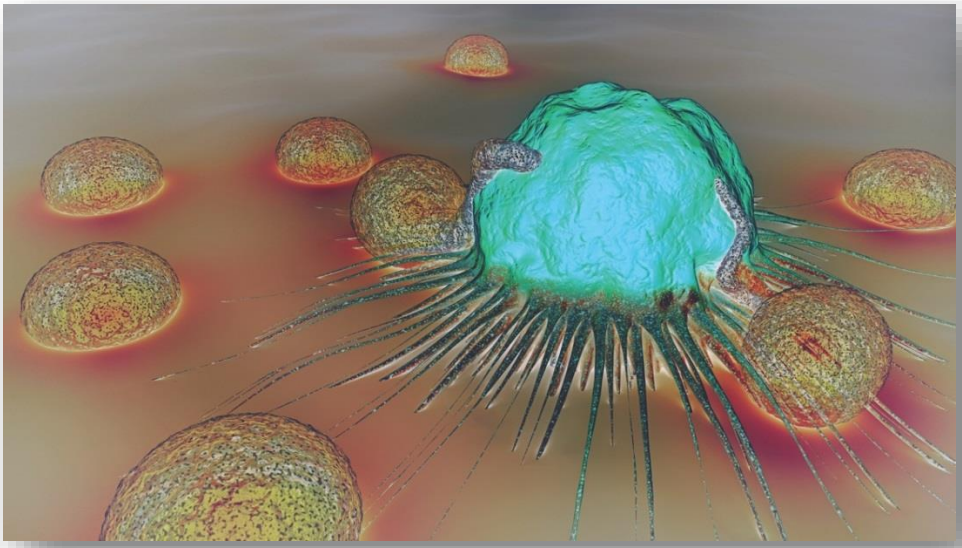

# Abstract Book of the Workshop

## Towards Novel Anticancer Strategies: It's Time to Build a New Research Community

18th November 2019, Naples

CESTEV

via Tommaso De Amicis, 95

University of Naples Federico II

- https://sites.google.com/view/anti-cancer-strategy/home - anti_cancercommunity

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# Scientific Program

08.30 - 09.00 **Registration**

09.00 - 09.10 **Welcome**

09.10 - 09.15 **Giorgia Oliviero**, *Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II "***Biochip per la diagnosi rapida e il follow-up della leucemia linfatica cronica nella popolazione in territorio a rischio***"**

**MORNING SESSION**

Chairpersons **Gennaro Piccialli** and **Daniele Passarella**

09.15 - 09.30 **Andrea P. Falanga**, *Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II "***Synthesis of PNA Probes for Chronic Lymphocytic Leukemia Detection***"**

09.30 - 09.35 Discussion

09.35 - 09.50 **Elena Cesaro**, *Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II "***The Zinc-Finger Protein ZNF224 as Potential Prognostic Marker and Therapeutic Target for Chronic Lymphocytic Leukemia**"**

09.50 - 09.55 Discussion

09.55 - 10.15 **Ilaria Rea,** *Institute for Microelectronics and Microsystems, CNR, Naples "***ZnONWs-Based Electrical Biosensor for Chronic Lymphatic Leukemia Diagnosis**"**

10.15 - 10.20 Discussion

10.20 - 10.35 Coffee Break

10.35 - 10.50 **Giacomo Ianniello**, *TME srl - Test and Manufacturing Engineering* "**Cloud-Based Measurement System for the Management of Data from Chronic Lymphatic Leukemia Diagnosis Biosensor"**

10.50 – 10.55 Discussion

10.55 - 11.10 **Carla Lucia Esposito**, *Center for Experimental Endocrinology and Oncology (IEOS), CNR, Naples "***Targeting Cancer Cells with Nucleic Acid Aptamers**"**

11.10 - 11.15 Discussion

11.15 - 11.50 **Daniela Gaglio**, *Institute of Molecular Bioimaging and Physiology, National Research Council (IBFM-CNR) "***Cancer Metabolic Rewiring: Target for Precision Medicine**"**

11.50 – 12.00 Discussion

12.00 - 12.15 **Alessia Caso**, *Department of Pharmacy*, *University of Naples Federico II* "I**n Search of New Anticancer Lead Compounds: Combined Use of Molecular Networking and Spectroscopic Techniques for the Fast Detection of New Skeletons**"

12.15 – 12.20 Discussion

12.20 - 12.35 **Stefano Cinti**, *Department of Pharmacy, University of Naples Federico II "***Signal ON and Signal OFF Paper-Based Electroanalytical Strips for Nucleic Acids Detection: Breast Cancer as Case of Study**"

12.35 - 12.40 Discussion

12.40 - 12.55 **Alessandro D'Urso**, *Department of Chemical Sciences, University of Catania "***Interaction of Porphyrins and Biomolecules as Potential Anticancer Strategy***"*

12.55 - 13.00 Discussion

13.00 -14.00 Brunch

**AFTERNOON SESSION**

Chairpersons **Giorgia Oliviero** e **Valeria Costantino**

14.00 - 14.30 Laura **Cipolla**, *Department of Biotechnologies and Biosciences, University of Milano-Bicocca* "**Biomaterial Design for Regenerative Medicine, Drug Delivery and 3D Microenvironment Mimicry with Potential in Cancer Research**"

