Characterization of tumour-infiltrating lymphocytes from non-small cell lung cancer

Zuzanna Urban-Wójciuk[1]*, Elżbieta Chruściel[1]*, Mateusz Gliwiński[2], Jacek Kowalski[1,3], Tomasz Marjański[4], Witold Rzyman[4], Ted Hupp[1,5], Rafał Dziadziuszko[6], Wojciech Biernat[3], Natalia Marek-Trzonkowska[1,7]§

[1] International Centre for Cancer Vaccine Science (ICCVS), University of Gdańsk, Gdańsk, Poland; [2] Department of Medical Immunology, Medical University of Gdańsk, Gdańsk, Poland; [3] Department of Pathomorphology, Medical University of Gdańsk, Gdańsk, Poland; [4] Department of Thoracic Surgery, Medical University of Gdańsk, Gdańsk, Poland; [5] Cancer Research UK Edinburgh Centre, MRC Institute of Genetics & Molecular Medicine, The University of Edinburgh, Edinburgh, UK; [6] Department of Oncology and Radiotherapy, Medical University of Gdańsk, Gdańsk, Poland; [7] Laboratory of Immunoregulation and Cellular Therapies, Department of Family Medicine, Medical University of Gdańsk, Gdańsk, Poland; * These authors contributed equally to the study; § Corresponding author: natalia.marek-trzonkowska@ug.edu.pl

INTRODUCTION

Lung cancer is a disease of unmet clinical needs

Lung cancer is the most prevalent cancer worldwide (over 2 million new cases estimated in 2018) and the one with the highest mortality, according to the World Health Organization. Non-small-cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases and its main cause is cigarette smoking [1]. Despite all the recent advancements in treatment only 18% of patients with lung cancer survive 5 years after diagnosis [2]. It was shown that high level of tumour-infiltrating lymphocytes (TILs) in NSCLC correlates with improved disease-free survival and decreased risk of recurrence [3].

RESEARCH QUESTIONS

- **What is the composition of immune cell infiltrate in NSCLC (T2-T3 and N0-N1)?**
- **Which T cell subset has the highest anti-tumour potential in patients with NSCLC?**
- **What is the best source for T cell isolation if cellular therapy of NSCLC is planned?**

AIM

Selection of the most potent T cell subsets that can be used for T cell-based therapy of non-small cell lung cancer

WORKFLOW

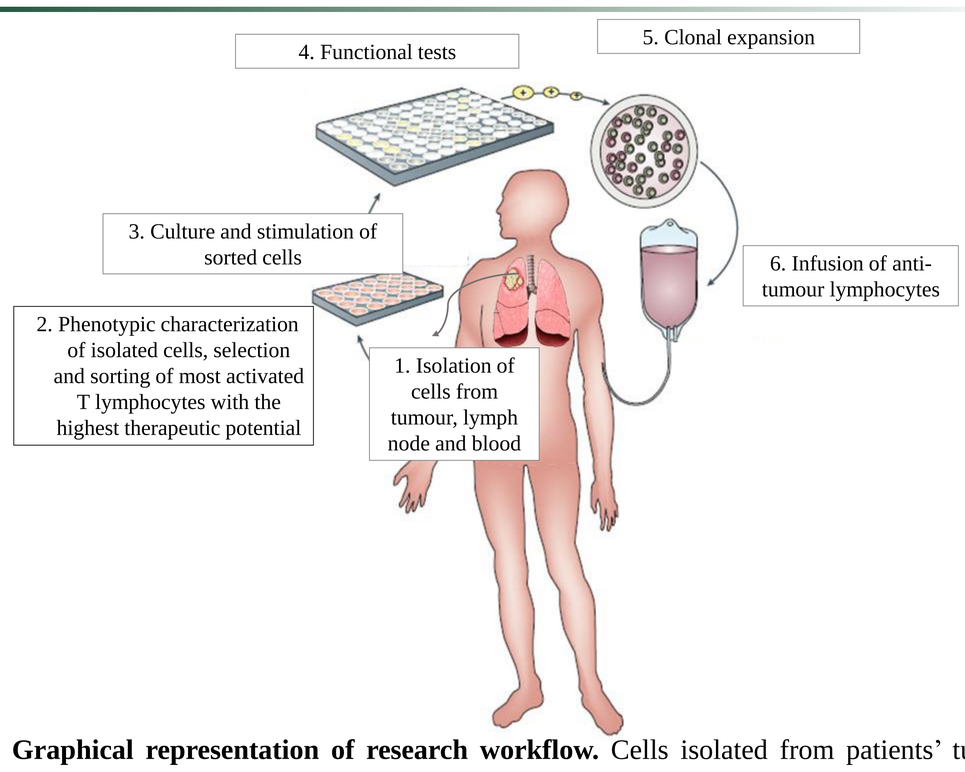

Figure 1. Graphical representation of research workflow. Cells isolated from patients' tumour, lymph node and peripheral blood are characterized. The most activated lymphocyte populations are selected, sorted, expanded and tested in functional tests as potentially useful for the future T cell based therapy.

