IT-03. IMMUNOTHERAPY WITH TUMOR LYSATE-PULSED DENDRITIC CELLS FOR NEWLY-DIAGNOSED GLOBLASTOMA FOLLOWING FLUORESCENCE-GUIDED RESSECTION
Ricardo Diez Valle1, Sonia Tejeda1, Suana Inogics2, Miguel Angel Idaho3, Ascension Lopez Diaz de Cerro2, Jaime Espinos1, Javier Aristu1, Jaime Gallego1, Javier Perez Calvo1, and Maurizio Bendandi1; 1Clinica Universidad de Navarra, Pamplona, Spain; 2CIMA, Pamplona, Spain

BACKGROUND: Immunotherapy is a promising therapy for glioblastoma, however, different strategies and potential selection biases make it difficult to evaluate efficacy. We hypothesized that treatment with tumor lysate-pulsed autologous dendritic cells would be effective when added to gross total resection and standard radio-chemotherapy in newly diagnosed glioblastoma. We designed a trial recruiting patients from the time of surgery to avoid selection biases. METHODS: All patient candidates for resection during the study period were screened, and an attempt at maximum resection was made in every case using fluorescence-guided surgery; less than 1 cm² residual tumor and glioblastoma pathology were required for entry confirmation. Adjuvant treatment included radio-chemotherapy with temozolomide up to 12 cycles. Vaccines were prepared as soon as possible after surgery using autologous dendritic cells pulsed with tumor lysate and matured ex vivo. The vaccination calendar started before radiotherapy. Overall survival was compared to a historic cohort and to European Organization for Research and Treatment of Cancer (EORTC)-published nomograms. RESULTS: We screened 32 patients and included 31 (96.9%); one was excluded because of the presence of residual tumor. The mean age was 58.6, and Karnofsky performance status score was 90-100 in 28% of the patients, 70-80 in 63%, and 60 in 6%; 10% of the patients were in RPA III, 42% RPA IV, and 48% RPA V. Immunotherapy was well tolerated and induced specific immune responses. Median overall survival at the moment of this writing is 27.4 months versus 14.7 months in patients treated with the control (p = 0.003). Median survival for patients in RPA class V is 26.9 versus 10.7 (p = 0.007). Compared to EORTC nomograms, 23 patients have lived longer than predicted, 3 have lived shorter, and 5 do not have enough follow-up information (p < 0.001, binomial distribution). In multivariate analysis, vaccination was the most significant variable (p = 0.012, odds ratio 2.7; 95% confidence interval: 1.24-5.77).

CONCLUSION: Tumor lysate-pulsed, autologous dendritic cell vaccination is safe and effective when added to standard therapy after gross total resection in glioblastoma.

IT-04. IMMUNE THERAPY OF BRAIN MEDULLOBLASTOMA WITH TH17 CELLS
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INTRODUCTION: Patients with medulloblastomas after primary therapy have a particularly poor prognosis. The aim of this study was to investigate the role of regulatory T (Treg) and IL-17- producing T-helper (Th17) cells in medulloblastoma immune pathology and therapy. METHODS: Fresh medulloblastoma cells were cultured during the serum-free status after surgery. ELISPOT analyses, completed in 13 children, showed GAA responses in 11, most commonly to IL15Rα. We conclude that peptides from the gangliosides of medulloblastoma is generally well tolerated, although distinguishing pseudoprogression from true progression can be challenging. Immunological and clinical responses have been obtained. More extensive analyses of efficacy in a multi-institutional context are warranted.

IT-02. TUMOR-INFLITRATING LYMPHOCYTES AND IMMUNE ESCAPE IN HUMAN MALIGNANT GLIOMAS AFTER WT1 VACCINATION
Yasuyoshi Chiba, Naoya Hashimoto, Naoki Kagawa, Manabu Kinoshita, Yoshiyuki Kijima, Ryushi Hiryama, Yusuuke Oji, Akihiro Tsuboi, Yoshihiro Oka, Haruo Sugiyama, and Toshiaki Yoshimine; Osaka University Graduate School of Medicine, Suita, Japan

In cancer immunotherapy, tumor cells try to escape from antitumoral immunity, whereas tumor-infiltrating lymphocytes (TILs) try to eliminate tumor cells. Many in vitro studies have demonstrated those phenomena indirectly, but few studies have reported this competition between TILs and tumor cells in immunotherapy. The purpose of this study is to investigate what happened to immune cells and tumor cells in glioma specimens by Wilms’ tumor 1 (WT1) peptide vaccination. Fourteen malignant glioma specimens, removed surgically before and after WT1 vaccination, were stained immunohistochemically with CD3, CD4, CD8, CD79α/β, FOXP3, Ki-67, WT1, HLA class I-ABC, and TGF-beta antibodies. The average CD3+, CD4+, and CD79α/β+ lymphocyte count was significantly reduced (P < 0.01). The FOXP3+ CD4+ proportion was significantly increased (P < 0.05). The CD4+/CD8+ ratio tended to decrease (P = 0.12), and the FOXP3+ /CD8+ ratio was stable. The WT1 expression score and HLA class I staining index were significantly reduced (P = 0.028 and P = 0.031, respectively). The TGF-beta expression score tended to increase, but not significantly (P = 0.066). No tumor specimen expressed FOXP3 beyond WT1 vaccination, but four specimens expressed it after WT1 vaccination. In this study, the patient selection was biased (85% of patients defined as progressive disease). This study suggested that in the ineffective cases of WT1 vaccination, malignant glioma cells escaped from antitumoral immunity by various mechanisms, such as down-regulation of WT1 and HLA class I, induction of FOXP3+ regulatory T cells, production of TGF-beta, and expression of FOXP3.

