Dr Roberto Nilo

Born:
Nationality:
Address:
Phone: +
e-mail address:
PEC address:

EDUCATION

MASTER'S DEGREE IN DRUG BIOTECHNOLOGY
University of Studies of Naples "Federico II", Naples, Italy
Title of the Experimental Thesis in Molecular Oncology: Aptamer-decorated and siRNA-loaded nanoparticles for PD-L1 gene silencing in triple-negative breast cancer cells
Period of activity: October 2020 – October 2022
Achievement Date: 10/26/2022
Votation: 110/110 *cum laude*

BACHELOR'S DEGREE IN BIOTECHNOLOGY FOR THE HEALTH, PHARMACEUTICAL CURRICULUM
University of Studies of Naples "Federico II", Naples, Italy
Title of the Experimental Thesis in Organic Chemistry: Synthesis and Purification of a Monophosphate Precursor of Cyclic Inosine Diphosphate Ribose (cIDPR)
Period of activity: October 2017 – September 2020
Achievement Date: 30/09/2020
Votation: 96/110

HIGH SCHOOL DIPLOMA (CLASSICAL STUDIES)
Liceo Classico "D. Cirillo", Aversa (CE), Italy
Period of activity: September 2012 – July 2017
Votation: 78/100

PROFESSIONAL EXPERIENCES

TUTORING ACTIVITY
University of Studies of Naples "Federico II" – Department of Pharmacy, Naples, Italy
Period of activity: September 2022

FORMATIVE INTERNSHIP AND EXPERIMENTAL THESIS ACTIVITY (MASTER'S DEGREE)
IEOS – Institute for Endocrinology and Experimental Oncology "G. Salvatore" – CNR, Naples, Italy
Period of activity: April 2021 – April 2022

FORMATIVE INTERNSHIP AND EXPERIMENTAL THESIS ACTIVITY (BACHELOR'S DEGREE)
University of Studies of Naples "Federico II" – Department of Pharmacy, Naples, Italy
Period of activity: September 2019 – November 2019

RESEARCH ACTIVITY TOPICS

From 2021 to date, my research activity, carried out in the laboratory directed by Dr. Laura Cerchia at the Institute of Endocrinology and Experimental Oncology "G. Salvatore" (IEOS) – National Research Council (CNR) of Naples (Italy), is focused on the characterization of RNA aptamers directed against triple-negative breast cancer (TNBC) cells, evaluating their antitumor effects *in vitro* and *in vivo* in animal models, the ability to inhibit the growth of cells resistant to conventional chemotherapy, and the ability to specifically deliver anticancer drug-loaded nanocarriers.

From 2019 to 2021 my research activity concerned the synthesis and purification of analogues of Cyclic Ribose Adenosine Diphosphate (cADPR)

Napoli, 22/11/22

PUBLICATIONS

ARTICLES IN JOURNALS

Camorani S, Tortorella S, Agnello L, Spanu C, d'Argenio A, **Nilo R**, Zannetti A, Locatelli E, Fedele M, Comes Franchini M, Cerchia L. Aptamer-Functionalized Nanoparticles Mediate PD-L1 siRNA Delivery for Effective Gene Silencing in Triple-Negative Breast Cancer Cells. *Pharmaceutics*. 2022 Oct 18;14(10):2225

Camorani S, d'Argenio A, Agnello L, **Nilo R**, Zannetti A, Ibarra LE, Fedele M, Cerchia L. Optimization of Short RNA Aptamers for TNBC Cell Targeting. *Int J Mol Sci.* 2022 Mar 23;23(7):3511

Marzano M, Terracciano M, Piccialli V, Mahal A, **Nilo R**, D'Errico S. O6-[(2″,3″-*O*-Isopropylidene-5″-*O*-tbutyldimethylsilyl)pentyl]-5′-*O*-tbutyldiphenylsilyl-2′,3′-*O*-isopropylideneinosine. *Molbank*. 2022; 2022(1):M1345

