No grade III/IV fluid retention or cardiac events were observed. Therapy was stopped preterm in 36 P (ADOC 17, AC-DOC 19) because of toxicity (17 P), progression (4 P), death (1 P), other causes (5 P), and for lack of compliance (9 P). In 26 of 193 (13.5%) P a pCR with no detectable viable tumor cells was confirmed.

Conclusion: Dose-dense combination or conventional sequence of adriamycin and docetaxel are feasible, well tolerated, and highly effective as preoperative CHT in primary operable breast cancer. The trial is planned to close in September 2001.

537 POSTER DISCUSSION

Preoperative trastuzumab (T) and paclitaxel (P) for HER2-overexpressing (HER2+) stage II/III breast cancer

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We conducted a phase II study of preoperative T&P, followed by definitive breast surgery and postoperative doxorubicin/cyclophosphamide (AC). The primary study endpoint was pathological complete response to preoperative therapy, defined as absence of invasive disease. Eligible women had HER2+ breast cancer (either +2 or +3 by IHC), clinical stage II or III disease, and LVEF > 50%. Preoperative treatments were T (4 mg/kg x 1, then 2 mg/kg weekly x 11) and P (175 mg/m2 every 3 weeks x 4 treatments). Adjuvant AC at standard doses of 60/600 mg/m2 respectively, every 3 weeks x 4, was begun after surgery and no less than 6 weeks after the final T dose. Cardiac function was assessed at baseline, following preoperative T&P, and after cycles 2 and 4 of AC. 40 patients (median age 49) were accrued to the study, having clinical stage II (55%) or III (43%) cancer (one patient had ipsilateral supraclavicular node involvement as sole site of metastatic disease). Initial biopsies were HER2 positive, 2+ (20%) or 3+ (80%). Asymptomatic grade 2 cardiac toxicity was seen in 4 patients, 1 following H&T, 3 during AC therapy. All 4 patients developed LVEF between 40 and 50%. One patient came off study following first T dose for hypersensitivity reaction. No other unexpected toxicity was observed. Pathological complete response was observed in 7 of 40 patients (18%). Objective clinical response (CR and/or PR) was observed in 27 of 40 patients (68%). Neoadjuvant T & P appears feasible in women with stage II/III HER2+ breast cancer, and has substantial clinical activity, particularly among women with HER2 3+ tumors. Cardiac function merits close surveillance in patients receive preoperative T & P followed by adjuvant AC.

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538 POSTER DISCUSSION

Efficacy and safety of three-weekly herceptin with paclitaxel in women with her2-positive metastatic breast cancer: preliminary results of a phase II trial

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Herceptin in combination with chemotherapy has been shown to increase survival in women with HER2 positive metastatic breast cancer (MBC). Herceptin has so far been administered weekly in most studies. A less frequent, 3-weekly treatment schedule would be more convenient for patients, doctors and treating institutions.

In this phase II study, patients received Herceptin at a dose of 8 mg/kg (loading) followed by 6 mg/kg every 3 weeks (maintenance) in combination with paclitaxel (175 mg/m2) every 3 weeks for 8 cycles. At this point, Herceptin was continued as monotherapy until progression of disease.

32 patients were recruited with a median age of 53 years (31-70). The majority (94%) of patients had metastatic disease: 50% lung metastases, 47% liver metastases and 19% a malignant pleural effusion at baseline. 81% of patients had 2 or more sites or organs involved. 68% had received previous treatment for MBC; 70% anthracyclines, 59% hormone therapy and 72% radiotherapy; 90% of patients were taxane naïve.

The median number of cycles (range) for paclitaxel was 6 (1-8) and for Herceptin 7 (1-22), counting 3 weekly doses as a cycle for Herceptin. 3 patients experienced infusion reactions during Herceptin infusion but were able to continue treatment. No serious cardiac events were reported: 5 patients experienced a decrease of more than 15% of their LVEF. The most common moderate to severe adverse events were (% of patients): myalgia (44%), arthralgia (31%), dyspnoea (16%), fatigue (6%), mucositis (6%), paresthesia (6%), headache (9%) and diarrhoea (9%). Grade 3 and 4 haematological toxicity was limited to neutropenia (grade 3, 13% of patients; grade 4, 3%).

