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The "Inverse" Translational Research: A Proposed New Concept for a Better Clinical Application

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Abstract

Pulmonary medicine is a dynamic discipline. Degenerative and malignant lung disorders have a serious impact on health services all over the world and tremendous efforts are being made for developing new diagnostic technologies and therapeutic options. In translational research, knowledge from basic science is transferred to clinical medicine. However the diagnostic and therapeutic challenges we face in "real life" daily medical practice require a more pragmatic approach. The suggested concept of "inverse" translational research is based on a process of presenting these unsolved dilemmas to the academic and bio-pharma industries scientific communities. This methodology may save research time and financial resources both in diagnostic and therapeutic procedures. Diagnostic methods in pulmonary medicine are based on clinical and physiological examinations of the disease process. Imaging by various radiologic techniques are usually a step towards the biopsy of the lesion and the final diagnostic relies on staining techniques for microscopy and observer experience. The latest technological advances are reaching now the imaging in real time of cellular morphology and functions by using the fluorescent properties of tissues when irradiated by LASER. Improved survival following oncological surgery is based on the "free margins" diagnosis at pathological examination. Real time (intra-operatory) examination of the tissues margins following resection may improve results by preventing local relapse of disease. Special fluorescent dyes administered immediately after the tumor resection and traced by special bio-photonic technologies are tested for this purpose. Therapeutic options are now switching from new drugs and medical devices development towards tissue engineering by cell engraftment in

Biological compatible scaffolds. This technology may theoretically apply in chronic diffuse disorders as emphysema or replacing "damaged" areas in localized diseases as lobar bronchiectasis or agenesis. "Lung in a chip" is a real alternative for animal models in studying new drug properties and tissue activity. Theranostics combine diagnostic and therapeutic methods for personalized or precise medicine.

Keywords: Translational research; Pulmonary; Radiologic techniques

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Abbreviations

CLM: Confocal Laser Microscopy; VOC: Volatile Organic Compounds

Introduction

Chronic lung diseases represent a major cause of disability. They are the third cause of death in the western world, surpassed only by cancer and cardiovascular diseases [1]. The disability inflicted by pulmonary diseases is the consequence of years of evolution, paucity and non-specificity of symptoms, delayed diagnosis and the lack of a significant therapy that may impact their prognosis. While excluding lung cancer, these chronic lung diseases progress for years and represent a serious burden for health and social frameworks [2]. Individuals, families and nations are affected by the lack of early diagnosis and available therapies. Medical technology advances in the recent years, mainly in radiologic and endoscopic imaging allowed a better understanding of disease evolution. Pharmaceutical industries develop new drugs that try to affect disease mechanism rather than treating symptoms. However, both diagnostic and therapeutic procedures lack definitive disease specificity and are associated with significant side effect morbidity and even mortality. Medical science and industry interaction has the purpose of providing better solutions in both diagnosis and therapy. Transferring knowledge from basic sciences to clinical medicine and improving access, organization and coordination of systems of care is defined as translational research. The Institute of Medicine's Clinical Research Roundtable described two "translational blocks" in the clinical

research enterprise labeled as T1 and T2. T1 represents the transfer of new understanding of disease mechanisms gained in the laboratory into the development of new methods for diagnosis, therapy and their first testing in humans. T2 represents the translation of results from clinical studies to every day clinical practice and heath decision making [3]. A more realistic and practical approach may be to recognize major diagnostic and therapeutic challenges in clinical practice and present them for solutions to the scientific/industrial communities. So instead of trying to apply the laboratory advances into clinical applications (from bench to bedside) it may be better to identify the bedside clinical dilemmas and try to resolve them in the lab. In this article, I shall try to describe this "inverse" translational research perspective based on the latest diagnostic and therapeutic advances.