CONGRESS PROCEEDINGS WITH DOI

Agnello L, Camorani S, Tortorella S, d'Argenio A, **Nilo R**, Fedele M, Zannetti A, Franchini MC, Cerchia L. Nano-Immunotherapy in TNBC: Aptamer-Based Nanoparticles for PD-L1 siRNA Delivery to Cancer Cells. *Proceedings of the American Association for Cancer Research Annual Meeting 2022. 22-A-3061-AACR. April 08-13 2022, New Orleans, USA. Cancer Res 2022;82(12_Suppl). Abstract nr 367. https://doi.org/10.1158/1538-7445.AM2022-367*

ABSTRACT PRESENTED AT THE CONFERENCES

Ibarra LE, Camorani S, Agnello L, Chesta CA, Palaios RE, d'Argenio A, **Nilo R**, Zannetti A, Fedele M, Cerchia L. Optimizing photo-assisted eradication of triple-negative breast cancer through novel doped conjugated polymer nanoparticles. *EACR 2022. June 20-23, 2022, Seville, Spain*

Camorani S, Agnello L, Tortorella S, Ibarra LE, d'Argenio A, **Nilo R**, Fedele M, Zannetti A, Franchini MC, Cerchia L. Development of Innovative Drug-Loaded and Aptamer Targeted Nanosystems for Treatment of Triple-Negative Breast Cancer. *National Research Council-Department of Biomedical Sciences Conference, "Target discovery for unmet medical needs and precision/personalized medicine", CNR Headquarters, January 24-25, 2022, Rome, Italy*

Ibarra LE, Camorani S, Agnello S, Pedone E, Pirone L, d'Argenio A, **Nilo R**, Chesta CA, Palacios RE, Fedele M, Cerchia L. Development of Aptamer-Conjugated Polymer Nanoparticles for Selective Photodynamic Therapy of Triple-Negative Breast Cancer Cells. *National Research Council-Department of Biomedical Sciences Conference, "Target discovery for unmet medical needs and precision/personalized medicine", CNR Headquarters, January 24-25, 2022, Rome, Italy*

CERTIFICATES

EIPASS
Achievement Date: 06/12/2014

TECHNICAL SKILLS

Since 2021: Maintenance of tumor cell lines in monolayer and in co-cultures. Cell viability and proliferation assays. Essays on migration and invasion. Analysis of apoptosis by flow cytometry. Evaluation of the binding capacity of the aptamer to its target by ELISA and FACS assays. Analysis of the expression of surface proteins and evaluation of the binding of fluoresceined aptamers to the target cell and their internalization through the use of a confocal microscope. Evaluation of the efficacy of tumor targeting and tumor growth inhibition of nanocarriers *in vitro* and *in vivo*. Evaluation of protein expression by immunoblot. DNA, RNA and protein extraction. qRT-PCR. Aptamer selection using SELEX technology. Using UV light for photodynamic therapy approaches.

Since 2019: Chromatographic techniques, including HPLC and columns. Several organic syntheses steps. Different extractions. Principles of NMR. Synthesis of oligonucleotides by automatic synthesizer.

Napoli, 22/11/22

ATTACHED:

- Graduation certificate with exams with grade and date
- Degree thesis title page
- Abstracts presented at the conference
- Copy of a personal identity document
- EIPASS Certificate
- Substitute declarations (art.46/47 D.P.R. n. 445/2000), Allegato B

Napoli, 22/11/22

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

WE2022N78528000023 Matricola n°:

SEGRETERIA STUDENTI AREA DIDATTICA
SCIENZE BIOTECNOLOGICHE

Si certifica che :

il dott. NILO ROBERTO
nato il a

ha superato in data 26/10/2022, con voti 110/110 E LODE, l'esame di laurea in BIOTECNOLOGIE DEL FARMACO, LM-9 - CLASSE DELLE LAUREE MAGISTRALI IN BIOTECNOLOGIE MEDICHE, VETERINARIE E FARMACEUTICHE.
Lo studente e' in possesso della laurea di durata triennale in BIOTECNOLOGIE PER LA SALUTE.
Si certifica, inoltre, che la durata del corso di studi e' di due anni.