Investigator assessed responses were: complete response 9.4%, partial response 43.8% and overall response rate 53% (95% CI 35-71); 25% of patients had stable disease. The median response duration was 6.3 months (1.5-13.4+) and 15 patients continue on treatment; the estimated median TTP is 10.9 months.

Preliminary data indicate that the efficacy and safety of this 3-weekly regimen of Herceptin with paclitaxel are similar to the standard, approved weekly regimen of Herceptin with paclitaxel (NEJM 2001;344;783-92). Further investigation of this 3-weekly Herceptin regimen is ongoing/planned in the metastatic setting (monotherapy) and it will be used in the HERA adjuvant study.

539 POSTER DISCUSSION

Cyclophosphamide (C) - Epirubicine (E) - Capecitabine (X) combination, CEX: A safe and active regimen in the treatment of locally advanced/inflammatory (LA/I) or large operable (LO) breast cancer (BC). An EORTC-IDBBC study

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Purpose: To evaluate the maximum tolerated dose (MTD) of X in combination with fixed doses of E (100 mg/m^2) and C (600 mg/m^2) q 3 weeks. To have preliminary information on the antitumor activity of the regimen by treating a cohort of 15 LA/I or LO BC patients (pts) at the MTD, for a maximum of 6 cycles.

Methods: Four dose-escalation levels (L) of X were planned: L1: 1500, L2: 1800, L3: 2100 and L4: 2400 mg/m^2/day from day 1 to day 14. Dose escalation was allowed if $\leq$1/3 or 1/6 pts experienced dose-limiting toxicity (DLT: febrile neutropenia: grade 4 neutropenia lasting $\geq$7 days; grade 4 thrombocytopenia; grade 3–4 non-hematological toxicity (NHT) other than alopecia; discontinuation of X for more than 8 doses due to toxicity). Eligible pts were $\geq$18 and $\leq$70 years old, had LA/I or LO BC and a WHO performance status (PS) 0–1.

Results: From February to December 2000, 23 pts entered the study (L1 = 3 pts; L2 = 3 pts; L3 = 15 pts: L4 = 2 pts). Major pts characteristics were: median age 48 years (range 33–68), PS 0 (23 pts): LA/1 BC (9 pts/9 pts); LO BC (5 pts). The MTD was identified at L3 since 2/2 pts treated at L4 experimented a DLT [grade 3 mucositis (1 pt) and grade 3 fatigue that led to X discontinuation for more than 8 doses (1 pt)].

Dose Level 3. *Drug administration* (15 pts/61 cycles): median number of cycles: 4. range 2–6; median relative dose intensity: 100%, 100%, and 96% for C, E, and X, respectively. *Safety data* (15 pts/60 cycles): G4 neutropenia (9 pts); febrile neutropenia (2 pts); no grade 4 NHT. Grade 3 NHT that occurred in >1 pts were nausea and palmar-plantar-crythrodysesthesia (2 pts each). *Activity data* (15/15 pts; WHO criteria): 1 CR; 10 PR; 4 NC. Median time to response was 44 days (range 30–83).

Conclusions: CEX is a safe regimen with a promising antitumor activity (RR 73%) in LA/1 and LO BC pts. Planned next step is to confirm the high activity of this association in a phase II trial.

