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

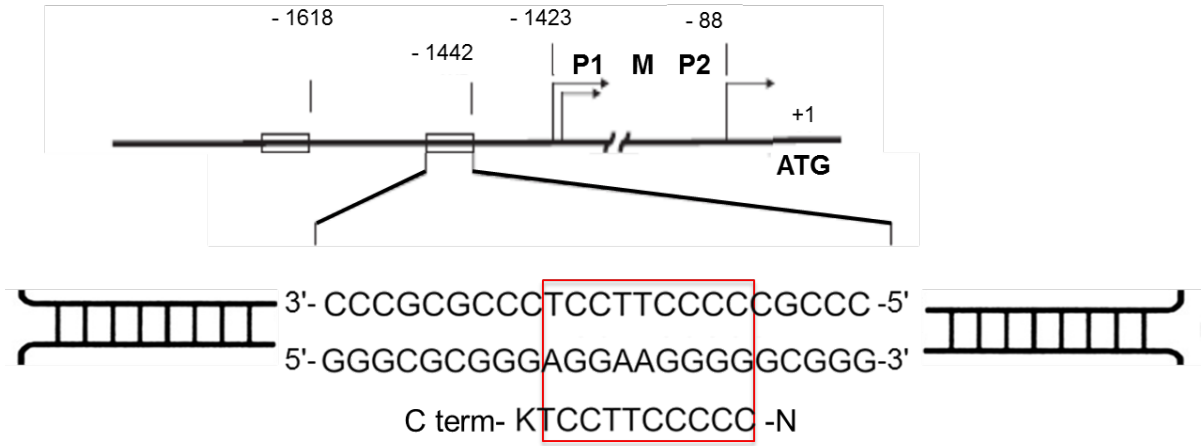
Heterotriplex-forming PNA in the targeting of the Bcl-2 P1 promoter in anticancer treatment

Andrea P. Falanga [1,*], L. Tripodi [2,3], M. Marzano [4], S. D'Errico [1], G. Piccialli [1], N. Borbone [1], L. Pastore [2,5] and G. Oliviero [5]

[1] Dipartimento di Farmacia, Università degli Studi di Napoli 'Federico II'.
[2] CEINGE-Biotecnologie Avanzate, Napoli.
[3] Scuola Europea di Medicina Molecolare (SEMM), Naples site
[4] Istituto di Cristallografia (IC) CNR, Bari
[5] Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli Federico II, Napoli, Italia

* Corresponding author: andreapatrizia.falanga@unina.it

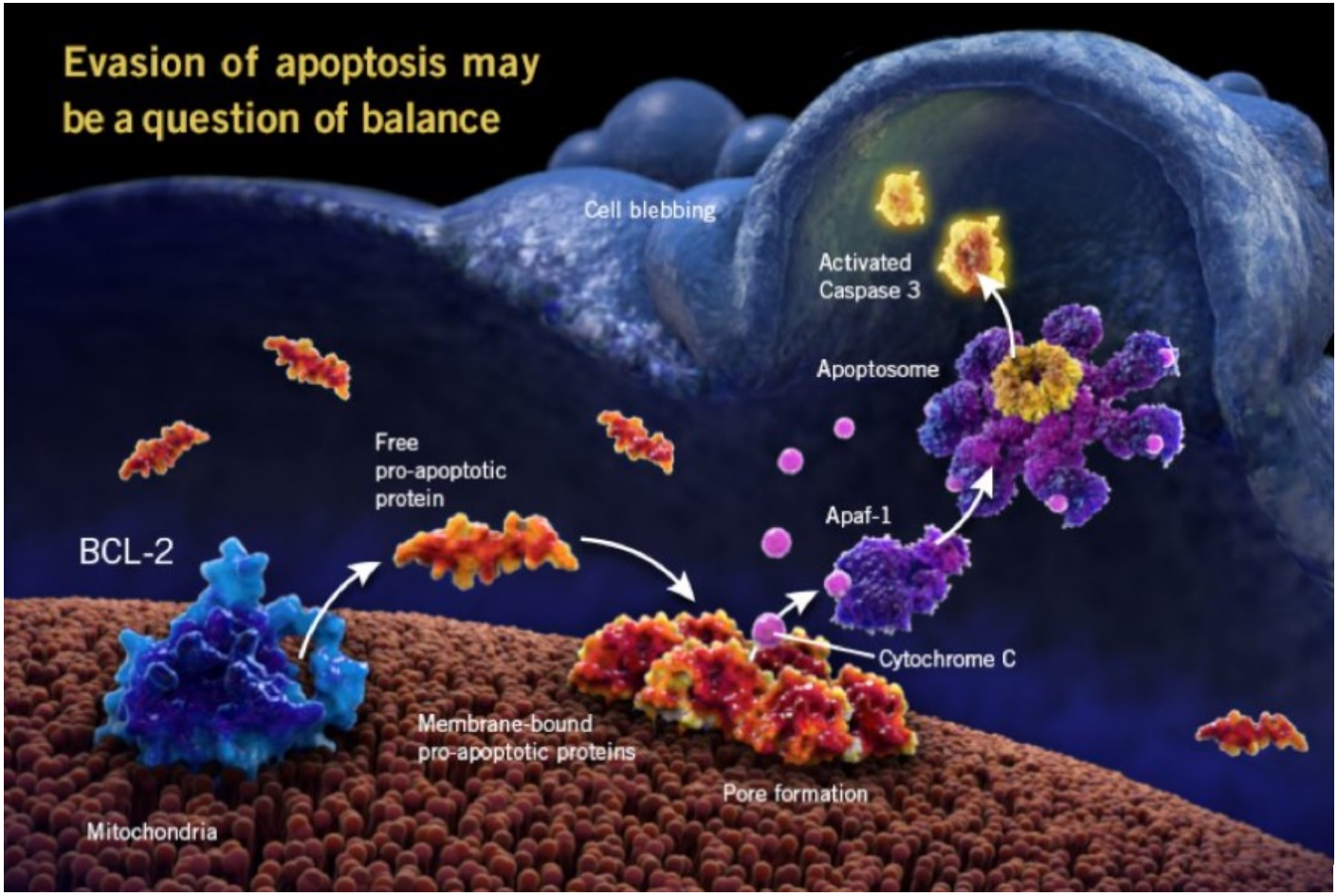
Heterotriplex-forming PNA in the targeting of the Bcl-2 P1 promoter in anticancer treatment



