

# Intratumoural Delivery of mRNA Loaded on a Cationic Hyper-Branched Cyclodextrin-Based Polymer Induced an Anti-Tumour Immunological Response in Melanoma

Yousef Khazaei Monfared <sup>1,†</sup>, Mohammad Mahmoudian <sup>2</sup>, Parvin Zakeri-Milani <sup>2,\*</sup>, Claudio Cecone <sup>1</sup>, Tomoya Hayashi <sup>3</sup>, Ken J. Ishii <sup>3</sup>, João Conde <sup>4</sup>, Adrián Matencio <sup>1</sup> and Francesco Trotta <sup>1,\*</sup>

<sup>1</sup> Department of Chemistry, University of Turin, 10125 Turin, Italy;  
yousef.khazaeimonfared@unito.it (Y.K.M.); claudio.cecone@unito.it (C.C.);  
adrian.matencioduran@unito.it (A.M.)

<sup>2</sup> Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz 5165665931, Iran;  
mahmoodian.nano@gmail.com

<sup>3</sup> Division of Vaccine Science, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo (IMSUT), Tokyo 113-8654, Japan;  
tomoya-h@ims.u-tokyo.ac.jp (T.H.); kenishii@ims.u-tokyo.ac.jp (K.J.I.)

<sup>4</sup> ToxOmics, NOVA Medical School (NMS), Faculdade de Ciências Médicas (FCM),  
Universidade Nova de Lisboa, 1099-085 Lisboa, Portugal; joao.conde@nms.unl.pt

\* Correspondence: pzakeri@tbzmed.ac.ir (P.Z.-M.); francesco.trotta@unito.it (F.T.)

