*proceedings*

*Abstract*

# TITAN Project: Microfluidic and Sensing Tools for Immunotherapy †

**Maria Serena Chiriacò [1,*], Elisabetta Primiceri [1], Antonio Turco [1], Valeria Garzarelli [1,2], Giulia Siciliano [1], Alessia Foscarini [1], Ahmed Alsadig [1], Annunziata Carbonara [3], Benedetta Stampone [2,4], Gianluca Trotta [4], Marco Cereda [5], Marco de Tullio [3], Giuseppe Gigli [1,2] and Francesco Ferrara [1,*]**

1 CNR NANOTEC—Institute of Nanotechnology, Via per Monteroni, 73100 Lecce, Italy; elisabetta.primiceri@nanotec.cnr.it (E.P.); antonio.turco@nanotec.cnr.it (A.T.); valeria.garzarelli@nanotec.cnr.it (V.G.); giulia.siciliano@nanotec.cnr.it (G.S.); alessia.foscarini@nanotec.cnr.it (A.F.); ahmed.alsadig@nanotec.cnr.it (A.A.); giuseppe.gigli@unisalento.it (G.G.)
2 Department of Mathematics & Physics E. de Giorgi, University of Salento, Via Arnesano, 73100 Lecce, Italy; benedetta.stampone@stiima.cnr.it
3 Department of Mechanics, Mathematics and Management and Centre of Excellence for Computational Mechanics (CEMeC), Politecnico di Bari, 70125 Bari, Italy; a.carbonara5@studenti.poliba.it (A.C.); marcodonato.detullio@poliba.it (M.d.T.)
4 CNR STIIMA—Institute of Intelligent Industrial Systems & Technologies, Via P Lembo 38-F, 70124 Bari, Italy; gianluca.trotta@stiima.cnr.it
5 STMicroelectronics S.r.l., Via Olivetti 2, 20864 Agrate Brianza, Italy; marco.cereda@st.com
* Correspondence: mariaserena.chiriaco@nanotec.cnr.it (M.S.C.); francesco.ferrara@nanotec.cnr.it (F.F.)
† Presented at the XXXV EUROSENSORS Conference, Lecce, Italy, 10–13 September 2023.

**Abstract:** The 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 appropriate corrective actions on 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

check for updates

**Citation:** Chiriacò, M.S.; Primiceri, E.; Turco, A.; Garzarelli, V.; Siciliano, G.; Foscarini, A.; Alsadig, A.; Carbonara, A.; Stampone, B.; Trotta, G.; et al. TITAN Project: Microfluidic and Sensing Tools for Immunotherapy. *Proceedings* **2024**, *97*, 214. https://doi.org/10.3390/proceedings2024097214

Academic Editors: Pietro Siciliano and Luca Francioso

Published: 10 May 2024



## 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 achieve continuous sampling of critical quality attributes, to quickly recognize deviations from the desired range and take appropriate corrective actions [1]. Parameters to be verified and related tools include bacterial contamination [2]; counting of cells by microfluidic and electrical detection; ratio of 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 microfluidics, different technologies have been addressed, having the common purpose of using as low a volume of

sample as possible, since it would originate from patient-derived tissues. In particular, we developed the following topics:

1. *Bacterial contamination check.*
2. *Automatic cell counting.*
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 of nucleic acid extraction was optimized for a point-of-care (POC) instrument (scheme depicted in Figure 1). The optimized extraction-free protocol in minimal analysis volume, 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+ *Staphylococcus aureus* was performed in a 5 µL total volume of reaction, reaching the limit of detection of 1 CFU/mL.