RESULTS

Histological characterization of NSCLC patient

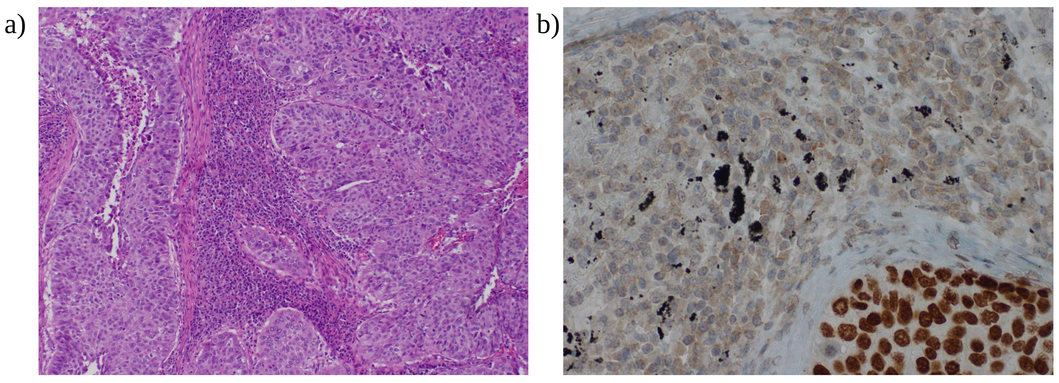

Figure 2. Staining of formalin-fixed parafin-embedded (FFPE) NSCLC sample. a) H&E staining - phenotype of squamous-cell carcinoma; immune cell infiltrate in the stroma; b) IHC nuclear staining for p40 (marker of squamous-cell carcinoma); note black anthracotic pigment within macrophages.

Culture of cells isolated from NSCLC patient

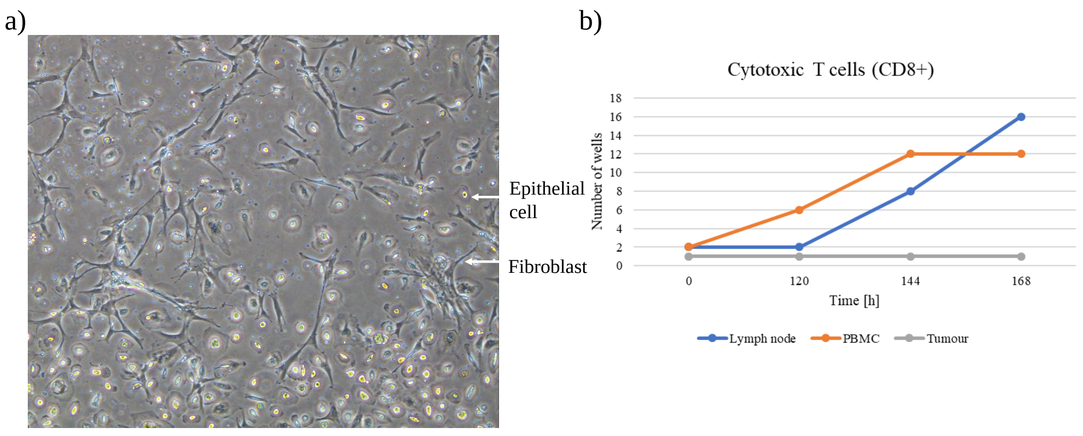

Figure 3. Cells isolated from NSCLC patients grow well *in vitro*. a) tumour cells were isolated with the use of collagenase-based method, after removal of CD45+ cells the remaining cells were seeded into a plate in DMEM low glucose medium with human AB serum; at least two phenotypically different cell types can be observed in the culture (fibroblast-like and epithelial-like cells); b) CD45+ cells from the tumour, alongside those extracted from peripheral blood and lymph node, were sorted into CD4+ and CD8+ subsets and cultured. An example of proliferation dynamics of CD8+ T cells isolated from different sources is depicted.