Per il conseguimento del predetto titolo ha sostenuto e superato i seguenti esami :

Insegnamento		Data Esame	Voto	CFU	Ateneo (*)	SSD
20133	PROVA FINALE	26/10/2022	Superato	10	016	0
32331	BIOTECNOLOGIE MICROBICHE E DELLE FERMENTAZIONI	29/09/2022	27	16	016	
32333	GENOMICA, TRANSCRITTOMICA E PROTEOMICA	19/07/2022	25	12	016	
U1999	FORME FARMACEUTICHE INNOVATIVE	18/07/2022	27	10	016	
02224	CHIMICA FARMACEUTICA	10/06/2022	30	6	016	CHIM/08
15870	TIROCINIO	29/04/2022	Superato	8	016	
32334	FARMACOLOGIA E BIOTECNOLOGIE FARMACOLOGICHE	20/01/2022	28	10	016	
32332	CHIMICA FARMACEUTICA BIOTECNOLOGICA	05/01/2022	30	6	016	CHIM/08
04755	FARMACOLOGIA E FARMACOTERAPIA	29/09/2021	26	6	016	BIO/14
U3749	BIOCHIMICA DEL METABOLISMO TUMORALE E APPROCCI	18/06/2021	30	5	016	BIO/10
U1361	BIOCHIMICA DELL'APOPTOSI	07/06/2021	29	5	016	BIO/10
18393	IMMUNOLOGIA CLINICA ED IMMUNOTERAPIA	18/02/2021	28	6	016	MED/04
05049	FISIOLOGIA CELLULARE	26/01/2021	29	6	016	BIO/09
17439	PROGETTAZIONE E SINTESI DI BIOMOLECOLE	14/01/2021	30	14	016	

(*) 016: UNIVERSITA' DEGLI STUDI DI NAPOLI FEDERICO II

Ai sensi dell'art. 15 della L. 183/2011 il presente certificato non puo' essere prodotto agli organi della pubblica amministrazione o ai privati gestori di pubblici servizi.
I dati riportati nel presente certificato sono estratti dall'archivio informatizzato del Centro di Ateneo per i Servizi Informativi (CSI).
La firma del responsabile dell'ufficio e' omessa ai sensi dell'art. 3 del Decreto Legislativo n. 39 del 12/2/1993.

Napoli, 08/11/2022

Il Capo dell'Ufficio
AMALIA TADDEO

Si rilascia il presente certificato in carta libera per gli usi per i quali la legge non prescrive il bollo (D.P.R. 26/10/72 N. 642, ALL. B e successive modificazioni).

Napoli, 22/11/22

UNIVERSITA' DEGLI STUDI DI NAPOLI FEDERICO II

Dipartimento di Farmacia

CORSO DI LAUREA MAGISTRALE IN

BIOTECNOLOGIE DEL FARMACO

TESI DI LAUREA SPERIMENTALE

IN

ONCOLOGIA MOLECOLARE

Aptamer-decorated and siRNA-loaded nanoparticles for PD-L1 gene silencing in triple-negative breast cancer cells

Relatore
Ch.ma Dott.ssa
LAURA CERCHIA

Relatore Interno
Ch.mo Prof.
GENNARO PICCIALLI

Candidato
ROBERTO NILO
Matr. [redacted]

ANNO ACCADEMICO 2021/2022

Napoli, 22/11/22

Abstract: EACR22-0827

P2-368: OPTIMIZING PHOTO-ASSISTED ERADICATION OF TRIPLE-NEGATIVE BREAST CANCER THROUGH NOVEL DOPED CONJUGATED POLYMER NANOPARTICLES

L.E. Ibarra[1,2], S. Camorani[3], L. Agnello[3], C.A. Chesta [4], R.E. Palacios[4], A. d'Argenio[3], **R. Nilo[3]**, A. Zannetti[5], M. Fedele[3], L. Cerchia[3]

[1]Institute of Experimental Endocrinology and Oncology "G. Salvatore" IEOS- CNR- Naples- Italy, National Research Council, Naples, Italy

[2]Instituto de Biotecnología Ambiental y Salud INBIAS, Universidad Nacional de Río Cuarto UNRC y CONICET, Rio Cuarto- Córdoba, Argentina

[3]Institute of Experimental Endocrinology and Oncology "G. Salvatore", National Research Council, Naples, Italy

