation levels respective to their function. Indeed 85% of protein phophorylation was affected. So they used permeability enhancer, phosphatase and kinase inhibitors to stabilize the phophorylation levels. More rigorous studies are ongoing to test the accuracy of this fixative also for RNA levels and other post-translational modifications; is the fixative available? Tissues can be used for both frozen and paraffin and also for FACS analysis. *Biomarker harvesting using nanoparticles and discovery of phophoproteins in breast cancer* - "Smart" core shell affinity bait nano-porous particles; great picture. These particles amplify the biomarker concentration; the biomarker is concentrated because there is no gradient as it is taken from the solution. Different Baits are used for different king of proteins: cationic proteins, anionic proteins, Affinity crhomatografy, and other small molecules et etc; the bait used determines the type of proteins selected. Also selection is based on size selection. They work in less than 1 minute, also they protect degradation (ex: from trypsin that cannot get into the bead). Urine example and the breast cancer example with L Esserman.

L Butterfield - *Assay standardization and expectations* - CLIA test needs to be accurate, precise: this paper proposed guidelines please look at it Landay Fleisher Altman Maino; Ask Lisa for slide with summary of papers that talk about blood collection, timing, transport and cryopreservation. Other issues are fresh vs frozen and this may be very important for APC while it does not affect T cell responses to peptides. ELISPOT have been extensively discusses including sources of variability; Several guidelines have been presented including the need to rest, normal donor control cells need to be included, automated counting is more objective and back ground should be less that 50 spots per million cells; have appropriate positive controls such as peptide pool of common immune responses such as CMV etc. What is the criteria for a positive response? Establish SOP. Tetramer analysis (Britten et al CVC 2009: study at least 100,000 cells). Several recommendations and guidelines. Examples are the TCR zeta assay that is an activation signal believe to be defective in cancer patients; donors studied several times to see variability over multiple assays: this is done at Pittsburgh by Lisa: Butterfield LH Gooding W and Whiteside TL J Immunotherapy 2`: 81, 2008 and Xu T and Depalma and Perricone JTM). Use of central laboratories may help overcome the extensive cost of the see the Pittsburgh model.

N Disis - *Steps in biomarker validation* - Phase I-V hypothesis generating (first three are hypothesis generating and the last two are hypothesis validating. Phase I: exploratory studies in the target population; correlation studies associating into outcome so that can be used to be added to the study prospectively (association with prognosis). *Association with controls* Transgenic models can help for this phase. We under power the studies on biomarker studies; most cancer vaccines are not toxic. Phase III Salazar and Disis 2009 correlation between survival and development of memory response. McShane et al JNCI 2005; discusses data mining. Important is to use multivariate analysis and showed that epitope spreading was predictive of the highest survival. This was validated prospectively in a HER2 TH vaccination plus Trastuzumab. Changes in TGF-β may be good serological marker with bad prognosis in breast cancer (patients with metastatic breast had very high level) as for the mouse model patients changed the level of TGFb during therapy. Also there was a good correlation between epitope spreading and decreases in TGF β. Thus candidate immune correlates of response where very highly correlated to survival with the magnitude of Th1 responses to the specific antigen (HER2) as well as the magnitude of epitope spreading. Multiple biomarkers improve prediction. SNPs to Fc-gamma predict response to Her2 new.

D Stroncek - Discuss micro-RNAs and also papers about potency. Maturation paper is very interesting; ask Dave for some good example. Also the mechanism of stem cell mobilization and the differences: Nervi B link DC J cell Biochem 2006; 99: 690. Important to show that the general markers are the same but the genes are different when using the global analysis. "Gold standard studies of potency assays" study.

H Nishikawa - *Immune monitoring of protein vaccine-induced immune responses; are we ready*? – (Mie University); Immune monitoring of NY-ESO-1 protein vaccine. A good correlation between antibody responses and T cell responses. Thus we may be able to predict T cell responses by analyzing antibody responses. Development of a detection system to identify antibodies against NY-ESO-1; there is a very good quality assay. Good inter institute cross validation and therefore is a standard assay. Using this assay using the previously described beads nanoparticles to test the induction of immune response. All patients had esophageal cancer and expressed the NY-ESO-1. Sero negative patients developed strong antibody responses after vaccination while the patients who had pre-existing responses did not change much of an enhancement in all cases but only in a few. Correlation between immune responses at antibody and T cell level was evaluated in two patients using an in vitro sensitization assay and tetramer analysis; thus in this two cases there was a good correlation.

Raj Puri - *Biomarkers and Monitoring* - The FDA perspective by Puri Director of the Division; office of cellular tissues and gene therapy CBER. Definition can also be used to evaluate product quality; the may have the same phenotype but different genetic characteristics; also they may help identify markers that can eventually be associated with clinical outcome.

