Cancer Immunol Immunother (2006) 55:1406–1411
DOI 10.1007/s00262-006-0187-x

SYMPOSIUM PAPER

International Society for Cell and Gene Therapy of Cancer (ISCGT) annual meeting: conference overview and introduction to the symposium papers

Barbara-ann Guinn · James S. Norris · LaiQiang Huang · Farzin Farzaneh · Noriyuki Kasahara · Albert B. Deisseroth

Received: 10 May 2006 / Accepted: 13 May 2006 / Published online: 17 June 2006

Keywords Gene therapy · Immunotherapy · Cancer research · Meeting report · International Society for Cell and Gene Therapy of Cancer

Abbreviations

Ad	Adenovirus
AML	Acute myelogenous leukemia
CRAd	Conditionally replicative adenoviruses
CMV	Cytomegalovirus
CTL	Cytotoxic T lymphocyte
DC	Dendritic cell
EGFR	Epithelial growth factor receptor
GT	Gene therapy
HbcAg	Hepatitis B core antigen
HbsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular cancer
HNSCC	Head and neck small cell carcinoma
HOT	HSP-mediated oncolytic therapy
HSP	Heat shock protein
hTERT	Human telomerase reverse transcriptase
ISCGT	International Society for Cell and Gene Therapy of Cancer
MK	Midkine
NPC	Nasopharyngeal carcinoma
pIX	Adenovirus capsid protein IX
RT	Radiation therapy
SFDA	State Food and Drug Administration, China
shRNA	Small hairpin RNA
SOCS1	Suppressor of cytokine signaling 1
TAA	Tumor associated antigens
TK	Thymidine kinase
Tregs	Regulatory/suppressor T cells
VP	Viral particles

B. Guinn (✉) · F. Farzaneh
Department of Haematological Medicine,
King's College London School of Medicine,
The Rayne Institute, 123 Coldharbour Lane,
London, SE5 9NU, UK
e-mail: barbara.guinn@kcl.ac.uk

J. S. Norris
Department of Microbiology and Immunology,
BSB Medical University of South Carolina,
173 Ashley Avenue, Room 201, P.O. Box 250504,
Charleston, SC 29425, USA

L. Huang
The Center for Biotech and Bio-Medicine,
Graduate School at Shenzhen, Tsinghua University,
Shenzhen 518055, China

N. Kasahara
Department of Medicine, David Geffen School of Medicine,
University of California Los Angeles,
Los Angeles, CA 90095, USA

A. B. Deisseroth
Sidney Kimmel Cancer Center, 10835 Road to the Cure,
San Diego, CA 92121, USA

The 2005 International Society for Cell and Gene Therapy of Cancer (ISCGT) meeting was organized by Jim Norris (BSB Medical University of South Carolina, USA) and its smooth running was greatly aided by Katherine Lindley (BSB Medical University of South Carolina), Suzanne McGrath (King's College London, UK) and the ISCGT Secretariat Farzin Farzaneh (King's College London). The meeting was staged in China where the first commercial gene therapy agent, an adenovirus

(Ad) vector expressing the p53 tumor suppressor gene (Ad-p53; trade name Gendicine), was developed. Zhaohui Peng (President & CEO, SiBiono Genetech Co. Ltd.) presented the results of clinical trials for a range of cancers including nasopharyngeal carcinoma (NPC) and head and neck squamous cell carcinoma (HNSCC) (350 patients so far), as well as off-label use in 400 hepatocellular (HCC) patients (reviewed in [10]). To date the predominant side effects has been self-limited fever in 32% of patients although one patient has received 85 doses of up to 10^{13} VP total with no adverse effects. One interesting finding is that the response does not seem to correlate with the p53 mutational status of the tumor, perhaps suggesting an immunological effect of the Ad-p53 treatment rather than genetic augmentation of a dysfunctional p53.

