



Abstract

# TITAN Project: microfluidic and sensing tools for immunotherapy †

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**Abstract:** Titan project aims to improve immunotherapy, targeting the efficiency of methods to obtain genetically engineered T cells. Immunotherapy has achieved great success in clinical trials, but it is currently very expensive in terms of time required for analysis, reagents and samples. TITAN aims to the continuous sampling of critical quality attributes, in order to quickly recognize deviations from the desired range and take appropriated corrective actions of process parameters, for an optimal outcome. To achieve its aims, TITAN is currently developing microfluidic and sensing tools for the accurate and efficient real-time monitoring of the T-cells amplification process.

**Keywords:** CAR-T cell immunotherapy; on-chip quality control; microfluidic and sensing platform.

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## 1. Introduction

Immunotherapy with genetically engineered T cells has achieved some spectacular success in clinical trials and has obtained marketing approval. A key need is the widespread availability of small-scale bioreactors providing in-process monitoring. TITAN aims to the continuous sampling of critical quality attributes, to quickly recognize deviations from the desired range and take appropriated corrective actions[1]. Parameters to be verified and related tools include: bacterial contamination[2]; counting of cells by microfluidic and electrical detection; ratio live/dead cells to be identified by gold nanoparticles on a capacitive sensor; cytokines production identified by electrochemical methods; T-cell function tests through the production of spheroids into droplet microfluidic devices and the electrochemical detection of metal nanoparticles (NPs) through anodic stripping voltammetry.

## 2. Materials and Methods

For each of the developed strategies for sensing and microfluidic, different technologies have been addressed having the common purpose of using a volume of sample as low as possible, since it would originate from patient-derived tissues. In particular, we developed the following topics:

2.1 Bacterial contamination check

2.2 Automatic cell counting

2.3 Functional assays

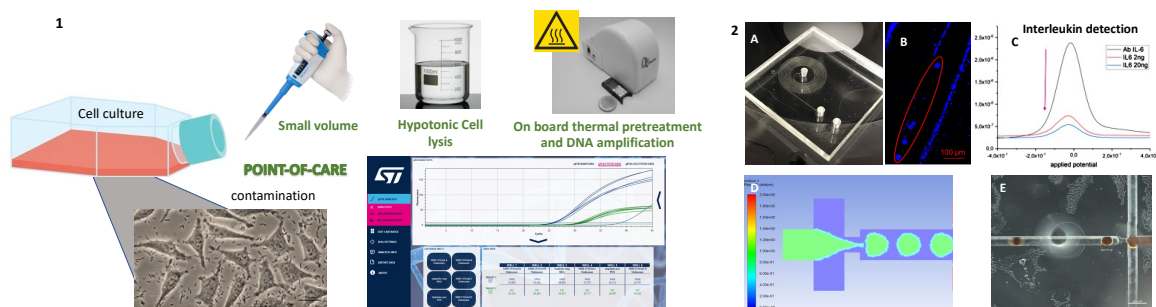
### 3. Results and Discussion

#### 3.1. qPCR for contamination check on a POC platform

An innovative protocol for qPCR working with a very low volume of raw sample (0,25 µl) and avoiding the step nucleic acid extraction was optimized for a Point-Of-Care (POC) instrument. The optimized extraction-free protocol in minimal volume analysis combined with the possibility to perform the qPCR assay on a portable device, demonstrates the possibility to avoid the time-consuming bacterial cell culturing. The achieved amplification of the Gram+ *S. aureus* was performed in a 5 µl total volume of reaction, reaching the limit of detection of 1 CFU/ml.

#### 3.2. CAR-T cells efficiency tests

Lymphocytes spilled out from bioreactor will be focused and counted through a simulated and optimized spiral channel (Fig. 2A-B) which will help in the single cell identification. Detection of Interleukins IL6 and IL10 has been performed down to 2 ng/ml by immobilizing antibodies on the surface of microfabricated gold electrodes (Fig. 2C), but this limit can be improved by nanostructuring the surface of electrodes. Interleukins and cytokine detection will be integrated into functional assays to evaluate the efficiency of CAR-T cells in killing tumor cells. To improve reproducibility and standardization of the assays, a microfluidic method to produce spheroids of the same dimensions has been improved, based on computational simulation and fabrication of a droplet generator working with 2-phase emulsion of matrigel containing cells and oil (Fig.2D-E). To complete the functional test for CAR-T, tumor cells were loaded with gold NPs and their release after action of activated lymphocytes will be detected through anodic stripping voltammetry.



**Figure 1.** Sketch of the qPCR assay procedure. **Figure 2 (right) A-B.** Spiral channel for cell focusing. **C.** Interleukin detection by DPV. **D-E.** Simulated and realized device for droplets generation.

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**Conflicts of Interest:** Declare conflicts of interest or state “The authors declare no conflict of interest.”

### References

1. Ferrara F., Zoupanou S., Primiceri E., Ali Z., Chiriaco M.S., Beyond liquid biopsy: Toward non-invasive assays for distanced cancer diagnostics in pandemics, *Biosensors and Bioelectronics*, Volume 196, 2022, 113698, ISSN 0956-5663, <https://doi.org/10.1016/j.bios.2021.113698>.
2. Garzarelli, V., Chiriaco, M.S., Cereda, M., Gigli, G., Ferrara, F., Ultrasensitive qPCR Platform for Rapid Detection of Bacterial Contamination of Raw Biological Samples at the Point of Care. Available at SSRN: <https://ssrn.com/abstract=4350274> or <http://dx.doi.org/10.2139/ssrn.4350274>