Antineoplastic effects of liposomal short interfering RNA treatment targeting *BLIMP1/PRDM1* in primary effusion lymphoma

Giovanni Riva,[1] Ivana Lagreca,[1]* Adriana Mattiolo,[2] Daniela Belletti,[3] Laura Lignitto,[2] Patrizia Barozzi,[1] Barbara Ruozi,[3] Daniela Vallerini,[1] Chiara Quadrelli,[1] Giorgia Corradini,[1] Fabio Forghieri,[1] Roberto Marasca,[1] Franco Narni,[1] Giovanni Tosi,[3] Flavio Forni,[3] Maria Angela Vandelli,[3] Alberto Amadori,[2,4] Luigi Chieco-Bianchi,[4] Leonardo Potenza,[1°] Maria Luisa Calabrò,[2°] and Mario Luppi[1°]*

**Contributed equally to this manuscript.*
°Contributed equally to this manuscript.

[1]*Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia; Hematology Unit, AOU Policlinico, Modena;* [2]*Immunology and Molecular Oncology, Veneto Institute of Oncology, IOV-IRCCS, Padova;* [3]*Department of Life Sciences, University of Modena and Reggio Emilia, Modena; and* [4]*Department of Surgery, Oncology and Gastroenterology, Oncology and Immunology Section, University of Padova, Italy.*

Funding: this work was supported by grants from the Ministero della Salute (Ricerca Finalizzata, GR-2010-2313609, to LP and ML), the Ministero dell'Istruzione, Università e della Ricerca (MIUR, PRIN2009, to ML), the Associazione Italiana per la Ricerca sul Cancro (AIRC, IG 14797-2013, to ML), AIRC and Fondazione Cariverona (grant n. 6599, to MLC), and the Associazione Italiana Lotta alle Leucemie, Linfoma e Mieloma (AIL) - Sezione 'Luciano Pavarotti' Modena-ONLUS (to LP and FForghieri). AM and LL were recipients of a fellowship from the Veneto Institute of Oncology.

Correspondence: mario.luppi@unimore.it
doi:10.3324/haematol.2015.126854

Supplementary Information

Sequences of siRNAs against BLIMP1/PRDM1.

In this work, we used the following set of 3 validated anti-BLIMP1/PRDM1 siRNAs (Stealth RNAi™ siRNA, Invitrogen, Life Technologies, Carlsbad, CA, USA), in equal amounts:

5'-GCGACGAAGCCAUGAAUCUCAUUAA-3' (ref. PRDM1-HSS101017),

5'-GGCCUUUCAAAUGUCAGACUUGCAA-3' (ref. PRDM1-HSS101018),

5'-CAGAACGGGAUGAACAUCUACUUCU-3' (ref. PRDM1-HSS184611).

