BILAGA 02

FNA-based immune profiling of tumor microenvironment

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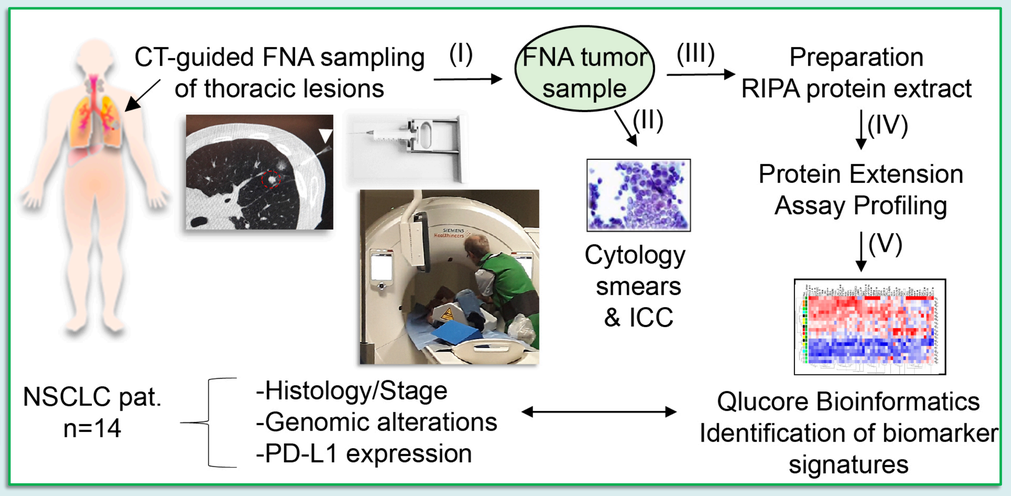
We describe development of Fine Needle Aspiration (FNA) based molecular cytology for Personalized Cancer Medicine#.

Conclusions:

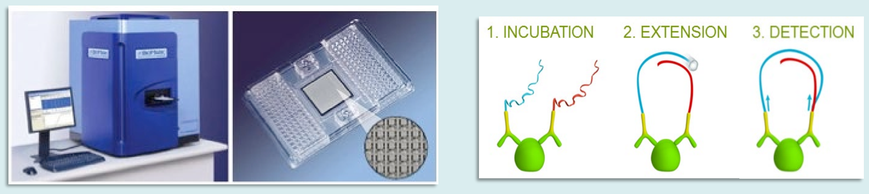
- **Repeated FNA samples for analysis can be obtained from cancers of breast, lung, thyroid, prostate, etc. via simplified routine.**
- **For precision placement of FNA needles (Ø 0.5-0.7 mm), sampling may be combined with imaging (*e.g.* CT, US, MR, fusion imaging).**
- **Only tiny amounts of FNA material are needed and can be prepared for extensive targeted protein, RNA and mutation profiling.**
- **Predictive molecular signatures can be identified.**

→This minimally traumatic methodology is suitable for longitudinal evaluation of treatment efficacy and may identify targets in the tumor microenvironment at therapy resistance.

Project outline *(e.g.* lung cancer)



Profiling of 167 proteins by PEA (Proximity Extension Assay**) with high specificity *and* high sensitivity**



Background

There are increasing demands for precise, rapid, informative, and minimally invasive biomarker based diagnostics in biopsy material for personalized cancer therapies. Guidance of treatment with *e.g.* immune checkpoint inhibitors (ICI) by biomarker signatures using such procedures would be important already at the diagnostic step.

- We present the combination of minimally invasive FNA sampling and ultra-sensitive techniques for targeted analysis of molecular biomarkers in minute materials from breast and lung tumors.
- We have shown that this methodology; (1) is highly sensitive and reproducible, (2) correlates with results from routine analysis, and (3) permits extensive immune- and tumor protein profiling with assessment of putative biomarkers of important for ICI treatment selection.

Aim

To improve FNA based molecular diagnostics for prediction and evaluation of precision cancer medicine treatments.

Materials & methods

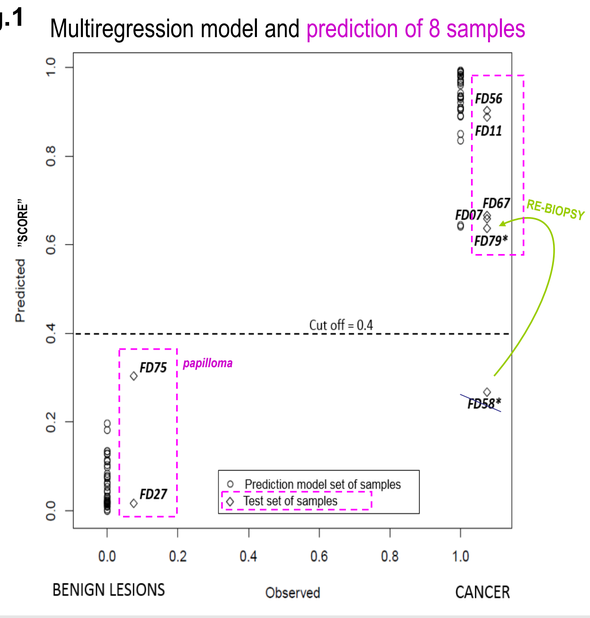
Breast tumors: FNA samples from 25 patients with cancer and 33 patients with benign lesions. Cancer subtypes: Luminal A (n=9), Luminal B (n=4), Luminal B/HER2+ (n=5), HER2+ (n=3), and Triple negative (TNB, n=4). Lung cancer: Transthoracic FNA samples from 14 patients with non-small-cell lung cancer (NSCLC).

