230. Novel approaches to lung transplantation

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Mesenchymal cells isolated from the airways of BOS patients as targets of innovative drug-loaded nanoparticles
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Our group has recently shown that proliferating mesenchymal cells (MC), obtained from BAL of BOS patients, express CD44 and that this expression correlates with mTOR expression and with a high proliferative rate. By these results we have designed an innovative approach based on biocompatible nanoparticles loaded with the mTOR inhibitor everolimus and functionalized with anti CD44 MoAb, for the selective targeting to the specific cells (targNP). Fluorescent labeled targNP have been used to assess cell uptake by confocal microscopy. Cell apoptosis/death (annexin V/7AAD) and proliferation (CTSEI) were evaluated by flow cytometry.

We used primary MC isolated from 2 BOS patients (grade 1 and 2) with the following phenotype: BOS 1: 85% CD90+ of which 33% co-expressing CD9; BOS2: 93% CD90+ with 38% co-expressing CD146, 25% CD9, and 38% both CD146 and CD9. Both MC samples were negative for CD45RO and CD34 and positive for CD44 (98%). TargNP were shown to adhere to membrane within 15 min and completely enter into the cells after 45 min. Drug free nanoparticles, as control, were completely inert. TargNP treated cells showed a significantly higher mean rate of annexin V at 4 h (17.4 versus 4.5%) and 24 h (44 versus 5.3%) respect to control cells while mean 7AAD expression at 24 h was 4.1 versus 1.3%. Likewise, cell proliferation was significantly inhibited at 24 and 48 h (mean: 41 and 37.2%, respectively). This is, by far, the first proof of concept that an innovative approach based on drug coated NP can be used to selectively address MC which proliferate in the airways. Further in vitro and in vivo studies will investigate possible efficacy of this new treatment strategy.

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Structural differences in airways during chronic rejection after lung transplantation: A (micro)-CT analysis
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Chronic rejection is a major problem after lung transplantation (Tx) and is accepted to be a small airway disease. Recently a distinction between an obstructive (fBOS) and restrictive phenotype (RAS) has been made. We aimed to investigate structural differences. Human explant lungs from 5 fBOS and 3 RAS patients were collected during reTx, starting at generation 5. In patients with fBOS α-smooth muscle actin (αSMA) expression and with in vitro proliferative rate. By these results we have been able to assess a feasible method the balance between IFN-gamma/IL17- producing clones and IL10-producing cells/Treg cells in the peripheral blood was observed in patients who developed BOS (p= 0.03 and 0.04 respectively) while Treg cell count decreased significantly (p= 0.002) and IL10 showed a non significant trend toward a decrease. Moreover the ratio between IL 17 and IL 10 or Treg cell count was significantly increased in BOS (0.85 vs 0.21; 10.4 vs 1.9 respectively) while IFNγ/IL10 ratio did not significantly change.

In conclusion detection of IL17/Treg ratio in the peripheral blood of LTR represents a feasible and useful tool in the identification of patients at higher risk of BOS development. By this way the role of Th17 axis in BOS pathogenesis is further confirmed.

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Mechanistic differences of chronic lung allograft dysfunction phenotypes in lung transplantation
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Purpose: The nonsmaller azithromycin, is now widely used in the treatment of Bronchiolitis Obliterans Syndrome after lung transplantation. However, only a proportion of patients respond by improving their lung function. This study aimed to evaluate differences in airway microenvironment between azithromycin responsive (52.9% improvement in FEV1,) and azithromycin resistant BOS patients.

Methods: Bronchoalveolar lavage (BAL) from recipients identified as stable n=10 (control), azithromycin responsive n=10 and azithromycin resistant n=10 were evaluated for cell differential, IL-1α, IL-1β, IL-6, IL-9, TNF-α proteins. BAL was then added to primary bronchial epithelial cells (BECs) and tested for viability by XTT assay.

Results: BAL neutrophilia (%) was increased in responders (50% p<0.0001) and non-responders (52.9% p<0.0001) compared to the control (0.8%). IL-1α, IL-1β, IL-6, IL-9, TNF-α were increased in both groups (all proteins <0.05) compared to the control. The levels of IL-1α, IL-1β and TNFα showed increasing trend in responders compared to non-responders. PBEC viability in response to BAL was reduced in non-responders (p=0.012) but not in the responders group (p=0.64). Moreover, there was a negative correlation between PBEC viability and IL-1α (p=0.042), IL-8 (p=0.0017), TNFα (p=0.039), IL-1β (p=0.045) and IL-6 (p=0.045). Three

Conclusions: Unlike in responders, where azithromycin blocks IL-17 T cell mediated neutrophilia, azithromycin resistant phenotype is associated with epidermal growth factor receptor (EGFR) expression. A unique EGFR-targeting therapy could be considered for BOS patients who develop an azithromycin resistant phenotype.

