

for protein extraction after 20 hours of severe hypoxia and the expression of cellular protein was measured by densitometry of western blots.

Treatment of human glioblastoma multiforme cells U87 with a fluorescent (FAM-tagged) LNA/phosphorothioate gapmer confirmed a very efficient uptake (>95%) of oligonucleotide into the cells.

Using U87 cells we found that the most potent of the screened oligonucleotides was a 4 LNA, 8 phosphorothioate, 4 LNA gapmer (Cur813) targeting the 3'-coding region of the HIF-1 $\alpha$ -gene. For further in vitro investigation of Cur813 we treated human glioblastoma cell lines U87 and U373 and the human prostate cancer cell line 15PC3 with the gapmer oligonucleotide. We were able to establish a dose-dependent down-regulation of hypoxia induced HIF-1 $\alpha$  protein and the HIF-1-regulated protein Glut1 in all the cell lines using Cur813. Relative to untreated controls a down-regulation of 75-90% was obtained by 100 nM Cur813 and a down-regulation of 85-90% was obtained by 400 nM, the effect of the treatment varying between the three different cell lines.

Since the proposed molecular target was effectively inhibited without any apparent in vitro toxicity, we are currently investigating the in vivo efficacy of Cur813 in U373 xenografts on nude NMRI mice. Initial experiments have shown a significant inhibition of tumor growth by a 1 daily i.p. injection of 5 mg/kg Cur813 for 7 days.

707

ORAL

### Identification and characterization of DEGA, a novel leucine-rich repeat family member differentially expressed in human gastric adenocarcinoma: effects on cell cycle and tumorigenicity.

D.S. Pereira, K.E. Rabenau, J. O'Toole, K.A. Kobylarz, S.B. Kaplan, H. Kotanides, R. Bassi, L. Witte. *ImClone Systems New York, Department of Molecular and Cell Biology and Department of Immunology, New York, USA*

Using a cDNA microarray approach to search for genes involved in a variety of cancers, we discovered DEGA, a novel cDNA differentially expressed in human gastric adenocarcinomas. Cloning of this cDNA from A549 cells revealed a 2070 bp fragment containing an open reading frame of 1569 bp that encodes a 522 amino acid protein. This protein contains a signal peptide, five leucine-rich repeat motifs (protein-protein interaction domains) and an IgG and transmembrane domain, suggesting that it resides on the plasma membrane. Transfection of 293 cells with an EGFP fusion construct confirmed cell surface localization. Although the cytosolic portion of this protein does not possess signal transduction-related domains, approximately 1/5 of the cytosolic amino acids are either a serine or a threonine. Blast searches for sequence similarity to this protein revealed an exact match to AMIGO-2, a very recently identified, but functionally uncharacterized protein related to AMIGO, a leucine-rich repeat family member implicated in axon tract development (Kuja-Panula et al., JCB 160:963-973, 2003). Aside from being highly expressed in normal tissue of the breast, ovary, uterus, and lung, in this report, we show that DEGA/AMIGO-2 is differentially expressed in approximately 1/3 of tumor versus normal tissue from gastric adenocarcinomas patients. It is also expressed in some gastric adenocarcinoma cell lines (ex. AGS) as well as other cancer cell lines. When compared to empty vector controls, stable expression of an DEGA/AMIGO-2 anti-sense construct in the gastric adenocarcinoma cell line, AGS, led to an accumulation of cells in the G2/M phase of the cell cycle and a nearly complete abrogation of tumorigenicity in athymic (nu/nu) mice. Although we have not defined a precise role for DEGA/AMIGO-2, our data suggest that it may be an etiologic factor functioning as a signaling cell adhesion molecule to regulate the cell cycle as well as tumorigenesis in a subset of gastric adenocarcinomas.