Left-over FNA sample materials were lysed (RIPA buffer) and used for protein analysis by Proximity Extension Assay (PEA, Olink Proteomics, Oncology II® and Immuno-Oncology® panels, for technical information, see www.olink.com). Data was analysed using *e.g.* Qlucore Omics Explorer (qlucore.com).

Results

- Breast cancer (BC); benchmarking of key markers (*e.g.* HER2/ERBB2) showed good correlation to established routine analyzes [1].
- Multiple regression modelling of PEA-data produced a tentative signature for 100% discrimination between breast cancer and benign lesions [1] (**Fig. 1**).

Fig.1 Multiregression model and prediction of 8 samples



Key proteins in the signature "cancer vs benign"

Relative *increase* in model:
FUR: Furin, e.g. cell motility,
HO-1: Heme Oxygenase, e.g. hypoxia
GPNMB: GlycoProtein Non-Metastatic B, e.g. metastasis,
CXCL9: T-cell chemoattractant chemokine

Relative *decrease* in model:
FGF-BP1: FGF-binding protein 1, e.g. cell proliferation
DCN: Decorin, e.g. vascular/tissue remodelling

- Several immune related proteins in BC samples showed significant correlation to ER expression and prolifieration (Ki67) (**Fig. 2A**).
- Examples of immune proteins associated to immune therapy and immune microenvironment are shown in **Fig. 2B** in relation to BC subtypes [2].

Fig.2A

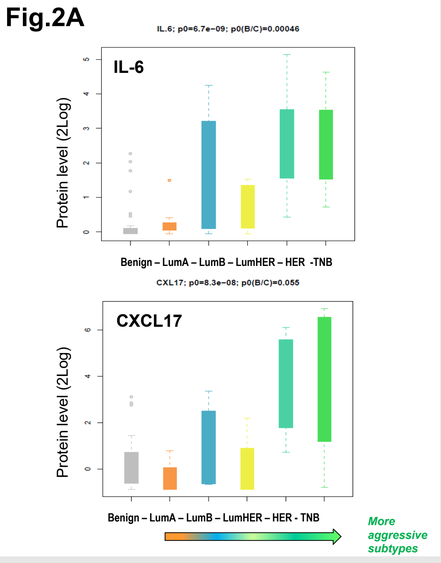
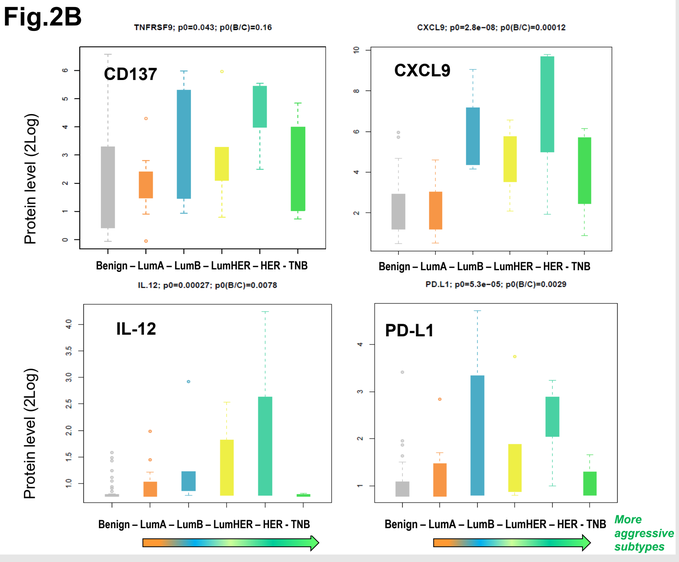


Fig.2B



- Lung cancer (LC): Consecutive FNA samples från one tumor (#20) showed similar cytology and PD-L1 levels (**Fig. 3A**).
- PD-L1 was detected by PEA in all LC samples [3] (**Fig. 3B**).