540 POSTER DISCUSSION

A single, fixed-dose of Pegfilgrastim given once-per-chemotherapy cycle is as effecitve as daily Filgrastim in the management of neutropenia in high-risk breast cancer

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Purpose: Prophylactic use of Filgrastim (F) reduces the incidence and duration of chemotherapy-induced neutropenia (CIN), thereby decreasing the associated risk of infectious complications and compromised outcomes due to chemotherapy treatment delays and dose reductions. Pegfilgrastim (PegF) is a unique sustained-duration cytokine with self-regulating, neutrophil dependant pharmacokinetics. This randomized, double-blind,

multicenter phase 3 trial compared a single 6-mg fixed dose of PegF given once-per-chemotherapy cycle with daily injections of F for the reduction in the duration of CIN in patients with breast cancer.

Methods: Patients with stage II–IV breast cancer receiving doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2 at 35 centers in Europe, Australia, and the United States (n = 157) were randomized to receive either a single, fixed dose (6 mg) of PegF and daily placebo or daily F (5 mcg/kg/day) until ANC 10×10^9/L or for 14 days starting 24 hr post-chemotherapy. The primary endpoint was the duration of severe neutropenia (SN, ANC $< 0.5 \times 10^9$/L) in cycle 1 of chemotherapy.

Results: In patients treated per protocol, the incidence of severe neutropenia was 82% with PegF (n = 68) and 84% with F (n = 62), with mean DSN of 1.8 and 1.6 days, respectively. Duration of SN in all body weight quartiles groups were similar between both treatment groups. Over all cycles, the incidence of febrile neutropenia (temp. > 38.2° with SN) was 13% in patients receiving PegF and 20% in patients receiving F. The chemotherapy dose received by both groups was comparable with only ~5% of patients experiencing a ≥25% dose-reduction in any cycle. Side effects, including bone pain, were similar for both groups and across the weight range.

Conclusions: A single, 6-mg fixed dose of PegF is as effective as a course of daily F injections in prophylactically reducing the risk of neutropenia, and is similarly well tolerated. Fixed-dose, once-per-cycle PegF has the potential to simply the managent of CIN for healthcare professionals and patients.

Immunobiology and biological therapies

541 POSTER DISCUSSION

Induction of antitumor immunity with rna-pulsed dendritic cells vaccine in mice

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Purpose: Dendritic cells (DCs) are identified as the most effective antigen presenting cells (APC). DCs posses an exceptional capacity to capture antigens, process and present antigenic peptides and induce response of host T cells. Several strategies of pulsing tumor antigens have been shown to be effective methods. However, these approaches are currently limited for clinical application, as few human tumor antigens have been identified. The advantages of vaccinating with RNA from tumor cells instead of tumor antigens are that it may contain multiple antigens and can be isolated from a small number of tumor cells. In this study, we examined whether RNA pulsed DCs could diminish established tumors. Methods: DCs were generated from bone marrow cells of C57BL/6 mice with GM-CSF and IL-4 for 3 to 8 days. DCs pulsing with RNA isolated from Lewis lung cancer (LLC) cells was performed using lipofectin method. Results: Luciferase-RNA pulsed DCs produced luciferase protein, actually, and the dose of protein product from DCs was more than that of 3T3 cells. Cultured DC strongly expressed MHC class I, class II, CD40, CD86 and CD11c by flow cytometry analysis, indicating a satisfactory maturation process as APC. DCs pulsed RNA from tumor resulted in similar patterns of cell surface antigen expression as non-pulsed DCs. Both pulsed DCs and non-pulsed DCs strongly phagocyted antigens using latex beads. Moreover they acted as powerful stimulators of the mixed lymphocyte reactions and they were 50 folds more potent than fresh splenocytes. However RNA from LLC pulsed DCs had stronger CTL induction on DCs itself than B16 melanoma cells. Next, To treat established tumors in vivo, 7 days post tumor challenge mice were immunized with irradiated tumor cells, non-pulsed DCs or pulsed DCs. Pulsed DCs induced a significant reduction in tumor growth compared to other treatments. Conclusion: These results support the use of DCs pulsed with RNA vaccines for the treatment of cancer.