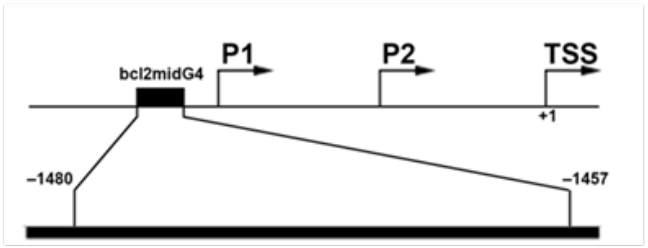
Abstract: Among several anti-gene strategies, the use of DNA analogues such as Peptide Nucleic Acids (PNAs) represents a promising tool for the modulation of gene transcription thanks to their chemical and enzymatic stability and the absence of charges in their backbone. PNAs can downmodulate oncogene expression successfully. We have recently demonstrated that the 7-mer PNA was able to selectively bind the longest loop of the G-quadruplex formed upstream of the P1 promoter of the Bcl-2 gene. Results demonstrated the ability of PNA-coated OAd5 oncolytic vectors to load and transfect their PNA cargo with a high efficiency and, also the synergistic cytotoxic effect against human A549 and MDA-MB-436 cancer cell lines. We have also demonstrated that the synthesized PNA does not interact with the corresponding duplex. To improve the target specificity towards the Bcl-2 P1 promoter, we extended the length of the pyrimidine-rich PNA from seven to ten bases. Herein, the 10-mer PNA and its FITC labelled analogue were synthesized, and the interaction with the N10–19 tract of bcl2midG4 in double strand was investigated by PAGE, CD and CD melting experiments. The druggability of the new PNAs was supported by fluorescence microscopy, which showed that the FITC-PNA specifically enters the cell nuclei. Finally, cytotoxicity assays confirmed the biological activity of the new anti Bcl-2 PNA. Overall, the studies here reported provide the basis for the development of new PNA-based anticancer agents for the treatment of human cancers.

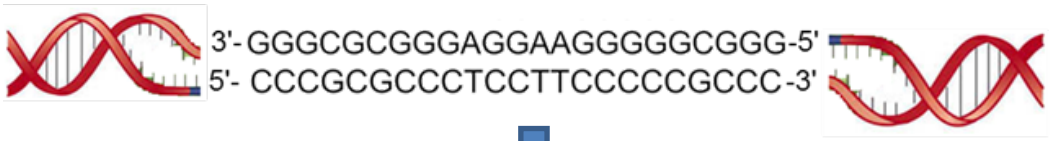
Keywords: antigene; Bcl-2; cancer therapy; heterotriplex; PNA; tumor