Diagnostic advances

Following clinical information and physical examination, disease diagnosis is based on cellular morphology identification and meticulous analysis of changes in physiological pathways. In lung disorders, morphology investigation is performed mainly by imaging techniques – radiology or endoscopy. Functional investigation relies usually on pulmonary function tests. The technology of positron emission tomography (PET) is aimed to diagnose in both fields (morphology and physiology) by adding the glucose pathway changes in disease with anatomical changes by computed tomography (CT) [4]. However these *in vivo* tests fail in reaching the cellular resolution and the definitive diagnosis is made at last by the microscopic examination of *ex vivo* tissue. Novel diagnostic technologies were developed and they are able to visualize cell morphology *in vivo*. The 2014 Nobel Prize for chemistry honored the pioneers of the super resolution fluorescence microscopy. "The prize is about how the optical microscope became a nanoscope" said Staffan Normark, Permanent Secretary of the Royal Swedish Academy of Sciences in announcing the decision. Indeed tissue –light interaction is the fundamental of microscopy [5]. Tissue components have the unique feature of fluorescence when irradiated with adequate length light beams. These components are named fluorophores and they may exist in cellular structures (autofluorescence) like mitochondria and lysosomes or extracellular matrix due to proteins like collagen and elastin. Fluorophores may be also used as tracers when attached to a macromolecule (dyes, bioreactive agents and etc). The popular fluorescein derivate FITC (fluorescein isothiocyanate) and indocyanine green have been used in various medical indications for years. They induce tissue fluorescence. These properties opened the field of biophotonic microscopy and the ability of laboratory investigation of both cell morphology and function [6]. But the clinical application was still lacking. The confocal laser microscopy (CLM) is combining the advantages of the confocal microscopy over "classical" microscopy with the ability of some laser beams to provoke tissue autofluorescence or fluorescence induced by dyes. A low power laser is usually used to focus on a tissue point and the irradiated light is returning through a pinhole (confocal) to the detector. While scanning this two-dimensional imaging plane, it rejects the light outside the point and the result is a non-blurred image. CLM use in endoscopy is named confocal laser endomicroscopy [7]. Like in *ex vivo* situation, in endomicroscopy the lens has to be close to the investigated tissue and even touch it *in vivo* ("touch and see" procedure). Two types of devices are commercially available: one based on a built in scanner at the tip of an endoscope (Optiscan/ Pentax colonoscope Australia/Japan) and the other based on a probe

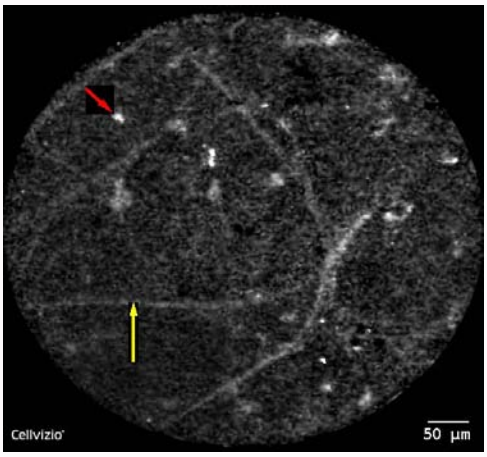

Figure 1: Probe Confocal Laser Endomicroscopy with Cell vizio Alveoscopy image of normal lung tissue: alveolar septae (yellow arrow) and cellular morphology, possible macrophage (red arrow).

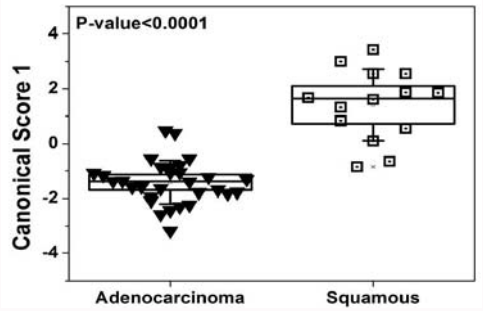

Figure 2: Graphical representation of the first canonical score values for patients with malignant nodules that differentiate between adenocarcinoma and squamous cell lung cancer that were obtained from a chemical nano array containing gold nanoparticle sensors. Each point represents one patient. The positions of the mean values are marked with □, the boxes correspond to the 95% confidence limits and the error bars correspond to the standard deviation. The confidence intervals are significantly separated (p< 0,0001) (reproduced with author permission from [10]).