[4]Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados IITEMA, Universidad Nacional de Rio Cuarto y CONICET, Río Cuarto- Córdoba, Argentina

[5]Institute of Biostructures and Bioimaging, National Research Council, Naples, Italy

Introduction

Triple-negative breast cancer (TNBC) has a poor prognosis because of the aggressive clinical behavior and limited targeted treatment options. In recent years Photodynamic therapy (PDT) has been examined experimentally in TNBC and it has been proposed as a therapeutic option to bypass and inhibit escape pathways in multidrug resistant breast cancer cells. Here, we investigated the tumor targeting and the antitumorigenic effectiveness of novel photosensitizers-based on conjugated-polymers-nanoparticles (CPNs) whose cancer selectivity is ensured by the conjugation to novel RNA aptamers recognizing human TNBC cells at high efficiency.

Material and Methods

We prepared CPNs by using the CP F8BT due to its exceptional features as a donor antenna to collect excitation energy and funnel it towards molecular dopant PS acceptors, such as the porphyrin PtOEP. Two functional polystyrene-based polymers (PS-PEG-COOH and PSMA) were chosen and incorporated in ~ 20% mass ratio to F8BT mass in order to improve colloidal stability and introduce COOH groups on the surface of CPNs for EDC reaction with NH2-modified aptamers (the previously validated anti-EGFR CL4 and the recently optimized sTN58 and sTN29 aptamers). Aptamer-decorated CPNs were characterized by size and zeta potential by DLS. Cell targeting/uptake, citotoxicity and photodynamic evaluation of the nanovectors were tested in TNBC cells and cisplatin-resistant derivatives.

Results and Discussions

Our results show the selectivity of recognition for TNBC membrane receptors and cell uptake of doped conjugated PNPs decorated with CL4, sTN58 or sTN29 aptamer in different TNBC cells. A significant improvement in PDT efficacy was obtained in the presence of CPNs functionalized with the aptamer compared to CPNs unconjugated or scrambled aptamer-conjugated. In cisplatin-resistant cells, sTN58 was the best candidate for improving labelling and PDT efficacy with CPNs. We propose sTN58, sTN29 and CL4 aptamers as valuable tools for selective TNBC cell internalization and therapeutic improvements for CPNs in PDT protocols.

Napoli, 22/11/22

Conclusion

Our study proposes novel and safe photosensitizers-loaded and aptamer-decorated nanosystems with excellent potential for the application in TNBC therapy. The availability of a panel of TNBC cell-type targeting aptamers, will supply multiple tumor-targeting agents with the possibility of using them in different combinations depending on distinct molecular and/or clinical TNBC phenotypes.

Napoli, 22/11/22

AACR: Abstract nr 367

< NANO-IMMUNOTHERAPY IN TNBC: APTAMER-BASED NANOPARTICLES FOR PD-L1 SIRNA DELIVERY TO CANCER CELLS >

Lisa Agnello[1,2]; Simona Camorani[1]; Silvia Tortorella[3]; Annachiara d'Argenio[1]; **Roberto Nilo[1]**; Monica Fedele[1]; Antonella Zannetti[4]; Mauro Comes Franchini[3]; Laura Cerchia[1]

[1]Institute of Experimental Endocrinology and Oncology "G. Salvatore" (IEOS), CNR, Naples, Italy.
[2]Università degli studi della Campania "Luigi Vanvitelli", Caserta, Italy.
[3]Department of Industrial Chemistry "Toso Montanari", University of Bologna, Bologna, Italy.
[4]Institute of Biostructures and Bioimaging, CNR, Naples, Italy.

