

pancreatic tumor cells, exogenous MDA-7 protein activates STAT3 and kills cells via engagement of IL-20 receptors. The specificity of bystander killing is demonstrated using neutralizing anti-MDA-7 antibodies and anti-receptor antibodies, which inhibit the apoptotic effects. In sum, we show that Ad-mda7 is able to induce growth inhibition and apoptosis in pancreatic cancer cells via inhibition of the Wnt/PI3K pathways and identify a novel bystander mechanism of MDA-7 killing in pancreatic cancer that functions via IL-20 receptors. These combination of activities makes Ad-mda7 a promising novel strategy for treating pancreatic cancer.

Sunil Chada is an employee of Introgen Therapeutics.

810. *Ex-Vivo* Activation and Genetic Manipulation of Human T Cells Results in Consistent *In-Vivo* Expansion and Lethal GvHD in a Novel Murine Xenotransplant Model

Bruno Nervi,¹ Michael P. Rettig,¹ Julie K. Ritchey,¹ Gerhard Bauer,¹ Jon Walker,¹ Phillip E. Herrbrich,¹ Todd E. Meyerrose,¹ Mark Bonyhadi,² Jan A. Nolta,¹ John F. DiPersio.¹
¹Oncology, Washington University School of Medicine, Saint Louis, MO; ²Xcyte Therapies, Inc., Seattle, WA.

Our group has previously reported the development of novel CD34-TK chimeric suicide genes for optimal T cell engraftment, graft versus leukemia effect and minimal graft versus host disease (GvHD) in murine BMT models (Rettig et al., Mol. Ther. 2003; Rettig et al., J. Immunol. 2004). These studies have demonstrated critical functional impairment of murine T cells after selected methods of activation, transduction and selection. Unfortunately no *in-vivo* models exist to consistently examine the impact of *ex-vivo* manipulation of human T cells (HuT) on T cell function *in-vivo*. NOD SCID β 2M null mice (β 2 mice) were conditioned with 250cGy TBI on day -1 (n=31), or 300cGy on day 0 (n=22). 10⁷ naive HuT or CD3/28 bead activated (Xcyte™DYNABEADS®) with 50 U/ml IL-2 for 4 days (Act 4d) or 8 days (Act 8d) HuT were injected retroorbitally (ro), intravenous tail vein injection (iv) or lower HuT doses resulted in no expansion or GvHD. Engraftment of HuT in peripheral blood (PB) of recipient mice was evaluated weekly by FACS and euthanasia was performed if mice lost > 20% body weight.

Table 1. NOD SCID β 2M null mice

	250cGy		300cGy	
	Naive (n=31)	Naive (n=8)	Act 4d (n=9)	Act 8d (n=5)
PB engraftment	20%±15	33.2%±21	59.3%±19 *	33%±18
Lethal GvHD	65%	75%	100%	80%

* p < 0.02

All mice receiving 300cGy and succumbing to lethal GvHD had well preserved CD4/CD8 ratios (1-1.2). Infiltration of murine tissues was greatest in those mice receiving 300cGy and Act 4d HuT (spleen, liver, lung, kidney: 50-70%). In mice receiving naive T cells, expression of activation markers (CD25, CD30, CD69) increased from 1% on day 0 to 28-39% on day 15. In contrast, *ex-vivo* Act 4d and Act 8d T cells expressed high levels of these same markers on day 0 (62-99%) followed by rapid down regulation on day 3 (2.2-7.8%) and subsequent increased expression similar to naive T cells. Of interest, serum human IFN γ levels dramatically increased over time in all mice who went on to develop lethal GvHD (day 3=0.27ng/ml and day 15=36ng/ml) compared to mice who did not develop lethal GvHD (day 10=0.04ng/ml and day 17=1.02ng/ml). Finally, we evaluated the HuT engraftment and GvHD potential of naive and Act 4d in RAG2 γ null mice conditioned with chlodronate liposomes on day -1 and 350cGy on day 0, as previously described by others. We injected 10⁷ and 1.5x10⁷ naive or Act 4d HuT iv. All mice exhibited low HuT engraftment and no lethal GvHD.