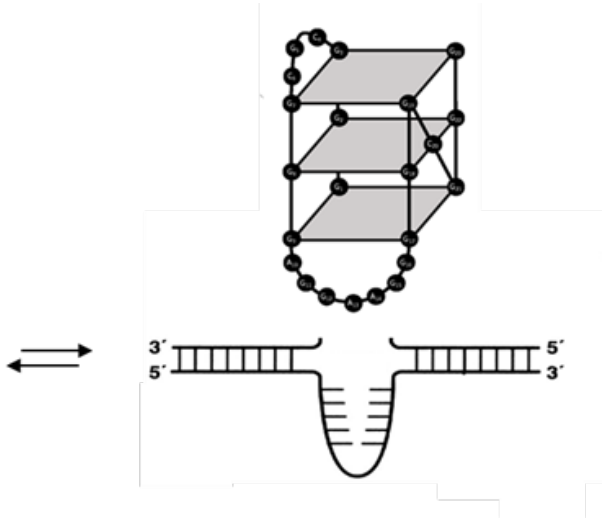
Introduction



Introduction



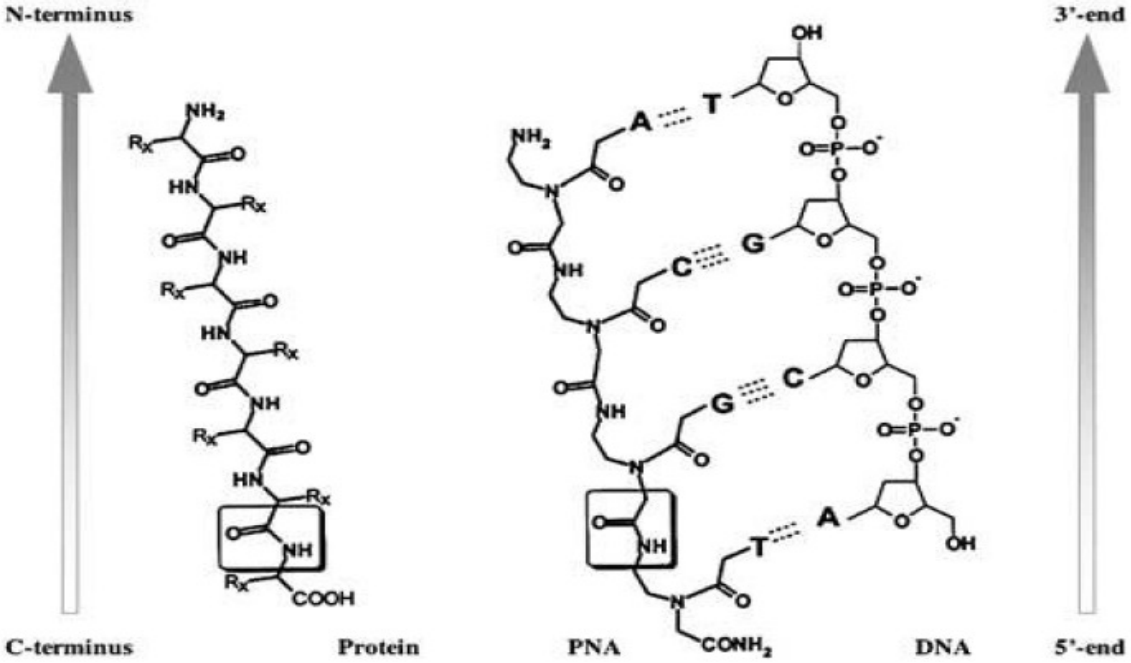




Numerous trascription factors bind this region

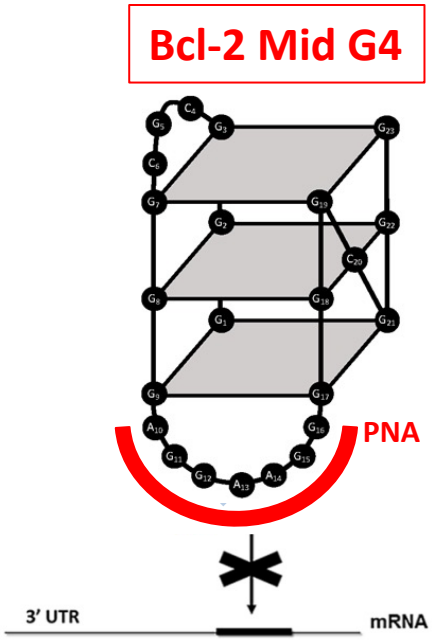
Peptide Nucleic Acid (PNA)

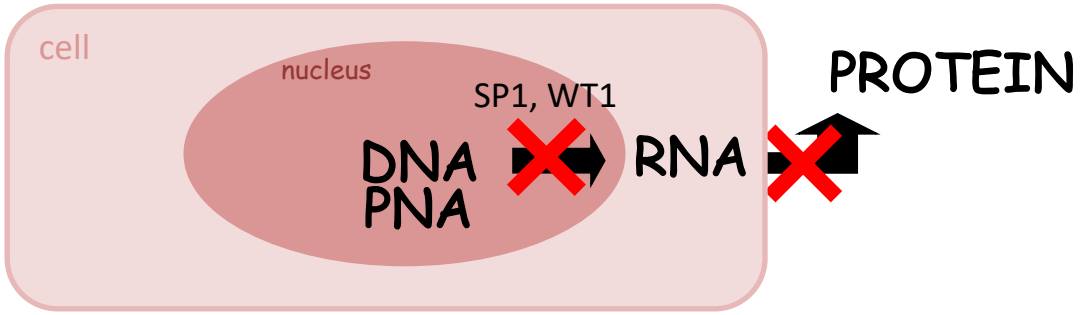
«What DNA could do, PNA can do it better»

