725. Correction of Laminin-5-Deficient Junctional Epidermolysis Bullosa by Transplantation of Genetically Modified Epidermal Stem Cells. A Phase-I Clinical Trial

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Mutations in genes encoding the laminin-5 heterotrimer, a key component of the epidermal-dermal junction, cause junctional epidermolysis bullosa (JEB), a severe and often fatal skin adhesion defect. Epidermal stem cells isolated from patients affected by β3 chain-deficient JEB were transduced with a retroviral vector expressing a β3 cDNA, and used to generate uniformly transduced cultured skin implants. The transgene was steadily expressed for >160 cell doublings in culture, leading to restoration of normal laminin 5 levels, assembly of functional hemidesmosomes, and full phenotypic correction. Cloning and sequencing of vector integrations showed that <20 stem cells are responsible for long-term maintenance of a transplantable skin culture. A phase-I clinical trial started in October 2005, aimed at proving the safety of the transduction/transplantation procedure, and analyzing persistence of transgene expression and long-term survival of transduced stem cells. The first patient was a 30-yr-old male affected by non-lethal JEB, carrying a null mutation in one LAMB3 allele and a point mutation (E212K) in the other one. The mutation affects the assembly of the laminin-5 heterotrimer, present at residual levels (<5%) in vitro and in vivo. Six genetically modified, cultured epidermal sheets of 100 sq cm were transplanted on both legs after removal of the outer skin layer using a minimally invasive technique. The procedure was well tolerated, and the patient discharged after five days. Engraftment was completed after 10 days, and transplanted skin remained stable on both legs in the absence of blistering or inflammation for the duration of the follow-up (4 months at the time of writing). 3-mm punch biopsies were taken 1 and 3 months after transplantation, and analyzed for vector presence by quantitative PCR and for protein expression by immunohistochemistry. A vector signal compatible with full transduction of the transplanted epidermis was observed at both time points. Synthesis and assembly of normal levels of heterotrimeric laminin-5 and α6β4 integrin was observed at the level of the basal lamina in all biopsies, together with the development of a firmly adherent, fully differentiated epidermis. Epidermal stem cells ($p64^+$) were detected in the basal layer of the transplanted skin in normal numbers. These data show that gene therapy of JEB by transplantation of genetically corrected stem cells is feasible, and leads to full phenotypic correction of the adhesion defect in vivo. Safety studies are under way, which include detection or humoral or cytotoxic immune responses against laminin-5, and ex vivo cloning and sequencing of the integrated proviruses.

726. Clinical Trial of Recombinant Adenovirus-p53 (Gendicine) Combined with Radiotherapy in Nasopharyngeal Carcinoma Patients

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The study was to evaluate the safety and the efficacy of recombinant adenovirus-p53 (Adp53, trademarked as Gendicine) combined with radiotherapy in nasopharyngeal carcinoma (NPC) patients. A controlled clinical trial on Gendicine combined with radiotherapy, that is, gene therapy + radiotherapy (GTRT), was conducted in 24 patients, and 25 patients treated with radiotherapy alone (RT) formed the control group. In the GTRT group, Gendicine $1x10^{12}$ virus particle (vp) was intratumorally injected once a week over to eight weeks, and concurrent irradiation was given. For both groups, the conventional fractionation 2Gy/day, five fractions a week, with a total dose of 70Gy in 35 fractions were given to either primary tumor or neck lymph node. Patients were monitored for adverse event and serum anti-adenoviral antibody, and tumors were monitored for response. A comparative study was performed between both groups on the immediate response rate by CT at the end of 4^{th} week, 7^{th} week and 2 months after the treatment. The median follow-up interval of surviving patients was 34.5 months (24~45 months). Wild-type p53 gene therapy enhanced significantly radiotherapy efficacy by 1.59 times in patients with NPC at 4-week time point. Two months after the treatment complete response rate of GTRT group was 2.1 times that of RT group (62.5% v.s. 29.6%). The 3-year overall survival rate of GTRT group was 14.4% higher than that of GT group. No dose-limiting toxicity and adverse events were noted, except for transient fever after Gendicine administration. Intratumoral injection with Gendicine was safe and effective for patients with NPC.

727. Improvements in Clinical and Radiographic Outcomes in a Phase I Study of AAV-GAD Gene Therapy for Parkinson's Disease

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Parkinson's disease (PD) is a movement disorder characterized by difficuly initiating movements (bradykinesia), muscular rigidity and tremor. This is associated with pathologically increased activity of the subthalamic nucleus (STN) and the downstream globu pallidus interna (GPi) leading reduced activity of pre-motor cortex (PMC). To normalize this pattern of activity, the STN was injected unilaterally with an adeno-associated virus vector expressing glutamic acid decarboxylase (AAV-GAD) in 12 patients with advanced PD, while the untreated side served as a control. GAD is the rate-limiting step in the synthesis of GABA, the major inhibitory neurotransmitter in the normal brain. Patients were divided into 3 groups of 4, and each group receiving 35μl of 10^{11}(low), $3x10^{11}$(middle) or 10^{12}(high-dose) packaged genomes/ml. All 12 patients have now been followed for 6 months. There have been no study-related adverse events. 2 patients had high neutralizing antibody titers prior to surgery, but there was no change in titers in any patient following treatment. Using the