Other presenters of Ad-p53/Gendicine clinical trial data included Demin Han (Beijing Tongren Hospital, The Capital Medical University of China) who has recently completed a phase I clinical trial of Ad-p53 in laryngeal squamous cell carcinoma patients, with 11 out of 12 patients surviving with a mean survival time of 5.9 years following treatment compared with 46–71% surviving (depending on stage) when treated with conventional therapy. Demin Han also reported that viral infection was observed in biopsy specimens primarily along the needle track but was associated with considerable lymphocytic infiltration, again implicating the immune response as a contributory mechanism. Shanwen Zhang (Beijing University School of Oncology, China) reported on a phase II/III clinical trial in which 40 patients with NPC received radiation therapy (RT) and Ad-p53 gene therapy (GT), increasing survival rates by 13% compared with radiation therapy alone (survival rate 78.8% RT+GT vs. 66% RT alone). In advanced HCC, Yongsong Guan (West China Hospital of Sichuan University, China) showed that gastrointestinal symptoms were lower ($P < 0.05$) and 1-year survival rates were significantly higher in patients treated with chemoembolization combined with Ad-p53/Gendicine (43%) compared to those treated with chemoembolization alone (24%).

Jack Roth (The University of Texas, MD Anderson Cancer Centre, USA), who developed the clinical application of p53 gene therapy [4] said they now treat their gene therapy patients as out-patients and have two randomized clinical trials in progress, one using Ad-p53/ADVEXIN® as a monotherapy and one in combination with chemotherapy. To summarize in phase I–II trials, they found that 10% of patients had major responses in the multi-cycle monotherapy group. The majority of patients showed stable disease for approximately 3 months or longer with 59% of patients having disease control (response or stable disease). Longer term survival was associated with the higher dose treatment.

Replication-defective adenoviruses were a major focus of the meeting and Marie Lin (China PLA General Hospital, Beijing, China) described the effects of Ad-delivered epithelial growth factor receptor (EGFR) antisense RNA on breast cancer to inhibit EGFR protein expression in both MDA-MB-231 breast cancer cells and HMEC cells. Intratumoral injection of the recombinant adenovirus in mice caused growth arrest of MDA-MB-231 tumors. Patrick Arbuthnot (University of Witwatersrand, South Africa) described their use of adenovirus to express small hairpin RNA (shRNA) in cell and mouse models to counter replication of HBV infection, which is associated with a 100-fold elevated risk of HCC development. Two of the ten shRNAs screened were able to specifically knock down HBV gene expression by over 80%, more effective than equivalent synthetic RNA duplexes. Injection of the recombinant adenoviral particles into HBV transgenic mice transduced 70% of the hepatocytes, diminished serum hepatitis B surface antigen (HbsAg) concentrations by up to 90% which lasted for 4 weeks, and also reduced intrahepatic production of hepatitis B core antigen (HbcAg). Chuan-Yuan Li (Duke University Medical Centre, USA) described an adenovirus-mediated GRP94 tumor vaccine, Ad-sGRP94, which is modified and secreted. Despite poor results when used alone, vaccination and radiation therapy led to marked inhibition of tumor growth and in 20% of the cases complete tumor regression was observed.

Robert Hoeben (Leiden University Medical Centre, The Netherlands) investigated the feasibility of engineering adenovirus capsid protein IX (pIX), which is abundant and small, as an anchor for incorporating peptide ligands into the capsid for tumor specific targeting. Victor van Beusechem (VU University Medical Centre, Amsterdam) also described strategies for changing adenovirus tropism by genetically replacing its entire fiber protein with a fusion molecule comprising the virion-anchoring tail domain of the fiber linked to the oligomerization domain from reovirus attachment protein sigma 1 and a His-tag, which enabled the virus to propagate efficiently and target highly selectively to cells expressing a His-tag binding receptor. With the additional mutation of the integrin-binding site in the penton base protein, the adenovirus showed even higher specificity of targeting to cells expressing His-tag receptor, markedly reduced transduction of HepG2 cells or liver slice models (i.e., enhanced liver de-targeting), and prolonged persistence in the circulation due to shielding from the humoral response.

Physical techniques to cloak Ad and hide it from the immune system were also described. Len Seymour (University of Oxford, UK) detailed the need to extend the plasma circulation of Ads to promote greater access to disseminated disease. It was shown that the circulating half-life of Ads (about 2 min in mice and 15 min in humans) could be increased by predosing animals with $10^{10–11}$ VP to first saturate the Kupffer cells. A multivalent polymer coating, pHPMA, was also reported to increase circulating half-life and decrease liver infection by four to five logs by preventing all mechanisms for viral infection of hepatocytes.