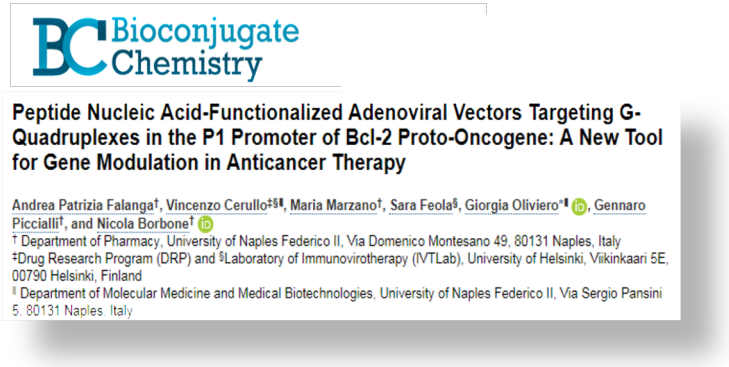


- Increased affinity of hybridization
- Increased biological stability
- Increased chemical stability

Aim





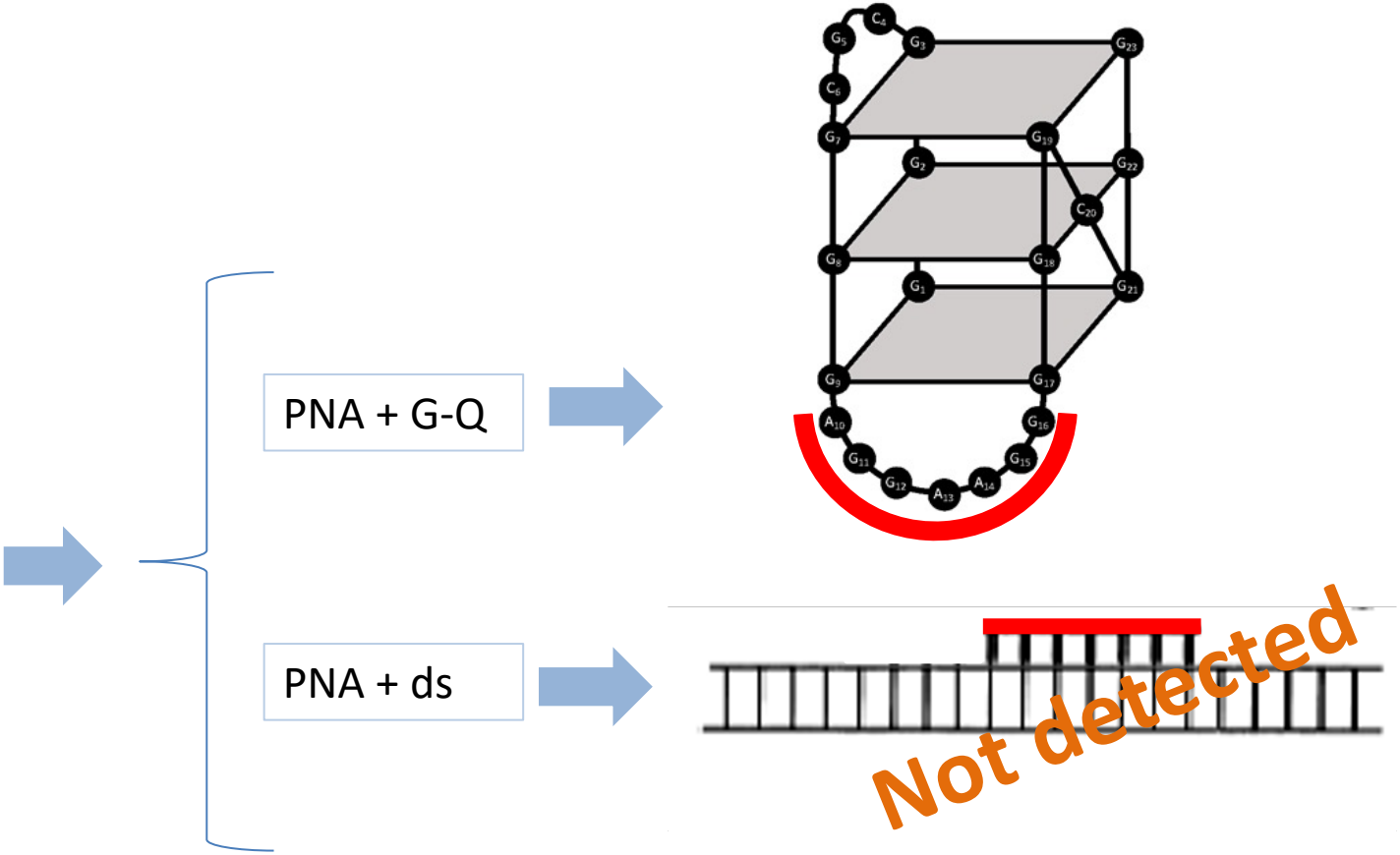

Bioconjugate Chemistry

Peptide Nucleic Acid-Functionalized Adenoviral Vectors Targeting G-Quadruplexes in the P1 Promoter of Bcl-2 Proto-Oncogene: A New Tool for Gene Modulation in Anticancer Therapy