Figure S1

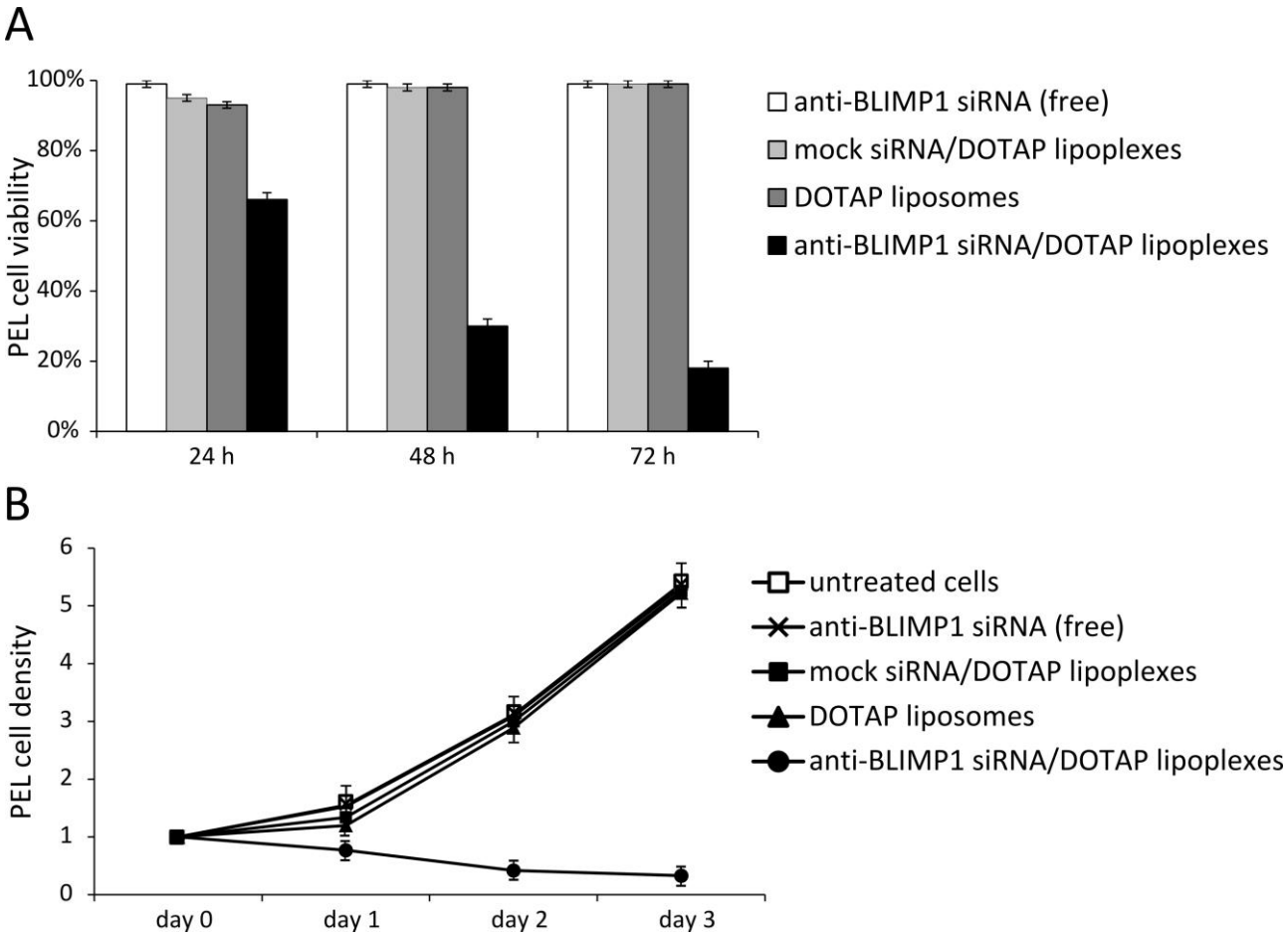

Figure S1. Antineoplastic effect of anti-BLIMP1 siRNA/DOTAP lipoplexes in HBL-6 cell line. (**A**) Proportions of viable PEL cells and (**B**) modifications in cellularity over time after treatment with anti-BLIMP1 siRNA/DOTAP lipoplexes, compared with different controls (i.e. untreated cells and cells treated with empty DOTAP liposomes, mock siRNA/DOTAP lipoplexes, or not-vehiculated (free) anti-BLIMP1 siRNAs). Results were obtained as described in Figure 1A and 1B.