Quite a number of studies on conditionally replicative adenoviruses (CRAds) were presented. Among them, Victor van Beusechem described AdDelta24-p53 which is derived from AdDelta24 (with a deletion in the pRb-binding domain of E1A-CR2) and expresses functional human p53, which exhibits stronger anti-cancer potency than its parent AdDelta24 both in vitro and in vivo [1, 5, 6] with a considerably improved safety profile compared to Ad5 and AdDelta24. Imre Kovesdi (VectorLogics Inc., USA) (Hedley et al. 2006: symposium paper) presented their work to improve AdDelta24-RGD, a variant of AdDelta24 displaying an integrin-binding RGD motif inserted in the fiber knob in order to expand viral tropism, which will be tested in clinical trials in the US and Netherlands. It was reported that genetic incorporation of large proteins such as GFP and thymidine kinase (TK) into the viral capsid by fusing their sequences with pIX offers a means to provide steric shielding from the humoral response. Proof of principle was demonstrated by ELISA experiments in which sera from mice that had been immunized with wild type Ad vector showed 60% less binding to Ad vectors displaying pIX-TK, suggesting that the shielding concept is feasible.

The use of tumor-specific promoters was illustrated by Bingliang Fang (University of Texas, M.D. Anderson Cancer Center, USA) who presented results from studies of a TRAIL-expressing oncolytic adenovector, Ad/TRAIL-E1, in which expression of both TRAIL and viral E1A genes is under the control of a synthetic promoter consisting of sequences from the human telomerase reverse transcriptase (hTERT) promoter and a minimal cytomegalovirus (CMV) early promoter. Ad/TRAIL-E1 elicited a much stronger therapeutic effect in various human cancer cell lines than replication-defective adenovirus expressing TRAIL (Ad/TRAIL-RGD) or an oncolytic adenovector without TRAIL (Ad/GFP-E1). Intratumoral administration of Ad/TRAIL-E1 eliminated all xenograft tumors established from a human non-small cell lung cancer cell line, H1299, in nude mice, and resulted in longer-term tumor-free survival with no treatment-related toxicity found.

Masatoshi Tagawa (Chiba Cancer Research Institute, Japan) (Tagawa et al. 2006: symposium paper) reported the development of Ad-midkine (MK), a CRAd combining transcriptional targeting and tropism modification, in which E1A expression is controlled by the MK promoter and the fiber knob of Ad serotype 5 has been replaced with that of serotype 35, thereby enhancing tumor selectivity. Ad-MK was highly cytotoxic to HCC cells but not normal fibroblasts, and intratumoral injection in immunodeficient rodent models of HCC significantly retarded subsequent tumor growth compared with wild type Ad.

Another notable development from China was reported by Ronghua Zhou from Shanghai Sunway Biotech, who showed results with their E1B 55K-deleted oncolytic adenovirus H101 in clinical trials for HNSCC and NPC. H101 combined with chemotherapy showed 86.5% overall response rate versus 59% with chemotherapy alone, and in late 2005 received State Food and Drug Administration (SFDA) approval, representing the world's second commercial gene therapy product and first approved CRAd. John Neumanaitis (Mary Crowley Cancer Institute, USA) reported follow-up studies with the first E1B 55K-deleted oncolytic adenovirus, ONYX-015, to which Shanghai Sunway Biotech has now acquired licensing rights. Si-Yi Chen (Baylor College of Medicine, USA) also described a strategy developed in collaboration with Sunway Biotech to combine viral oncolysis and heat shock protein (HSP)-mediated local immunotherapy (HSP-mediated oncolytic therapy (HOT) vaccine). They found that an oncolytic virus expressing HSP, which chaperones tumor antigens to dendritic cells (DCs), can eradicate tumors and induce anti-tumor immunity, a result which could not be achieved by oncolytic virus alone. However, the treatment was found to have little effect on distal tumors in a phase I clinical trial, and so their current efforts focus on combining this approach with siRNA-mediated inhibition of Suppressor of cytokine signaling 1 (SOCS1), a pseudosubstrate of cytokine-activated JAKs which normally causes their degradation. With siRNA targeting SOCS1, DCs presenting the murine melanocyte antigen TRP2 were found to break self tolerance and induce vitiligo, and could inhibit the growth of weakly immunogenic melanomas.