Andrea Patrizia Falanga†, Vincenzo Cerullo‡§∥, Maria Marzano†, Sara Feola§, Giorgia Oliviero*∥, Gennaro Piccialli†, and Nicola Borbone†

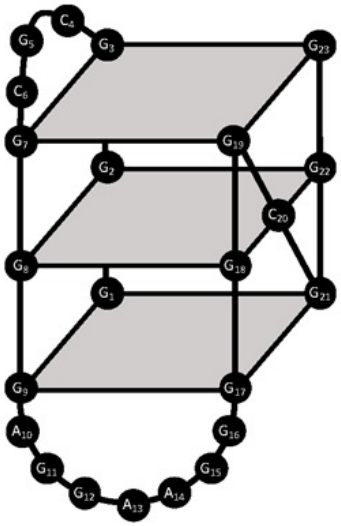
† Department of Pharmacy, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy
‡Drug Research Program (DRP) and §Laboratory of Immunovirotherapy (IVTLab), University of Helsinki, Viikinkaari 5E, 00790 Helsinki, Finland
∥ Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, Via Sergio Pansini 5, 80131 Naples, Italy

Peptide Nucleic Acid (PNA)



Falanga et al. (2019) *Bioconjug. Chem.*, **30**, 572–582.

PNA strategy



GQ-Bcl-2

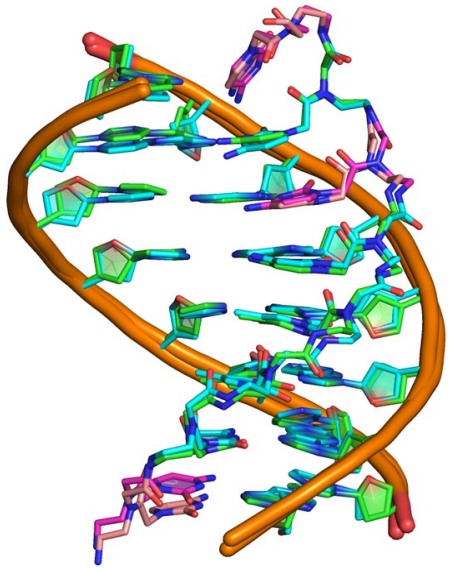
C-rich

5' 3'
G C
G C
G C
G C
C G
G C
C G
G C
G C
G C
A T
G C
G C
A T
A T
G C
G C
G C
G C
G C
G C
C G
G C
G C
G C
3' 5'

PNA

ds-Bcl-2

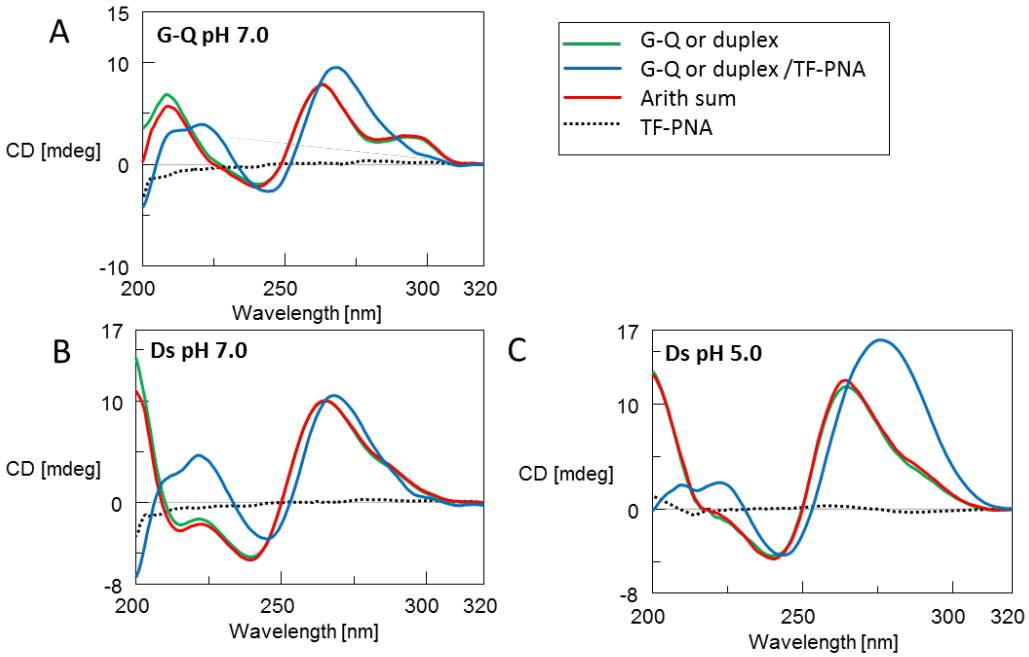
5' 3'
G C
G C
G C
G C
C G
G C
C G
PNA
G C
G C
G C
A T
T
C
C
T
T
C
C
C
C
C
A T
G C
G C
G C
G C
G C
C G
G C
G C
G C
3' 5'