Figure S2

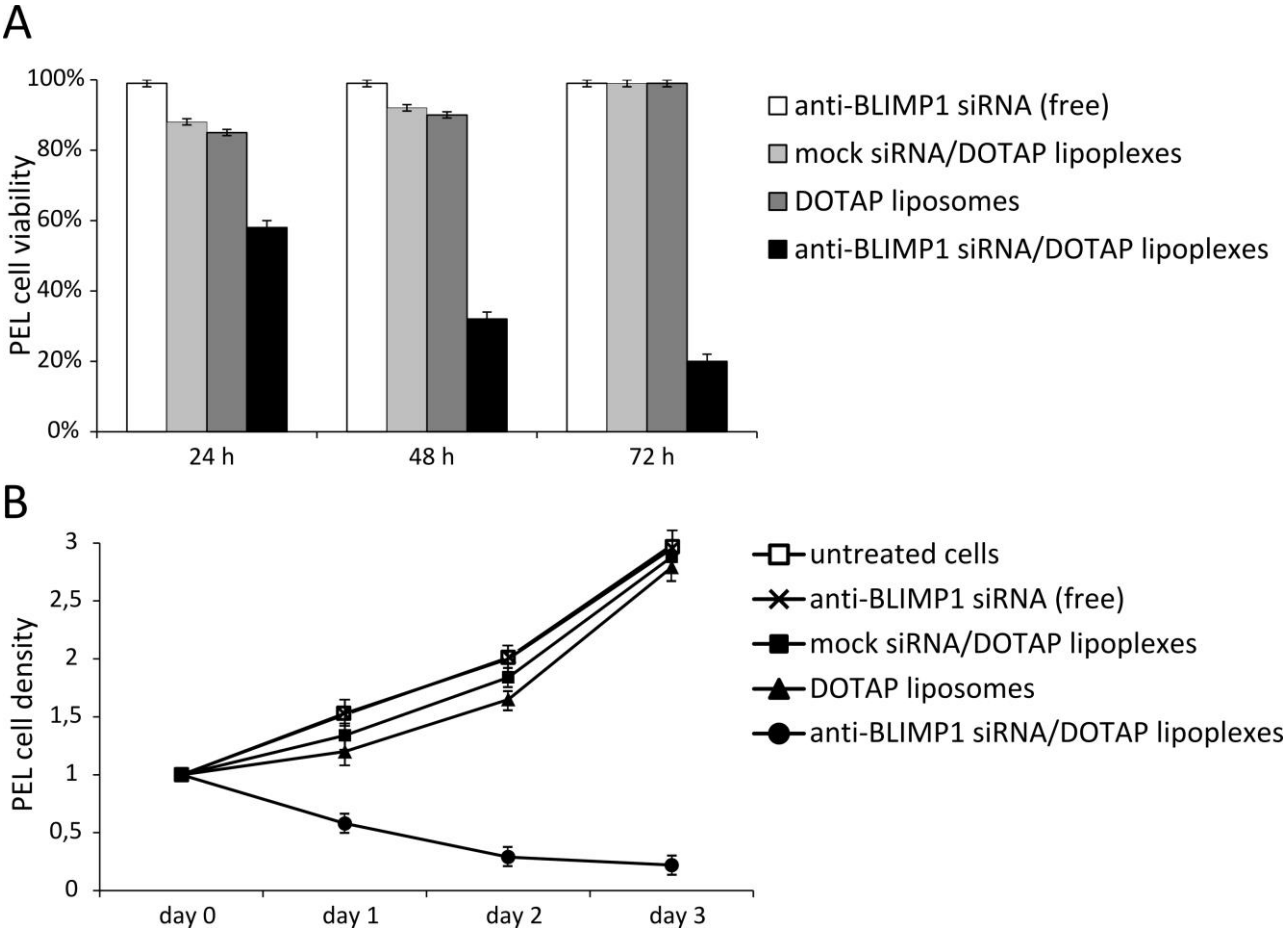

Figure S2. Antineoplastic effect of anti-BLIMP1 siRNA/DOTAP lipoplexes in CRO-AP/3 cell line. (**A**) Proportions of viable PEL cells and (**B**) modifications in cellularity over time after treatment with anti-BLIMP1 siRNA/DOTAP lipoplexes, compared with different controls (i.e. untreated cells and cells treated with empty DOTAP liposomes, mock siRNA/DOTAP lipoplexes, or not-vehiculated (free) anti-BLIMP1 siRNAs). Results were obtained as described in Figure 1A and 1B.

