



# PhD in CHIMICA INDUSTRIALE E INGEGNERIA

## CHIMICA / INDUSTRIAL CHEMISTRY AND CHEMICAL ENGINEERING - 39th cycle

**PNRR 117 Research Field: NON-VIRAL MRNA VECTORS FOR CAR-T PRODUCTION IN GLIOBLASTOMA MODELS**

**Monthly net income of PhDscholarship (max 36 months)**

**€ 1400.0**

In case of a change of the welfare rates during the three-year period, the amount could be modified.

### Context of the research activity

**Motivation and objectives of the research in this field**

The tremendous success of CAR-T cells (CAR=Chimeric Antigens Receptor) in the treatment of B-cell malignancies has revolutionized the field of cancer immunotherapy. However, CAR-T cells have not been yet effective in treating solid tumors. CAR-T cells for adoptive transfer have been developed also for glioblastoma (GBM), the most aggressive and lethal primary brain tumor and have been evaluated in preclinical studies with encouraging results. Although these encouraging results, developing engineered CAR-Ts at clinical levels by viral-based gene transfer is costly and logistically difficult since it requires GMP facilities designed to manufacture GMP-grade viral vectors and transduced CAR-T cells. Although transient expression of CAR through RNA modification of T cells may result in reduced anti-tumor efficacy due to limited presence of CAR-Ts, this application remains a valid option in brain tumors since these cells can be locally delivered to avoid cell loss or dilution when these cells are administered intravenously. In addition, intratumoral administration of these cells can easily be achieved in these patients through the insertion of specific devices during the surgical removal of the tumor. We propose the use of T-cells, in which B7-H3 CAR expression will be obtained by non-viral transfection agents, demonstrating the efficacy of mRNA-B7-H3 CAR-Ts in a preclinical model of GBM. The aim of this project is



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|  | <p>to design and investigate two different mRNA chemical nanovectors (NVs):</p> <ol style="list-style-type: none"> <li>1. Biocompatible NVs encapsulating mRNA either encoding B7-H3 CAR or green fluorescent protein (GFP) to be used for in-vitro T-cell transfection.</li> <li>2. Biocompatible NVs coated with CD137, to specifically target tumor reactive infiltrating T-cells (trTILs). These NVs will also be injected in-vivo. Once the NVs will be implanted intratumorally, they will transfer the transgenes into the TILs expressing CD137 and transiently program the cells to express B7-H3 CAR.</li> </ol>  |
| <p><b>Methods and techniques that will be developed and used to carry out the research</b></p> | <p>This research project will include the preparation and physico-chemical characterization of lipid-based NVs for mRNA by different techniques (at Polimi) and their ability to transfect the genetic material in GBM models to produce CAR-T cells (at FINCB). The size distribution and morphology of the NVs will be tested by dynamic light scattering (DLS) and small angle X-ray scattering (SAXS), while mRNA encapsulation efficiency will be determined by fluorescent assays (<i>i.e.</i>, RiboGreen RNA assays) and agarose gel. Their colloidal stability will also be tested in cell culture media. Peripheral blood mononuclear cells (T-cells) will be transfected with mRNA loaded NVs. mRNAs either encoding B7-H3 CAR or GFP will be used for <i>in-vitro</i> T-cell transfection. The persistence of the expression of CAR on the surface of the NV-transfected T-cells will be tested. Their functional activity will be evaluated both <i>in-vitro</i> against GBM primary cell lines and <i>in-vivo</i> in xenograft models. We will test different lipid mixtures and the best candidate will be selected in terms of structural properties, mRNA encapsulation efficiency, <i>in-vitro</i> and <i>in-vivo</i> biological function. The optimized NVs formulation will also be modified by coating the NV surface with CD137 (CD137-NV), to specifically target tumor reactive infiltrating T-cells (trTILs). CD137-NVs will be modified with fluorinated lipids to obtain traceable NVs through Fluorine-19 Magnetic Resonance Imaging (<math>^{19}\text{F}</math>-MRI). The obtained <math>^{19}\text{F}</math>-CD137-NVs will be characterized in terms of size, colloidal stability, and biological function, and then they will be injected <i>in-vivo</i>. Once the NVs will be implanted intracranially using the same tumor</p> |



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|  | coordinates, they will transfer the transgenes into the TILs expressing CD137 and transiently program the cells to express B7-H3 CARs.   |
| <b>Educational objectives</b>            | <ul style="list-style-type: none"> <li>• Learn how to design and develop non-viral gene delivery vectors</li> <li>• Learn how to assemble and characterize nano-scale materials</li> <li>• Learn cell culture practices</li> <li>• Learn how to design a non-viral CAR construct</li> <li>• Learn how to test the CAR-T potency using preclinical <i>in-vitro</i> and <i>in-vivo</i> models</li> <li>• Learn to consider how to transfer research activities into clinical practice</li> </ul> |
| <b>Job opportunities</b>                 | <ul style="list-style-type: none"> <li>• R&amp;D positions in biotech or pharmaceutical companies</li> <li>• Biomaterial scientist in chemical and biomedical companies</li> <li>• R&amp;D positions in Drug and Gene Delivery Technology companies</li> <li>• R&amp;D position in biotech company focused on research, development, manufacturing, and clinical validation of innovative therapies</li> <li>• Post-doc position in a translational laboratory</li> </ul>                      |
| <b>Composition of the research group</b> | 2 Full Professors<br>3 Associated Professors<br>5 Assistant Professors<br>6 PhD Students   |
| <b>Name of the research directors</b>    | Proff. Baldelli Bombelli, Metrangolo   |

| <b>Contacts</b>  |
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| Additional support - Financial aid per PhD student per year (gross amount) |    |
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| Housing - Foreign Students   | -- |
| Housing - Out-of-town residents (more than 80Km out of Milano)             | -- |

| Scholarship Increase for a period abroad |         |
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| Amount monthly                           | 700.0 € |
| By number of months                      | 6       |

| National Operational Program for Research and Innovation   |   |
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| Company where the candidate will attend the stage (name and brief description)                       | Fondazione Istituto Neurologico Carlo Besta (FINCB) - IRCCS Via Giovanni Celoria, 11, 20133 Milano MI <a href="https://www.istituto-besta.it/">https://www.istituto-besta.it/</a>                               |
| By number of months at the company   | 6   |
| Institution or company where the candidate will spend the period abroad (name and brief description) | University of Copenhagen Department of Pharmacy, Universitetsparken 2 København <a href="https://pharmacy.ku.dk/research/vaccine-design-delivery/">https://pharmacy.ku.dk/research/vaccine-design-delivery/</a> |
| By number of months abroad   | 6   |

**Additional information: educational activity, teaching assistantship, computer availability, desk availability, any other information**

**Confidentiality (in case of DM 117 – Agreement with company):** since this is a thematic scholarship, the management of Confidential Information, Results and their publication is subordinate to the restrictions agreed upon with the funding company. Upon acceptance of the scholarship, the beneficiary must sign a specific commitment.

**Individual budget for research** (5.700 euro): 1<sup>st</sup> year: 1.900 euro; 2<sup>nd</sup> year: 1.900 euro; 3<sup>rd</sup> year: 1.900 euro

**Teaching assistantship (availability of funding in recognition of supporting teaching activities by the PhD student):** there are various forms of financial for activities of support to the teaching practice. The PhD student is encouraged to take part in these activities within the limits allowed by the regulation.