Heterotriplex: ds + PNA

CD analysis

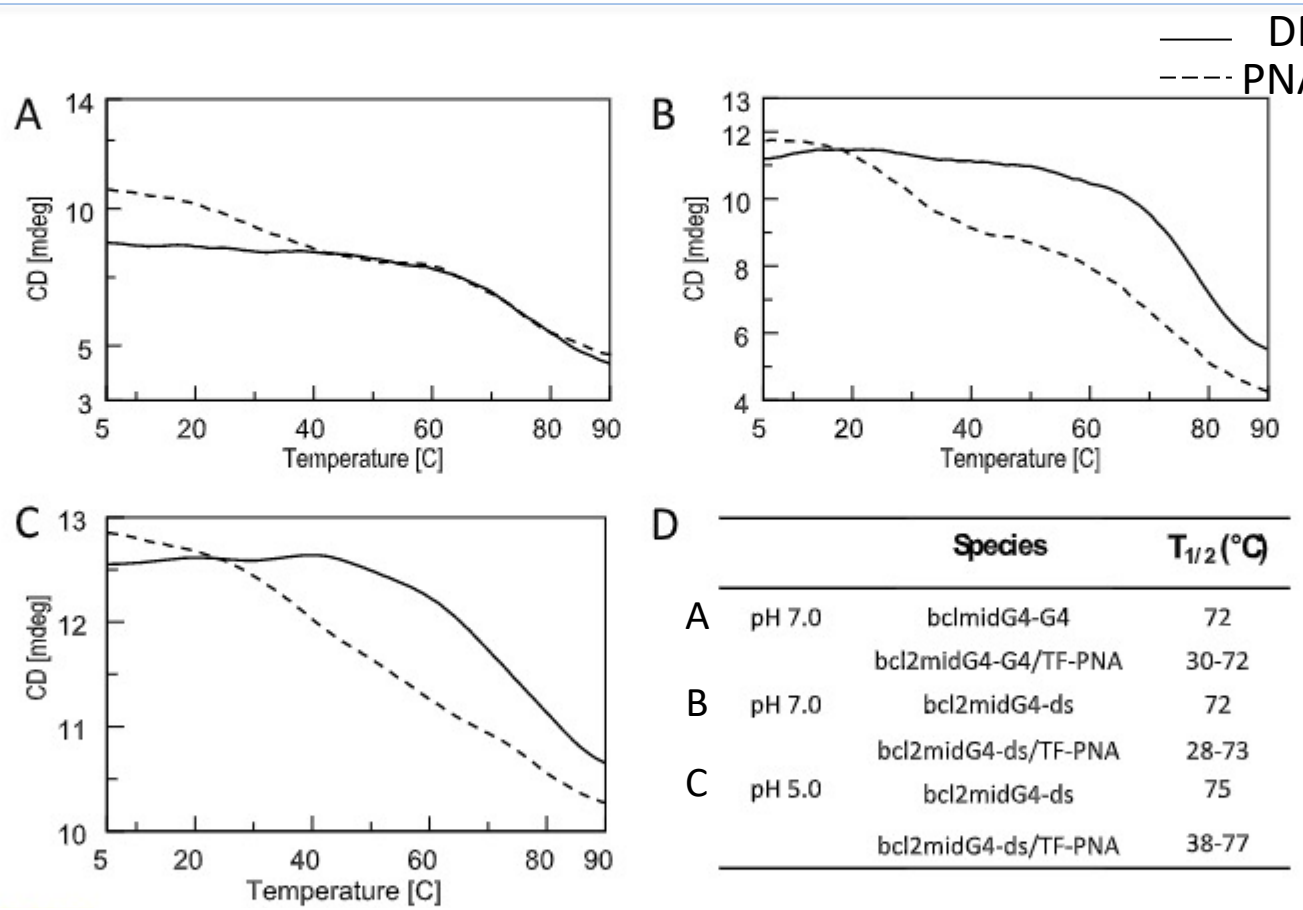
Results and discussion



	Sample	λ min (nm)	λ max (nm)
pH 7.0	bcl2midG4-G4	240	208-263-293
	bcl2midG4-G4/TF-PNA	244	220-268
pH 7.0	bcl2midG4-ds	240	265-290
	triplex	245	222-268
pH 5.0	bcl2midG4-ds	240	265-290
	triplex	243	223-276

