The 7th International Electronic Conference on Medicinal Chemistry (ECMC 2021)
01–30 NOVEMBER 2021 | ONLINE

Exploring a Peptide Nucleic Acid-based Antisense Approach for CD5 Targeting in Chronic Lymphocytic Leukemia

Francesca Greco [1,*], Andrea Falanga[1],Giorgia Oliviero[2],*Rosa Catapano[2], Simona Romano[2], Nicola Borbone[3], Arianna Pastore[2], Maria Marzano[4], Gennaro Piccialli[1], Paola Costanzo[2] and Michela Grosso[2]*

[1] Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, Napoli, 80131, Italy, ;

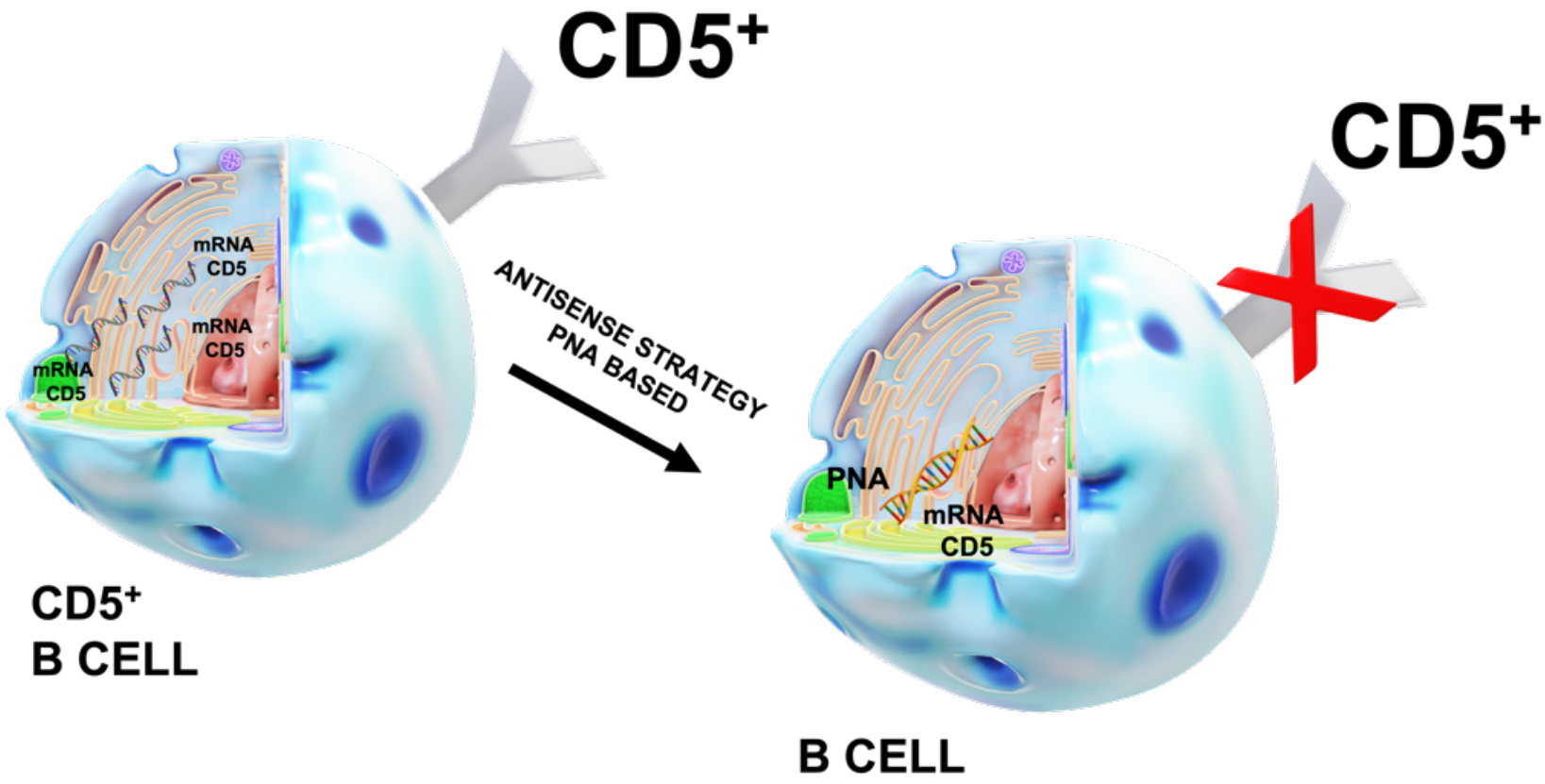
[2] Department of Molecular Medicine and Medical Biotechnology, University of Naples Federico II, Via Sergio Pansini 5, Napoli, 80131, Italy

[3] ISBE-IT, University of Naples Federico II, Corso Umberto I, Napoli, Italy

[4] Institute of Crystallography, National Council Research of Italy, Bari, 70126, Italy

- Corresponding author: *francesca.greco@unina.it*

Exploring a Peptide Nucleic Acid-based Antisense Approach for CD5 Targeting in Chronic Lymphocytic Leukemia



Abstract:

Chronic Lymphocytic Leukemia (CLL) is characterized by the overexpression of the transmembrane protein CD5 in B cells. To assess the downregulation of the protein and modulate the CLL aggressiveness we focused on developing an antisense approach Peptide Nucleic Acid (PNA) based.

