Biological response modifiers

222

QUANTITATION OF CYTOKINE mRNA LEVELS IN TISSUES FROM *IN VIVO* SOURCES USING REVERSE TRANSCRIPTION (RT) / POLYMERASE CHAIN REACTION (PCR) ASSAYS. Berleth, E.S., Ehrke, M.J., Dolnick, B.J., and Mihich, E. (Roswell Park Cancer Institute, Buffalo, New York).

These studies were initiated to measure steady-state levels of multiple cytokines (IL1α, IL1β, TNFα, IL2, IL6, IFNγ, and TGFβ) in cells from various murine tissues: spleen, thymus, and peritoneal exudate cells (PECs). These data will be compared with changes in cytokine activity previously documented in these cell types in response to different variables [presence of tumor and/or treatment with doxorubicin (DOX)]. There is a paucity of information addressing absolute amounts of mRNAs from *in vivo* tissue sources, reflecting the difficulty of reproducibly isolating RNA from the complex mixtures of cells present in tissues, as well as the difficulty in accurately quantitating low abundance cytokine RNAs. The RT/PCR assays developed for these studies utilize a competitive template technique for quantitation. Cellular RNA and a specific *in vitro* transcribed standard RNA are subjected to RT/PCR. The standard and cellular RNAs compete identically for all reactants; the amount of a specific RNA in the cellular RNA is calculated based on the ratio of standard to cellular product and the known amount of standard RNA in the assay. This technique has been optimized for accurate quantitation from *in vivo* sources in two ways. First, variations in the efficiency of RNA extraction between samples are corrected by normalization to recovery of an unrelated ^{3}H-RNA included as a "spike" in all samples. Second, the internal standard used in each assay competes identically for all the reactants, and in both the RT and PCR reactions, therefore ensuring that amplification of the cellular and standard RNA is identical. The results of this quantitation of cytokine mRNA levels in spleen, thymus, and PECs of untreated C57BL/6 mice, with and without EL4 lymphoma and with or without DOX treatment will be presented. (NIH grant #CA15142 and DHHS grant #CA16056).

223

THE FEASIBILITY OF MICE TRANSGENIC FOR ONCOGENES AS THERAPEUTIC MODELS IN CYTOKINE RESEARCH

H Thomas, P Jolicoeur*, FR Balkwill

Biological Therapies Laboratory, Imperial Cancer Research Fund, London, U.K.
*Institut de Recherches Cliniques de Montreal, Montreal, Canada.

A transgenic mouse model of mammary cancer has been developed with the c-neu oncogene under the control of the MMTV-promoter. Mice develop poorly differentiated mammary tumours stochastically at 10-14 months of age, most frequently in non-virgin mice. Heterogeneous tumours histologically similar to human comedo-type ductal breast carcinomas arise, are often polyclonal, resemble human tumours in their natural history, metastasise to lung and are not immunogenic. The mice are being progressively inbred on a Balb/c background and six generations have now been reached. We report on the feasibility of using these mice as a model for experimental cytokine prophylaxis and therapy. In addition to our studies in transgenic mice, seven tumours, from early generations, have been successfully transplanted into nude mice and the direct action of IFN-α A/D hybrid and rat IFN-γ measured. There has been marked intertumour heterogeneity in histology, tumour biology, cytokine sensitivity and tumour progression with successive passages as well as disseminated metastases in some mice.

224

A BROAD SPECTRUM PHASE II TRIAL WITH CONTINUOUS INFUSION (CI) OF RECOMBINANT INTERLEUKIN-2 (IL-2) IN METASTATIC TUMORS.

Le Cesne A[1], Berthaud P[1], Brandely M[2], Toussaint C.[1] Rixe O[1]., Kayitalire L[1], Mezlini H[1], Spielmann M[1], Le Chevalier T[1], Tursz T.[1]

(1) Service de Médecine B, Institut Gustave-Roussy, Villejuif, France
(2) Roussel Uclaf Laboratories,Romainville.

In order to determine an antitumor activity of IL-2, in non immunosensitive chemoresistant metastatic malignancies, we performed a broad spectrum phase II study of rIL-2 Roussel-Uclaf from 7.90 to 12.91 including patients with tumors poorly or not studied for sensitivity with IL-2. Pts received induction rIL-2 at 20 10^{6}UI/m²/d on days 1-5, 15-19 and 28-30 in CI and maintenance rIL-2 at the same dose 5 consecutive days every 4 weeks. 46 pts entered this study including 11 soft tissue sarcomas, 7 mesotheliomas (M), 7 bone sarcomas, 4 thymic carcinomas (TC), 4 breast cancers (BC), 3 non small cell lung cancers (NSCLC), 3 metastases from unknown primary, 3 undifferentiated nasopharyngeal tumors (UCNT), 1 pheochromocytoma, 1 cutaneous lymphoma (CL), 1 thyroid cancer and 1 parotidis carcinoma.
102 cycles of rIL-2 were administered with a median number of 2 cycles/pt (1-5). All pts were treated in a non intensive care unit. We observed 2 rIL-2 related deaths 1 pt by cardiorespiratory failure and 1 pt with CL by cardiogenic sepsis failure with dramatic increase of circulating leukemic T cells. Toxicities (T) were similar to other high dose rIL-2 regimens, however sepsis (4 episodes), renal (3gr II episodes) and hepatic T (6gr III-IV episodes) were uncommun with this CI schedule. We observed 1 PR in a TC (pt alive in disease free status 24 months after surgery of pleural residual lesions) (Lancet, 1990), and 3 MR (1 BC, 1 NSCLC and 1 M). 2 rapidly progressing osteosarcomas remained stable for serveral weeks.
Despit a major response in a TC pt, the activity of rIL-2 in non melanoma and renal tumors is disappointing.This data suggests that pts with these advanced/metastatic malignancies are not the ideal candidates to rIL-2-based immunotherapeutic protocols.