CD analysis

Results and discussion

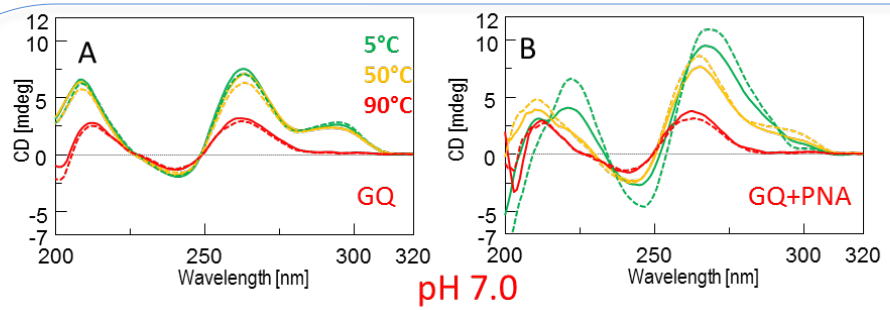


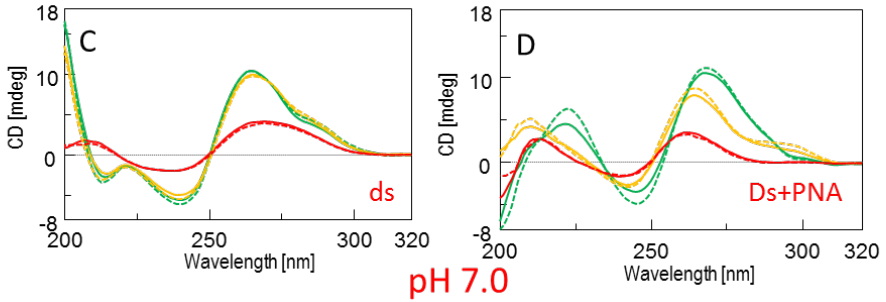
D

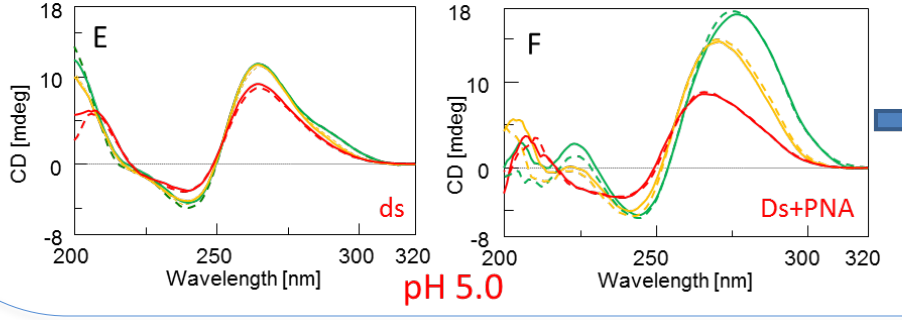
		Species	$T_{1/2}$ (°C)
A	pH 7.0	bclmidG4-G4	72
		bcl2midG4-G4/TF-PNA	30-72
B	pH 7.0	bcl2midG4-ds	72
		bcl2midG4-ds/TF-PNA	28-73
C	pH 5.0	bcl2midG4-ds	75
		bcl2midG4-ds/TF-PNA	38-77

CD analysis

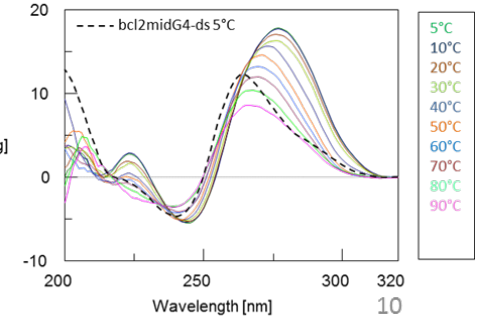
Results and discussion





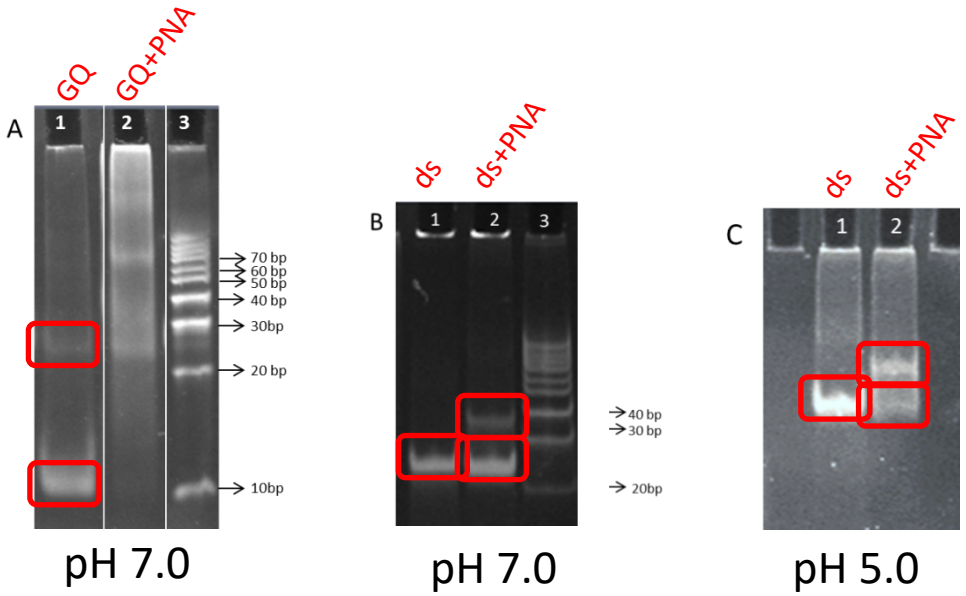


	Species	T_m (°C)
pH 7.0	bclmidG4-G4	72
	bcl2midG4-G4/TF-PNA	30-72
pH 7.0	bclmidG4-ds	73
	triplex	28-73
pH 5.0	bclmidG4-ds	75
	triplex	38-77