David Klatzmann (Hopital Pitie-Salpetriere, France), however, presented a cautionary and highly thought-provoking discussion on immunotherapy, citing a review by Rosenberg et al. [11] which had reported that out of 440 patients receiving 541 different tumor vaccines, to date only 4 patients (1%) have shown a

complete response, 9 patients (2%) a partial response and 427 (97%) have shown no response. Increasing evidence indicates that the difficulties encountered in realizing the potential of immunotherapy may be attributed in large part to the action of regulatory T cells (Tregs). Tregs were first described by Gershon in 1972 [7], and in 1995, Sakaguchi et al. [12] showed that these CD4+ CD25+ cells controlled autoimmunity through their adoptive transfer into T cell null mice leading to multi-organ autoimmunity. However, the Treg population cannot be defined solely by CD4+CD25+ as this population also includes activated effector cells and it is FoxP3 that is one of the best markers for this population. Tregs prevent fetus rejection, atherosclerosis, regulate anti-infectious immunity and inhibit anti-tumor immunity. According to data from studies by David Klatzmann, Tregs account for 8% of the T lymphocytes in regional lymph nodes prior to tumor implantation, but post-implantation there is a massive expansion in the frequency of Tregs of up to 25% in the draining (but not contralateral) lymph nodes. Depletion of Tregs by intraperitoneal injection of anti-CD25 antibodies at day 2 post-tumor implantation is sufficient to allow tumor rejection, and Treg depletion allows increased activation of CD4+ CD25- T cells in draining lymph nodes (13 to 27% CD69+), intratumoral T cells (33 to 96% IFNγ+) and intratumoral natural killer cells (23 to 86% IFNγ+).

In this regard, Albert Deisseroth (Sidney Kimmel Cancer Center, San Diego, CA, USA) reported on an Ad-sig-TAA/ecdCD40L adenoviral vector prime-TAA/ecdCD40L protein boost cancer vaccine strategy. The subcutaneous injections of the vector encoding a chimeric transcription unit composed of a tumor associated antigen linked to the CD40 ligand (which is usually presented on activated CD4 cells) induced in anteric mice an increase of the level of tumor associated antigen (TAA) specific T cells in the tumor tissue, and at the same time decreased the level of CD4CD25FOXP3 positive CD4 negative regulatory T cells. The TAA specific antibodies induced by the vaccine in test mice reacted with human breast and prostate cancer biopsy specimens. This vaccine induced a memory response for up to a year, and was active in old (18 month old) as well as young (2 month old) test mice.

Farzin Farzaneh (King's College London) explained another mechanism of immunoevasion by acute myelogenous leukemia (AML) blasts, which express both class I and class II major histocompatibility antigens and both DC and tumor-specific markers, but not the critical co-stimulatory molecule B7.1. Accordingly, Farzin Farzaneh has developed an HIV-based lentiviral vector, SFFVpro-IL2-2Aprot-B7.1, for co-expression of IL-2 and B7.1. The woodchuck hepatitis virus WPRE element, which has frequently been used to enhance gene expression from lentivirus vectors but has recently been implicated as a potential cause of HCC after administration of EIAV-based lentiviral vectors in fetal mice, has been removed. This has led to a four to five-fold reduction in expression but is still more than adequate, and transduction of murine AML cells has shown these to be as effective as irradiated cell vaccines in vivo. Transduced human AML cells show positive T cell responses by ELISpot (>250/10^5 T cells) and lysis of autologous target tumor cells by chromium lysis assays, and this engineered tumor cell vaccine strategy has been approved in the UK for testing in clinical trials, representing the world's second approved clinical trial employing HIV-based lentivirus vectors. Farzin Farzaneh noted that chronic exposure of tumor antigens in the natural course of disease may result in clonal exhaustion and that the strongest antigen may not be the best for vaccination [2], hence the use of a whole cell vaccine would present the immune system with all possible tumor antigens in an unbiased manner. Farzin Farzaneh's group has also developed a technique in which retroviral and lentiviral vectors can be biotinylated and then concentrated more than 1,000× using streptavidin-paramagnetic beads, and allows ligand incorporation for vector targeting [3]; they are currently pursuing the application of this method for the improvement of vectors for cellular vaccine engineering.

Also using a whole cell vaccine Bao-En Shan (The Fourth Hospital of Hebei Medical University, China) (Hao et al. 2006: symposium paper) showed that the modification of murine colon26 cells to express IL-23 led to their rejection in vivo. They evaluated a range of immunological responses and showed that IL-23 secretion by colon26 cells led to suppressed tumor growth, prolonged survival of the host, proliferation of splenocytes, cytotoxic T lymphocyte (CTL) activity, increased DC production and Th1 cytokines.

