Update on cystic fibrosis and lung transplantation

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Relevant Financial Relationship Disclosure Statement

I will not discuss off label use and/or investigational use of the following drugs/devices

No relevant financial relationships exist related to this presentation.
Cystic Fibrosis: What we know

- The most common life-limiting recessive genetic disease affecting children and young adults.

- Cystic fibrosis gene (Cystic fibrosis transmembrane conductance regulator-CFTR-) isolated in 1989 and mapped to chromosome 7.

- More than 1300 different mutations of this gene identified. Delta F508 mutation the most frequent and responsible for the most severe forms.
Cystic Fibrosis: What we know

- It is responsible for most cases of elevated concentrations of chloride in sweat, exocrine pancreatic insufficiency and major cause of severe chronic lung disease in children

- Pathobiology: gene defect (mutation) changes a protein that regulates the movement of salt in and out of cells. The result is thick, sticky mucus in the respiratory, digestive and reproductive systems, as well as increased salt in sweat.
Cystic Fibrosis: What we know

Pathology: bronchiectasis, airway mucus plugging, infection, inflammation, fibrosis, vessel remodelling
Cystic Fibrosis and Lung Transplantation: What we know

- The predominant negative prognostic factor in CF is chronic progressive lung disease.
- Most commonly, patients are treated with CFTR modulator therapies and inhaled medication like mucolytics and antibiotics used to better reach the airways and fight infections.
- Lung transplantation is an established therapy for patients with end-stage CF improving survival and quality of life (estimated 5-year survival without transplantation of 33 +/- 14%, compared with a 5-year post-transplant survival of 68.2 +/- 5.6%, Zurich experience).
- Careful timing is key to achieving the best long term outcomes for any patient presenting for consideration of LTx (Thabut G et al Am J Respir Crit Care Med 2013;187:1335–40)
LUNG TRANSPLANT: The Pathologist

The timeline

PreTransp/Transplant

- Study of native lung
- Explant lungs

Post Transplant

- Monitoring of recipients
- Post-transplant complications

THE END

- Study of graft failure
  - Autopsy
  - Explant
Cystic Fibrosis and Lung Transplantation (Pre)- transplant time: study of native lung

Unique opportunity to carefully study whole lungs without the superimposed agonal complications seen in autopsy specimens.
Cystic Fibrosis: What we don’t know

• The exact role of CFTR causing structural damage: The salt controversy....: High salt hypothesis; low volume hypothesis; low secretion hypothesis

• The CFTR deficiency influencing numerous innate and acquired immune dysfunctions (altered inflammation before structural damage and infection)

• Genotype-phenotype correlations (the same mutation with different clinical manifestations, thus highlighting intervention of environmental factors and modifier genes)
Cystic fibrosis: Animal models

MOUSE

<table>
<thead>
<tr>
<th>Identifier</th>
<th>Mutation</th>
<th>Detectable CFTR mRNA</th>
<th>Salient features</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFTR&lt;sub&gt;tm1EMO&lt;/sub&gt;</td>
<td>Exon 10 replacement</td>
<td>No detectable WT CFTR mRNA</td>
<td>Severe intestinal complications</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm1CAM&lt;/sub&gt;</td>
<td>Exon 10 replacement</td>
<td>No detectable WT CFTR mRNA</td>
<td>Pancreatic ductal blockage; Severe intestinal pathology</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm1CAM&lt;/sub&gt;</td>
<td>ΔF508 Exon 10 replacement</td>
<td>Mutant mRNA 30% of WT CFTR levels</td>
<td>No pancreatic abnormalities; Longer survival than null models</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm1BAY&lt;/sub&gt;</td>
<td>Exon 3 insertion/duplication</td>
<td>&lt;2% of WT CFTR mRNA</td>
<td>Severe intestinal complications</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm1BAY&lt;/sub&gt;</td>
<td>Exon 2 replacement</td>
<td>No detectable WT CFTR mRNA</td>
<td>Severe intestinal complications</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm1EUR&lt;/sub&gt;</td>
<td>ΔF508 Exon 10 insertion (hit and run)</td>
<td>Mutant mRNA at normal WT levels</td>
<td>Nonlethal intestinal abnormalities; no pancreatic or liver abnormalities</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm1KTH&lt;/sub&gt;</td>
<td>ΔF508 Exon 10 replacement</td>
<td>Decreased mutant mRNA in intestine</td>
<td>Impaired sperm transport within the female reproductive tract; no gallbladder pathology</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm1HGU&lt;/sub&gt;</td>
<td>Exon 10 insertion</td>
<td>10% of WT CFTR mRNA</td>
<td>Mild intestinal complications; longer survival</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm2HGU&lt;/sub&gt;</td>
<td>G486C Exon 10 insertion (hit and run)</td>
<td>Mutant mRNA at normal WT levels</td>
<td>Nonlethal intestinal abnormalities</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tm1HSL&lt;/sub&gt;</td>
<td>Exon 1 replacement</td>
<td>No detectable WT CFTR mRNA</td>
<td>Severe intestinal complications</td>
</tr>
<tr>
<td>CFTR&lt;sub&gt;tmG51D&lt;/sub&gt;</td>
<td>G51D Exon 11 replacement</td>
<td>Mutant mRNA 53% of WT CFTR levels</td>
<td>Absent or mild (nonlethal) intestinal obstruction</td>
</tr>
</tbody>
</table>