IT-01. PEPTIDE VACCINE THERAPY FOR CHILDHOOD GLIOMAS: UPDATED RESULTS OF A PILOT STUDY
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Diffuse brainstem gliomas (BSG), other malignant astrocytomas, and multiform recurrent low-grade gliomas carry a poor prognosis, and new therapies are needed. Having gained experience with immunotherapy for adult gliomas, we extended these insights to childhood gliomas on the basis of our observations regarding their profiles of glioma-associated antigen (GAA) expression. We initiated a pilot trial of subcutaneous vaccination with peptides for GAA epitopes emulsified in montanide-ISA-51 given every 3 weeks for 8 courses along with intramuscular injections of poly-ICLC in HLA-A2+ children with newly diagnosed BSG, high-grade gliomas (HGG), or recurrent gliomas. The recurred GAA was EPHA2, IL13Rα2, and survivin. Primary endpoints were safety and T cell responses against vaccine-targeted GAs, assessed by ELISPOT analysis. Treatment response was evaluated clinically and by MR imaging. To date, 28 children have been enrolled, 16 with newly diagnosed BSG, 5 with newly diagnosed HGG, 4 with recurrent low-grade gliomas, and 3 with recurrent HGGs. No dose-limiting non-central nervous system toxicity has been encountered. One child with a BSG had transient tumor enlargement in association with acute neurological deterioration 4 months after beginning vaccination, but that later regressed and culminated in a sustained partial response (PR), consistent with pseudoprogression. Two other children with BSG had symptomatic pseudoprogression, with transient neurological deterioration and tumor enlargement followed by stabilization. Among 24 patients evaluable for response, 3 had rapidly progressive disease, 16 had stable disease for >2 cycles, 3 had PRs, 1 had a molecular response, and 1 had prolonged disease-stasis. Among 24 patients evaluable for pseudoprogression, with transient neurological deterioration and tumor enlargement followed by stabilization. Among 24 patients evaluable for response, 3 had rapidly progressive disease, 16 had stable disease for >2 cycles, 3 had PRs, 1 had a molecular response, and 1 had prolonged disease-free status after surgery. ELISPOT analyses, completed in 13 children, showed GAA responses in 11, most commonly to IL13Rα2. We conclude that peptides from the gangliosides of medulloblastoma is generally well tolerated, although distinguishing pseudoprogression from true progression can be challenging. Immunological and clinical responses have been obtained. More extensive analyses of efficacy in a multi-institutional context are warranted.

CLIN-Immunotherapy/Biologic Therapies

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VACCINATION IT-02. TUMOR-INFILTRATING LYMPHOCYTES AND IMMUNE ESCAPE IN HUMAN MALIGNANT GLIOMAS AFTER WT1 VACCINATION

IT-03. IMMUNOTHERAPY WITH TUMOR LYSATE-PULSED DENDRITIC CELLS FOR NEWLY-DIAGNOSED GLOBLASTOMA FOLLOWING FLUORESCENCE-GUIDED RESSECTION

IT-04. IMMUNE THERAPY OF BRAIN MEDULLOBLASTOMA WITH TH17 CELLS

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expansion of human Th17 cells, which reveals a novel role for IL-2 in controlling the balance between IL-17+ and Treg cells and provides new insight on the role of IL-17+ T cells in tumor immune pathology and therapy.

IT-05. OVERALL SURVIVAL AND PROGRESSION-FREE SURVIVAL WITH BEVACIZUMAB AND NON-BEVACIZUMAB-BASED REGIMENS IN PATIENTS WITH RECURRENT OR PROGRESSIVE GlioBLASTOMA FROM UNITED STATES COMMUNITY PRACTICES

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BACKGROUND: Bevacizumab (BV) as monotherapy and in combination with irinotecan has demonstrated safety and efficacy in recurrent glioblastoma (GBM). Few studies have evaluated BV use and effectiveness in large, community-based practices. METHODS: Adult patients with recurrent histologically confirmed GBM or grade IV astrocytoma starting second-line treatment from 7/1/2006 to 6/30/2010 were identified using electronic medical records from United States community practices (McKesson Specialty Health; The US Oncology Network). Patients with prior BV exposure were excluded. Treatment patterns and outcomes data were analyzed through 7/1/2011 or last follow-up. Overall survival (OS) and progression-free survival (PFS) estimates for BV (monotherapy [mono] or combination [combo]) and non-BV regimens were determined by the Kaplan-Meier method and compared using the log-rank test. A Cox proportional hazards regression model assessed the effects of patient and treatment characteristics on outcomes, adjusting for covariates. RESULTS: We identified 159 GBM patients initiating second-line treatment with BV combo (n = 79), BV mono (n = 57), or non-BV (n = 23) regimens. Patient characteristics were similar across groups. OS (hazard ratio [HR] = 0.38, 95% confidence interval [CI]: 0.21-0.68) median: 9.5 mos with BV versus 4.8 mos without) and PFS (HR = 0.39, 95% CI: 0.21-0.71, median: 6.0 mos with BV versus 2.8 mos without) were significantly longer with BV than with non-BV regimens. Patients treated with BV combo had a trend toward longer overall OS (HR = 0.51, 95% CI: 0.41-1.00, median: 11.5 mos with combo versus 6.9 mos with mono) and longer PFS (HR = 0.54, 95% CI: 0.34-0.88, median: 8.0 mos with combo versus 4.8 mos with mono) than those receiving BV mono. Multivariate analyses showed that BV treatment (mono or combo) was associated with a reduced risk of death (BV mono versus non-BV: HR = 0.51, 95% CI: 0.27-0.96; BV combo versus non-BV: HR = 0.31, 95% CI: 0.17-0.57). The incidence of BV-related toxicities was consistent with clinical trial reports. CONCLUSIONS: BV-containing regimens are associated with significant improvements in OS and PFS in the second-line treatment of GBM in the community practice setting based on this retrospective analysis. These findings are consistent with published trial data.