708

ORAL

### MSD-EACR Research Award. Celecoxib activates a novel mitochondrial apoptosis signaling pathway

V. Jendrossek, R. Handrick, C. Belka. *Dept. Radiation Oncology, Experimental Radiotherapy Group, Tuebingen, Germany*

**Background:** The cyclooxygenase-2 inhibitor Celecoxib has been shown to inhibit tumor cell growth independently from its capacity to block the COX-2 enzyme and to increase the efficacy of ionizing radiation in experimental settings. The growth inhibitory effects of celecoxib had been attributed to its pro-apoptotic action.

**Methods:** To gain insight into the mechanisms of celecoxib induced apoptosis and to differentiate between death receptor and mitochondrial pathways the activation of caspases-9, -8 and -3, the cleavage of the

caspase-3 substrates PARP and ICAD as well as the induction of mitochondrial alterations and of nuclear changes were tested in Jurkat T- and BJAB B-lymphoma cells with defects in either pathway as well as in embryonic fibroblasts of Apaf-1 expressing and Apaf-1 knock out mice. For comparison, apoptosis was also induced by death receptor stimulation and irradiation.

**Results:** Celecoxib induced dose and time dependent apoptosis in Jurkat and BJAB cells. Activation of caspases-9, -8 and -3 was detectable in parallel with cleavage of PARP and inactivation of ICAD. The celecoxib action was associated with a breakdown of the mitochondrial membrane potential and release of cytochrome c. Lack of FADD, overexpression of a dominant negative FADD, lack of caspase-8 and treatment with caspase-8 specific inhibitors had no influence celecoxib induced apoptosis. In contrast, overexpression of a dominant negative caspase-9 mutant or inhibition of caspase-9 interfered with celecoxib induced cell death. Similarly, lack of Apaf-1 expression abrogated Celecoxib induced apoptosis. Surprisingly, overexpression of anti-apoptotic members of the Bcl-2 protein family did not abrogate caspase-9, -8, and -3 activation, PARP cleavage, breakdown of the mitochondrial membrane potential and release of cytochrome c upon treatment with celecoxib, while inhibiting radiation induced apoptosis.

**Conclusion:** We conclude that celecoxib induces apoptosis via a novel caspase-9 and Apaf-1 dependent, but Bcl-2 and death receptor independent mechanism.

## Adult leukemia

709

ORAL

### Outcome and patterns of failure in solitary plasmacytoma: a multicenter rare cancer network study on 258 patients

M. Ozsahin<sup>1</sup>, R. Tsang<sup>2</sup>, P. Poortmans<sup>3</sup>, Y. Belkacemi<sup>4</sup>, M. Bolla<sup>5</sup>, F. Oner<sup>6</sup>, C. Landmann<sup>7</sup>, B. Castelain<sup>8</sup>, J. Buijsen<sup>9</sup>, D. Knobel<sup>1</sup>. <sup>1</sup>Centre Hospitalier Universitaire Vaudois (CHUV), Radiation Oncology, Lausanne, Switzerland; <sup>2</sup>Princess Margaret Hospital, Toronto, Canada; <sup>3</sup>Dr. Bernard Verbeeten Institute, Tilburg, The Netherlands; <sup>4</sup>Hopital Tenon, Paris, France; <sup>5</sup>Centre Hospitalier Universitaire, Grenoble, France; <sup>6</sup>Cerrahpasa School of Medicine, Istanbul, Turkey; <sup>7</sup>University Hospital, Basel, Switzerland; <sup>8</sup>Centre Oscar Lambret, Lille, France; <sup>9</sup>MASTRO, Maastricht, The Netherlands