No CFTR mouse model developed spontaneous lung changes limiting their usefulness in the study of CF development/progression.
## Cystic fibrosis: Animal models

### Anatomical and physiological similarities between the human and porcine lung

- Long life span to study the pathological outcomes and prognosis of CF disease
- **Limitation:** Meconium ileus present in 100% vs 15% in infants: high risk of surgery complication

### Anatomical and physiological similarities between the human and ferret lung

- CFTR pharmacologic and bioelectric functionality in ferret epithelia is similar to that of human epithelia
- Short gestation period and time to reach adolescence
- Small size
- **Limitation:** Lung infections in CF ferrets is rapid and if animals are not treated with antibiotics, they succumb to polymicrobial lung infections within the first week of life.

### Table: Comparative Analysis of Animal Models for Cystic Fibrosis Study

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous lung infection</th>
<th>Pancreatic disease</th>
<th>Intestinal disease</th>
<th>Liver and gallbladder disease</th>
<th>Reproduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Yes</td>
<td>PI</td>
<td>MI</td>
<td>Biliary cirrhosis</td>
<td>Severe vas deferens defect</td>
</tr>
<tr>
<td>Mouse*</td>
<td>No</td>
<td>No</td>
<td>Intestinal obstruction, often fatal</td>
<td>No</td>
<td>Reduced fertility in females</td>
</tr>
<tr>
<td>Pig</td>
<td>Yes</td>
<td>PI</td>
<td>100% MI</td>
<td>Biliary cirrhosis</td>
<td>Severe vas deferens defect</td>
</tr>
<tr>
<td>Ferret</td>
<td>Yes</td>
<td>PI</td>
<td>75% MI</td>
<td>Liver disease</td>
<td>Severe vas deferens defect</td>
</tr>
</tbody>
</table>

*Mouse: Includes C57BL/6 and BALB/c strains.
Micro-CT and serial histological sections showed extensive airway dilatation and obstruction in all airway generations from generation 6 onward, with an enormous reduction in the number and size of terminal bronchioles.
The findings are of high clinical relevance for the treatment of CF lung disease: The maintenance treatment of chronic CF lung infection and airway obstruction aims to improve airway clearance. These drugs as well as chest physiotherapy are unlikely to reach the peripheral airway generations where airway obstruction is most prominent. Targeting peripheral airway disease in an early stage to prevent destruction seems appropriate.
This study both pathologically and radiologically confirms that emphysema is common in end-stage CF lungs, and is age related. This finding is of particular importance: life expectancy of CF patients has dramatically increased in the last decades.
Cystic Fibrosis: lesson from explant lungs
Detected infections unknown before transplantation

**ISHLT CONSENSUS**

A consensus document for the selection of lung transplant candidates: 2014

An update from the Pulmonary Transplantation Council of the International Society for Heart and Lung Transplantation

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**Relative contraindications**

- Age >65 years in association with low physiologic reserve and/or other relative contraindications. Although there cannot be endorsement of an upper age limit as an absolute contraindication, adults >75 years old are unlikely to be candidates for lung transplantation in most cases. Although age by itself should not be considered a contraindication to transplant, increasing age generally is associated with comorbid conditions that are either absolute or relative contraindications.

- Class I obesity (BMI 30.0–34.9 kg/m²), particularly truncal (central) obesity.

- Progressive or severe malnutrition.

- Severe, symptomatic osteoporosis.

- Extensive prior chest surgery with lung resection.

- Mechanical ventilation and/or extracorporeal life support (ECLS). However, carefully selected candidates without other acute or chronic organ dysfunction may be successfully transplanted.