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Results of a phase 2b multi-center, randomized, double-blind, placebo-controlled study of an RNA therapeutic, ALN-RSV01, in respiratory syncytial virus (RSV)-infected lung transplant patients
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1, 2, 3, 4, 5, 6, 7, 8, 9

In conclusion: in RAS lungs, both airway>1 mm and terminal bronchiolies shrink and disappear while in fBOS lungs, the airways become obstructed. There was no difference in terminal bronchiolies between fBOS and normal lung.

IL-17- and Th17-associated cytokines have been linked to the development of acute and chronic rejection after lung transplantation in both animal models and humans. An increase in IL-17 mRNA expression and of IL-17 levels in BAL have been described in LTR with BOS and during AR episodes in comparison with Stables Recipients. On the other hand a decrease in IL-10 producing clone number and in Treg cell frequency has been described in BOS patients.

AIM of the present study was to assess with a feasible method the balance between IFN-gamma/IL17- producing clones and IL10-producing cells/Treg cells in the peripheral blood of 26 LTR (13 stable recipients, 13 BOS Patients). IFN-gamma IL17 and IL10 producing clones were assessed by ELISPOT. CD4/CD8 cells were assessed by flow-cytometry. A significant increase of IL17 and IFN-gamma producing cells in the peripheral blood was observed in patients who developed BOS (p= 0.03 and 0.04 respectively) while Treg cell count decreased significantly (p= 0.002) and IL10 showed a non significant trend toward a decrease. Moreover the ratio between IL 17 and IL 10 or Treg cell count was significantly increased in BOS (0.85 vs 0.21; 10.4 vs 1.9 respectively) while IFNγ/IL10 ratio did not significantly change.

In conclusion detection of IL17/Treg ratio in the peripheral blood of LTR represents a feasible and useful tool in the identification of patients at higher risk of BOS development. By this way the role of Th17 axis in BOS pathogenesis is further confirmed.

RSV infection after lung transplantation is an independent risk factor for the development of bronchiolitis obliterans syndrome (BOS). ALN-RSV01 is a small interfering RNA targeting the RSV nucleocapsid gene that is critical for viral replication. Previously, we performed a Phase 2 randomized, double-blind, placebo (PBO)-controlled trial in 24 RSV-infected lung transplant patients administering
aerosolized ALN-RSV01 or PBO for 3 days. The primary endpoint of safety and tolerability was attained. In addition, there was a significant reduction in the secondary endpoints of incidence of new or progressive BOS at day 90 (p=0.027) and patient’s symptom scores in the ALN-RSV01 group compared to PBO. To extend these results, we performed a Phase 2b multi-center, multinational, randomized, double-blind, PBO-controlled trial in RSV-infected lung transplant patients in which the primary endpoint was the effect of ALN-RSV01 on the incidence of new or progressive BOS at Day 180. Secondary endpoints included the impact of ALN-RSV01 on symptom scores, antiviral activity and safety. RSV positive subjects were randomized (1:1) to receive either aerosolized ALN-RSV01 or PBO for 5 days, alongside the hospital’s standard-of-care. Subject stratification to treatment arms was based on two binary factors: 1) time from symptom onset to treatment start and; 2) pre-infection BOS grade. Of the 3,985 subjects preselected at 33 centers, 218 were RSV positive, of which 87 were randomized. Enrollment is completed and subjects are now in the follow-up phase. Final study results will be presented at this meeting.

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BAL neutrophil levels in a randomised controlled trial of azithromycin therapy in bronchiolitis obliterans syndrome post lung transplantation
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Open studies have reported an improvement in FEV1 in some BOS patients treated with Azithromycin (Azith). We recently demonstrated that Azith is superior to placebo treatment, improving FEV1 in patients with BOS. It is suggested that a BAL neutrophil reduction is associated with treatment benefit. We have investigated this in our trial.
Methods: A prospective, randomised double blind placebo controlled study of Azith 250mg o.d. or placebo on alternate days, in BOS patients. The primary outcome was change in FEV1 at 12 weeks and a secondary outcome BAL neutrophil %.
Results: 46 patients were randomised (23 Azith, 23 placebo) stratified for pre op %.
Conclusion: Azith is superior to placebo improving lung function in patients with BOS. It is suggested that a BAL neutrophil reduction is associated with treatment benefit. We have investigated this in our trial.