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Adenovirus-mediated gene therapy for superficial bladder cancer: successful transduction of normal and malignant human urothelium

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Purpose: One of the principal challenges in treating transitional cell carcinoma (TCC) of the bladder is to avoid progression of superficial tumours (Ta, T1 and Tis), for which cure rates are high, to muscle-invasive tumours (T2 and beyond), with a far worse prognosis. Intra-vesical delivery of recombinant adenovirus vectors is an attractive strategy for local treatment of these superficial tumours. In human bladder tumour cell lines, the efficiency of transduction is variable, and correlates with expression of the human coxsackie/adenovirus receptor (hCAR) - Li et al (1999) Cancer Research 59:325-330. We have studied adenoviral transduction of normal and malignant human urothelial cells, both as primary monolayer cultures and as intact organotypic raft cultures.

Methods: Normal human urothelial (NHU) cells were stripped from underlying stroma and maintained in vitro as monolayer cultures. Alternatively, intact pieces of full-thickness urothelium were maintained ex vivo as organotypic raft cultures, at an air-liquid interface. Patient samples were treated with Ad-lacZ at varying multiplicity of infection (MOI), up to MOI of 10. Similar experiments were performed with a variety of human TCC tumour cell lines and with freshly explanted TCC tumours from human bladders.

Results: All 15 of 15 primary NHU cell lines have been infected with efficiency at least as great as the high hCAR-expressing cell line A549, and approaching the efficiency for human embryonic kidney 293 cells. Efficiency was reproducibly higher than for most human TCC cell lines and was independent of passage number, from only 24 hours in culture onwards. In addition, 7 of 7 fresh human TCC bladder tumour explants have been successfully infected. In contrast, in organotypic raft cultures from the same patients, efficiency of infection was much reduced, and occurred only in the most superficial layers. Transduction of freshly-explanted human TCC tumours was more efficient than with raft cultures of intact normal urothelium.

Conclusions: We demonstrate for the first time that transduction by adenovirus of both normal and malignant human urothelial cells in monolayer culture is efficient and reproducible. Experiments with organotypic raft cultures from the same patients suggest that a physical barrier, rather than hCAR status, is the main obstacle to transduction of intact tissue. Higher efficiency in human TCCs suggests possible tumour-selectivity.

543 POSTER DISCUSSION

Production of EIAV based lentiviral vectors for gene therapy

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Lentiviruses are a family of complex retroviruses. Equine Infectious Anaemia Virus (EIAV) has the simplest genomic structure of all of the lentiviruses. We have codon-optimised the gag/pol gene rendering it Rev/RRE independent. An added advantage of this is the removal of any packaging motif expression. This limits the amount of gag/pol RNA that is encapsidated thus preventing homologous recombination to allow the generation of high titre stocks that are RCR free.

We have inserted the cPPT/CTS elements into these vectors to give increased transduction efficiency in target cells. The polyadenylation enhancer, WPRE, has also been included to increase expression levels. Real time quantitative PCR (TaqMan) analysis was performed using the ABI PRISM 7700 sequence detection system to asses the level of expression (RNA) per integrated copy of genome (DNA) of various vector constructs. This has allowed us to optimize the expression configuration for specific cell types.

The envelope of choice for pseudotyping lentiviral vectors has been VSV-G due to its wide tropism and pseudotyping efficiency. However, its use is limited by the need to regulate expression because of toxicity. We have utilized a temperature sensitive VSV-G cell line in which VSV-G expression is induced at 32°C for 72hrs and suppressed at 37°C. This temperature regulation of VSV-G expression is compared to the Tet inducible systems. Here we present in vitro and in vivo data showing EIAV transduced neurons, epithelia, haematopoietic and cancer cells.

In conclusion EIAV vectors are rapidly becoming an invaluable gene transfer tool of high efficiency and biosafety. They function in most primary target cells (dividing and non-dividing cells) and in the tissues of experimental animals in vivo. This opens new avenue for scientific research and clinical applications has allowed us to optimize the expression configuration for specific target cell types.