- Colonization or infection with highly resistant or highly virulent bacteria, fungi, and certain strains of mycobacteria (e.g., chronic extrapulmonary infection expected to worsen after transplantation).

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**Table 1. Referral**

<table>
<thead>
<tr>
<th>Referral</th>
<th>CA</th>
<th>major discrepancies, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPLDs:</td>
<td>75</td>
<td>4 (13): 2 HP; 2 DIP; 1 CA; 1 Aspergillus; 1 TB</td>
</tr>
<tr>
<td>IPF</td>
<td></td>
<td>1 UnILP + CA; 1 UnILP</td>
</tr>
<tr>
<td>Non-IPF/IPFs</td>
<td>1/2</td>
<td>(50): mycobacterial lymphadenitis</td>
</tr>
<tr>
<td>IPH</td>
<td>1/4</td>
<td>(25): bronchiectasis</td>
</tr>
<tr>
<td>LCH</td>
<td>1/8</td>
<td>(12): CA</td>
</tr>
<tr>
<td>LAM</td>
<td>1/3</td>
<td>(33): UIP/IPF</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emphysema</td>
<td>3/31</td>
<td>(10): 1 TC; 2 Aspergillus</td>
</tr>
<tr>
<td>Cystic fibrosis</td>
<td>2/23</td>
<td>(9): 1 TC; 1 Aspergillus</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td>18/175 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

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**Notes:**

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Cystic Fibrosis and *Aspergillus Fumigatus*

- Less frequent than bacterial infection but not rare in end stage CF (isolation rate varies widely from 6-to 58%)
- Different forms: ABPA: 1-25%; colonization:20-30% (significantly improved in the last decade), airway invasive form: 15%
- Many LT centres used Aspergillus prophylaxis
- Not rare false negative microbiological tests: serum (steroid treatment), culture (not sensitive medium)

Aspergillus infection unknown before transplantation in about 10%. Aspergillus both as colonization and invasive form significantly impact on graft survival
BLTX N° 70 (July 2001):
Cystic Fibrosis (F, 24 yrs)
Aspergillus infection
(unknown before transplantation)

Perioperative Aspergillus prophylaxis was switched to Aspergillus invasive treatment (in addition to immunosuppressive therapy)
Cystic Fibrosis and non tuberculous mycobacterium

- Isolated from the respiratory tract of approximately 5% to 40% of individuals with cystic fibrosis
- Responsible for more rapid decline in lung function and even death in certain circumstances
- Difficult to treat (prolonged treatment: risk of further increase of antibiotic resistance and adverse drug effects)
- Pre-transplant colonisation with M abscessus considered as a strong relative contraindication to lung transplantation (no indication for treatment)
- Severe infection (fatal) described after lung transplantation (Gilljam M 2010; Taylor JL 2006; Sanguinetti M 2001; M. Gilljam Trulock EP 1989)
- Crucial role of microbiologist and pathologist
Cystic fibrosis: F; 23 yrs (BLTX 200; August 2008)

NMT (abscessus) unknown before transplantation

6 months later: post-transplant transbronchial biopsy

Not treated with antibiotic prophylaxis “Wait and see”
Cystic Fibrosis: lesson from explant lungs
Detected tumours unknown before transplantation

- Rare in lung without underlying disease (1-2%), increased in CF/bronchiectasis.
- Pathogenesis: Hyperplasia of pulmonary neuroendocrine cells as adaptive response to hypoxia
- Older CF recipients at higher risk
- Careful investigation of bronchial lymph nodes

Carcinoid and tumorlet:
INCIDENCE IN OUR CENTER: 6%
LUNG TRANSPLANT: The Pathologist

The timeline

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Post-Transplant complications

THE END

- Study of graft failure
  - Autopsy
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CF: News from post-transplant monitoring biopsies
Higher risk of immunological disorders

Higher Risk of Acute Cellular Rejection in Lung Transplant Recipients with Cystic Fibrosis