IT-06. INTRACEREBRAL CPG IMMUNOTHERAPY WITH CARBON NANOTUBES ABBROGATES THE GROWTH OF SUBCUTANEOUS MELANOMAS IN MICE

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Recently, we showed that intratumoral delivery of low-dose, immunostimulatory CpG oligodeoxynucleotides (CpG) conjugated with carbon nanotubes (CNTs) was more effective than free CpG and not only eradicated intracranial (i.c.) gliomas but also induced antitumor immunity that protected mice from subsequent i.c. or systemic tumor rechallenge. To examine if the same “intracerebral immunotherapy” strategy could be applied to the treatment of metastatic brain tumors, mice that had intracranial s.c. and subcutaneous (s.c.) melanomas were injected intratumorally with CNT-CpG into either location. When applied to s.c. melanomas, CNT-CpG response was mostly local, and it only modestly inhibited the growth of i.c. melanomas. However, when delivered to i.c. melanomas, CNT-CpG inhibited the growth of not only brain but also s.c. tumors. Furthermore, compared to s.c. injections, i.c. CNT-CpG elicited a stronger inflammatory response that resulted in more potent antitumor cytotoxicity and improved in vivo treatment outcomes compared to both i.c. and s.c. tumors. To investigate factors that accounted for these observations, CNT-CpG biodistribution and cellular inflammatory responses were examined in both tumor locations. I.c. melanomas retained the CNT-CpG particles longer and were infiltrated by TLR-9-positive macrophages. In contrast, myocardial-derived suppressive cells were more abundant in s.c. tumors. Although depletion of these cells prior to s.c. CNT-CpG therapy enhanced cytotoxic response, antitumor responses to brain melanomas were unchanged. These findings suggest that intracerebral CNT-CpG immunotherapy is more effective than systemic therapy in generating antitumor responses that target both brain and systemic cancers.

IT-07. A BI-INSTITUTIONAL PILOT STUDY OF PEPTIDE-BASED VACCINES IN COMBINATION WITH POLY ILC2 IN PATIENTS WITH WHO GRADE 2 LOW-GRADE GLIOMA

Hideko Okada1, Lisa H. Butterfield2, Ronald L. Hamilton1, Arlan H. Mintz1, Johnathan A. Engh1, Jan Drappatz2, Mark O. Lively3, Michael D. Chan1, Andres M. Salazar1, Douglas M. Potter1, Edward G. Shaw4, and Frank S. Lieberman5; 1University of Pittsburgh Cancer Institute, Pittsburgh, PA; 2Wake Forest School of Medicine, Winston-Salem, NC; 3OncoVir, Inc, Washington, DC

On the basis of promising data from our phase I/II study targeting multiple glioma-associated antigen (GAA) epitopes in patients with recurrent high-grade gliomas (HGGs), we initiated a pilot study of subcutaneous vaccinations with synthetic peptides for GAAs epitope emulsified in montanide-ISA-51 every 3 weeks for 8 courses as well as intramuscular administration of poly-IIC2 in HLA A2+ patients with newly diagnosed high-risk WHO grade 2 low-grade glioma (LGG) without prior radiation therapy (RT) (cohort 1), newly diagnosed high-risk LGG with prior RT (cohort 2), or recurrent LGG (cohort 3). Primary endpoints were safety and CD8+ T-cell responses against vaccine-targeted GAAs, assessed by enzyme-linked immunospot (ELISPOT) assays. Treatment response was evaluated clinically and by MRI. GAAs for these peptides are IL-13Ralpha2, Epha2, WT1, and survivin. A pan-HLA-DR tetanus toxoid peptide (TetA830) was included to enhance general helper CD4+ T-cell response. To date, 12, 1, and 30 patients have been enrolled in cohorts 1, 2, and 3, respectively. No dose-limiting toxicity has been encountered except for one case with grade 3 fever (cohort 1). ELISPOT assays, completed in 7 and 1 patients in cohorts 1 and 2, respectively, demonstrated robust and sustained interferon (IFN)-gamma (type-1) responses against at least 3 of the GAA epitopes in all cases, whereas IL-5 (type-2) responses were absent or transient in all cases. The magnitude of the IFN-gamma ELISPOT responses in this study is significantly higher than those observed in our previous phase I/II study in HGG patients. Currently, of the patients who received all 8 vaccinations, 5 of 10, 1 of 1, and 2 of 8 patients in cohorts 1, 2, and 3, respectively, are stable (median follow-up of 16.2 months), although evaluation of progression-free survival would require a longer observation period. Our preliminary results demonstrate that the regimen in these patients is well tolerated and induces a robust type-1 anti-GAA T-cell response.

IT-08. MIR-124 AS A NOVEL IMMUNOTHERAPEUTIC MOLECULE TO REVERSE GLIOMA-MEDIATED IMMUNE SUPPRESSION AND ENHANCE ANTI-TUMOR IMMUNE RESPONSE

Jun Wei, Ling-Yuan Kong, Fei Wang, Shuo Xu, Tiffany A. Doucette, Sherise D. Ferguson, Yuhui Yang, Kayla McEnery, Krishan Jethwa, Olsi Gjyshi, Wei Qiao, Frederick F. Lang, Ganeshe Rao, Greg N. Fuller, George A. Calin, and Amy B. Hemberger; The University of Texas MD Anderson Cancer Center, Houston, TX

MicroRNAs (miRNAs) have been shown to modulate critical gene transcripts involved in tumorigenesis, but their role in tumor-mediated immune suppression is unknown. In this study, we evaluated miRNAs that are preferentially downregulated in malignancy and that interact with immune suppressive pathways as potential new therapeutics. On the basis of miRNA-gene expression profiles of gliomas using tissue microarrays, in situ hybridization, and molecular modeling, we selected mir-124 as the lead candidate for modulating signal transducer and activator of transcription 3 (STAT3), a key molecular hub in tumor-mediated immune suppression. In a glioma tissue microarray, miR-124 expression was significantly downregulated in all grades and types of gliomas relative to normal brain. Upon upregulating mir-124 in glioma cancer stem cells (gSCs), STAT3 was inhibited; this inhibition reversed tumor-mediated immune suppression, as reflected by an increase in T-cell proliferation, FoxP3+ regulatory T-cell (Treg) inhibition, and pro-inflammatory immune response upregulation. Treatment of immune-suppressed glioblastoma patient T cells with mir-124 induced a marked efferent response. Furthermore, the in vivo local or systemic administration of mir-124 in multiple murine models of glioma, including intracranially transplanted heterogeneous high-grade gliomas, exerted potent anti-glioma therapeutic effects secondary to STAT3 inhibition in the immune cell population and secondarily enhanced effector responses in the local tumor microenvironment. In summary, mir-124 may be a novel immune-activating agent for glioma treatment (including all grades and types); by exploiting the
immune system to mediate direct tumor cytotoxicity, the vexing problem of miR delivery to tumors has been overcome.

