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ESGCT 2010 Oral Presentations

Or 1

Homologous recombination

Toni Cathomen

See supplement

Or 2

Emerging use of transposons for gene therapy

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Effective gene therapy requires robust delivery of the desired genes into the relevant target cells, long-term gene expression, and minimal risks of secondary effects. The development of efficient and safe non-viral vectors would greatly facilitate clinical gene therapy studies. However, non-viral gene transfer approaches typically result in only transient gene expression in most primary cells. The use of non-viral gene delivery approaches in conjunction with the latest generation transposon technology based on Sleeping Beauty (SB) or piggyBac transposons may potentially overcome some of these limitations. In particular, a large-scale genetic screen in mammalian cells yielded novel hyperactive SB transposases, resulting in robust and stable gene marking in vivo after hematopoietic reconstitution with cord blood-derived CD34+ hematopoietic stem/progenitor cells in mouse models. Moreover, the first-in-man clinical trial has recently been approved to use redirected T cells engineered with SB for gene therapy of B-cell lymphoma.Finally, induced pluripotent stem cells (iPS) could be generated after genetic reprogramming with transposons encoding reprogramming factors. Moreover, transposons can be used to "coax" the differentiation of iPS into transplantable cell types. These recent developments underscore the emerging potential of transposons in gene therapy applications and induced pluripotent stem generation for regenerative medicine. (VandenDriessche et al., Blood. 2009;114:1461–1468; Mates, Chuah et al., Nature Genetics, 41(6):753–61, 2009; VandenDriessche et al., Hum Gene Ther. 20(12):1559–61, 2009; Belay, Matrai et al., Stem Cells, in press 2010).

Or 3

Antisense oligonucleotides mediated exon skipping therapy for Duchenne muscular dystrophy

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Antisense-mediated reading frame restoration is presently one of the most promising therapeutic approaches for Duchenne muscular dystrophy (DMD). In this approach, antisense oligoribonucleotides (AONs) induce specific exon skipping during pre-mRNA splicing of mutated dystrophin transcripts. This is aimed to restore the disrupted open reading frame and allow synthesis of internally deleted, partly functional Becker-like dystrophin proteins. The approach is theoretically applicable to over 70% of all patients, with exon 51 skipping being applicable to the largest group of patients (13% of all mutations). Proof of concept has been achieved in cultured muscle cells from patients carrying different mutation types, in the *mdx* mouse model, and recently in DMD patients in clinical trials after local treatment of PRO051, a 2′-O-methyl phosphorothioate modified AON targeting exon 51. In each case AON treatment resulted in skipping of the targeted exon and dystrophin restoration in the absence of adverse effects. For therapeutic application, long term systemic delivery would be preferred. Therefore, current work focuses on different systemic delivery methods and long term treatment of dystrophic animal models. Following encouraging results in mice, a subsequent trial where patients are treated systemically has recently been completed successfully and a 6-months follow-up trial using the most effective dosage is underway by Prosensa.

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Cancer immunotherapy with genetically modified lymphocytes

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Adoptive cell transfer using anti-tumor antigen reactive T cells has proven to be a useful strategy for the treatment of metastatic melanoma, with objective response rates of up to 72%, with 16% of patients rendered disease free. These studies involved the extraction and ex-vivo expansion of naturally occurring tumor-infiltrating lymphocytes (TIL). Only one-half of patients are able to receive this therapy due to lack of harvestable tumors, inability to grow TIL, or a lack of cellular reactivity. As an alternative approach to TIL therapy, high-affinity T cell receptors (TCR) can be introduced into normal T cells and the adoptive transfer of these cells into the lymphodepleted patients has been shown to

gene therapy for WAS patients using lentiviral-vector transduced HSC in combination with reduced intensity conditioning.

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Lentiviral vector-mediated gene transfer of FOXP3 into CD4+T cells isolated from patients with IPEX Syndrome generates potent suppressor regulatory T cells

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IPEX (Immune Dysregulation, Polyendocrinopathy, Enteropathy, X-linked) is a genetic disease caused by mutations of the transcription factor FOXP3, the master gene for naturally occurring (n) $CD4^+CD25^+$ regulatory T (Treg) cell fate and function. It is well established that nTreg cells play a central role in the control of immune responses to autoantigens, allergens, and tumor antigens. In IPEX patients the lack of functional nTreg cells leads to the development of early onset life-threatening systemic autoimmunity. Current therapies for the cure of patients with IPEX are limited. The majority of patients are treated with immunosuppressive drugs, with only partial control of the clinical manifestations. At present, haematopoietic stem cell transplantation (HSCT) is the only definitive cure, but it is available for a limited number of patients.

An alternative strategy to restore tolerance could aim at generating high numbers of human Treg cells by lentiviral-vector- (LV-) mediated gene transfer of FOXP3 (LV-FOXP3). We previously showed that transduction of human $CD4^+$ T cells generates a homogeneous population of Treg cells that can be propagated in vitro. Here we demonstrate that high an stable FOXP3 expression can be induced in $CD4^+$ T cells isolated from patients carrying different FOXP3 mutations. FOXP3-mutated effector T (Teff) cells isolated from severely affected patients could be converted into functional Treg cells displaying potent in vitro suppressive activity. Preliminary results further suggest that LV-FOXP3 transduced cells can efficiently inhibit Teff cell responses in vivo in a xenogeneic graft versus host disease model (xeno-GvHD).

These results demonstrate that suppressive capacity is conferred to human T cells upon high and stable FOXP3 expression even in the presence of FOXP3 mutations, as in the case of IPEX patients. Overall, these findings pave the way for the development of a gene therapy approach using adoptive transfer of Treg-converted mature lymphocytes for the treatment of patients with IPEX Syndrome.

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Enhancement of Ad-CRT/E7-mediated antitumor effect by preimmunization with L. lactis expressing HPV-16 E7

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Background: Developing effective vaccines that target HPV-16 E6 and/or E7 has become a necessity, because current prophylactic vaccines against HPV infection do not protect against cervical cancer already established. We previously showed the effectiveness of adenovirus expressing CRT/E7 (Ad-CRT-E7) in a cervical cancer animal model. We also demonstrated that intranasal immunization of *Lactococcus lactis* encoding HPV-16 E7 (*Ll*-E7) anchored to its surface, induced significant HPV-16 E7-specific immune response. In this study we assessed the combination of both approaches in a cervical cancer animal model.

Method: Groups of mice were immunized intranasally with Ll-E7 on days 0, 14, and 28. One week later, the mice were challenged with HPV-16 E7-expressing murine tumour cells (TC-1) in the right leg. Ad-CRT-E7 vector was injected intratumorally once they reached a diameter of 6 mm. Tumor growth and survival were monitored. Infiltration of CD8 + cytotoxic T lymphocytes and the presence of apoptosis within the tumour were analyzed.

Results: A single dose of Ad-CRT/E7 was able to reduce the tumour size 60%, with a 20% of survival, compared with the controls. Interestingly, this antitumour and survival effect were increased to 80% and 70% respectively by the intranasal preimmunization with Ll-E7. Significant CD8 + cytotoxic T lymphocyte infiltration was detected in the tumours of mice treated with Ll-E7 + Ad-CRT/E7. *In situ* TUNEL apoptosis analysis showed a strong correlation between tumour regression and a higher number of apoptotic cells.

Conclusion: Preimmunization with Ll-E7 enhances the antitumour and survival effect of the Ad-CRT/E7 in a cervical cancer animal model.

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Targeting lentiviral vectors to dendritic cells by the Nanobody display technology

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