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1 (44)*</th>
<th>Group 2 (89)**</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACR ≥A1 index (%)***, median, Q1–Q3</td>
<td>29, 12.5–50</td>
<td>25, 0–40</td>
<td>0.08</td>
</tr>
<tr>
<td>ACR ≥A2 index (%)****, median, Q1–Q3</td>
<td>22.5, 0–33</td>
<td>0, 0–25</td>
<td>0.02</td>
</tr>
<tr>
<td>ACR****** ≥1</td>
<td>75%</td>
<td>67%</td>
<td>0.37</td>
</tr>
<tr>
<td>BOS ≥1</td>
<td>26%</td>
<td>30%</td>
<td>0.60</td>
</tr>
<tr>
<td>BOS ≥2</td>
<td>15%</td>
<td>19%</td>
<td>0.65</td>
</tr>
<tr>
<td>Status at 1 year (dead)</td>
<td>9%</td>
<td>17%****</td>
<td>0.17</td>
</tr>
<tr>
<td>Status at follow-up (dead)</td>
<td>49%</td>
<td>77.5%****</td>
<td>0.0004</td>
</tr>
<tr>
<td>Post-transplant viral infection/pneumonia</td>
<td>86%</td>
<td>89%</td>
<td>0.79</td>
</tr>
</tbody>
</table>

✓ CF showed higher ACR frequency, especially for more severe forms (ACR>A2)
✓ Younger CF patients had a higher risk of rejection.
CF: News from post-transplant monitoring biopsies
Higher risk of immunological disorders

Plausible Hypothesis:

✓ Enhanced immune activation due to genetic background and young age may increase the reaction against self/non-self antigen, thus increasing the risk of rejection.

✓ Altered pharmacokinetic cyclosporine profile. Patients with CF have higher clearance, a shorter mean residence time, and more erratic absorption of the drug than those without CF.

Multivariable generalized linear models showed that higher values of ACR index were more significantly related to the pre-transplant diagnosis of CF.
CF: News from post-transplant monitoring biopsies
Higher risk of immunological disorders

- CF recipients showed a higher frequency of antibody mediated rejection-AMR- (Roux A et al 2016; Levine D et al. 2016)
- Lung AMR is increasingly recognized as a cause of pulmonary allograft failure
- Caused by donor specific antibodies -DSA -and non DSA (the last more difficult to recognize)
Case Z.G., 34 M, yrs old (BLTX N° 354 for Cystic Fibrosis; June 2015)

After LT: acute respiratory failure, severe pulmonary hypertension with right ventricle dysfunction. Unresponsive to any medical treatment

Death: 7 days after LTx

Autopsy: ALI (AMR) + foci of ACR (A2B0), no infections

Re-evaluation of early (2 days after LTX) biopsy: AMR

(Previously generically as mild edema/capillaritis consistent with ischemia-reperfusion injury):

C4d

S6 kinase (p-S6K)
High levels of anti-AT1R and ETAR antibodies measured retrospectively: revealed the presence of both types of antibodies prior to transplantation which increased on p.o. day 4 from 12.8 to 15.2 Units/ml and from 15 to 18.4 Units/ml,
CF: News from post-transplant monitoring biopsies
Higher risk of Post-Transplant Lymphoproliferative disorders

The largest study: 30,598 recipients (transplanted from 1999 to 2011) from ISHLT registry

- PTLD developed in 2% of CF recipients compared to 1% for non-CF recipients ($p < 0.001$)

- Risk factors identified in the CF population: Negative EBV serostatus and human herpes viruses 1–3, 6 and 8 in recipients, episodes of acute rejection in the first 3 months following transplantation, young patient age, and intensity of immunosuppression

- Stratified multivariable analysis controlling for age revealed EBV negative non-CF recipients have an almost 2 fold increased risk of developing PTLD, whereas EBV negative CF recipients had an almost 6.5 fold increased risk. Age is an important factor BUT increasing age is protective only in CF

- Not rare involvement of the graft (difficult to recognize)
CF: F; 20yrs (BLTX Nº 381; 26 August 2016)

11 months later:
Progressive dyspnea, fever, bilateral nodules (high suspicious of infection)
Final Diagnosis: monomorphous B monoclonal EBV-related

IHC: CD20
EBV ISH (mRNA EBER)
Update on cystic fibrosis and lung transplantation
Summary and Key Remarks for pathologists

- Careful study of explant lungs provides:
  - New pathogenetic information: appropriate sampling including fresh lung fragments for biobank are mandatory.
  - Information concerning unknown pre-transplant associated lesions: audit for physicians and at the same time incentive to improve the sensitivity of such diagnostic tests.

- Post-Transplant monitoring biopsies highlight:
  - High frequency of immunological complications: why? A great challenge for us!!
  - High frequency of PTLD: graft involvement require high expertise/experience for precise diagnosis.

My final thoughts
CF patients are now better treated and are transplanted older.
CF have now a better post-transplant outcome than other recipients.
Improving our knowledge their outcome could be even more better...

When you know better you do better
(Maya Angelou)