Table 2. RAG2 γ null mice

	Naive T cells iv		350cGy Act 4d cells iv	
	10e7 (n=10)	1.5x10e7 (n=4)	10e7 (n=4)	1.5x10e7 (n=4)
PB engraftment	11.8%±7.6	11.9%±3.5	6.1%±6.3	9.9%±5.8
Lethal GvHD	0%	0%	0%	0%

In conclusion, we have developed a consistent and informative xenograft model of human T cell induced GvHD in NOD SCID β 2M null mice. This will allow us to study the effects of specific *ex-vivo* T cell manipulation including transduction, selection, expansion, and the depletion or addition of various T cells and other cellular subsets on GvHD.

MB is an employee of Xcyte Therapies, Inc. and has equity interest in the company.

GENE THERAPY APPROACHES TO PULMONARY DISEASE

811. ACE-Targeted eNOS and BMPR2 Gene Therapy Attenuates Pulmonary Hypertension in a Chronic Hypoxia Rat Model

Ann M. Reynolds,¹ Mark D. Holmes,¹ David T. Curiel,² Andrew H. Baker,³ Paul N. Reynolds.¹

¹Thoracic Medicine, Royal Adelaide Hospital, Adelaide, SA, Australia; ²Div Human Gene Therapy, UAB, Birmingham, AL; ³Cardiovasc & Med Sci, Uni of Glasgow, Glasgow, United Kingdom.

Idiopathic pulmonary arterial hypertension is a fatal disease characterized by vascular remodeling and right ventricular failure. Modern vasodilator therapies improve prognosis but ultimately most patients succumb or require heart-lung transplantation. We previously developed targeted adenoviral (Ad) strategies for pulmonary vascular gene delivery based on the use of a bispecific conjugate (Fab-9B9) that targets Ad infection to angiotensin converting enzyme (ACE) expressed on pulmonary vascular endothelium. In the current study we determined whether targeting achieved therapeutic gains when using Ad gene delivery of endothelial nitric oxide synthase (eNOS), then evaluated a more novel approach using targeted delivery of the gene for bone morphogenetic protein receptor type 2, based on the knowledge that mutations in this receptor and defects in BMPR2 signaling account for many of the inherited forms of IPAH. Rats (n=6) were injected with 5x10⁹ pfu of either irrelevant virus, AdCMVeNOS, AdCMVeNOS+Fab9B9 or saline (PBS), then exposed to 10% oxygen atmosphere for three weeks, then right ventricular systolic pressure (RVSP), mean pulmonary artery pressure (PAP) and right ventricular hypertrophy (Fulton Index, FI) were determined. A concurrent group of rats maintained in normoxic conditions was used as a control. Hypoxia lead to increased RVSP (42.2±4.6mmHg), PAP (34.5±2.8 mmHg) and FI (0.48±0.02) vs normoxic controls (22.3±0.8, 19.4±1.2 and 0.26±0.01 respectively). AdCMVeNOS attenuated RVSP(33.9±1.9), PAP(27±2.3) and FI(0.41±.03), with a further therapeutic gain seen with Fab-9B9 targeting (RVSP 28.0±1.8). A separate experiment using irrelevant virus+Fab-9B9, AdCMVBMPR2+Fab-9B9 or PBS showed that BMPR2 gene delivery attenuated the hypoxia-induced increase in RVSP, PAP and FI by 27%, 34% and 50% respectively. Irrelevant Ad alone or with Fab-9B9 had no effect. Thus, the novel findings are that Ad-mediated eNOS delivery attenuates chronic hypoxia-induced PHT, and this effect is improved with targeting. This is also the first demonstration that upregulation of BMPR2 signaling attenuates pulmonary hypertension, suggesting a therapeutic strategy for patients with BMPR2 mutations.