**IT-09. EX VIVO GENERATION OF CENTRAL MEMORY-LIKE ANTI-TUMOR T CELLS TO TREAT MALIGNANT BRAIN TUMORS**

Shicong Yang, Gary E. Archer, Hongsheng Miao, Xinyu Cui, Weihua Xie, David Snyder, Andrea J. Pretorius, Anjelika Dechkovskaia, Elizabeth Reap, Luis A. Sanchez Perez, Pamela Norberg, Robert Schmittling, Duane A. Mitchell, and John H. Sampson; Duke University, Durham, NC

Compared to vaccines using RNA-loaded dendritic cells (DCs), adoptive cell transfer (ACT) using ex vivo expanded anti-tumor cells seems to be more potent in eradicating established tumors. At present, the consensus for the best type of T cells designated for ACT is central memory T cells (Tcm), which express high levels of CD62L and secrete IL-2. In this study, we chose total tumor RNA (tRNA) from glioblastoma (GBM) and murine brain tumor lines to load onto DCs as a platform to ex vivo expand anti-tumor T cells and optimize the culture conditions with the use of cytokines to generate Tcm cells. To optimize the platform, we utilized peripheral blood mononuclear cells (PBMC) from patients with GBM and murine brain tumor lines. Among the cytokines tested (IL-2, IL-7, IL-12, and IL-21), only IL-2 and IL-7 supported the growth of T cells in vitro; when the DCs were added in the co-culture, we observed a synergy of cytokines and DCs in supporting the growth of T cells, and the presence of DC favored the expansion of CD4+ T cells. IL-2 preferentially expanded one subset that was identified as CD4+CD25highFoxP3high regulatory T cells (Treg) during ex vivo culture; meanwhile, IL-7 enabled multiple-subset proliferation from the original precursors. Interestingly, supplementing the cultures with a cytokine cocktail (IL-7, IL-12, and IL-21) greatly increased the percentage of CD62L+ central memory-like T cells, which specifically recognized tRNA-loaded DCs as a surrogate target and engrained better in a xenogeneic murine model. Moreover, the antitumor T cells generated from the use of this cytokine cocktail exhibited superior antitumor activity compared to T cells from the use of IL-2 in a syngeneic murine IC model. At present, we are investigating the molecular mechanism of how the central memory-like anti-tumor T cells generated by the cytokine cocktail convey such superior anti-tumor activity in vivo.

**IT-10. THE MICRORNA REGULATORY AXIS OF STAT3: IMPLICATIONS FOR GLIOMA STEM CELL BIOLOGY**

Fei Wang, Jun Wei, Oks Gyshli, Ling-Yuan Kong, Shuo Xu, Frederick Lang, George Calvin, and Amy B. Heimberger; The University of Texas MD Anderson Cancer Center, Houston, TX

We have previously demonstrated that the signal transducer and activator of transcription 3 (STAT3) is a key molecular hub for gliomagenesis and tumor-mediated immune suppression. Over the last decade, microRNAs (miRs) have emerged as playing fundamental roles in cancer, including differentiation. To determine if these miRs are interacting with and regulating the STAT3 pathway, target scan analysis, real-time quantitative PCR, mutational analysis, and forced overexpression and inhibition were used in glioma cancer stem cells (gCSCs) to dissect these interactions. We have found that miR-124 is significantly down-modulated and that miR-21 is specifically enhanced within the gCSC population; a direct inverse correlation is observed between miR-21 and miR-124 expression. Forced overexpression of miR-124 in gCSCs inhibited IL-6 receptor and STAT3 protein expression, inhibited miR-21, and decreased their immunosuppressive properties. In contrast, the forced overexpression of miR-21 markedly enhanced the immunosuppressive properties of gCSCs. These miRNA-mediated alterations of the immunological properties of the gCSCs may be in part related to their differentiation state and “stemness.” Mechanistic studies demonstrate that miR-124 controls the STAT3 pathway proximally while STAT3 regulates miR-21, thus demonstrating a complex regulatory axis of miRNAs on transcriptional pathways. Manipulating these miRNAs may be an alternative or supplementary approach for therapeutically targeting STAT3 in glioma patients.

**IT-11. THE ANTI-TUMOR AND IMMUNOLOGICAL PROPERTIES OF MI-R142-3P IN GLIOMA**

Shuo Xu, Jin Wei, Ling-Yuan Kong, Fei Wang, George Calvin, and Amy B. Heimberger; The University of Texas MD Anderson Cancer Center, Houston, TX

MicroRNAs (miRNAs) have been shown to play a critical role in tumorigenesis, but their role in tumor immunity remains largely unknown. Recent data have shown that the glioma-associated macrophage/microglia are immune-suppressive, tumor-supportive, and participate in therapeutic resistance. On the basis of miRNA-gene expression microarrays of glioblastoma-infiltrating microglia and matched peripheral monocytes, miR-142-3p was identified as the most significantly downregulated miRNA candidate in the microglia population. Highly conserved binding sequences for miR-142-3p were identified in the 3’-untranslated regions (UTRs) of several TGFβ3 pathway-related genes including TGFB1, TGFB2, and TGFβ3, which were all upregulated in glioblastoma-infiltrating microglia and are known to modulate macrophage M1/M2 phenotype shift as well as function. An in vitro macrophage induction assay demonstrated a preferential lower miR-142-3p expression in the immune-suppressive M2 macrophages than in pro-inflammatory M1 macrophages. In vivo ablation of miR-142-3p within immune-competent GL261-bearing mice resulted in tumor growth suppression and enhanced anti-tumor immune responses. This administration was sufficiently immunologically potent to induce virus-like fever in mice without other evident toxicity. These data indicate a unique role of miR-142-3p in glioma immunity by modulating glioma-infiltrating microglia phenotype shift and function as well as revealing its transitional potential in glioma treatment.