Triple-negative breast cancer (TNBC) is a heterogeneous and aggressive group of breast cancers. The lack of specific actionable targets makes chemotherapy the main treatment for TNBC patients. However, chemotherapy has limited success due to scarce bioavailability, severe systemic side effects and drug resistance. Polymeric nanoparticles (PNPs) may efficiently deliver in vivo therapeutics to tumors when conjugated to specific targeting agents. Potential agents for targeting tumor cells are aptamers: short, single-stranded oligonucleotides that interact at high affinity with their targets. Here, we report the characterization of new multifunctional nanovectors consisting of safe and biodegradable PNPs, highly specific TNBC aptamers as delivery agents and artificial small interfering RNA (siRNA) as drug payload, designed to suppress programmed cell death-ligand 1 (PD-L1) expression, a major feature of immune evasion in cancer cells. We efficiently entrapped siRNA-PD-L1 into PNPs. To enable active targeting, siPD-L1-PNPs were functionalized with TN145-aptamer, which we previously generated by cell-SELEX and shown to bind with nanomolar affinity to TNBC cells distinguishing them from both normal breast cells and non-TNBC breast cancer cells. TN145 aptamer actively internalizes into target cells, thus representing a good candidate to deliver a therapeutic payload. We show that the aptamer-decorated nanovectors efficiently deliver fluorescein-labeled siRNA into TNBC MDA-MB-231 and BT-549 cells, as assessed by confocal microscopy. Unconjugated nanovectors or conjugated with scrambled aptamers were used as controls. Non-TNBC BT-474 and MCF7 breast cancer cells, were used to exclude unspecific binding. Importantly, a 30-min incubation of TN145-conjugated nanovectors on target cells, at a siPD-L1 concentration of 1 nM, results in stronger PD-L1 silencing than that achieved by siPD-L1 delivered via a commercial transfection reagent. Furthermore, TN145-PNPs loaded with both siPD-L1 and cisplatin were generated and the efficacy of combined treatment was tested on tumor cells and tumor and immune cell co-cultures. Chemotherapy, including cisplatin, has been reported to induce PD-L1 enrichment in TNBC cells, hence an aptamer-targeted nanosystem enabling the synergistic effect of siRNA that directly knocks down PD-L1 expression on tumor cells with a powerful chemotherapeutic drug could be the future way to eradicate TNBC cells.

Napoli, 22/11/22

<IEOS-CNR >

DEVELOPMENT OF APTAMER-CONJUGATED POLYMER NANOPARTICLES FOR SELECTIVE PHOTODYNAMIC THERAPY OF TRIPLE-NEGATIVE BREAST CANCER CELLS

Luis Exequiel Ibarra[1,2], Simona Camorani[1], Lisa Agnello[1,3], Emilia Pedone[4], Luciano Pirone[4], Annachiara D'Argenio[1], **Roberto Nilo[1]**, Carlos Alberto Chesta[5], Rodrigo Emiliano Palacios[5], Monica Fedele[1], Laura Cerchia[1]

[1]Institute of Experimental Endocrinology and Oncology "G. Salvatore" (IEOS), CNR, Naples, Italy.
[2]Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Río Cuarto (UNRC) y Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Río Cuarto (5800), Córdoba, Argentina.
[3]Università degli studi della Campania "Luigi Vanvitelli", Caserta, Italy.
[4]Institute of Biostructures and Bioimaging, CNR, Naples, Italy
[5]Instituto de Investigaciones en Tecnologías Energéticas y Materiales Avanzados (IITEMA), Universidad Nacional de Río Cuarto (UNRC) y Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Río Cuarto (5800), Córdoba, Argentina.

Background:

Photodynamic therapy (PDT) arises as an improved treatment tool for a variety of solid tumors because its highly localized action. Recently, we have developed photosensitizers-based on conjugated-polymers-nanoparticles (CPNs). A more selective tumor targeting for CPNs in triple-negative breast cancer (TNBC) could be achieved by the conjugation with novel aptamers.

Methods and Results:

CPNs were synthetized using poly(9,9-dioctylfluorene-alt-benzothiadiazole), Pt(II)octaethylporphyrin and polystyrene-(PS)-COOH stabilizer polymers to increase colloidal stability and allow functionalization with biomolecules through a carbodiimide reaction. CPNs were successfully conjugated to NH2-terminated aptamers CL4, sTN58, sTN29 binding selectively to TNBC membrane receptors, and also with the scrambled aptamer (SCR) used as negative control. A fully characterization of the resulting CPNs by size, zeta potential, and binding affinity using TNBC cell lines was performed. Finally, biocompatibility and PDT efficacy assays were conducted. Aptamer-decorated-CPNs maintained suitable nanoscale narrow-size distribution, were non-toxic in dark condition, with great binding affinity against TNBC compared to SCR-conjugated/non-conjugated CPNs.