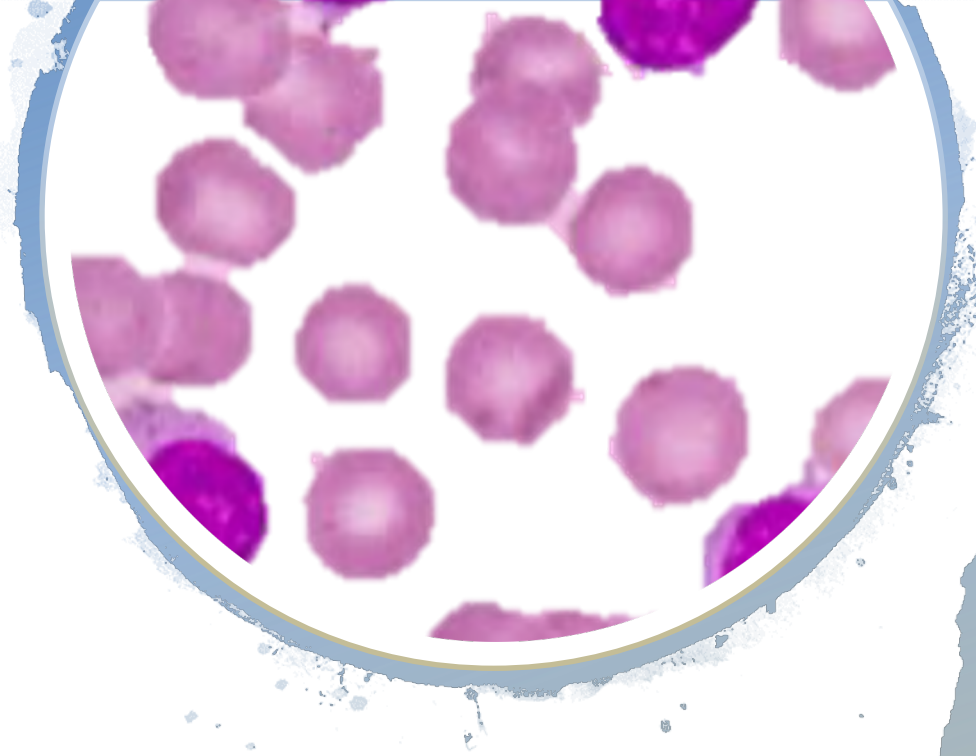
Using bioinformatics tools, we selected a tract of 12 mer of the CD5 transcript and synthesized the corresponding DNA (DNA wild type) tract to be used as a mimetic target. Moreover, we also synthesized the complementary 12 mer PNA strand and the scrambled one, named respectively PNA and PNA scrambled. Both the PNA compounds were decored with two residues of Serine Phospate (SerP) at its C-terminus interspaced by two glicine (Gly) spacers to favor the transfection process lipofectamine mediated for subsequent in vitro experiments.

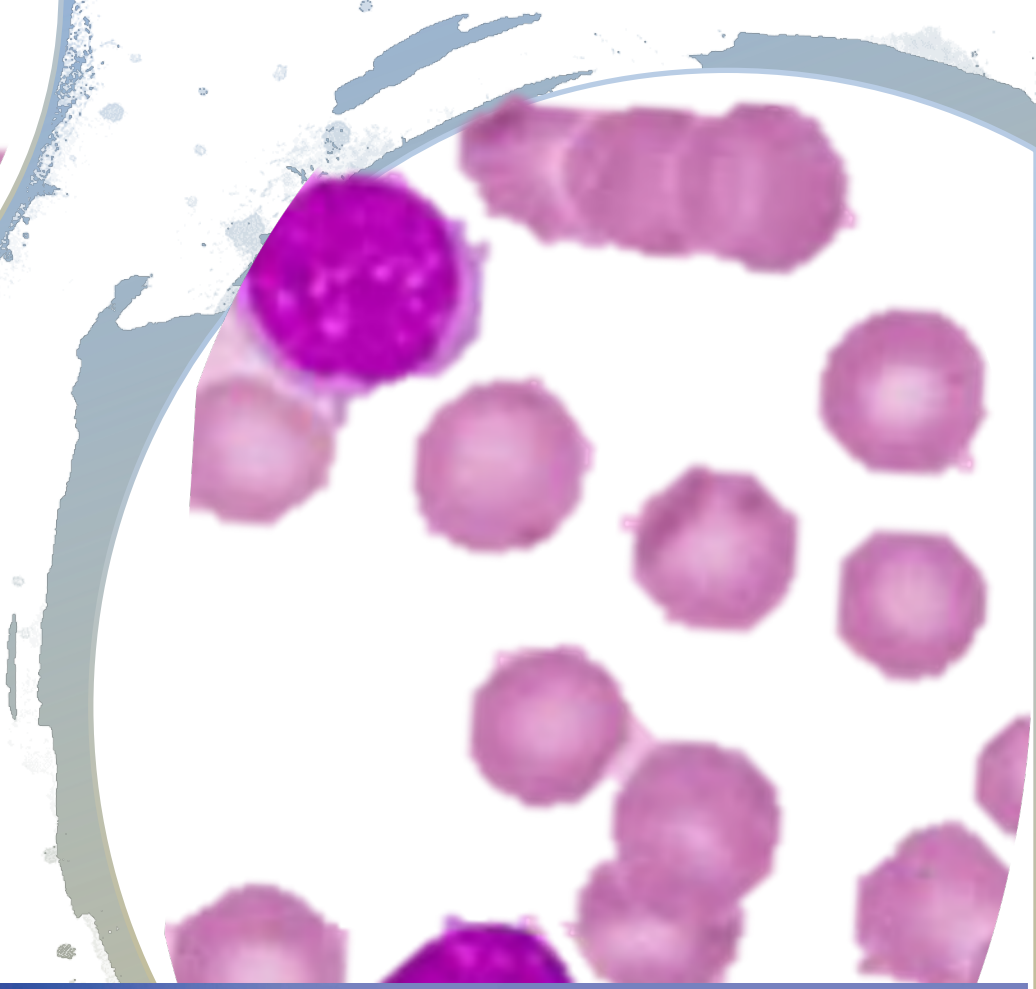
To evaluate the ability of the PNA to selectively bind its target we performed physical-chemical characterizations of PNA:DNA and PNA scrambled:DNA complexes. Circular Dichroism (CD), CD melting, TDS, and non-denaturing polyacrylamide gel electrophoresis (PAGE) analysis were performed. Each experiment confirmed that only the PNA and DNA wild type are specifically and selectively able to form a heteroduplex PNA: DNA in vitro, justifying further experiments to accomplish the antisense strategy in cells.