Characterization of lymphocytes from NSCLC patient

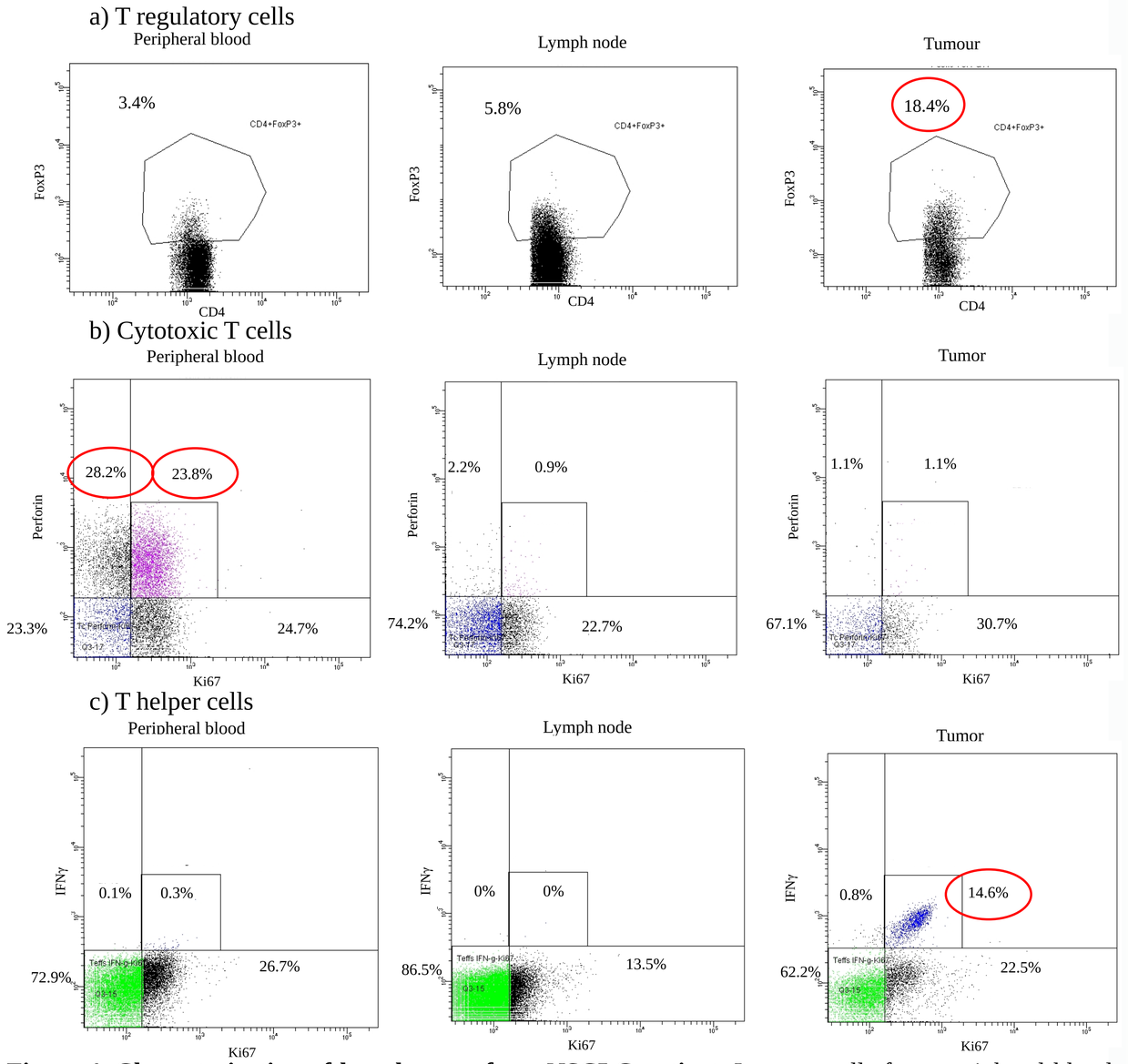

Figure 4. Characterization of lymphocytes from NSCLC patient. Immune cells from peripheral blood, local lymph node and tumour were stained and analysed with flow cytometry to assess number and activation status of a) regulatory T cells (% of CD4+FoxP3+ cells within CD4+ T cell population is shown); b) cytotoxic T cells (% of perforin and Ki67 expressing cells within CD8+ T cell population is depicted) and c). helper T cells (% of IFNγ and Ki67 positive cells within CD4+ T cell population is shown).

CONCLUSIONS

- Immune cell infiltrate of NSCLC is characterized by increased numbers of immunosuppressive regulatory cells (Tregs), anergic cytotoxic T cells and activated helper T cells.
- Proliferation rate of T cells isolated from patients with NSCLC strongly depends on their source (tumour, lymph node, peripheral blood).
- Activated tumour-derived CD4+ T cells and activated peripheral blood-derived CD8+ T cells are the most promising subsets for T cell-based therapy of non-small cell lung cancer.

Literature

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Corresponding author

natalia.marek-trzonkowska@ug.edu.pl