model are clinically achievable. In the case of silibinin, levels measured in the gut of colorectal cancer patients after daily consumption of formulated silibinin (silipide Indena; dose 1.44 g) for one week were about a fifth of the efficacious level of silibinin determined in the gut of *Apc*$^{Min+}$ mice, which had received silipide with their diet (0.2% silibinin) for their lifetime. Ultimately this type of work helps to identify promising efficacious and safe cancer chemopreventive substances.

Wednesday 8 November 10:15–12:00

WORKSHOP 6

Overcoming critical barriers in immunotherapy

22 INVITED

CTLA-1 and PD-1 abrogation as targets for therapy

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CTLA-4 is a negative regulator of T cell activity and inducer of T cell tolerance. It is highly up-regulated on activated CD4 T cells, particularly CD25+ T regulatory cells. It promotes the anti-tumor activity of GM-CSF transduced tumor cell vaccines in mouse tumor models (van Elsas et al *JEM 2001*, Hurwitz et al *Cancer Res 2000*. CTLA-4 gene knock-out mice die of profound lymphoid proliferation and infiltrative myocarditis. CTLA-4 antibody has been shown to induce clinically meaningful responses in metastatic melanoma and RCC. The response rates range from 5% to 22%; most sustained over >12 months. Immune breakthrough events (IBEs) are associated with response and time to relapse (clinical benefit). The IBEs are principally colitis, hypophysitis and skin related, and are generally self-limited or medically manageable. There are rare cases of colitis requiring colectomy, but no infusion reactions or immunogenicity have been noted with the molecule. In a recent trial of CTLA-4 antibody at 3 mg/kg given intravenously every eight months for a year and a peptide vaccine administered subcutaneously 12 times in a year in patients with very high-risk resected melanoma, high levels of immunity were seen to MART-1 and gp100 by ELISPOT assay, and IBEs were clearly associated with time to progression, with $p > 0.03$. Elevated levels of antibodies to OmpC, and E. Coli gut antigen, were observed, and the presence of a single CTLA-4 nucleotide polymorphism called AG49 was associated to relapse and onset of autoimmunity ($p > 0.04$). 24/25 patients with resected stage IV disease (15) or stage IIIB/C disease (10, with a mean of 7 positive lymph nodes) are alive with a median of 17 months of follow-up. These promising data will be followed up with a confirmatory trial with a higher dose of CTLA-4 antibody.

Another way to utilize T regulatory pathways to increase tumor specific effector T cell activity is via modulation of PD-1 (programmed death-1), a molecule up-regulated on activated T cells, both CD8 and CD4 which binds B7-H1/2 and is a *negative* T cell regulator. PD-1 pathways block TcR signal transduction, and PD-1 knockouts show lymphocytic myocardial infiltration, as well as arthritis and glomerulonephritis. An anti-PD-1 human antibody has been generated that abrogates the activity of PD-1. A variety of experiments on the use of an anti-PD-1 abrogating antibody in vitro show that:anti-PD-1 increases proliferation of and enriches for functional tumor-antigen specific CTL and Th cells. Post-vaccine and endogenous pre-vaccine CTL specific for antigens MART-1 and gp100 from melanoma patients are increased in function and avidity as well as in number by 3–30 fold after exposure to PD-1 antibody in vitro. No changes in T cell phenotype were observed, but by Ki-67 staining and by CFSE staining, significant increases in levels of proliferationwere observed. PD-1 abrogation does not appear to impact on programmed cell death *in vitro*. Anti-PD-1 antibody may have an important role in rescuing and amplifying anti-tumor immunity *in vivo* and *in vitro*. We feel that clinical trials of PD-1 abrogating antibody are warranted, alone and with a cancer vaccine approach.

23 INVITED

T regs in cancer

T. Curiel[1,2], B. Barnett[1], W. Zou[1,3], I. Kryczek[1,3], M. Brumlik[1,2], J. Cheng[1], J. Rueter[1]. [1]*Tulane Medical School, Medicine, New Orleans, USA;* [2]*San Antonio Cancer Institute, Medicine, San Antonio, USA;* [3]*University of Michigan, Surgery, Ann Arbor, USA*

Cancers actively evade immunity through a variety of mechanisms. Recent evidence implicates CD4+CD25+ regulatory T cells (Tregs) in tumor immune evasion. The origins of tumor Treg remain poorly defined, as they may arise from naturally-occurring Tregs following tumor microenvironmental conditioning, or they may arise through a distinct differentiation pathway. Likewise, their mechanism(s) of action are not clearly defined *in vivo* and may be through contact-dependent or –independent pathways. Substantial evidence from mouse models for cancer demonstrates that depletion of Tregs improves endogenous and vaccine-induced tumor-specific immunity with improved tumor clearance and host survival. Tregs appear to help defeat immunity in some human cancers as well. To test the hypothesis that Treg depletion would improve immunity in human cancer, we undertook a phase I study that demonstrated that denileukin diftitox (Ontak, a fusion toxin consisting of interleukin-2 genetically fused to diphtheria toxin, approved to treat cutaneous T cell leukemia/lymphoma) depleted Tregs in ovarian, breast, bladder and lung cancer with concomitant improvement in general measures of immunity. Metastatic ovarian cancer largely regressed in one patient, prompting a phase II efficacy trial. This trial enrolled patients with epithelial ovarian cancer, stages III or IV failing first-line therapy with optimal surgical debulking and platinum-based chemotherapy, who were treated with Ontak 12 micrograms/kilogram once monthly. Six patients have been treated to date with one partial response, three disease stabilizations and two progressions. Immune analyzes remain incomplete post-Katrina, but existing data confirm that Ontak treatment is associated with reductions in phenotypic CD4+CD25+ blood Tregs and increased CD3+IFN-γ T cells. These data suggest that Ontak depletes Tregs in ovarian cancer. The link between Treg depletion and any observed immune changes or clinical effects is under active investigation, and remains to be determined. In a mouse model for ovarian cancer we demonstrated that the IL-2 moiety of Ontak does not mediate clinical effects; preliminary data suggest cytotoxicity for tumor cells is not a major mode of Ontak action. Future work will test Treg depletion with vaccination. Other regulatory cells (CD8+ Tregs and B7-H4+ myeloid cells) are also under investigation.

24 INVITED

Novel ideas in DC therapy and DC crosstalk

L. Zitvogel. *France*

Abstract not received.

25 INVITED

Spotlight on IDO: an ancestral metabolic enzyme turns immune regulator

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Indoleamine 2,3-dioxygenase (IDO) degrades the indole moiety of tryptophan and initiates the production of neuroactive and immunoregulatory metabolites, collectively known as kynurenines. The functional expression of IDO by dendritic cells has emerged in recent years as a major mechanism of peripheral tolerance. IDO contributes to maternal tolerance in pregnancy, control of allograft rejection, and protection against autoimmunity, inflammatory pathology and allergy. In both humans and mice, IDO-expressing tolerogenic dendritic cells are found in tumor-draining lymph nodes, possibly resulting in antigen-specific anergy. The wide spectrum of physiopathologic conditions in which IDO appears at work suggests that multiple mechanisms are used by this effector system to down-regulate T cell and inflammatory responses. Two theories have been proposed to account for tolerance induction via tryptophan catabolism. One theory posits that tryptophan breakdown suppresses T cell proliferation by critically reducing availability of this indispensable amino acid in local tissue microenvironments. The other theory assumes that kynurenines act to suppress immune reactivity, probably through direct interaction with effector T cells. Recent data obtained in our laboratory suggest that both effects are required for an efficient tolerogenic crosstalk of dendritic T cells and T lymphocytes.