made of a bundle of optic fibers able to pass the working channel of usual endoscopes (Cellvizio, MaunaKea Technologies, France) [8]. When this probe is introduced through the working channel of a flexible bronchoscope it may conduct a 488 nm laser beam to touch tracheobronchial walls or alveolar tissue. The real time moving images at the alveolar level are the first *in vivo* examination obtained by an endoscopic technology. They have an optical area of 0, 28 mm^2 at a video frame rate of 12 images per second. The probe may analyze to a depth of 50 microns, with a lateral resolution of 3 microns. Dedicated software may assist in image interpretation. Based on autofluorescence properties of lung tissues, morphologic changes may be described at the tracheobronchial wall, alveolar ducts and septa. Cells are still difficult to identify with the exception of "activated" or tar laden macrophages (Figure 1). New dyes and probes may improve the real time diagnosis [9]. The search for non-invasive methods of diagnosis is not limited to endoscopy. One of the fascinating developments in recent years approaches pulmonary disease diagnosis by examining the exhaled breath. Exhaled volatile organic compounds (VOC) analysis is promising candidates for lung cancer biomarkers. VOCs are emitted from malignant cells membrane and surrounding microenvironment to the blood stream. The blood chemistry induced changes are analyzed by exhaled breath investigation through mass spectrometry techniques or with nano-

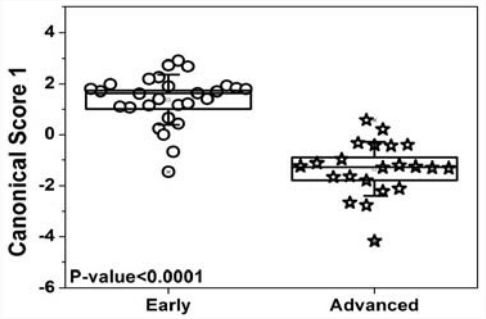

Figure 3: Graphical representation of the first canonical score values for patients with early or advanced non-small cell lung cancer nodules that were obtained from a chemical nanoarray containing seven gold nanoparticle sensors and an organically-functionalized carbon nanotube sensor. Each point represents one patient. The positions of the mean values are marked with ○, the boxes correspond to the 95% confidence limits, and the error bars correspond to the standard deviation. The confidence intervals are significantly separated (p<0,0001) (reproduced with author permission from [10]).

arrays of chemical sensors [10]. This technology made possible to discriminate between benign and malignant pulmonary nodules in a high risk cohort. It also allowed differentiation of adenocarcinoma from squamous cell carcinoma and localized from advanced disease (Figure 2,3). Gold nanoparticles and gas-chromatography - mass spectrometry analysis further differentiated between small cell and non- small cell lung cancer subtypes [11]. A volatile fingerprint assay was also developed to differentiate genetic mutations in cancer cells associated with targeted therapy in personalized oncologic therapy [12]. Hypericin loaded bioconjugated gold nanoparticles are sensitizers that may be used as optical probes for any imaging technology such as confocal laser endomicroscopy previously described, but also optical coherence tomography (OCT) or Surface Enhanced Raman Scattering (SERS) based bio-sensing [13]. The last mentionable outstanding technology refers to detection of lung cancer mutations in body fluids. In non-small cell lung cancer (NSCLC), epidermal growth factor receptor (EGFR) mutations have emerged as important biomarkers in predicting the response to the EGFR tyrosine kinase inhibitors. Tissue sampling is time consuming and technically difficult to perform (especially repeated biopsies) and requires expensive laboratory methods for analysis. The analysis of circulating tumor DNA in blood or tumor cell identification is both in validation studies. A novel core technology addresses these limitations by investigating the EGFR mutations in saliva samples of patients with NSCLC. It is named electric field–induced release and measurement (EFIRM) [14]. All these technologies need validation in large studies before deciding on their use in common clinical practice. If accepted, they may have a tremendous impact on real time diagnosis, surgical margins examination in operative procedure or targeting therapy.

