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

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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.

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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.