Fig. 3A CT-guided FNA-sampling of NSCLC

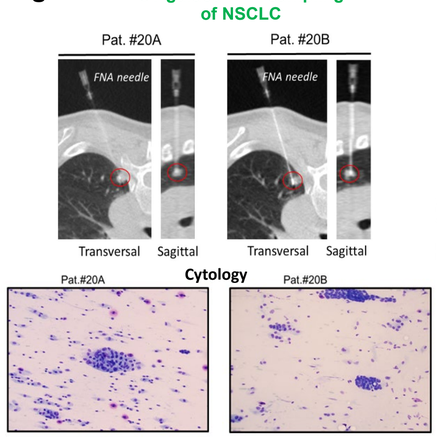
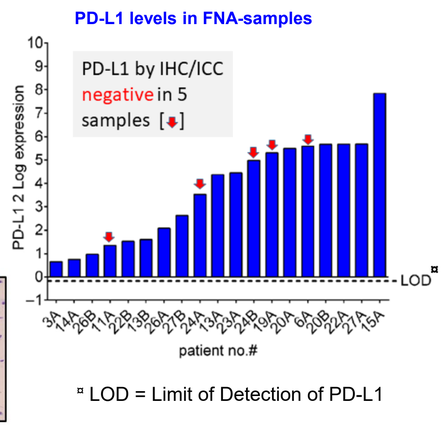


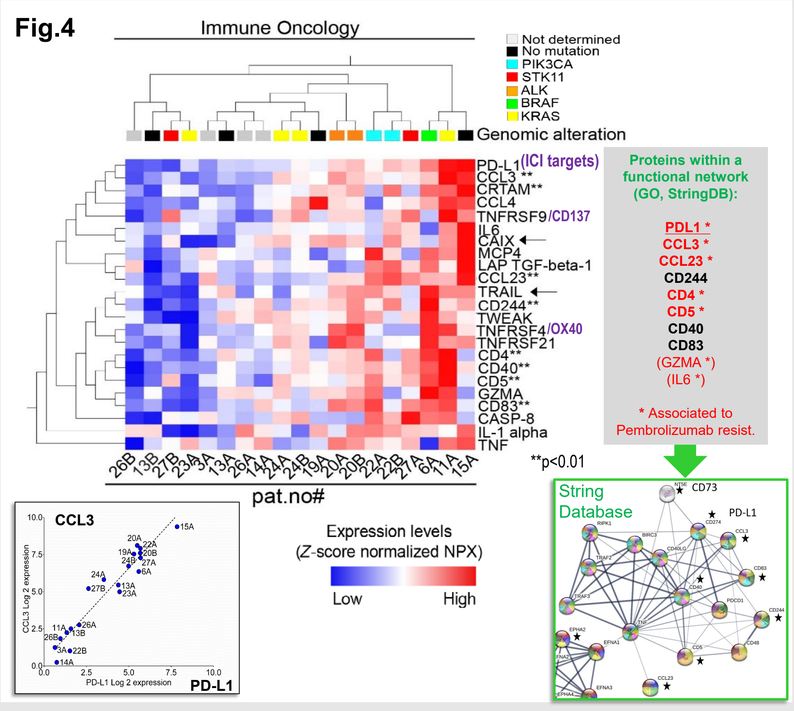
Fig. 3B PD-L1 levels in FNA-samples



¤ LOD = Limit of Detection of PD-L1

- PD-L1 associated protein signatures related to immune and tumor signaling were indentified in LC samples. Several of these proteins has been linked to immune therapy resistance by others (**Fig.4**).
- Proteins associated to tumor stage in LC were identified (not shown).
- Functional network analysis (String Database) showed associations between several proteins within the correlation analysis [3].

Fig.4



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§ ***All results described in three publications:***

[1] A fine-needle aspiration-based protein signature discriminates benign from malignant breast lesions.
Franzén B, Kamali-Moghaddam M, Alexeyenko A, Hatschek T, Becker S, Wik L, Kierkegaard J, Eriksson A, Muppani NR, Auer G, Landegren U, Lewensohn R. *Mol Oncol 2018 09;12(9):1415-1428*

[2] Protein profiling of fine-needle aspirates reveals subtype-associated immune signatures and involvement of chemokines in breast cancer.
Franzén B, Alexeyenko A, Kamali-Moghaddam M, Hatschek T, Kanter L, Ramqvist T, Kierkegaard J, Masucci G, Auer G, Landegren U, Lewensohn R. *Mol Oncol 2019 02;13(2):376-391*

[3] Multiplex immune protein profiling of fine-needle aspirates from patients with non-small-cell lung cancer reveals signatures associated with PD-L1 expression and tumor stage.
Franzén B, Viktorsson K, Kamali C, Darai-Ramqvist E, Grozman V, Arapi V, Hååg P, Kaminskyy VO, Hydbring P, Kanter L, Nyrén S, Ekman S, De Petris L, Lewensohn R. *Mol Oncol. 2021 Nov;15(11):2941-2957.*

Project 3: https://ki.se/en/onkpat/research-team-lewensohnviktorssonlindberg

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