† These authors contributed equally to this work

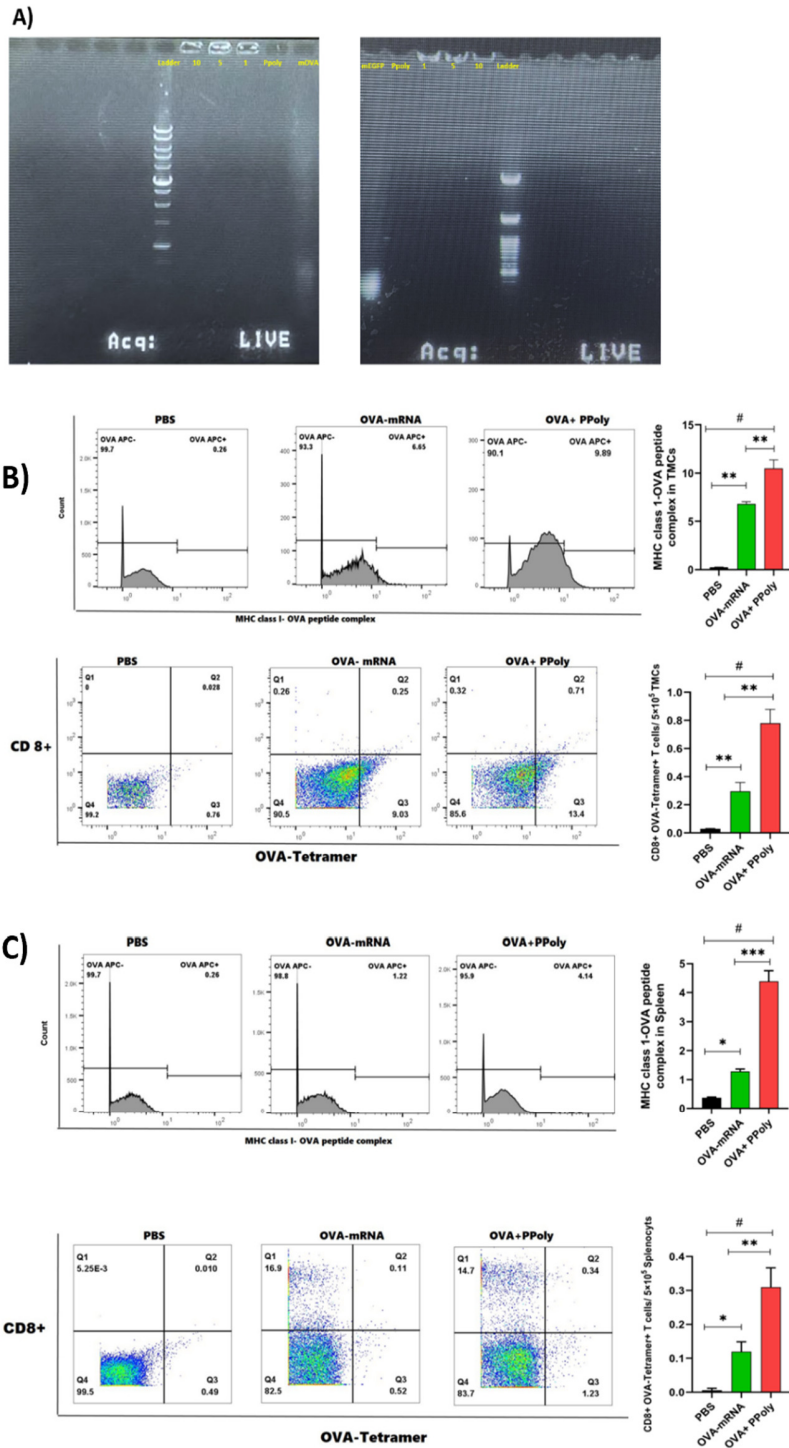


Figure S1. **A**, Gel retardation assay for OVA, left, and EGFP-mRNA, right. **B**, Quantification of H2-Kb SIINFEKL expression and SIINFEKL+ CD8+ T cells antigen specific T cell responses in tumor tissues, and **C**, Spleen tissues following treatment with formulations. (\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , #  $p < 0.0001$ ).

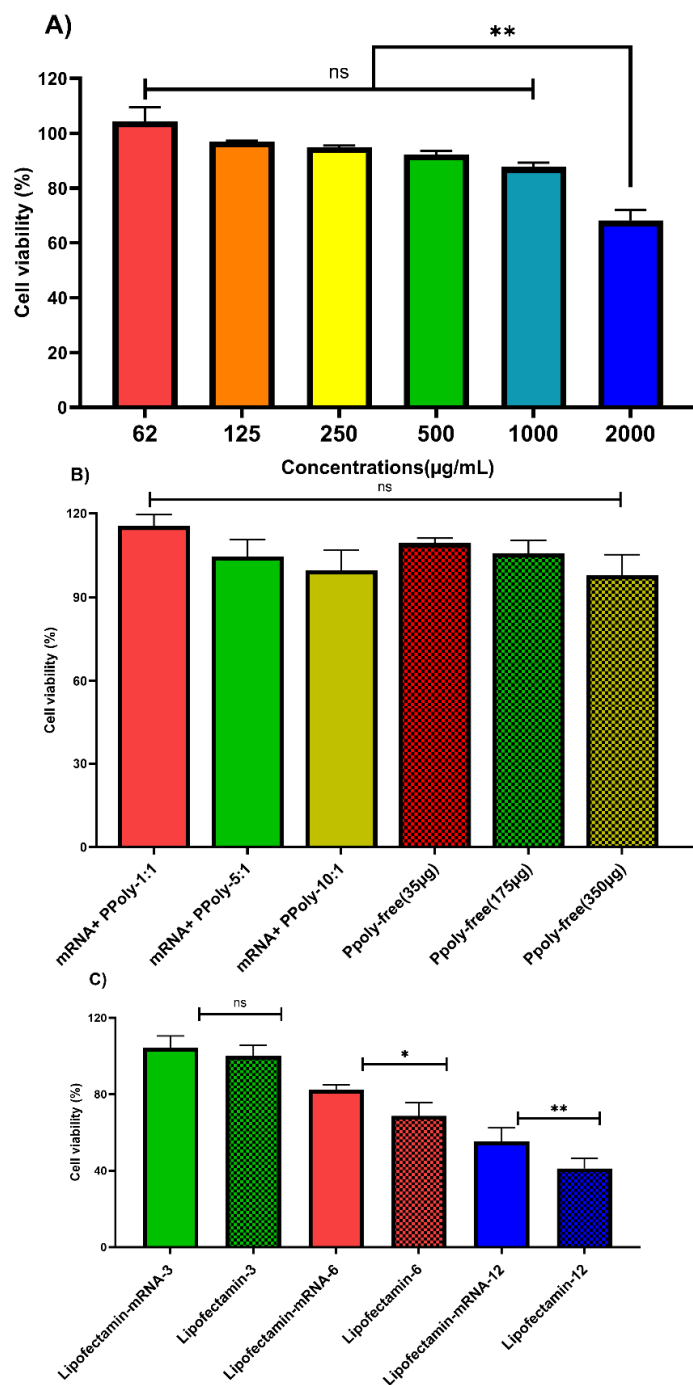


Figure S2. **A**, Cytotoxicity results for different concentrations of Ppoly. **B**, Different ratios (1:1, 1:5 and 1:10) of Ppoly free and complexes with mRNA. **C**, Different volumes of Lipofectamine (μL) free and complexes with mRNA against melanoma cancer cell lines. (ns, Not Significant; \*  $p < 0.05$ , \*\*  $p < 0.01$ )