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ENDOXY – Endothelialization of a gas permeable membrane for the development of a biohybrid lung assist device
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Methods: A prospective, randomised double blind placebo controlled study of Azith 250mg o.d. or placebo on alternate days, in BOS patients. The primary outcome was change in FEV1 at 12 weeks and a secondary outcome BAL neutrophil %.
Results: 46 patients were randomised (23 Azith, 23 placebo) stratified for pre op diagnosis of suppurative disease and operation (single or bilateral lungs). Of the 46 ITT patients, baseline and final visit BAL were available for 28 (13A, 1SP). In these patients the baseline % neutrophils, median (IQR), in the Azith group was 17 (4 to 69%) and in the placebo group 15 (2 to 56%). The baseline to final visit change in % neutrophils varied, with around half the patients in both treatment arms showing an increase from baseline and half a decrease. There was a median increase from baseline in the Azith group of 10%, IQR (~10% to 18%) and in the placebo group a median decrease from baseline of ~1% (~8% to 5%). These changes were statistically non significant (P=0.4 and 0.7 respectively, 1-sample Wilcoxon).
Conclusion: Azith is superior to placebo improving lung function in patients with BOS. In the same patients, whilst BAL neutrophil % was high, we observed no consistent fall with Azith. This may suggest the mechanism of benefit does not rely on a quantitative reduction in neutrophils.

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ENDOXY – Endothelialization of a gas permeable membrane for the development of a biohybrid lung assist device
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The interaction of stem cells with the surrounding matrix environment is crucial for cell fate. The development of biomaterials that recapitulate the in vivo environment is a key component to driving differentiation of pluripotent cells into lung endoderm precursors. We investigate whether decellularized lungs with intact matrix composition can promote the differentiation of embryonic stem cells (ESC) into distal lung epithelial cells. Rad cadericungs were decellularzed by sequential tracheal lavages and retrograde pulmonary arterial perfusion using a range of physical, chemical, and enzymatic treatments. Histological staining, immunofluorescence, electron microscopy, and tensile testing have confirmed decellularization and preservation of matrix proteins. Murine ESC (Foxa2/CD4; Bry/GFP cells) were seeded onto scaffolds following endoderm induction using activin, and analysed for lung lineage marker expression. Seeded ES cells maintained Foxa2 expression and adopted an epithelial-like tubular organization. This demonstrates the ability of acellular lung scaffolds to support the adherence, proliferation, and potential differentiation of murine embryonic stem cells. Current studies are analysing their potential as viable scaffolds for the unidirectional differentiation of human endoderm-induced ESC (Hes2 cell line) into distal lung epithelial cells.

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LSC 2012 Abstract – Lung tissue engineering: generation and characterization of decellularized lung scaffolds for stem cell differentiation
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The interaction of stem cells with the surrounding matrix environment is crucial for cell fate. The development of biomaterials that recapitulate the in vivo environment is a key component to driving differentiation of pluripotent cells into lung endoderm precursors. We investigate whether decellularized lungs with intact matrix composition can promote the differentiation of embryonic stem cells (ESC) into distal lung epithelial cells. Rad cadericungs were decellularzed by sequential tracheal lavages and retrograde pulmonary arterial perfusion using a range of physical, chemical, and enzymatic treatments. Histological staining, immunofluorescence, electron microscopy, and tensile testing have confirmed decellularization and preservation of matrix proteins. Murine ESC (Foxa2/CD4; Bry/GFP cells) were seeded onto scaffolds following endoderm induction using activin, and analysed for lung lineage marker expression. Seeded ES cells maintained Foxa2 expression and adopted an epithelial-like tubular organization. This demonstrates the ability of acellular lung scaffolds to support the adherence, proliferation, and potential differentiation of murine embryonic stem cells. Current studies are analysing their potential as viable scaffolds for the unidirectional differentiation of human endoderm-induced ESC (Hes2 cell line) into distal lung epithelial cells.