To figure the ability of the PNA to downregulate its complementary mRNA we transfected Jurkat cell line and peripheral blood mononuclear cells from B-CLL patients with PNAs. Cytofluorimetric assays and real-time PCR analysis demonstrated the downregulation of CD5 expression due to incubation with the anti-CD5 PNA for both cell lines.

Keywords: PNA, B-chronic lymphocytic leukemia (B-CLL), CD5, antisense gene silencing, peripheral blood mononuclear cells (PBMC)

Introduction

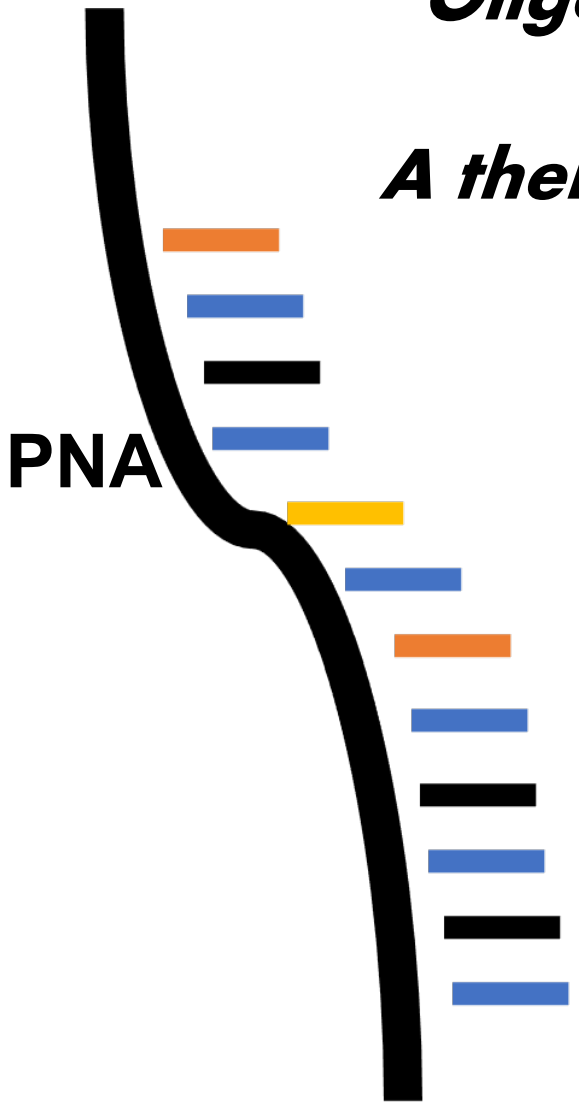




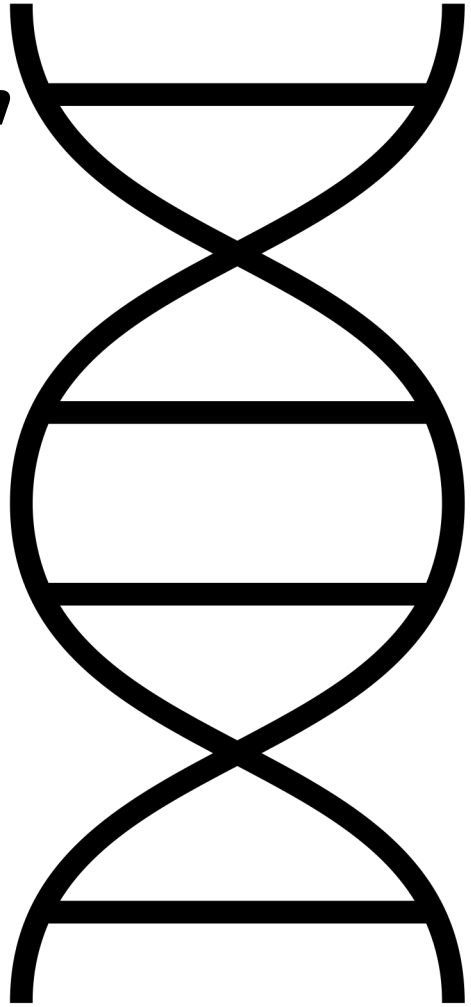
Chronic Lynfocytic Leukemia B Cell

Normal B Cell

Oligonucleotides and analogues: A therapeutic approach

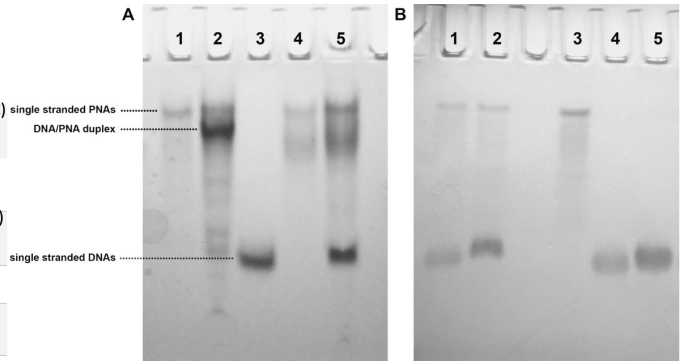


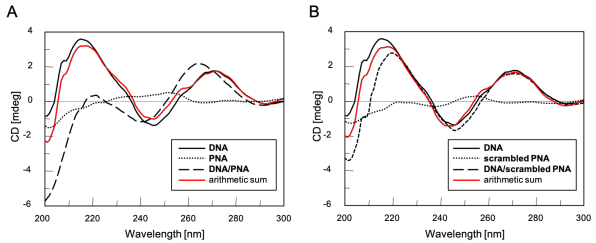
VS



Paper overview

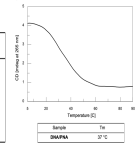
SAMPLE	SEQUENCE
PNA	tttctctcccaa-Gly-Ser(P)-Ser(P)-Gly-NH_2 (N→C)
PNA-FITC	FITC$(AEEA)_2$-tttctctcccaa- Gly-Ser(P)-Ser(P)-Gly-NH_2 (N→C)
scrambled PNA	cctattactcct-Gly-Ser(P)-Ser(P)-Gly-NH_2 (N→C)
Scrambled PNA-FITC	FITC$(AEEA)_2$-cctattactcct-Gly-Ser(P)-Ser(P)-Gly-NH_2 (N→C)
DNA	TTGGGAGAGAAA (5'→3')
C-rich control DNA	CCTCTGGTCTCC (5'→3')
G-rich control DNA	GGAGACCAGAGG (5'→3')