Jon Kyte (University of Oslo, Norway) (Kyte and Gaudernack 2006: symposium paper) described their transfer of whole tumor RNA into autologous DCs and their re-administration into patients in clinical trials. Pre-clinical studies in six patients with advanced melanoma showed that the T cell responses were specific to antigens in the transfected RNA. Two phase I/II clinical trials (androgen resistant prostate cancer and malignant melanoma) were conducted. The preparation of transfected DCs (tDCs) was possible from all elected patients and no serious side effects were seen. Immunological responses (ELIspot or T cell proliferation) were

demonstrated in 9 of 19 evaluable melanoma patients and 12 of 19 prostate cancer patients. The response rates were higher in patients vaccinated by intradermal injection, compared to intranodal vaccine administration.

Mark Tangney (Cork Cancer Research Centre, Ireland) (Tangney et al. 2006: symposium paper) described their in vivo electroporation technique in which tumors which were already established on the flank of mice were electroporated with plasmid DNA encoding B7.1 and GM-CSF. They showed regressions in 63% of mice using a fibrosarcoma model (JBS) and a significant reduction of secondary untreated tumors in a metastatic model. The mice show a specific response to the JBS tumor cells and a failure to reject an alternative tumor type (CT-26). Responding mice also showed CTL activity ex vivo and an in vivo memory response to challenge.

Another non-viral delivery technique that has proven effective in vivo was reported by Nagy Habib (Imperial College London, UK), who described their hydrodynamic gene transfer method which has been restricted to the liver via the use of a hepatic vein balloon catheter applied at the junction of the left hepatic vein and inferior vena cava. This has now been tested in clinical trials as a fluoroscopic guided outpatient procedure involving delivery of the TPO gene in a volume of 200 ml within 30 s for treatment of thrombocytopenia. This hydrodynamic gene therapy procedure achieved a 50% increase in platelet count which persisted for 2–3 weeks. For more long-term correction, Nagy Habib is now also using "OmniCytes", a novel type of pluripotent stem cell derived from the adherent CD34+ fraction of bone marrow. The use of OmniCyte stem cells for regenerative medicine has also now been tested in clinical trials for patients with liver failure. Highly promising results were obtained, with three of five patients showing improvement in serum bilirubin and four of five patients showing increased serum albumin. Nagy Habib also reported that OmniCytes were capable of cell–cell fusion and killing of cancer cells such as neuroblastoma, and additional clinical trials are now proceeding to test their ability to serve as a cellular delivery vehicle for IL-2 and IFNγ.

This meeting also witnessed reports of other highly promising cell-based therapies. Walter Gunzburg (Austrianova, and Research Institute for Virology and Biomedicine, Austria) described a novel cellulose sulfate-based cell encapsulation technology. Micro-encapsulated cells ("NovaCaps"), expressing a prodrug converting enzyme which converts ifosfamide to its active form, have shown considerable promise in a phase I/II clinical trial of non-resectable pancreatic cancer. A dose of 300 microcapsules delivered by arterial catheter led to 28.6% overall response rate, with 71% showing stable disease (which may be due to fibrosis of the tumor lesion), and no cases of local progression which is a highly unusual finding in pancreatic cancer. Median survival in these patients doubled from 20 to 40 weeks, and the 1 year survival rate was 36% compared to 11% for historical controls [9]. This therapy has now been given Orphan Drug status for non-resectable pancreatic cancer, and has been granted approval by the European FDA (EMEA) to proceed to pivotal phase III clinical trials in late 2006/early 2007.