A series of 258 adult patients with bone (n = 206) or extramedullary (n = 52) solitary plasmacytoma, consecutively treated between 1977 and 2001, was collected in a retrospective multicenter Rare Cancer Network study. Median age was 60 years (18-95), and male to female ratio was 1.87. Staging work-up included bone-marrow assessment (all patients), serum immunoglobulins (n = 149), immunosubtraction (n = 161), standard X-rays (n = 191), CT-scan (n = 163), MRI (n = 85), and bone scintigraphy (n = 73). HIV test was performed in only 7 patients, and all were negative. Inclusion criteria included solitary plasmacytoma in 18-year or older patients without evidence of multiple myeloma. Histopathologic diagnosis was obtained in all patients including biopsy in 160, partial resection in 85, and complete resection in 9 patients. Most (n = 215) of the patients benefited from RT alone; 34 had chemotherapy (mostly melphalan and prednisone) and RT, 8 had surgery alone, and one patient died before starting the RT. External RT volume included the clinical tumor volume with a sufficient margin. The median RT dose was 40 Gy (20-66) in 20 fractions (4-50) using 2 Gy (1.25-5) per fraction during median 29 days (4-74). RT was delivered using megavoltage photons in all patients. The median follow-up period was 56 months (7-245). Median time to multiple myeloma development was 21 months (2-135). One hundred seventeen (45%) patients developed multiple myeloma with a 5-year projected probability of 45%. The 5-year probability of overall survival, disease-free survival (DFS), and local control was 74%, 50%, and 86%; respectively. Six out of 9 patients treated with surgery alone presented a local relapse compared to 10 (10%) out of 105 treated with RT < 40 Gy or 21 (15%) out of 144 treated with RT > 40 Gy. In univariate analyses (logrank test), significant factors favorably influencing the survival were younger age (60 years or younger), extramedullary localization, and tumor size (< 4 cm). For DFS, in addition to the above-mentioned parameters, treatment with RT was a favorable factor as well. Local control was better in small tumors (< 4 cm). Unfavorable factors for myeloma development were older age (> 60 years) and bone localization. Multivariate analysis (Cox model) revealed that the best independent factors predicting the outcome were younger age and tumors < 4 cm. Bone localization was the only independent predictor for multiple myeloma development. In this multicenter retrospective study, extramedullary solitary plasmacytoma was found to have the best outcome,

especially when treated with RT. Novel therapies should be investigated in patients with solitary plasmacytoma localized in the bone.

710

ORAL

# **In vivo assessment of radiation induced apoptosis in follicular lymphoma patients by 99mTc-Annexin V scintigraphy.**

R.L.M. Haas<sup>1</sup>, R.A. Valdés-Olmos<sup>2</sup>, D. de Jong<sup>3</sup>, S.F. Zerp<sup>1</sup>, I. van den Heuvel<sup>2</sup>, C.A. Hoefnagel<sup>2</sup>, H. Bartelink<sup>1</sup>, M. Verheij<sup>1</sup>. <sup>1</sup> The Netherlands Cancer Institute, Radiotherapy, Amsterdam, The Netherlands; <sup>2</sup> The Netherlands Cancer Institute, Nuclear Medicine, Amsterdam, The Netherlands; <sup>3</sup> The Netherlands Cancer Institute, Pathology, Amsterdam, The Netherlands

**Purpose:** To study whether radiation-induced apoptosis in follicular lymphoma patients can be visualised in vivo by 99mTc-Annexin-V (TAV) scintigraphy. Annexin V is an endogenous human peptide with a high affinity for membrane-bound phosphatidylserine, which becomes exposed at the outer leaflet of the plasma membrane at an early stage of the apoptotic process.

**Methods and patients:** TAV scintigraphy was performed in 10 recurrent follicular lymphoma patients (median age 58 years, range 38-78 years) scheduled for local palliative irradiation within 1 week prior to treatment. Planar and SPECT images were performed. Patients were irradiated by 2 fractions of 2 Gy on days 1 and 3, and rescanned on day 4. Fine needle aspiration cytology (FNAC) was performed on days 1, 2, 3, 4 and 5 to evaluate early morphological changes compatible with apoptosis and to investigate the optimal timing for scintigraphy. The study was approved by the local ethical committee.