Figure S3

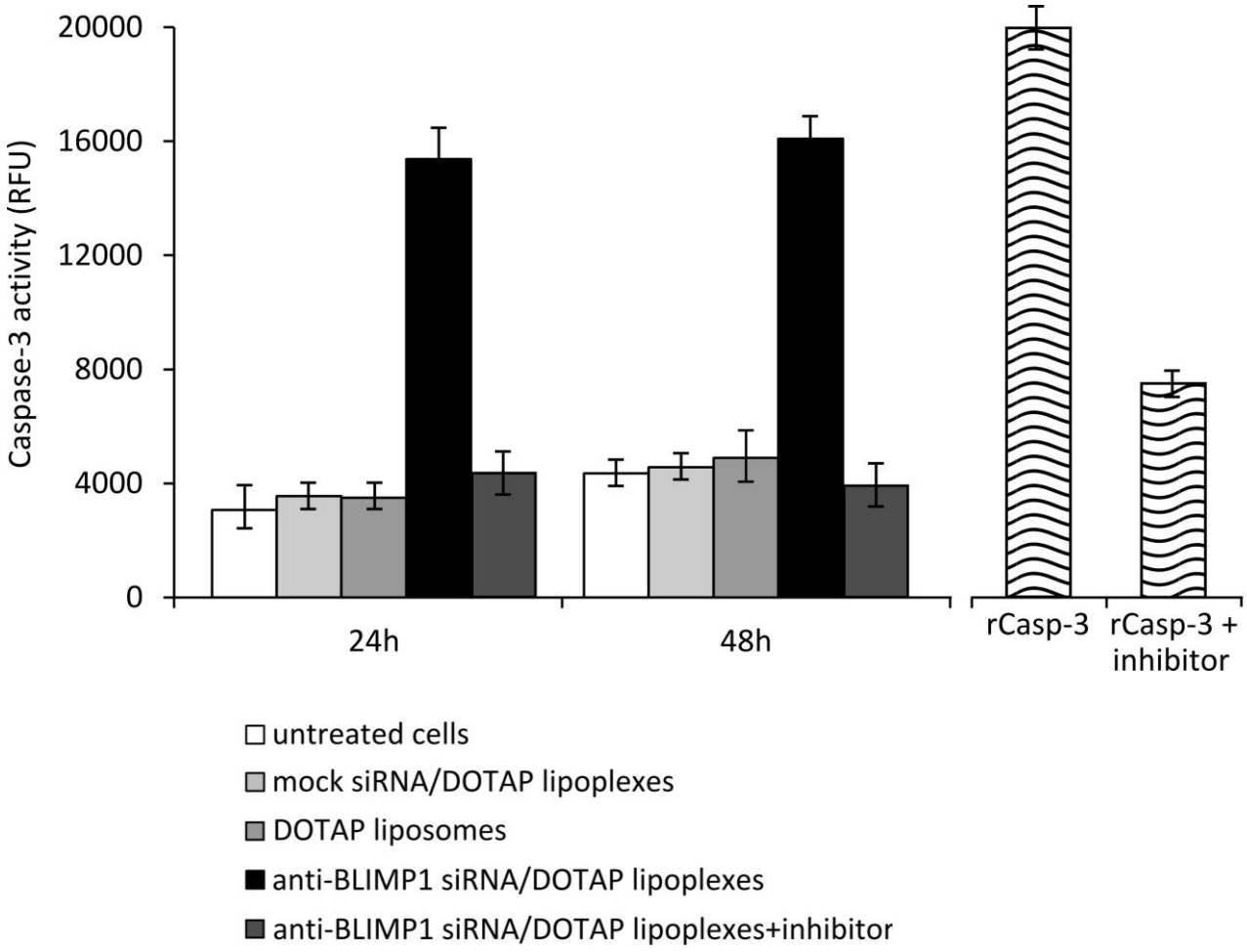

Figure S3. Caspase-3 activation. Specific activity of Caspase-3 expressed in relative fluorescence units (RFUs) was determined in BCBL-1 cells at 24 and 48 hours, after the treatments indicated above. On the right, recombinant Caspase-3 (rCasp-3; provided with Calbiochem assay kit) was also reported as positive control. In addition, a Caspase-3 inhibitor (DEVD-CHO; provided with the kit) was used as further control, both in cells treated with anti-BLIMP1 siRNA/DOTAP lipoplexes (at 24 and 48 hours) and rCasp-3. Data represent mean values of three independent experiments, performed in triplicate wells for each condition. Error bars represent standard error of the mean.