Therapeutic advances

The bottom line of any diagnostic algorithm is treatment in order to improve life quality. Like in other specialties, respiratory medicine is focusing on medication or surgical procedures for this purpose. However an important fraction of lung disorders are degenerative, meaning that tissue destruction led to the absence of a biologic target for drugs. If surgical procedures are not available, then we reach a dead end that frustrates both the medical professional and the patient. Lung transplant is a "limited" solution mainly due to worldwide shortage of available donors. This somber prediction is changing lately. Tissue engineering (TE) is becoming more clinically oriented and as techniques evolve, its application is expanding. TE is addressing the fascinating field of replacing non-functioning or absent tissues or even organs with cells that are populating specific three dimensional extracellular matrix and vasculature "built" in laboratory. The fundamental of this concept is that micro and nano-scale cues in the milieu around the cells influence their growth and metabolic activities. Thus they may be the basis of tissue morphogenesis [15,16]. Two main issues have to be addressed: the development of biological scaffolds and the delivery of reparative cells to the target sites of the lung. The first reparative study addressed the airways: acellular cadaveric trachea seeded with mesenchymal stem cells was used to engineer upper respiratory tract as a therapeutic strategy [17]. Distal lung tissue replacement seems to be more complicated both in cell population selection and scaffolds properties.

One of the first biological scaffolds was developed from afibrinogen–fibronectin–vitronectin hydrogel (FFVH) [18]. *In vitro*, FFVH scaffolds promoted attachment, histiocytic growth and expression of basement membrane proteins by primary ovine lung mesenchymal cells derived from lung biopsies. *In vivo* studies were then performed on animal model (sheep) with emphysema. Treatment with autologous cells delivered using FFVH was clinically well tolerated. Cells labelled with a fluorescent dye (PKH-26) were detected at treatment sites after 1 month. Tissue mass (assessed by CT imaging) and lung perfusion (assessed by nuclear scintigraphy) were increased at emphysema test sites. Post-treatment histology demonstrated cell proliferation and increased elastin expression without scarring or Collapse. No treatment-related pathology was observed at healthy control sites. FFVH scaffolds promote cell attachment, spreading and extracellular matrix expression *in vitro* and apparent engraftment *in vivo*, with evidence of trophic effects on the surrounding tissue.

Other studies analyzed acellular scaffolds obtained by removing cells from rat lungs. The extracellular matrix retained the hierarchical branching structures of airways and vasculature. A bioreactor was used to culture pulmonary epithelium and vascular endothelium on the acellular scaffold [19]. Solid-scaffold-based tissue engineering aims on building biomimetic 3D scaffolds that are biodegradable and porous polymers from natural or Synthetic origins. These scaffolds may deliver chemical cues able to "guide" cells to their desired fate (proliferation, differentiation, migration and metabolic function) [20]. Cellular engraftment was initially based on exogenous stem or progenitor cells. However during the last decade focus switched to immunomodulation and paracrine actions of mesenchymal stem (stromal) cells MSCs and endothelial progenitor cells EPCs. Lung is a complex organ and the identity and function of endogenous epithelial stem cells or progenitor cells is controversial in both mouse and human. The low constitutive epithelial turnover rate and the various signaling pathways (like Notch or beta-catenin) are part of the difficulties met at *in vivo* identification of the best way to engraft those cells in scaffolds. One of hypothesis explaining the pathogenesis of idiopathic lung fibrosis is based on the theory of the failure by endogenous airway stem cells to repair the damaged tissue. Theoretically the use of such "repair" cells may help in healing damaged interstitial tissue or blood vessels [21]. Mesenchymal stem (stromal) cells are active in a large number of inflammatory or immune mediated lung diseases. They originate in bone marrow, adipose or placental tissue. Some clinical trials were published describing their use in moderate to severe chronic obstructive pulmonary disease COPD, analyzing a safety end

point. Other studies currently evaluate their use in acute respiratory distress syndrome ARDS and septic shock [22].