**Results:** In 7/10 patients baseline scans were negative. In the 3 patients with minimal TAV uptake at the tumour site on the baseline scan, the pretreatment histopathological specimens confirmed the presence of low levels of spontaneous apoptosis. In 9/10 patients post-treatment scans showed a strong increase in TAV uptake at the irradiated tumour sites as compared with the baseline scans. In 1 patient the post-treatment scan remained negative. In 9/10 patients the cytological response confirmed the presence of apoptotic cells as visualised on the post-treatment scintigraphic images. In 1 patient the cytology was positive for apoptosis whereas the scan was negative. None of the post-irradiation images showed false-positive results. In 9/10 patients findings of apoptosis were predictive of radiotherapy response.

**Conclusion:** Radiation-induced apoptosis in follicular lymphoma patients can be visualised at early stages in vivo by TAV scintigraphy. TAV scintigraphy may prove an easy and rapid predictive assay for treatment outcome in follicular lymphoma patients.

711

ORAL

# **Localized mucosa-associated lymphoid tissue (MALT) lymphoma treated with radiation therapy has excellent clinical outcome**

R.W. Tsang<sup>1,5</sup>, M.G. Gospodarowicz<sup>1,5</sup>, M. Pintilie<sup>2</sup>, W. Wells<sup>1,5</sup>, D.C. Hodgson<sup>1,5</sup>, A. Sun<sup>1,5</sup>, M. Crump<sup>3,5</sup>, B.J. Patterson<sup>4,5</sup>. <sup>1</sup> Princess Margaret Hospital, Radiation Oncology, Toronto, Canada; <sup>2</sup> Princess Margaret Hospital, Biostatistics, Toronto, Canada; <sup>3</sup> Princess Margaret Hospital, Medical Oncology/Hematology, Toronto, Canada; <sup>4</sup> Princess Margaret Hospital, Pathology, Toronto, Canada; <sup>5</sup> University of Toronto, Toronto, Canada

**Background:** MALT lymphoma has unique clinical-pathological features but remains heterogeneous in etiology, sites of presentation, and biologic behavior. We report the outcome of patients with stage I/II disease treated with a policy of involved-field radiation therapy (RT).

**Material/Methods:** Between 1989-2000, 103 patients (stage IE, 91; stage IIE, 12) were referred for management, with data collected prospectively since 1992. Median age was 60 yrs, M:F ratio 1:1.9. Transformed MALTs (diffuse large B-cell lymphoma) were excluded. Presenting sites included: stomach 17, orbital 31, salivary glands 24, thyroid 13, other head and neck sites 5, lung 5, urinary bladder 3, skin 2, breast 2, and rectum 1. Staging included site-specific imaging, CT abdomen in 92% and bone marrow biopsy in 80%. Ninety-three patients received RT (RT alone, 85; chemotherapy and RT, 8). Five patients had surgical excision alone, 2 patients with gastric lymphoma received antibiotics alone, and 3 patients refused any treatment. The analysis focused on 93 patients who received RT. The median RT dose was 30 Gy (range 17.5-35 Gy) and the median follow up was 4.9 yrs (range 1.1-10.2 yrs).

**Results:** A complete response (CR/CRu) to RT was achieved in 92/93 patients, 1 patient had no response (orbit). To date, 3 patients have died (1 due to lymphoma, 1 from a second cancer, 1 from unrelated causes). The 5-yr disease-free survival (DFS) was 77%, and overall survival (OS) 98%. No relapses were observed in 26 patients with stomach or thyroid lymphoma, while 16/67 patients relapsed in the other sites. The 5-yr DFS for gastric and thyroid lymphoma was 94%, in contrast to 71% for other sites ( $p = 0.034$ ), although OS was the same. Among 92 patients with CR, 15 relapsed (4 salivary, 7 orbit, 2 nasopharynx, 1 larynx, 1 breast). Relapse sites: the untreated contralateral paired-organ only, 5 patients (3 orbit, 2 parotid); distant sites, 7 patients; and both local (both parotid) and distant sites, 2 patients. The overall local control rate with RT was 96% (89/93 patients). The locations of distant relapse were commonly in MALT sites rather than lymph nodes. Among the 16 patients with residual/recurrent MALT lymphoma, 10 were rendered disease-free again with additional courses of RT (7 patients), chemotherapy (1 patient), and CMT (2 patients), with a median follow up of 3.8 years from first relapse.