Various other novel and highly promising technologies were presented at this meeting, including additional improvements in adenovirus vectors for tumor apoptosis (John Dong, Medical University of South Carolina, USA; Xiang Liu, Medical University of South Carolina) and immunotherapy (John Neumanaitis, Mary Crowley Cancer Institute, USA; Yajun Guo, Shanghai Tumor Immune and Gene Therapy Center, China), development of adeno-associated virus vectors for cancer gene therapy (Marie Lin, University of Hong Kong) and hemophilia (Xiaobing Wu, VGTC, Beijing, China), improvements in retroviral vector targeting of transcription (Brian Salmons, Austrianova, Austria) and integration (Samson Chow, UCLA, USA), other tumor-selectively replication-competent virus systems such as vesicular stomatitis virus (Savio Woo, Mt. Sinai Medical Center, USA), measles virus (Kah-Whye Peng, Mayo Clinic, USA), Herpes virus (Robert Coffin, Biovex, UK; William Jia, University of British Columbia, Canada), and retrovirus (Nori Kasahara, UCLA, USA), and non-viral strategies for polyplex targeting (Yuhong Xu, Shanghai, China) and DNA repair (Depei Liu, Chinese National Academy of Sciences, Beijing, China) and approaches to understand the biology of cancer cells (Jianren Gu, Shanghai Cancer Institute, China; Qimin Zhan, Chinese Academy of Medical Sciences, Beijing, China), as well as other fascinating talks that cannot be fully described here due to space limitations. A full report on the 2005 ISCGT China meeting is in press [8] and further details regarding the ISCGT are available at http://www.iscgt.org.uk. The next ISCGT meeting will be held in Chiba, Japan from 13th to 15th October 2006 (http://www.iscgt-2006.jp).

References

1. van Beusechem VW, van den Doel PB, Grill J, Pinedo HM, Gerritsen WR (2002) Conditionally replicative adenovirus expressing p53 exhibits enhanced oncolytic potency. Cancer Res 62:6165–6171
2. Chan L, Hardwick NR, Guinn BA, Darling D, Gaken J, Galea-Lauri J, Ho AY, Mufti GJ, Farzaneh F (2006) An

immune edited tumour versus a tumour edited immune system: prospects for immune therapy of acute myeloid leukaemia. Cancer Immunol Immunother 55:1017–1024
3. Chandrashekran A, Gordon MY, Darling D, Farzaneh F, Casimir C (2004) Growth factor displayed on the surface of retroviral particles without manipulation of envelope proteins is biologically active and can enhance transduction. J Gene Med 6:1189–1196
4. Fujiwara T, Cai DW, Georges RN, Mukhopadhyay T, Grimm EA, Roth JA (1994) Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. J Natl Cancer Inst 86:1458–1462
5. Geoerger B, Vassal G, Opolon P, Dirven CM, Morizet J, Laudani L, Grill J, Giaccone G, Vandertop WP, Gerritsen WR, van Beusechem VW (2004) Oncolytic activity of p53-expressing conditionally replicative adenovirus AdDelta24-p53 against human malignant glioma. Cancer Res 64:5753–5759
6. Geoerger B, van Beusechem VW, Opolon P, Morizet J, Laudani L, Lecluse Y, Barrois M, Idema S, Grill J, Gerritsen WR, Vassal G (2005) Expression of p53, or targeting towards EGFR, enhances the oncolytic potency of conditionally replicative adenovirus against neuroblastoma. J Gene Med 7:584–594
7. Gershon RK, Cohen P, Hencin R, Liebhaber SA (1972) Suppressor T cells. J Immunol 108:586–590
8. Guinn BA, Norris JS, Farzaneh F, Deisseroth AB (2006) International Society for Cell and Gene Therapy of Cancer: 2005 meeting in Shenzhen, China. Cancer Gene Ther (in press)
9. Löhr JM, Kröger J-C, Hoffmeyer A, Freund M, Hain J, Holle A, Knöfel WT, Liebe S, Nizze H, Renner M, Saller R, Karle P, Müller P, Wagner T, Hauenstein K, Salmons B, Günzburg WH (2003) Safety, feasibility and clinical benefit of localized chemotherapy using microencapsulated cells for inoperable pancreatic carcinoma in a phase I/II trial. Cancer Therapy 1:121–131
10. Peng Z (2005) Current status of Gendicine in China: Recombinant human Ad-p53 agent for the treatment of cancer. Hum Gene Therapy 16:1016–1027
11. Rosenberg SA, Yang JC, Restifo NP (2004) Cancer immunotherapy: moving beyond current vaccines. Nat Med 10:909–915
12. Sakaguchi S, Fukuma K, Kuribayashi K, Masuda T (1985) Organ-specific autoimmune diseases induced in mice by elimination of T cell subset. I. Evidence for the active participation of T cells in natural self-tolerance; deficit of a T cell subset as a possible cause of autoimmune disease. J Exp Med 161:72–87