225

PRELIMINARY REPORT OF A FRENCH MULTICENTRIC RANDOMISED STUDY (CRECY) OF IMMUNOTHERAPY IN PATIENTS WITH METASTATIC RENAL CARCINOMA (MRC)

Investigator Board of the CRECY study (FNCLCC, CHRU, CH)
CRECY Coordinating Center, Centre L. Bérard 28 rue Laënnec, 69008 Lyon - France

A multicentric randomised study i.e Interleukin-2 (IL2) VS Interferon (IFN) VS both of them, has been set up in France as a phase II study in homogeneous groups (in terms of prognosis factors) of patients with MRC.
The rationale was first, the lack of available comparative data concerning these 3 types of treatment, although IL2 and IFN are now both registered drugs for this disease in Western Europe. A real randomised phase III trial has not appeared to be feasible since expected response rates should be very similar and too many patients should be required. A randomised study with a placebo group was judged unethical. The CRECY study consists of 3 pilot phase II studies conducted all together in 2 homogeneous groups of patients. Randomisation was proposed in order to avoid selecting the patients.The aims of these studies are first to determine response rate and survival respectively in each prognosis group and with each treatment type. Secondarily, to determine in each subgroup the induced toxicity, the quality of life and also the cross response rate of patients to whom the second cytokine was given after failure of the first one. Concurrently, the uneligible patients for these studies are taken into account to determine the exclusion rate.
Until now, 203 patients have been included and 320 exclusions have been registered. Unexpected toxic events were observed within the combined IL2-IFN trial leading to modify the planned dose.
A preliminary analysis will be performed in June 93 and results will be available to be exposed in details in November 93.

226

ETRETINATE (E) AND INTERFERON-ALPHA (IFN): A PHASE I-II STUDY IN SQUAMOUS CELL CARCINOMAS (SCC) AND TRANSITIONAL CELL CARCINOMAS (TCC).

§AD Roth, *W Bollag and §P Alberto,

§Div of Medical Oncology and Hematology, Geneva University Hospital, CH - 1211 Geneva 14, Switzerland, and *Div of pharmaceutical research, F. Hoffmann-La Roche Ltd, 4002 Basel, Switzerland.

Recent experimental and clinical investigations have demonstrated a synergism between retinoids and IFN regarding their growth inhibiting effects on epithelial tumors. Based on former encouraging results obtained in SCC with high dose E (300mg/d 5/7 d, 1/2w) (Alberto et al, unpublished results), we launched a pilot study investigating the efficacy and toxicity of a combination of E and IFN. E at 4mg/Kg/d was given every other week for 2 then 3 (3 pts), 3 (6 pts), 4 (6 pts) and 5 (5pts) consecutive days every other week. IFN was given at 6 Mio IU/d 5/7d in each group. 20 pts with advanced tumors of the lung (10), head and neck (2), bladder (2), esophagus (3), cervix (2) and penis (1) have been entered so far into the study. A total of 71 cycles of 2w have been given (2 to 10 cycles/pts). Withdrawal was due to grade 3 fatigue (2pts), pt request (2pts) or tumor progression. Responses are 2 PR (lungs, lasting 7w and 8 months), 1 MR (bladder, lasting 8 w) and 4 SD lasting 11 to 15 weeks (lung 1, esophagus 2, cervix 1). Apart from grade 3 fatigue in 4 pts, the toxicity was moderate (≤grade 2) involving fever, nausea/vomiting, muco-cutaneous changes and mild alterations in kidney and liver function tests. A significant increase in cholesterol or triglycerides was observed in 3 pts, 1 of them being diabetic on insulin therapy. We conclude that: 1) Intermittent high dose E and IFN in combination can be given safely for several months. 2) These results warrant further study (2 PR out of 10 pts with lung disease). 3) Compared with our previous experience with high dose E alone the combination of high dose E + IFN is less toxic. 4) IFN may attenuate retinoid side effects. The possibility of IFN induced changes in retinoid pharmacokinetic is currently under investigation.

227

THE TREATMENT OF EXTENSIVE LIMB SOFT TISSUE SARCOMA BY ISOLATION PERFUSION WITH HIGH DOSE r- TUMOR NECROSIS FACTOR ALPHA COMBINED WITH MELPHALAN

D. Lev, M. Inbar, P. Sorkin, S. Chaitchik & J.M. Klausner
Depts. of Surgery, Oncology and Intensive Care, Tel Aviv Sourasky Medical Center, Israel.

The synergistic anti-cancer activity of r-TNF alpha and melphalan administered via isolated limb perfusion (ILP) has been demonstrated in metastatic melanoma and only a very few cases of recurrent soft tissue sarcoma. 4 pts. with extensive soft tissue sarcoma of the lower limb, 3 with recurrent tumors and one with an advanced primary tumor, all candidates for amputations or hemipelvectomy, underwent ILP with hyperthermia via the external iliac vessels. r-TNF alpha 4mg followed by (30 min) melphalan 1.1mg/kg were administered to the affected limb. Marked tumor softening occurred within 48 hrs, and in tumors protruding through the skin hemorrhagic necrosis was evident within 24 hrs. Transient systemic toxicity included mild hypotension in 2, moderate respiratory failure in 1, and jaundice in 3 pts. Complete response was observed in 3 and partial response (near complete response) in 1 pt. Only 6 wks to 3 mths have elapsed and no disease progression was observed in any of the patients. The combination of r-TNF alpha and melphalan appears to be highly efficient in advanced soft tissue sarcoma confined to the limb.