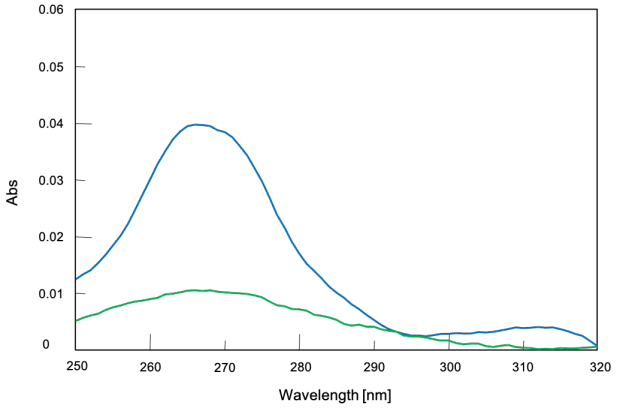


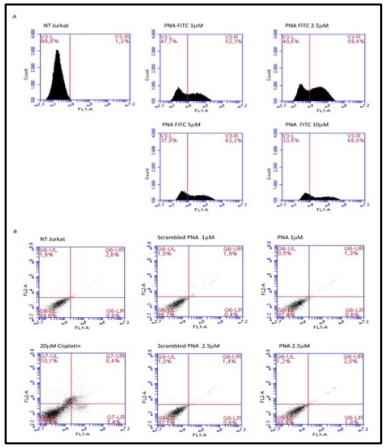


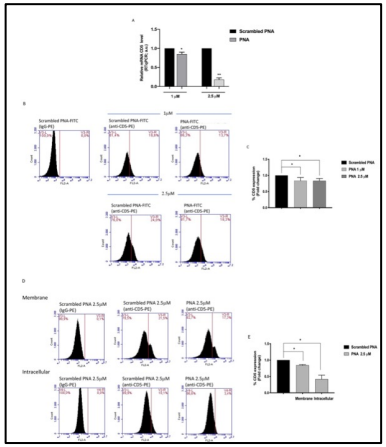
C

Sample	λ max (nm)	λ min (nm)
DNA	215-271	246
DNA/PNA	221-265	200-240
DNA/scrambled PNA	220-271	202-246







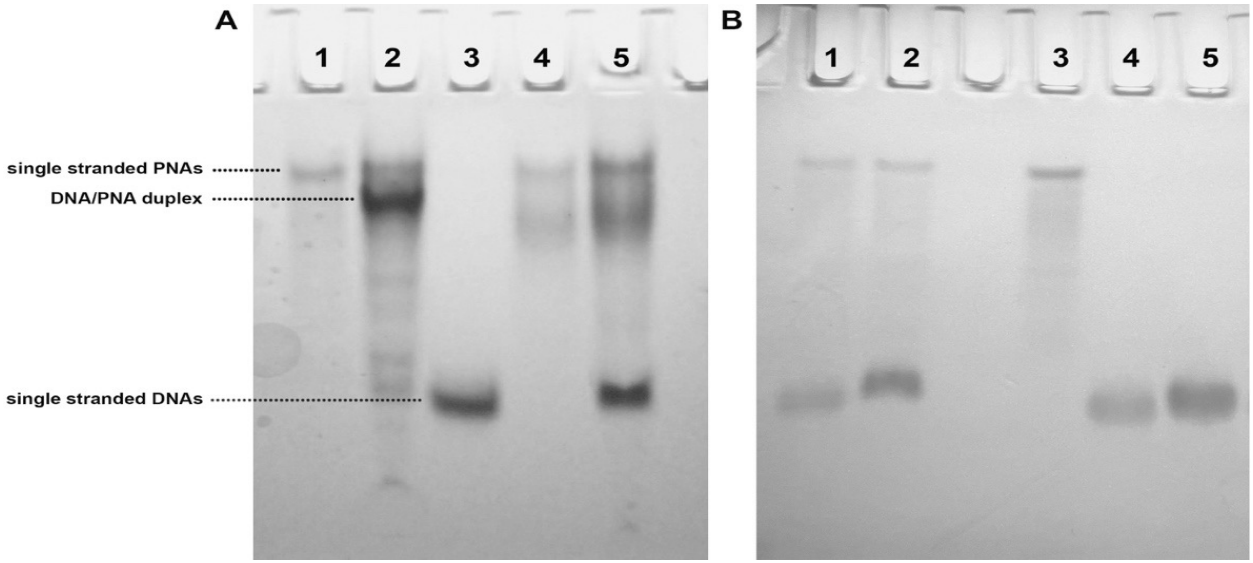


Result and discussion:

Selection of the Target CD_5 mRNA Sequence and Synthesis of DNA and PNAs

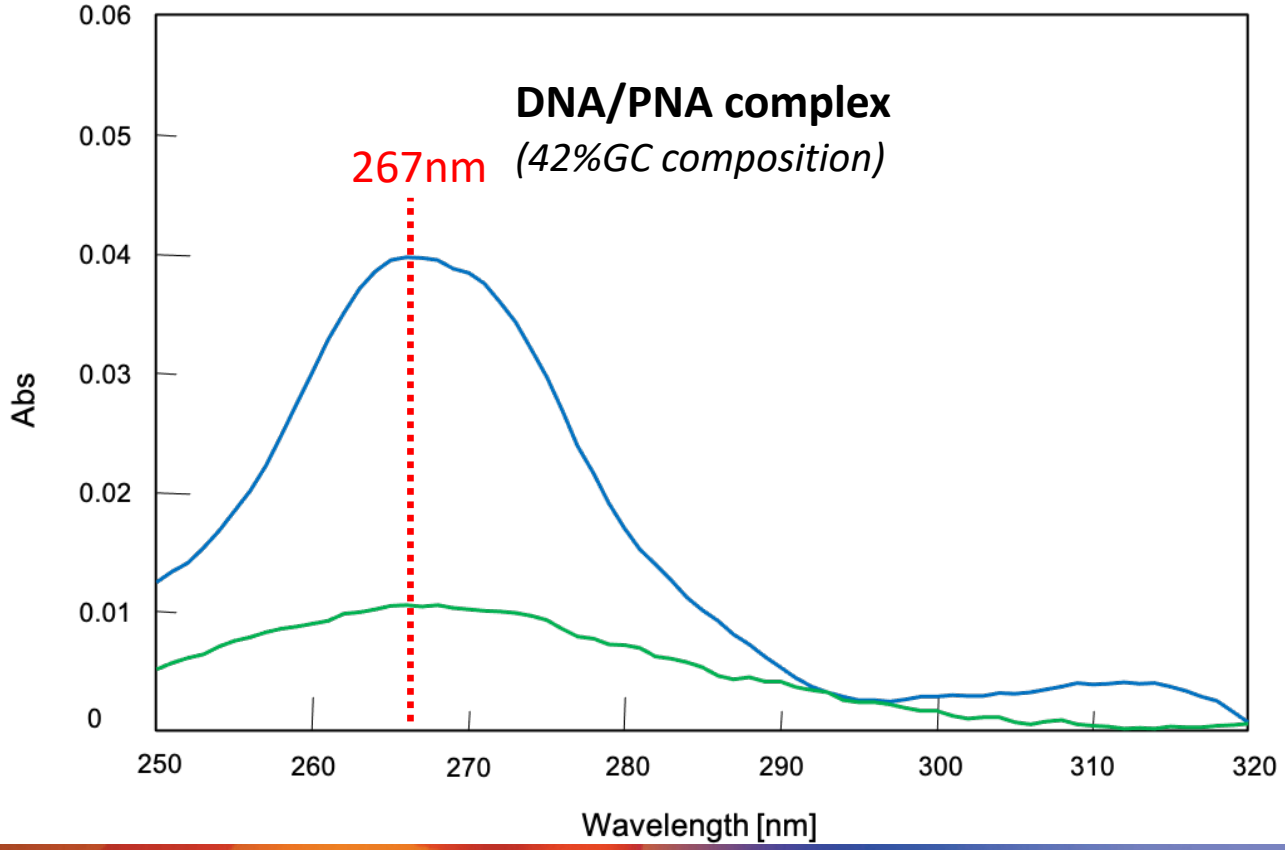
SAMPLE	SEQUENCE
PNA	tttctctcccaa-Gly-Ser(P)-Ser(P)-Gly-NH_2 (N→C)
PNA-FITC	FITC$(AEEA)_2$-tttctctcccaa- Gly-Ser(P)-Ser(P)-Gly-NH_2 (N→C)
scrambled PNA	cctattactcct-Gly-Ser(P)-Ser(P)-Gly-NH_2 (N→C)
Scrambled PNA-FITC	FITC$(AEEA)_2$-cctattactcct-Gly-Ser(P)-Ser(P)-Gly-NH_2 (N→C)
DNA	TTGGGAGAGAAA (5'→3')
C-rich control DNA	CCTCTGGTCTCC (5'→3')
G-rich control DNA	GGAGACCAGAGG (5'→3')

POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE)



PAGE in 100 mM PBS of A) ***PNA*** *(lane 1),* ***DNA*** *mixed with* ***PNA*** *(lane 2),* ***DNA*** *(lane 3),* ***scrambled PNA*** *(lane 4), and* ***DNA*** *mixed with* ***scrambled PNA*** *(lane 5); B)* ***C-rich control DNA*** *mixed with* ***PNA*** *(lane 1),* ***G-rich control DNA*** *mixed with* ***PNA*** *(lane 2),* ***PNA*** *(lane 3),* ***C-rich control DNA*** *(lane 4), and* ***G-rich control DNA*** *(lane 5). All mixtures were prepared at a 1:3 DNA/PNA ratio.*

THERMAL DIFFERENCE SPECTRA (TDS)

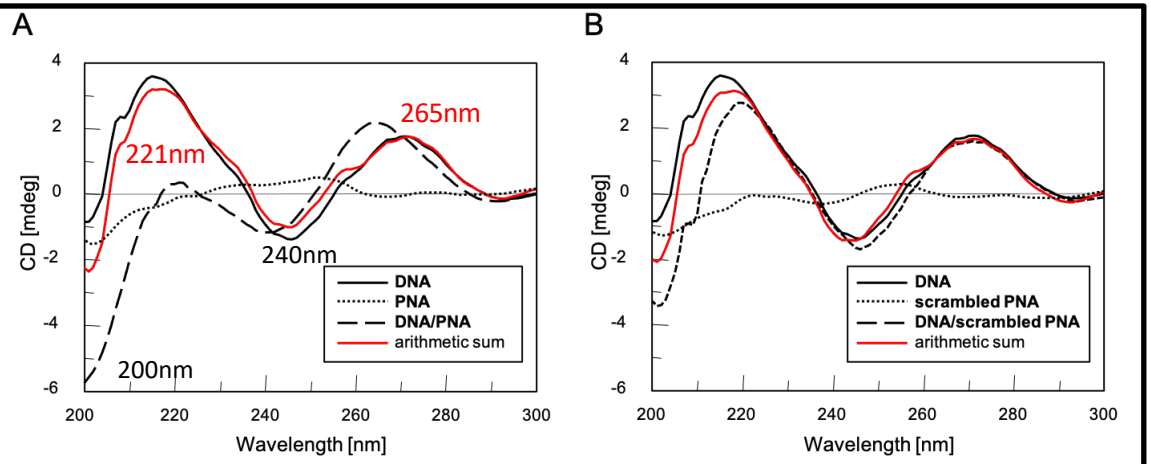


Structure	Major positive peak
Duplexes	
DNA duplex 0% GC	**259.5** ± 2 nm[a]
RNA duplex 0% GC	**259** ± 2 nm
DNA duplex 50% GC	**267** ± 2 nm ←
DNA duplex 100% GC	**276.5** ± 0.5 nm