Conclusions and significance:

The conjugation of therapeutic CPNs with specific aptamers against TNBC cells bring highly specific cell-targeting action. In the search of the development of new biomedical nanomaterials with a theranostic purpose, aptamer-conjugated polymers could represent a new nanoplatform for the treatment of TNBC using a PTD approach.

Keywords: conjugated polymer nanoparticles, photodynamic therapy, aptamers, TNBC

Napoli, 22/11/22

< IEOS-CNR >

< DEVELOPMENT OF INNOVATIVE DRUG-LOADED AND APTAMER TARGETED NANOSYSTEMS FOR TREATMENT OF TRIPLE-NEGATIVE BREAST CANCER >

Simona Camorani[1], Lisa Agnello[1,2], Silvia Tortorella[1,3], Luis Exequiel Ibarra[1,4], Annachiara d'Argenio[1], **Roberto Nilo[1]**, Monica Fedele[1], Antonella Zannetti[5], Mauro Comes Franchini[3], Laura Cerchia[1]

[1]Institute of Experimental Endocrinology and Oncology "G. Salvatore" (IEOS), CNR, Naples, Italy.
[2]Università degli studi della Campania "Luigi Vanvitelli", Caserta, Italy.
[3]Department of Industrial Chemistry "Toso Montanari", University of Bologna, Bologna, Italy.
[4]Instituto de Biotecnología Ambiental y Salud (INBIAS), Universidad Nacional de Río Cuarto (UNRC) y Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Río Cuarto (5800), Córdoba, Argentina.
[5]Institute of Biostructures and Bioimaging, CNR, Naples, Italy.

Text

Background:

Management of triple-negative breast cancer (TNBC) is still challenging because of its aggressive clinical behavior and limited targeted treatment options. Site-directed aptamer-based nanotherapeutics have the potential to overcome obstacles of chemotherapy. Here, we investigated the tumor targeting and the antitumorigenic effectiveness of novel drug-loaded and aptamer-decorated nanosystems in TNBC.

Methods and Results:

We generated short versions of three novel TNBC-specific aptamers, which preserve efficacious targeting, rapid cell uptake and anti-tumor properties as the parental moieties, thus representing good candidates to enable active targeting. siRNAs suppressing programmed cell death-ligand 1 (siPD-L1) expression, a major feature of immune evasion in cancer cells, were entrapped into TN145 aptamer-decorated polymeric nanoparticles (PNPs). Resulting targeted nanovectors efficiently delivered siRNA specifically to TNBC cells and caused strong PD-L1 silencing. We aim at testing combination therapy of siPD-L1-PNPs-TN145 with cisplatin-loaded and EGFR aptamer-targeted PNPs, we recently validated both in vitro and in TNBC xenografts.

Conclusions and Significance:

It has been reported that chemotherapy, including cisplatin, induces enrichment of PD-L1 in TNBC cells, thus, aptamer targeted nanosystems allowing the synergistic effect of siRNA, that directly knocks down the expression of PD-L1 on tumor cells, with a potent chemotherapeutic might be the future way for eradicating TNBC cells.

Keywords (max 5): Nucleic acid aptamers, precision nanomedicine, targeted drug delivery, TNBC, cancer immunotherapy

Napoli, 22/11/22

EIPASS CERTIFICATE

european informatics passport

Issued to **NILO ROBERTO**

Born in

EI-Card code **EIC00069084EU**

Date **12/06/2014**

has successfully completed the assessment for

EIPASS 7 MODULES

Fondamenti dell'Information Technology | Gestione funzioni di base del sistema operativo
Videoscrittura | Foglio elettronico | Gestione di dati strutturati | Presentazioni multimediali
Internet & Networking

certipass

Validate this certificate authenticity at www.eipass.com
certificate verification no. L3JB661T4L

Domenico Pontrandolfo
President & CEO
CERTIPASS

Firma autografa sostituita a mezzo stampa
ex Art. 3, comma 2 del D.Lgs 12/02/1993 n°39

Napoli, 22/11/22