As in other fields of medicine, pharma studies in pulmonary medicine require animal models for drug evaluation as safety and dosage. These studies are expensive and require specialized personnel. One of the most amazing scientific developments relates to the ability of a biomimetic microsystem to mimic the functional alveolar-capillary interface of the human lung [23]. This "lung on a chip" systemmay respond to mechanical, toxic or inflammatory strains and analyze the epithelial and endothelial uptake of nanoparticles. These chips may replace in future cell culture models as a low cost alternative for pharma studies.

The "Theranostic" concept

In the era of personalized medicine diagnostic methods and therapeutics interfere. There is an urgent clinical need of combining imaging technologies with diagnostic biomarkers for therapy results monitoring. Screening for disease requires profiling high risk patients. Diagnosis is then performed by imaging guiding and various biomarkers analysis. Drug delivery must be monitored by bio-imaging technologies. When this complex clinical problem was presented to the scientific community, the concept of combined diagnostic and treatment procedures at the same time (theranostics) was developed [24]. Theranostics is evaluated mainly in oncology. Platforms based on sound or light have been developed. High intensity ultrasound or ultrasound guided nanoparticles have been used [25]. Magnetism based platforms combine MRI diagnosis with magnetic nanoparticles that may be carriers of chemotherapy or generate hyperthermia [26]. Finally, light based platforms were long used for cancer diagnosis and therapy. Photodynamic (PDT) photothermal (PTT) and phototriggered drug release were developed as a necessity for precise therapy. These methods allow "collateral damage" limitation as therapy is focused on cancer tissue [27].

Conclusion

Personalised or Precise Medicine requires new diagnostic approach and monitoring of therapy. The classic "translational" research is short in providing the necessary answers to complex clinical settings. A more practical approach is based on trying to find technological and laboratory answers to real life medical dilemmas. This “inverse translational” concept comprises diagnostic and therapeutic advances mainly in endomicroscopy, molecular imaging and tissue engineering. The theranostics combine both diagnosis and therapeutics in an effort of simplifying and better targeting disease process. Although most of the progress was made in pulmonary oncology other chapters of respiratory medicine may benefit from this concept.