Mergny, Jean-Louis & Li, Jing & Lacroix, Laurent & Amrane, Samir & Chaires, Jonathan. (2005). Thermal difference spectra: A specific signature for nucleic acid structures. Nucleic acids research. 33. e138. 10.1093/nar/gni134.

Overlapped TDS spectra of DNA annealed with PNA (blue line) or scrambled PNA (green line).

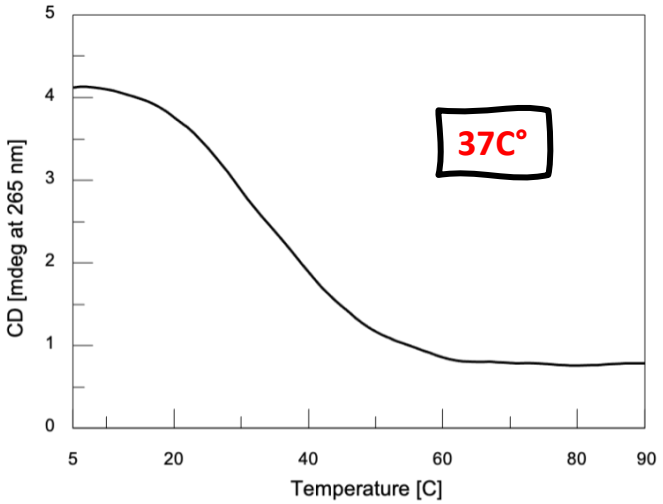
CIRCULAR DICHROISM AND CIRCULAR DICHROISM MELTING



C

Sample	λ max (nm)	λ min (nm)
DNA	215-271	246
DNA/PNA	221-265	200-240
DNA/scrambled PNA	220-271	202-246

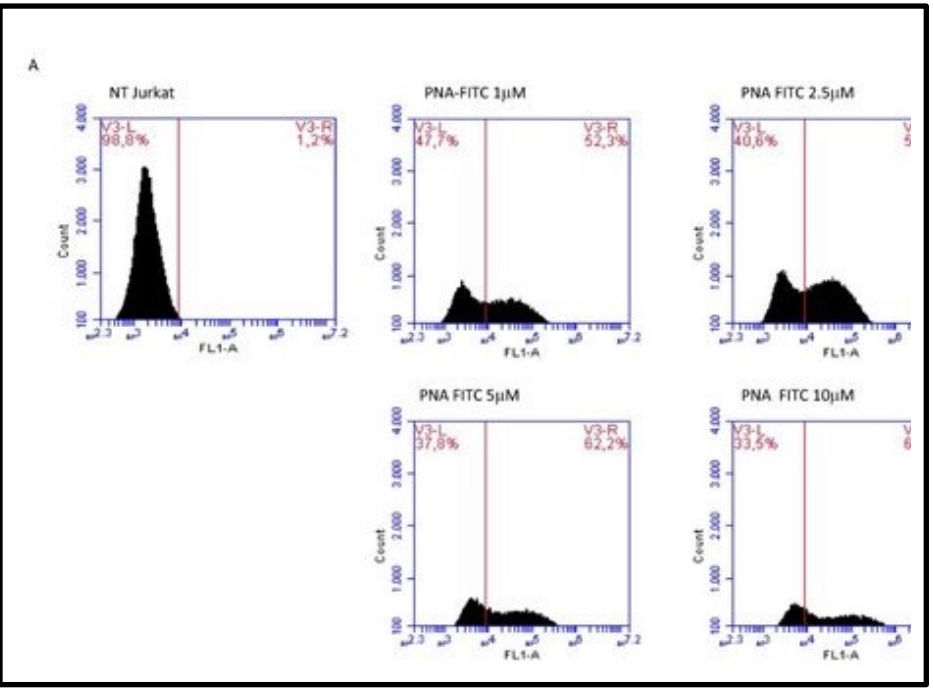
CD profile of the single-strand **DNA** alone (solid black line, panel A and B) and after annealing with **PNA** or **scrambled PNA** (dashed line, A and B respectively;. The arithmetic sum of **DNA** and **PNA** or **DNA** and **scrambled PNA** is reported as the red line (panel A and B, respectively). The CD profile of **PNA** or **scrambled PNA** alone is reported as the dotted line (panel A and B, respectively).