9. Other Workshop Activities (Reception, Excursion, other Meetings)

On March 25, we called a meeting regarding a report of this workshop. The participants are Dr. Hideaki Tahara, Dr. Francesco M Marincola Dr. Lisa H Butterfield, Dr. Magdalena Thurin, Dr. Ena Wang, Marimo Sato, Masahisa Jinushi, Akira Kanamoto, Yutaka Kawakami and Tomonori Yaguchi. We finished preparing a report in this day. We will submit this report to journal of transrational medicinre (http://www.translational-medicine.com/)

10. Comments and Opinions

With the support of the National Cancer Institute a workshop was organized in March 2009 to discuss biomarker discovery relevant to the biological therapy of cancer. The workshop was limited to USA and Japanese participation and focused on areas of overlapping interest focusing on model cancer systems of relevance to the understanding of tumor cell/host interactions. Emerging concepts where clearly identified that qualify as promising areas for future validation. Most advanced were the result of hypothesis generating investigations performed in human subjects and, therefore, likely to bear predictive, prognostic and therapeutic relevance in clinical application. The workshop included a strong representation of the leadership of the International Society for the Biologic Therapy of Cancer (iSBTc) and the Japanese Society for Biotherapy (JSB) and it was related to the task force launched by the iSBTc in concert with the United States Food and Drug Administration (FDA) to identify better strategies for biomarker discovery and validation in the field of biotherapy. This long-term effort will culminate with a Workshop tied to the Annual Meeting of the iSBTc to be held in Washington DC on October 28th 2009. From the present NCI-sponsored workshop it becomes evident that valid biomarker candidates predictive of responsiveness to treatment and/or overall disease outcome are emerging; among them interferon related signatures appear to bear a prominent role in several model systems and to be central to the modulation of immune-mediate tissue-specific destruction of which tumor rejection represents a representative facet. Interestingly, these emerging concepts and candidate biomarkers result from individual academic institutions emphasizing the creative role that academia can play in translational research and its potential in playing a significant role in academia/industry partnership.

US-Japan workshop on immunological molecular markers in oncology

March 23-24, 2009, Hawaii

Monday March 23, 2009

8:00 am - 8:15 am **Welcome and Introduction**
Hideaki Tahara and Francesco Marincola

8:15 am - 8:30 am **NCI Perspective on marker development and validation**
James Jacobson

8:30 am - 9:00 am **Plenary Session - Multi-functionality of tumor-specific T cells; just a biomarker?**
Hiroshi Shiku

9:00 am - 10:15 am **Session 1 (part A) - Immune response as prognostic signatures in cancer** *Chairs: Magdalena Thurin and Kohzoh Imai*

Markers of immune responses against tumor antigens expressed by human cancer-initiating cells - *Noriyuki Sato*

Impaired interferon signaling is a common defect in human cancer - *Peter Lee*

Study of NY-ESO-1 - data from patients vaccinated with NY-ESO-1 - *Yuichiro Doki*

10:15 am - 10:30 am *Break*

10:30 am - 12:10 pm **Session 1 (part B) - Immune response as prognostic signatures in cancer** Chairs: *Magdalena Thurin and Kohzoh Imai*

New diagnostic and prognostic markers in melanoma - *Mohammed Kashani-Sabet*

MFG-E8: a negative regulator and potential biomarker to predict clinical activities of GVAX - *Masahisa Jinushi*

A bottom up view of immunology - *Damian Chaussabel*

EpCAM, a tumor associated antigen to isolate circulating tumor cells in gastrointestinal cancers - *Hiroya Takeuchi*

12:10 pm - 1:30 pm *Lunch*

1:30 pm - 3:10 pm **Session 2 - Markers predicting response to immunotherapy**
Chairs: *Francesco Marincola and Noriyuki Sato*

You eat what you are: autophagy and immunity - *Michael T Lotze*

Molecular mechanisms for cancer cell induced immuno-suppression and resistance - potential biomarkers associated with response to immunotherapy - *Yutaka Kawakami*

Invasive and non-invasive monitoring for cancer immune therapy - *Toni Ribas*

Interleukin-13 receptor alpha 2 as a target for cancer immunotherapy - *Kazunori Kato*

3:10 pm - 3:30 pm *Break*

3:30 pm - 4:45 pm **Session 3 - Genetic Background in immune-relevant genes – relation to outcome** Chairs: *Stefan Ambs and Yutaka Kawakami*

Systematic evaluation of genetic variants in inflammation pathway as predictors for cancer risk and clinical outcome - *Xifeng Wu*

The role of epigenetic changes in cancer immunotherapy - *Minoru Toyota*

Differences in tumor immune biology by race/ethnicity - *Stefan Ambs*

4:45 pm - 6:00 pm Discussion

6:00 pm - *Dinner*

Tuesday March 24, 2009

8:30 am - 9:00 am **Plenary Session – Melanoma as immunotherapy models**
John M Kirkwood

9:00 am – 10:15 am **Session 4 - Immunotherapy Treatments and Markers in Clinical Trials Phase II and III** Chairs: *Howard Streicher and Yuichiro Doki*

A survival guide to correlating clinical results with immune responses and biomarkers - *Howard Streicher*

Immunologic and epigenetic approaches for the treatment of esophageal cancer - *Yasunori Akutsu*

Monitoring tumor-specific immune responses to undefined antigens - *Bernie Fox*

10:15 am – 10:30 am *Break*

10:30 am - 11:30 am **Session 6 (Part A) - Assay Validation and Clinical Evaluation – Are We Ready?** Chairs: *David Stroncek and Hisahiro Matsubara*

Novel Proteomics Approaches to Immune Monitoring - *Julia Wulfkuhle*

Assay Standardization and Expectations - *Lisa Butterfield*

	Steps in biomarkers validation - *Nora Disis*
11:30 am - 12:45 pm	**Session 6 (Part B) - Assay Validation and Clinical Evaluation – Are We Ready?** Chairs: *Lisa Butterfield and Hideaki Tahara*
	The use of molecular assays to assess cellular therapies - *David Stroncek*
	Immuno-monitoring of protein vaccine-induced immune responses - Are we ready? - *Hiroyoshi Nishikawa*
	Biomarkers and Monitoring - *Raj Puri*
12:45 pm - 2:00 pm	*Lunch*
2:00 pm - 7:00 pm	Free Discussion
7:00 pm -	*Dinner* (Closing Remarks)