**IT-12. EX VIVO FUNCTIONAL ANALYSIS, EXPANSION, AND ADOPTIVE TRANSFER OF CYTOMEGALOVIRUS-SPECIFIC T-CELLS IN PATIENTS WITH GliOBLASTOMA MULTIFORME**

David G. Walker1, Tania Crough2, Leone Beagley2, Corey Smith3, Linda Jones2, and Rajiv Khanna2; 1NEWRO Foundation, Brisbane, Australia; 2QIMR, Brisbane, Australia

The frequent detection of human cytomegalovirus (CMV) antigens in glioblastoma (GBM) multiforme brain tumor lines to load onto DCs as a platform to ex vivo expand anti-tumor T cells and optimize the culture conditions with the use of cytokines to generate Tcm cells. To optimize the platform, we utilized peripheral blood mononuclear cells (PBMC) from patients with GBM andmurine brain tumor lines. Among the cytokines tested (IL-2, IL-7, IL-12, and IL-21), only IL-2 and IL-7 supported the growth of T cells in vitro; when the DCs were added in the co-culture, we observed a synergy of cytokines and DCs in supporting the growth of T cells, and the presence of DCs favored the expansion of CD4+ T cells. IL-2 preferentially expanded one subset that was identified as CD4+CD25highFoxP3high regulatory T cells (Treg) during ex vivo culture; meanwhile, IL-7 enabled multiple-subset proliferation from the original precursors. Interestingly, supplementing the cultures with a cytokine cocktail (IL-7, IL-12, and IL-21) greatly increased the percentage of CD62L+ central memory-like T cells, which specifically recognized tRNA-loaded DCs as a surrogate target and engrained better in a xenogeneic murine model. Moreover, the antitumor T cells generated from the use of this cytokine cocktail exhibited superior antitumor activity compared to T cells from the use of IL-2 in a syngeneic murine IC model. At present, we are investigating the molecular mechanism of how the central memory-like anti-tumor T cells generated by the cytokine cocktail convey such superior anti-tumor activity in vivo.

**IT-13. WT1 PEPTIDE VACCINATION FOR NEWLY DIAGNOSED GliOBLASTOMAS: PHASE I CLINICAL TRIAL OF COMBINATION WITH TEMOZOLOMIDE**

Nana Hashimoto, Akihito Sato, Yasuyoshi Chiba, Noriyuki Kijima, Yoshihiro Oka, Yusuke Oji, Manabu Kinoshita, Naoki Kagawa, Toshiki Yoshimine, and Haruo Sugiyama; Osaka University Graduate School of Medicine, Suita, Japan

The phase II clinical trial of WT1 vaccination against recurrent glioblastoma (GBM) showed safety and efficacy of the vaccination. Despite the central dogma that chemotherapy and immunotherapy should not be combined, our basic research in gliomas and reports on other cancers revealed the possibility for combining those 2 modalities. Notably, our preliminary evaluation of WT1 specific T cells in peripheral blood gave us some justification of the combination, taking into consideration the concept of homostatic proliferation of effector cells. We recently performed a phase I clinical trial to assess the safety of combining temozolomide (TMZ) and WT1 vaccination against GBM. The study was designed using a 3+3 cohort method and started at level 2; we based our protocol on the Stupp regimen, and WT1 vaccination was added just after the end of combined radiotherapy.

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Abstracts
IT-15. INCREASED EXPRESSION OF TUMOR-ASSOCIATED antigens was well tolerated and may alleviate TMZ-induced lymphopenia in GBMs disease, and one had disease progression. Present findings showed that cing chemotherapy after RT (MRI) scan. Five patients (2 men and 3 women, mean age 55 years) cells, lymphocytes, and neutrocytes, and magnetic resonance imaging from peripheral blood mononuclear cells after having been activated and munotherapy using autologous lymphokine-activated killer cells (LAKs) in GBMs, immunotherapy is one of the more hopeful and attractive ap- stages of testing. We therefore investigated whether EphA2, IL-13Ralpha2, and survivin, and, additionally, Wilms’ Tumor 1 (WT1), are overexpressed in pe- phase of testing. We therefore investigated whether EphA2, IL-13Ralpha2, and survivin, and, additionally, Wilms’ Tumor 1 (WT1), are overexpressed in pe- phase of testing. We therefore investigated whether EphA2, IL-13Ralpha2, and survivin, and, additionally, Wilms’ Tumor 1 (WT1), are overexpressed in pe- phase of testing. We therefore investigated whether EphA2, IL-13Ralpha2, and survivin, and, additionally, Wilms’ Tumor 1 (WT1), are overexpressed in pe-
IT-18. INTERLEUKIN 13 RECEPTOR ALPHA-2 IS WIDELY OVER-EXPRESSED IN HUMAN AND CANINE PRIMARY BRAIN TUMORS AS DETECTED BY NOVEL BISPECIES-SPECIFIC MONOCLONAL ANTIBODIES