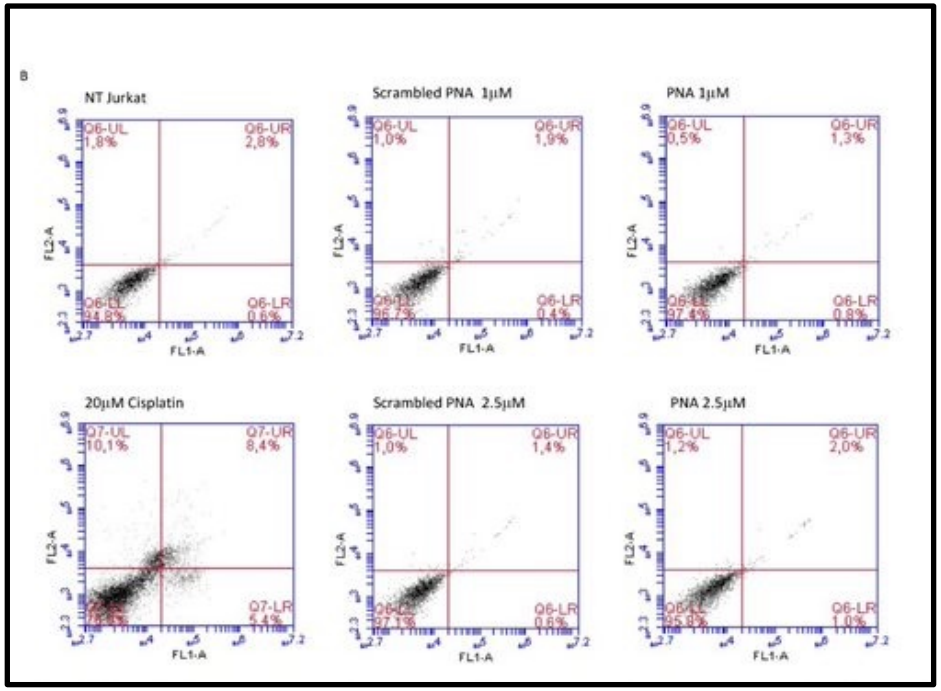


Sample	Tm
DNA/PNA	37 °C

CD melting curve of DNA/PNA mixture at 1:3 ratio obtained by monitoring the absorbance at 265 nm. Heating rate: 1 °C/min.

PNA transfection efficiency and cell death analysis

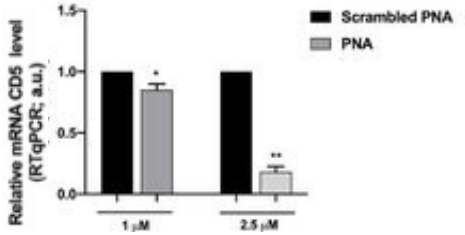




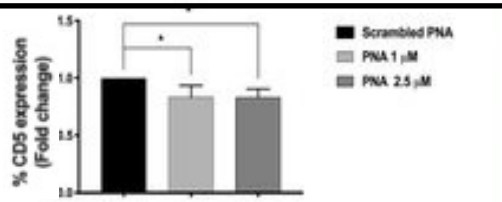
Flow cytometric histograms of Jurkat cells transfected with **PNA-FITC** to measure PNA delivery efficiency into the cells. Different concentrations of **PNA-FITC** were used (1, 2.5, 5, and 10 µM), and 48 h after transfection, the cells were harvested and analyzed by flow cytometry.

Two different concentrations (1.0 and 2.5 µM) of **PNA** or **scrambled PNA** were used for transfection. Necrotic and apoptotic cells were detected by annexin V and PI staining followed by flow cytometry analysis 48 h after transfection.

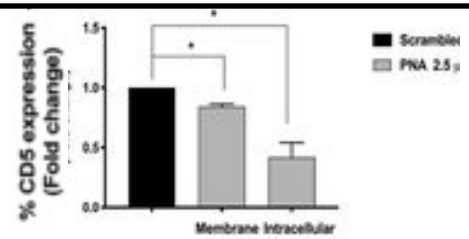
Evaluation of CD5 PNA treatment on PBMCs from B-CLL patients



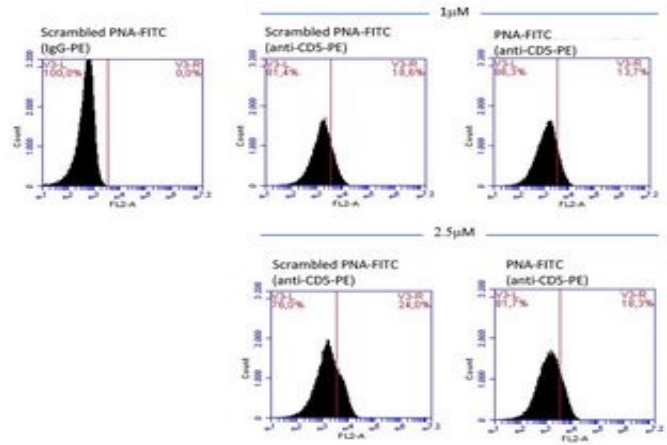
Representative dot plots of PBMCs characterization for the expression of CD19 and CD5



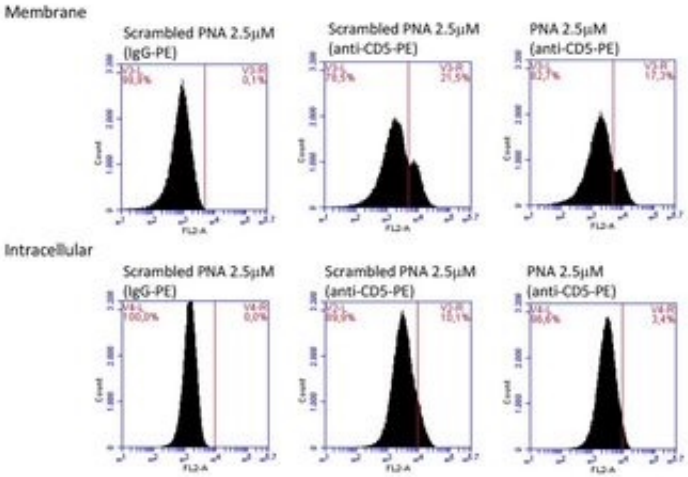
PNA transfection significantly decreased CD5 transcript levels compared to **scrambled PNA**



Data are shown as fold change of membrane and intracellular CD5 expression in Jurkat cells transfected with **PNA-FITC**,compared to the corresponding **scrambled PNA-FITC**, used as control.