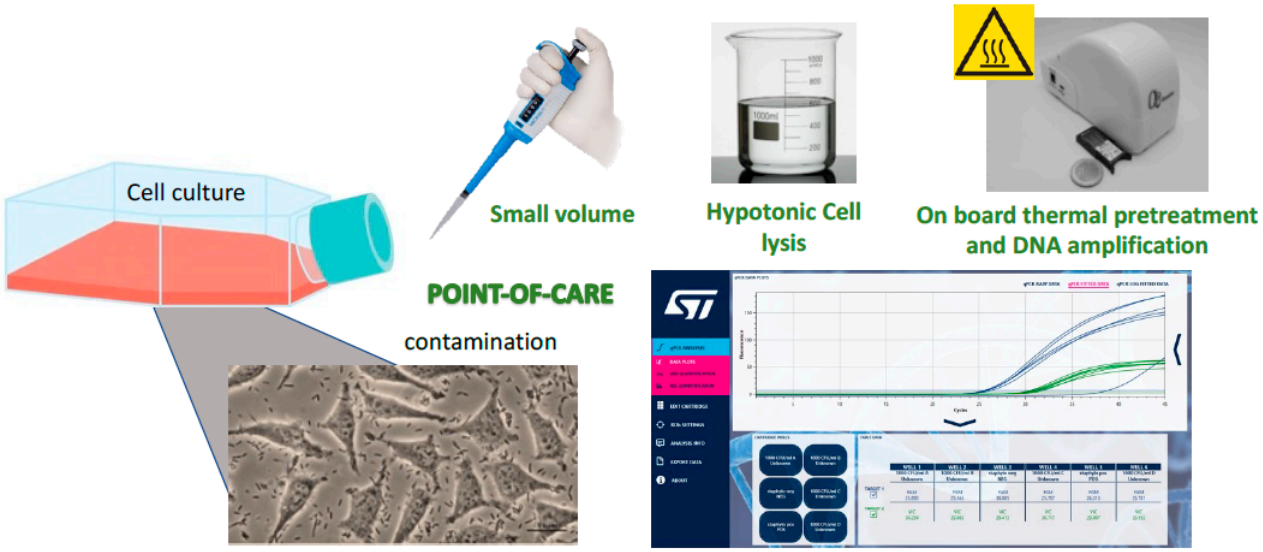


**Figure 1.** Sketch of the qPCR assay procedure.

### *3.2. CAR-T Cells Efficiency Tests*

Lymphocytes spilled out from bioreactor will be focused and counted through a simulated and optimized spiral channel (Figure 2A,B), which will help in the single cell identification. Detection of Interleukins IL6 and IL10 is performed down to 2 ng/mL by immobilizing antibodies on the surface of microfabricated gold electrodes (Figure 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 was improved, based on computational simulation and fabrication of a droplet generator working with 2-phase emulsion of Matrigel-containing cells and oil (Figure 2D,E). To complete the functional test for CAR-T, tumor cells are loaded with gold NPs and their release after action of activated lymphocytes is detected through anodic stripping voltammetry.

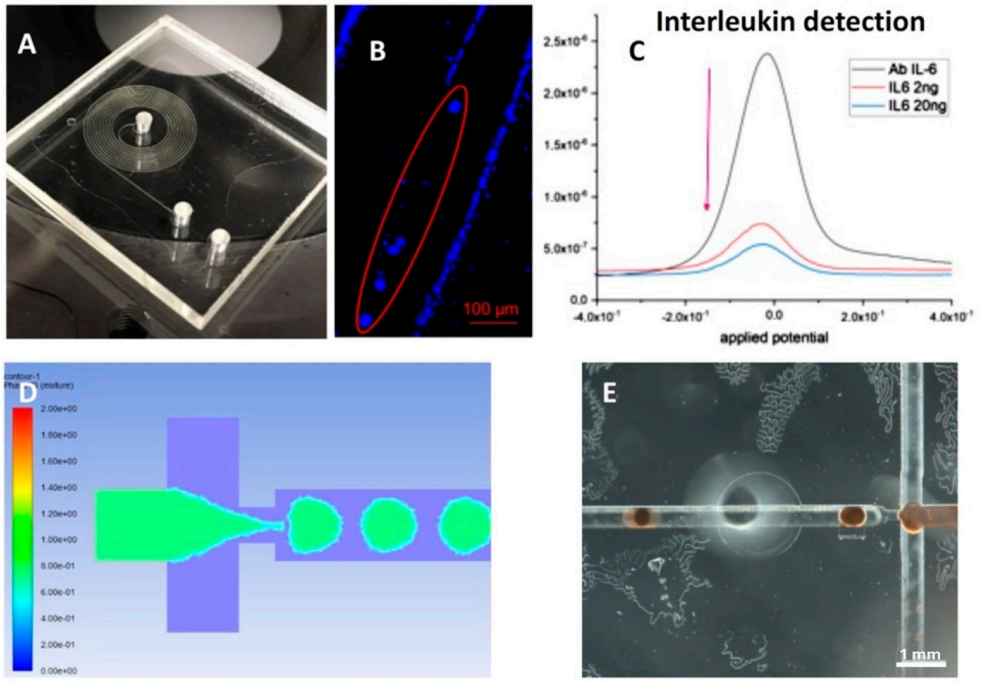


**Figure 2.** (**A**,**B**) Spiral channel for cell focusing. Red cycle highlight the position of cells in the channel (**C**) Interleukin detection by DPV. (**D**) Simulated device for droplets generation with emulsion of oil (green) in water (blue); (**E**) plastic device realized for droplet generation (red) in water.

**Author Contributions:** Conceptualization, F.F., M.S.C., E.P. and G.G.; methodology F.F., M.d.T., G.T., A.T. and M.C.; investigation: A.F., A.A., G.S., V.G., B.S. and A.C.; resources, F.F., M.S.C. and G.G.; data curation, E.P., M.C. and G.T.; writing—original draft preparation: M.S.C., F.F., A.F. and V.G.; writing—review and editing: F.F., M.d.T. and G.G.; supervision: F.F., M.S.C. and E.P.; project administration, F.F., G.T., E.P., M.d.T. and G.G.; funding acquisition, F.F., M.S.C. and G.G. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by the following funding program: Progetto PON ARS01_00906 "TITAN Nanotecnologie per l'immunoterapia dei tumori", funded within FESR Programme PON "Ricerca e Innovazione" 2014-2020 Azione II-OS 1.b).

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors and STMicroelectronics S.r.l. declare no conflict of interest.

## References

1. Ferrara, F.; Zoupanou, S.; Primiceri, E.; Ali, Z.; Chiriacò, M.S. Beyond liquid biopsy: Toward non-invasive assays for distanced cancer diagnostics in pandemics. *Biosens. Bioelectron.* **2022**, *196*, 113698. [CrossRef]
2. Garzarelli, V.; Chiriacò, 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. *Heliyon* **2023**, *9*, e16229. [CrossRef] [PubMed]