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Interleukin 13 receptor alpha 2 (IL-13Ralpha2) is overexpressed in a vast majority of patients with glioblastoma (GBM). It represents an attractive target for this and other solid tumors therapies. Recent reports indicated its association with glioma stem-like cells. We have thus generated monoclonal antibodies (mAbs) against the human receptor that would cross-react with a canine receptor because of the close similarities between brain tumors in human and canine patients. Peptides from three different regions of human IL-13Ralpha2, Peptides 1-3, showing 100% homology with canine receptors on the basis of 3-D structure were synthesized as immunogens. Standard protocols were used for generation and purification of monoclonal antibodies. Selected clones were examined for their immunoreactive abilities in Western blots, immunohistochemistry, immunofluorescence, cell binding, flow cytometry and internalization using human and canine tumor specimen sections, tissue lysates, and established cell lines. Several isolated mAbs detected a single protein band of expected molecular size for IL-13Ralpha2 in Western blots of cell and tissue lysates from human and canine GBM patients. Human and canine astrocytomas and oligodendrogliomas were all positive for IL-13Ralpha2 to various degrees. Interestingly, human meningiomas exhibited strong positivity (5/6). In addition, IL-13Ralpha2 was highly expressed in canine choroid plexus papillomas. Normal human and canine brain samples were virtually negative for IL-13Ralpha2 using the newly generated mAbs. One of the isolated mAbs, E10109 (Peptide 3), bound IL-13Ralpha2-positive live human and canine GBM cells and was internalized by these cells. The same antibody also worked in Western blots and immunohistochemistry. We have therefore obtained several monoclonal antibodies against IL-13Ralpha2 that cross-react with human and canine receptors. In addition to GBM, other brain tumors, such as high grade oligodendrogliomas, meningiomas, and canine choroid plexus papillomas, appear to express the receptor at high levels and thus may be appropriate candidates for IL-13Ralpha2-targeted imaging or therapies using novel antibodies.

IT-19. TVI-BRAIN-1 - A PHASE I STUDY TO TEST THE SAFETY OF A COMBINATION OF AUTOLOGOUS CANCER CELL VACCINATION, ADOPTIVE TRANSFER OF CANCER ANTIGEN-SPECIFIC T CELLS AND LOW-DOSE INTERLEUKIN 2 DURING TREATMENT OF PATIENTS WITH RECURRENT GRADE III/IV GLIOMA

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BACKGROUND: Glioblastoma (GBM) is one of the most aggressive human malignancies that has few effective therapies. Based on an earlier report on the tolerability and effectiveness of salvage therapy with TVI in patients with high-grade gliomas, we conducted a phase I trial of TVI-Brain-1, a modified, low dose protocol of the TVI regimen as a salvage therapy. METHODS: Patients with recurrent GBM or anaplastic glioma with Karnofsky performance status score (KPS) > 70 were eligible and had debulking craniotomy for diagnosis and tumor collection. Patients were vaccinated with attenuated autologous cancer cells followed by granulocyte/macrophage colony stimulating factor (GM-CSF). Immunologically active cells were then collected by apheresis, activated ex vivo with T cell stimulants and reinfused, followed by low-dose interleukin 2. The vaccine sequence was repeated a second time. RESULTS: Therapy was well tolerated by the 11 patients with GBM. The treatment resulted in a wide variety of grade 1 and 2 inflammatory-type side effects associated with cancer cell vaccination, immune cell infusion, and interleukin 2 treatment. Significant but unrelated toxicities seen during the protocol included myelodysplasia of tumor cells (1), head trauma from fall (1), operative site cyst (1), and steroid-induced psychosis. Five patients had tumor progression during the protocol and withdrew. CONCLUSIONS: TVI as salvage therapy for recurrent GBM appears safe and well tolerated. Treatment related toxicities were minimal. As a secondary outcome, median survival was 7.7 months for the entire cohort, which did not approach the median survival of 12 months seen in the original TVI study. Factors included poor patient prognostic factors at study entry and prior bevacizumab use. Additionally, the long interval from surgery to infusion of activated T cells, which represents the active part of this therapy, likely also limited efficacy. Currently, there is an ongoing phase II protocol for recurrent GBM and a planned phase III randomized trial for the treatment of patients with newly diagnosed GBM.

IT-20. DENDRITIC CELL VACCINES TARGETING HUMAN CYTOMEGALOVIRUS IN GliOBLASTOMA REVEAL LYMPH NODE HOMING AS A MAJOR AXIS FOR CLINICAL INTERVENTION

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BACKGROUND: Cytolethal distending virus (CDV) reactivation within glioblastoma (GBM) tumors provides a novel target for tumor-directed immunotherapy. METHODS: A phase I/II randomized clinical trial was undertaken to assess the immunogenicity and estimate efficacy of targeting the CMV integument protein, pp65, in patients with newly-diagnosed GBM using pp65 RNA transfected dendritic cells (DCs). Two cohorts of patients were treated. Cohort 1 received pp65 RNA-transfected dendritic cells (DCs) with or without autologous lymphocytes along with standard-dose temozolomide (TMZ) (200 mg/m2 x 5 days). Cohort 2 received pp65 RNA-pulsed DCs mixed with granulocyte/macrophage colony stimulating factor (GM-CSF) along with dose-intensified TMZ (100 mg/m2 x 21 days). At vaccine #4, all patients underwent single photon emission computed tomography (SPECT)/CT imaging of 111-Indium-labeled DCs. In cohort 1, the effects of prior vaccine-site skin preparation with inflammatory stimuli (tatanus toxoid versus unpulsed mature DCs) and佐y of prior vaccine-site skin preparation with inflammatory stimuli (tatanus toxoid versus unpulsed mature DCs) was assessed in a randomized fashion. RESULTS: Cohort 1 (n = 13): there were no vaccine-related, reportable serious adverse events. Expansion of pre-existing CMV-specific cellular and humoral responses was observed. There was a strong correlation between successful migration of DCs to vaccine-site draining lymph nodes and lymph node homing, and of CMV-specific T-cell responses (R = 0.73, P = 0.0005). Patients randomized to received tetanus toxoid skin preparation showed increased lymph node (LN) migration of DCs compared to patients receiving unpulsed mature DCs, and this was associated with superior DFS and OS within this cohort of patients (P = 0.011). Cohort 2 (n = 11): a grade 3 hypersensitivity after 8 vaccinations that was determined to be a reaction against GM-CSF was observed in one patient. Expansion of pre-existing and de novo induction of CMV-specific T cell responses was observed. Increased LN uptake of DCs was observed in all patients along with promising clinical responses (median DFS > 24 months and overall survival undefined at median follow-up of 26.8 months). CONCLUSIONS: DC migration may be an important functional biomarker and axis for improving the efficacy of CMV-targeted DC vaccines in patients with GBM.