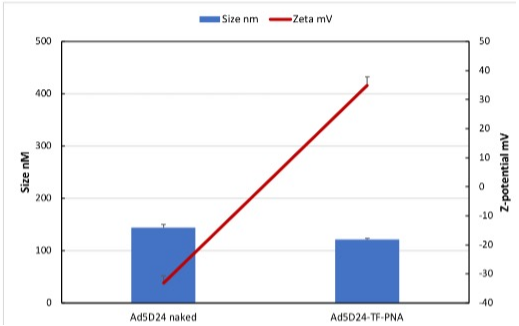
PAGE analysis

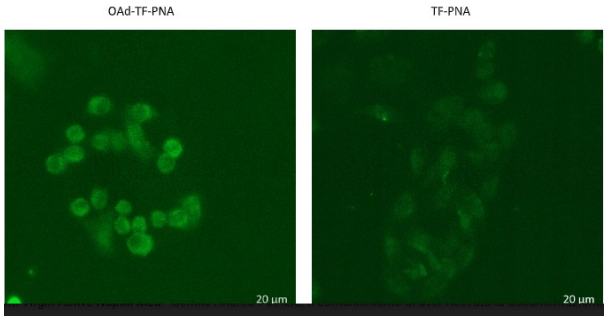
Results and discussion

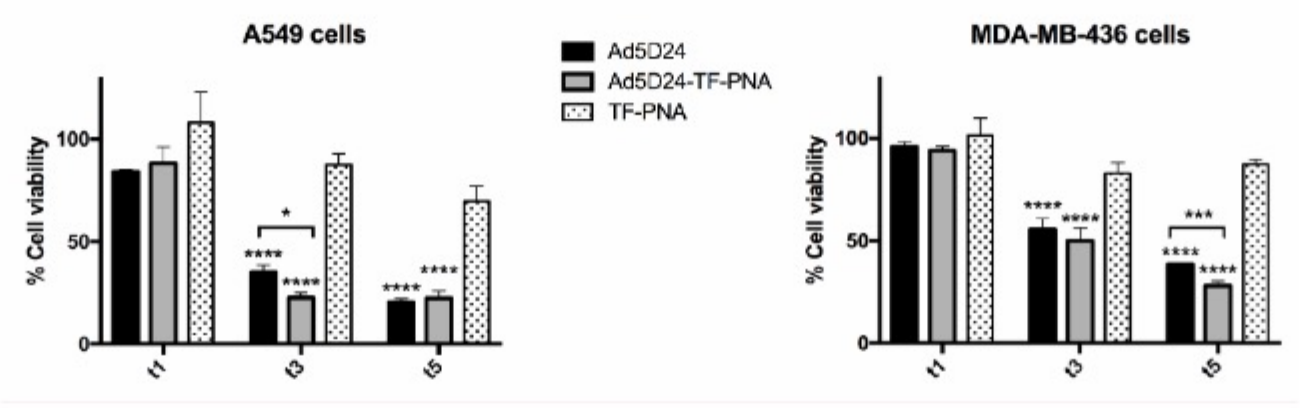


Biological characterization

Results and discussion







Conclusions

- PNA 10-mer interacts with the Bcl-2 G-Q promoting the formation of high order stuctures and with the Bcl-2 ds forming a PNA-DNA_2 heterotriplex
- The longer length of PNA-10 mer than 7-mer increases the chance of interaction with ds
- PNA-ds interaction is promoted at pH 5.0.
- Ad-TF PNA for tumor targeting-mediated release has been successfully developed for a selective transport of PNA in tumor cells.
- The produced Ad and Ad-PNA demonstrated a significant toxicity towards breast and lung human cancer cell types, with a stronger effect when Ad was functionalized with PNA. Interestingly, PNA/Ad induces a cooperative effect in cytotoxic activity.
- Safe transport of unbound PNA
- increasing the concentration in the target.

Thanks to my research group:

Prof. Giorgia Oliviero
Prof. Gennaro Piccialli
Prof. Nicola Borbone
Dr. Stefano D'Errico
Dr. Maria Marzano
Dr. Francesca Greco
Dr. Monica Terracciano

and:
Prof. Lucio Pastore
Dr. Lorella Tripodi

Thank you for your kind attention