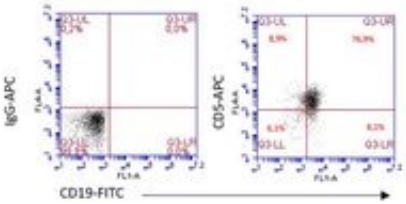


Flow cytometric analysis of CD5 expression in B-CLL PBMCs transfected with 1 μM **PNA** or **scrambled PNA**.

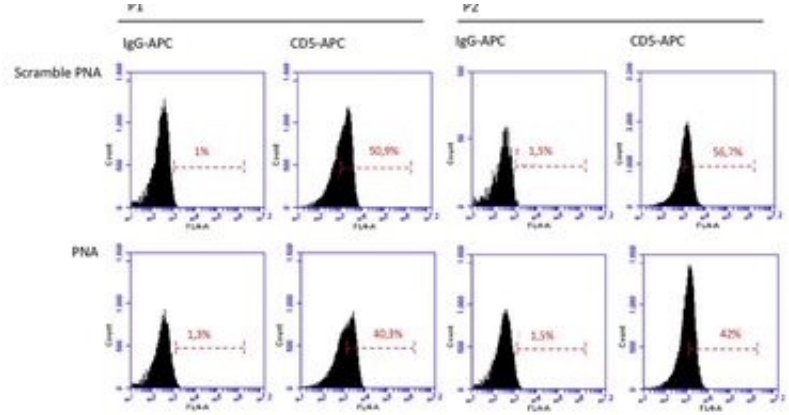


Representative flow cytometric histograms of Annexin V/PI staining. B-CLL PBMCs transfected with **PNA** and **scrambled PNA**

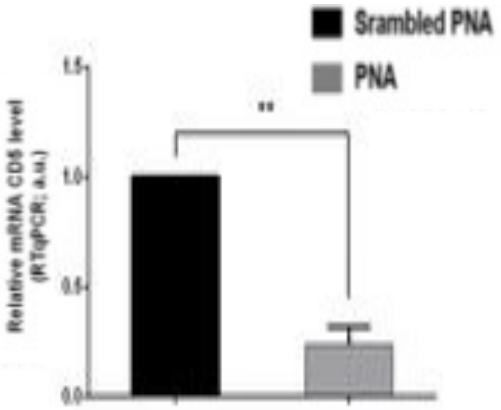
PNA impairs CD5 expression in B-CLL cells and sensitizes to fludarabine-induced cell death



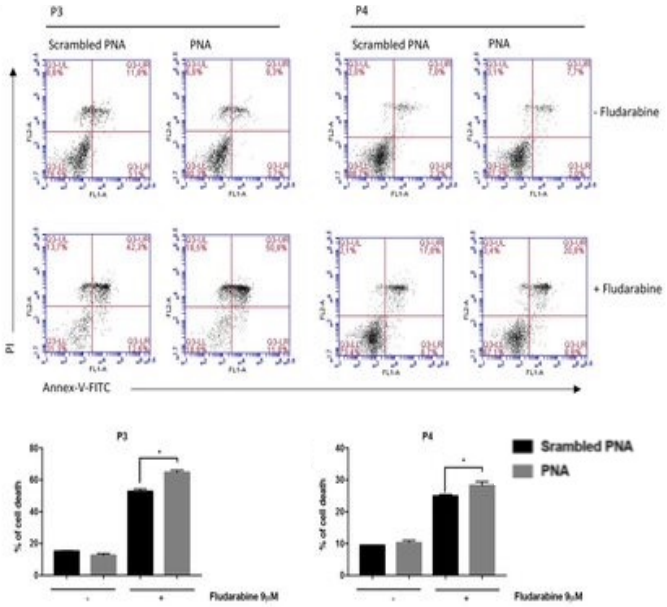
Representative dot plots of PBMCs characterization for the expression of CD19 and CD5



Flow cytometric analysis of CD5 expression in B-CLL PBMCs transfected with 1 μM **PNA** or **scrambled PNA**. CD5 staining was performed in two CLL patients (P1 and P2).



PNA transfection significantly decreased



Representative flow cytometric histograms of Annexin V/PI staining. B-CLL PBMCs transfected with **PNA** and **scrambled PNA**

Cell death values (Annexin V+/PI– cells and Annexin V+/PI+ cells) are also shown in the histogram

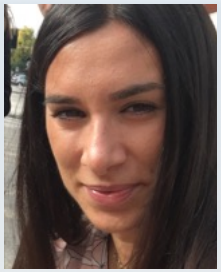
Conclusions

1. A suitable PNA molecule was synthesized to investigate its ability to bind the target sequence on the CD5 mRNA and downmodulate CD5 expression in CLL
2. Chemical-physical characterization showed a high PNA/DNA complex stability and this PNA molecule could hybridize with higher affinity to its mRNA target
3. Human T-leukemia Jurkat cell line and peripheral blood mononuclear cells were chosen for **PNA** treatment
4. Following the PNA treatment, Jurkat cells and PBMCs showed reduced CD5 levels both at RNA and protein levels
5. PNA-mediated mechanisms may involve inhibition of translation and possibly mRNA degradation
6. Peripheral blood mononuclear cells from B-CLL patients were treated combining PNA with a chemotherapeutic agent, FLUDARABINE, showing an enhancing of drug-induced apoptosis. The treatment sensitized CLL cells to chemotherapy treatment.

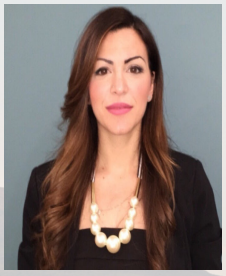
Thank you for your kind attention

Acknowledgments

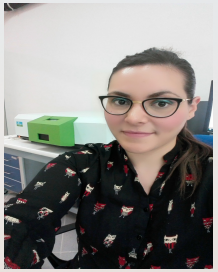

Gennaro Piccialli


Andrea Falanga


Nicola Borbone


Monica Terracciano


Stefano D'Errico


Maria Marzano


Giorgia Oliviero

Special thanks