IT-21. A TANDEM CHIMERIC ANTIGEN RECEPTOR THAT MEDIATES BISPECIFIC ACTIVATION AND TARGETING OF T-CELLS

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MOLECULAR TAXONOMY REVEALS THE EXISTENCE OF DENDRITIC CELL POPULATIONS IN TUMORS WHICH EXPRESS-specific CAR T-CELLS MEDIATES BISPECIFIC ACTIVATION AND TARGETING OF T-CELLS

Currently selected T-cell therapies are emerging as effective non-toxic modality for the treatment of cancer. However, malignancies are complex diseases in which multiple undetected antigens contribute to disease progression. In order to more comprehensively target cancer cells, a reprogramming strategy that utilizes a tandem chimeric antigen receptor (TCAR) is described. The TCAR contains a CD3ζ signaling domain and an antibody-activated extracellular domain of interest, which could be directed against various classes of tumor antigens. In this study, the ability of TCAR-D3E to mediate bispecific activation and tumor targeting was evaluated in vitro and in vivo. TCAR-D3E was demonstrated to activate autologous T-cells via CD3ζ in a manner dependent on antibody. TCAR-D3E also mediated cell killing of multiple cancer cell lines, including glioblastoma, lung cancer, and melanoma. Furthermore, TCAR-D3E killing was dependent on tumor cell expression of multiple epitopes. Finally, these findings suggest the potential of TCAR-D3E as a platform for developing bispecific T-cell immunotherapies that could be directed against multiple epitopes on tumor cells.

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pathogenesis through both distinct and redundant mechanisms. Hence, target-
ging different cancer-specific markers simultaneously could result in better therapeutic efficacy. However, developing two separate cellular prod-
ucts, each targeting a novel combination of markers, would be prohibitively costly and time-consuming.

...Toca 511 when delivered by bolus or slow infusion. Biodistribution of Toca 511 was observed with the 1.7 mL volume. PCR analyses of resected brain tumors was used to confirm the presence of vi48. Initial trials investigated delivery by a standard brain biopsy needle for subsequent intra-cerebral hemorrhage 4 weeks after the first treatment. Histologic examination of the tumor at surgery confirmed vi48 replication within the tumor. Several weeks after RRV delivery was observed in the peripheral blood from GAA-DC patients compared to baseline.

...pathologic correlated immune monitoring assay results were compared between pa-
tients who experienced a clinical benefit (CR, PR, SD) and those who did not. The objective of this study was to determine the feasibility of vi48 replication in GAA-DC patients and to evaluate vi48 biodistribution patterns and immunogenicity in vivo.

...gene transfer to target prostate cancer cells. To determine this, we assessed the expression of the vector and the prodrug using PCR analysis of tissue and plasma samples. RESULTS: The engineered prostate cancer cells were transduced with the vi48 vector at an initial multiplicity of infection of 50. PCR analysis of cell and plasma samples confirmed vi48 gene transfer and expression in vivo. There was no evidence of vi48 replication in any of the blood samples analyzed.

...of vi48 replication in vivo. There was no evidence of vi48 replication in any of the blood samples analyzed. DISCUSSION: This pilot study demonstrates that the vi48 system is safe and well-tolerated in non-malignant canine subjects. Further studies of this vector in a variety of tumor types and species will be needed to determine the potential of this system for cancer gene therapy.

...vector could be a useful therapeutic tool for the treatment of prostate cancer.

...dendritic cells (DC) loaded with vi48 can be used to induce antitumor immunity in vivo.

...vector was used to transduce prostate cancer cells ex vivo and to evaluate vi48 biodistribution patterns and immunogenicity in vivo.

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...in vivo. DISCUSSION: This pilot study demonstrates that the vi48 system is safe and well-tolerated in non-malignant canine subjects. Further studies of this vector in a variety of tumor types and species will be needed to determine the potential of this system for cancer gene therapy.

...use of vi48 for gene therapy in prostate cancer.

...vector could be a useful therapeutic tool for the treatment of prostate cancer.

...dendritic cells (DC) loaded with vi48 can be used to induce antitumor immunity in vivo.
with the ATL-DC patients. In addition, a significant correlation was observed between regulatory T lymphocyte (Treg) ratios (post-/pre-vaccination timepoints) and survival (p = 0.004) for patients in both trials. In fact, Treg ratios were independently prognostic for OS whereas tumor pathology was not in multivariate analyses. When patients from each trial were case-matched for tumor grade, tumor status (newly diagnosed versus recurrent), extent of resection, age, and Karnofsky performance score (KPS) status, the mean overall survival of patients on the ATL-DC trial was significantly longer than that of patients on the GAA-DC trial (p = 0.05). In conclusion, these results suggest that ATL-DC vaccination is associated with wider patient eligibility and prolonged survival compared with GAA-DC vaccination. Decreased post-/pre-vaccination Treg ratios and decreased frequencies of activated NK cells were associated with extended survival in patients from both trials, suggesting that these lymphocyte subsets may be relevant immune monitoring endpoints for DC-based vaccination strategies in malignant glioma patients.