**Conclusions:** Moderate-dose RT achieved excellent local control in localized MALT lymphomas. Relapses were observed in non-irradiated paired-organs or distant sites. Gastric and thyroid MALT lymphomas had better outcome. Patients with recurrence of lymphoma generally responded to further treatment with prolonged survival despite the relapse(s).

712

ORAL

# **Improved survival at the cost of neurotoxicity in primary CNS lymphoma (PCNSL). Long-term follow-up of a Phase 2 multicentre combined modality study (Trans-Tasman Radiation Oncology Group - TROG)**

P.C. O'Brien<sup>1</sup>, D.R. Roos<sup>2</sup>, G. Pratt<sup>3</sup>, K. Liew<sup>4</sup>, M. Barton<sup>5</sup>, M. Poulsen<sup>6</sup>, I. Oliver<sup>7</sup>, G. Trotter<sup>8</sup>. <sup>1</sup> Newcastle Mater Hospital, Radiation Oncology, NSW, Australia; <sup>2</sup> Royal Adelaide Hospital, Radiation Oncology, SA, Australia; <sup>3</sup> Royal Brisbane Hospital, Queensland Radium Institute, Qld, Australia; <sup>4</sup> Victorian Radiation Oncology Centre, Vic, Australia; <sup>5</sup> Liverpool Hospital, Radiation Oncology, NSW, Australia; <sup>6</sup> Mater Hospital, Queensland Radium Institute, Qld, Australia; <sup>7</sup> Royal Adelaide Hospital, Division of Cancer Services, SA, Australia; <sup>8</sup> East Coast Cancer Services, Qld, Australia

**Background:** Combined modality therapy has increasingly been used for patients with PCNSL but follow-up data beyond 5 years from prospective multicentre studies are few. This is an analysis of survival and neurotoxicity associated with a brief course of IV Methotrexate and cranial irradiation.

**Materials and methods:** Forty-six patients from 12 centres with biopsy-proven PCNSL were entered on a prospective Phase 2 study between 1991 and 1997. Patients had a median age of 58 (range 25-76) and were ECOG performance status 0-3. The treatment protocol consisted of Methotrexate 1gm/m<sup>2</sup> given day 1 and day 8, followed by radiotherapy, initially to the whole brain to total dose of 45 Gy in 25 fractions, followed by a 5.4 Gy boost. Intrathecal therapy was only given in patients with positive CSF cytology. The minimum potential follow-up for these patients is now 5 years, with a median follow-up for survivors of 7 years.

**Results:** The Kaplan-Meier estimate of survival at 5 years is 37%  $\pm$  14 (95% CI) and progression-free survival 38%  $\pm$  16 (95% CI). No patient has relapsed more than 5 years following treatment. We were unable to demonstrate any influence of age or performance status on overall survival or progression-free survival. Age, however, had a major impact on the risk of neurotoxicity. Patients older than 60 years had a 5 year probability of neurotoxicity of 47% compared with 13% for younger patients ( $p = 0.04$ ). The risk of neurotoxicity in older patients has continued to increase beyond 5 years. Ten of the 46 patients are alive, of whom 8 are without neurotoxicity.

**Conclusions:** A limited course of IV Methotrexate prior to radiotherapy appears to result in improved survival outcome in patients with primary CNS lymphoma. For patients younger than 60 these results may be inferior to more intensive combined modality regimens. Despite the limited course of IV Methotrexate and restriction of intrathecal therapy, the risk of neurotoxicity with this regimen is unacceptably high in older patients.