References

1. US Department of Health & Human Services.
2. World Health Organization – Fact Sheet nr.315. 2013.
3. Sung NS, Crowley WF, Genel M. The Meaning of Translational Research and Why It Matters. *JAMA*. 2008; 299: 3140-3148.
4. Mawlawi O, Townsend DW. Multimodality Imaging: an Update on PET/CT Technology. *Eur J Nuclear Med &MolImag.* 2008; 36: 15-29.
5. Nobel prize organization.
6. Nienhaus K, Nienhaus GU. Fluorescent Proteins for the cell imaging with Super-resolution. *Chem Soc Rev*. 2014; 43: 1088-1106.
7. Jabbour JM, Saldua MA, Bixler JN, Maitland KC. Confocal Endomicroscopy 2011 Instrumentation and Medical Applications. *Annals of Biomedical Engineering*. 2012; 40: 378-397.
8. Thiberville L, Moreno-Swirc S, Vercauteren T, Peltier E, Cave C, Heckly GB. *In Vivo* Imaging of the Bronchial Wall Microstructure Using Fibered Confocal Fluorescence Microscopy. *Am J Resp Crit Care Med*. 2007; 175: 22-31.
9. Yserbit J, Dooms C, Ninane V, Decramer M. Perspectives Using Probe-based Confocal Laser Endomicroscopy of the Respiratory Tract. *Swiss Med Wkly*. 2013; 43: 13764.
10. Peled N, Hakim M, Bunnet PA, Miller YE, Kennedy TC, Mattei J, et al. Non Invasive Breath Analysis of Pulmonary Nodules. J Thorac.Oncol. 2012; 7: 1528-1533.
11. Barash O, Peled N, Tisch U, Bunn PA, Hirsch FR, Haick H. Classification of Lung Cancer Histology by Gold Nanoparticle Sensors. *Nanomedicine: NBM*. 2012; 8: 580-589.
12. Peled N, Barash O, Tisch U, Lonescu R, Broza YY, Llouze M, et al. Volatile Fingerprints of Cancer Specific Genetic Mutations. *Nanomedicine: NBM*. 2013; 9: 758-766.
13. Pierce MC, Javier DJ, Richards-Kortum R. Optical Contrast Agents and Imaging Systems for Detection and Diagnosis of Cancer. *Int J Cance.r* 2008; 123: 1979-1990.
14. Wei F, Chieng-Chung L, Joon A, Feng Z, Troche G, Lira ME, et al. Noninvasive Saliva –based EGFR Gene Mutation Detection in Patients with Lung Cancer. *Am J RespCrit Care Med*. 2014; 190: 1117-1126.
15. Chun YW, Crowder SW, Mehl SC, Wang X, Bae H, Hak-joon S. Therapeutic Application of Nanotechnology in Cardiovascular and Pulmonary Regeneration. *Computational and Structural Biotechnology Journal*. 2013; 7: 1-7.
16. Shapira A, Deok-Ho K, Dvir T. Advanced Micro and Nanofabrication Technologies for Tissue Engineering. *Biofabrication*. 2014; 6: 2.
17. Ching S, Wong GL, Clark JR. Reconstruction of the trachea. *J.Reconstr. Microsurg*. 2014; 30: 153-162.
18. Ingenito EP, Sen E, Tsai LW, Murthy S, Hoffman A. Design and Testing of Biological Scaffolds for Delivering Reparative Cells to Target Sites in the Lung. *J Tissue EngRegen Med*. 2010; 4: 259-272.
19. Petersen TH, Calle EA, Zhao L, Lee EJ, Gui L, Raredon MB, et al. Tissue Engineered Lungs for *in Vivo* Implantation. *Science*. 2010; 329: 538-541.
20. Nichols JE, Cortiella JC. Engineering of a Complex Organ: Progress toward Development of a Tissue-engineered Lung. *Proc Am Thorac Soc*. 2008; 5: 723-730.
21. Weiss DJ. Concise Review: Current Status of Stem Cells and Regenerative Medicine in Lung Biology and Diseases. *Stem Cells*. 2014; 32: 16-25.
22. Mathay MA, Thompson BT, Read EJ, McKenna DH, Liu KD, Calfee CS, et al. Therapeutic Potential of Mesenchymal Stem Cells for Severe Acute Lung Injury. *Chest*. 2010; 138: 965-972.
23. Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. Reconstituting Organ-Level Lung Functions on a Chip. *Science*. 2010; 328: 1662-1668.
24. Jean-Marc I, Louguet S, Ballet S, Corot C. Theranostics and Contrast Agents for Medical Imaging: a Pharmaceutical company view point. *Quant Imaging Med Surg*. 2013; 3: 292-297.
25. Moon H, Kang J, Sim C, Kim J, Lee H, Chang JH, et al. Multifunctional theranostic contrast agent for photoacoustics and ultrasound-based tumor diagnosis and ultrasound-stimulated local tumor therapy. *J Control Release*. 2015; 218: 63-71.
26. Xie J, Zhang Y, Yan C, Song L, Wen S, Zang F, et al. High-performance PEGylated Mn-Zn ferrite nanocrystals as a passive-targeted agent for

magnetically induced cancer theranostics. *Biomaterials*. 2014; 35: 9126-9136.

27. Amoury M, Bauerschlag D, Zeppernick F, von Felbert V, Berges N, Di Fiore S, et al. Photoimmunotheranostic agents for triple-negative breast cancer diagnosis and therapy that can be activated on demand. *Oncotarget*. 2016.