**IT-26. FEASIBILITY OF AMPLIFIED CD133+ BRAIN TUMOR STEM CELL RNA-PULSED DENDRITIC CELLS FOR THE TREATMENT OF PATIENTS WITH RECURRENT GLIOBLASTOMA**

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INTRODUCTION: Malignant primary tumors of the brain remain essentially lethal despite aggressive and incapacitating conventional therapy. This poor prognosis may be tied to the presence of a recently identified subset of CD133+1 brain tumor stem cells (BTSCs) that appear resistant to radiation and chemotherapy. BTSCs represent a subpopulation of tumor cells that cannot be reliably propagated in sufficient quantity to serve as a direct source of antigen for vaccination. However, mRNA can be isolated from BTSCs and amplified with high fidelity to provide an abundant antigen template for loading of dendritic cells (DCs) for use in immunotherapy of recurrent glioblastomas (GBM). METHODS: The feasibility and safety of amplification of RNA from patients with first recurrences of GBM was explored in a phase I/II clinical trial (Re-START trial FDA IND BB-13,630, Duke institutional review board #0000667). Tumor tissue was removed and immediately dissociated into single cells and viably frozen. Tumors from outside institutions were placed into sterile media and delivered priority overnight before dissociation. BTSCs were isolated by sterile sorting for CD133, RNA was isolated from the BTSCs and unsorted tumor cells for the production of cDNA libraries and mRNA for antigen loading of mature DCs. If there was insufficient mRNA from the BTSC fraction, then unsorted tumor mRNA was used (tRNA). The fidelity of gene amplification was demonstrated by real-time PCR and gel electrophoresis. RESULTS: Sufficient quantities (>2.0 mg) of usable mRNA was successfully made in 13 of 17 attempts (77%). CONCLUSIONS: We found that mRNA can be isolated from BTSCs and provides an abundant antigen template for antigen loading of DCs for use in immunotherapy of patients with recurrent GBM. The feasibility and efficacy of this therapy is currently being evaluated in an ongoing dose-escalation trial of DC vaccination in combination with bevacizumab.

**IT-27. ADOPTIVE CELLULAR THERAPY TARGETING RECURRENT MEDULLOBLASTOMA AND PRIMITIVE NEUROECTODERMAL TUMORS AFTER MYELOABLATIVE AND NON-MYELOABLATIVE CONDITIONING**

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BACKGROUND: The use of total tumor RNA-loaded dendritic cells (DCs) was pioneered at our institution as a novel platform for inducing potent immunologic responses against the variety of uncharacterized and patient-specific antigens present within malignant tumor cells. We are currently exploring adoptive cellular therapy using amplified tumor RNA-pulsed DCs to expand tumor-specific lymphocytes ex vivo for the treatment of recurrent medulloblastoma (reMB) and primitive neuroectodermal tumors (rePNETs). OBJECTIVE: To explore the use of amplified tumor RNA-pulsed DCs to expand tumor-specific lymphocytes. METHODS: A single-arm, prospective phase I/II clinical trial of DC + SALT therapy is ongoing in pediatric and young adult patients with reMB or rePNETs (Re-MATCH trial - FDA IND BB-14058, Duke IRB 18020). The maximum tolerated dose of adoptively transferred tumor-specific lymphocytes will be established during phase I using a 3+3 study design in two cohorts of patients. Cohort A consists of patients with localized relapse and will receive induction chemotherapy followed by myeloblastic consolidation, autologous stem cell rescue, and tumor-specific immunotherapy. Cohort B consists of patients with disseminated disease and will receive salvage chemotherapy followed by myeloblastic conditioning (cyclophosphamide/fludarabine) prior to adoptive cellular therapy. The primary endpoint of the phase I trial is safety and defining the maximum tolerated dose (MTD). The primary endpoint of the phase II trial is progression-free survival at 12 months (PFS-12). This trial has 90% power to distinguish a historical PFS-12 of 33% from 55% after adoptive cellular therapy. RESULTS: Amplification of tumor mRNA and in vitro expansion of tumor-specific lymphocytes to clinical scale from six enrolled subjects (cohort A n = 1 and cohort B n = 5) has been completed, and four subjects have received adoptive cellular therapy after myeloblastic (n = 1) and nonmyeloblastic (n = 3) chemotherapy. Safety and clinical evaluation is ongoing, but no dose-limiting toxicities have been observed.

**IT-29. CLINICAL APPLICATION OF CHAPERONE HSP70 IN THE TREATMENT OF MALIGNANT BRAIN TUMORS**

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Molecular chaperones, particularly Hsp70, were shown to efficiently stimulate anti-cancer immune response. We previously reported that intratumoral application of Hsp70 in a model of intracranial glioma in rodents can enhance innate and adaptive immune response. As a next step, a pilot study for the local intratumoral delivery of Hsp70 was investigated for feasibility, safety, side effects, and possible systemic anti-tumor immune responses in patients with brain tumors. Patients (4.5-14 years old) with untreated newly diagnosed malignant brain tumors (n = 12) were enrolled in the pilot study. Following tumor resection, a silicone catheter was implanted into the residual tumor cavity, and the next day injections of chaperone Hsp70 were performed through the catheter (with a total of 2.5 mg per course). During the Hsp70 course, patients received no other treatment. Before Hsp70 administration and after the last injection, specific immune responses were evaluated in a delayed hypersensitivity test (DHT). Furthermore, peripheral blood samples were collected from lymphocyte subpopulations, cytokine levels, and cytolytic activity of natural killer (NK) cells. After the Hsp70 course, patients received conventional treatment (radio- and chemotherapy). Response to Hsp70 therapy was evaluated on MRI post-contrast scans before and four weeks after the last injection of Hsp70. The follow-up period for this trial was 12 months. Intratumoral injections of Hsp70 were well tolerated in patients. One patient had complete response, and one patient had partial clinical response documented by radiological findings. In three patients, we observed a positive DHT. In peripheral blood, we observed a shift from cytokines of humoral immunity provided by Th2-helpers (IL-4 and IL-6) toward cytokines of Th1-mediated response (TNF-α and INF-γ). This pilot study for the first time demonstrated the feasibility and safety of intratumoral delivery of recombinant Hsp70 in cancer patients. Our results suggest that purified Hsp70 can induce specific effective anti-tumor immune response and warrants further investigation in randomized clinical trials.