14.30 - 14.40 Discussion

14.40 - 14.55 **Antonella Accardo**, *Department of Pharmacy*, *University of Naples Federico II "***Supramolecular Nanostructures for the Selective Delivery of Anticancer Drugs and/or Contrast Agents**"

14.55 - 15.00 Discussion

15.00 - 15.15 **Elisa Michelini**, *Department of Chemistry, University of Bologna "***3D Bioluminescent Cancer Models**"

15.15 - 15.20 Discussion

15.20 - 16.00 **Maxim Berezovski**, *Department of Chemistry and Biomolecular Sciences, University of Ottawa "***Cancer Diagnostics and Therapeutics with Aptamer Nanotechnologies**"

16.00 - 16.10 Discussion

16.10 - 16.30 Concluding Remarks

# Scientific Contributes

**OC-01**

# Synthesis of PNA Probes for Chronic Lymphocytic Leukemia Detection

Andrea P. Falanga,[a,*] Stefano D'Errico,[b] Maria Marzano,[b] Nicola Borbone,[b] Gennaro Piccialli[b] and Giorgia Oliviero[a]

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Chronic lymphocytic leukemia (CLL) is a neoplastic disease characterized by the accumulation of small mature B lymphocytes in the blood, bone marrow and peripheral lymphoid organs (lymph nodes and spleen). The first indication for CLL diagnosis is to carry a complete blood count. In the case of altered results, other tests may be required, including bone marrow biopsy. For this reason, we focused our attention on development of a less invasive and faster diagnostic techniques. B lymphocytes from CLL patients express the CD5 transmembrane protein[1]. This protein is the main marker used for the diagnosis of CLL. It was decided to select a sequence within the CD5 mRNA that acts as a template for the synthesis of nucleic acid peptide probes (PNAs), capable of recognizing and selectively binding the CD5 diagnostic marker mRNA with high sensitivity. The ability of the synthesized PNA to hybridize the target sequence was investigated by using Circular Dichroism (CD), CD melting and Non denaturing electrophoresis in presence and in absence of the probe conjugated to the DNA sequence. Results demonstrated the interaction between the PNA and DNA sequence. However the presence of the probe on DNA sequence destabilizes the interaction.

***Acknowledgments**: This work was supported by: POR FESR 2014-2020. PROGETTI TRASFERIMENTO TECNOLOGICO E DI PRIMA INDUSTRIALIZZAZIONE PER LE IMPRESE INNOVATIVE AD ALTO POTENZIALE PER LA LOTTA ALLE PATOLOGIE ONCOLOGICHE. CAMPANIA TERRA DEL BUONO. Biochip per la diagnosi rapida e il follow-up della leucemia linfatica cronica nella popolazione in territorio a rischio.*

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**OC-02**

# The KRAB Zinc-Finger Protein ZNF224 as a Potential Prognostic Marker and Therapeutic Target for Chronic Lymphocytic Leukemia

Elena Cesaro,[a] Rosa Catapano,[a] Arianna Pastore,[a] Raffaele Sessa,[a] Marialuigia Iannalfo,[b] Federico Chiurazzi,[b] Michela Grosso,[a] Paola Costanzo[a,*]

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B-cell chronic lymphocytic leukemia (CLL) is a chronic leukemia resulting from the proliferation of a neoplastic clone of monoclonal B-lymphocytes with a very characteristic immunophenotype (CD19+/CD5+/CD23+) (1). The clinical course of CLL is highly variable. In some cases, the disease has an indolent course and the patients do not require therapy for years. In other cases, the disease progresses rapidly and the patients die despite treatment. Indeed, although in recent years chemo-immunotherapeutic combinations have resulted in significant progress in the treatment modalities, CLL remains incurable and drug resistance is a major cause of treatment failure. Therefore, the identification of novel prognostic factors may contribute to improve the CLL classification and predict outcomes for patients with CLL (2). ZNF224 is a transcription factor, belonging to the Kruppel-like zinc-finger protein family, that exerts an oncogenic role in CLL, contributing to impaired growth of CLL cells and apoptosis resistance (3). ZNF224 expression affects cell cycle progression in CLL through the transcriptional control of cyclin D3, a cell cycle regulatory factor with protumorigenic function. ZNF224 is expressed at high levels in CLL patients and represents a promising novel prognostic factor in CLL (4). We aim to analyze the correlation between ZNF224 expression levels and chemotherapy responsiveness in a large cohort of CLL patients, to validate ZNF224 as a useful marker to monitor the clinical outcome in CLL patients undergoing chemotherapy.
The development of an extensive screening system using a platform of PNA biosensors, that allows the simultaneous and rapid analysis of several molecular markers associated with the development and progression of CLL, could allow a detailed pathological staging of this pathology. Thus, patients cohorts may be divided into distinct prognostic categories for early prediction of pathology evolution and for selection of the best therapeutic strategy to reduce the risk of drug resistance.
For this reason, we will validate the use of the BioChip for CLL diagnosis with a large panel of biomarkers, including ZNF224, in RNA samples from CLL patients.

***Acknowledgments****: This work was supported by: POR FESR 2014-2020. PROGETTI TRASFERIMENTO TECNOLOGICO E DI PRIMA INDUSTRIALIZZAZIONE PER LE IMPRESE INNOVATIVE AD ALTO POTENZIALE PER LA LOTTA ALLE PATOLOGIE ONCOLOGICHE. CAMPANIA TERRA DEL BUONO. Biochip per la diagnosi rapida e il follow-up della leucemia linfatica cronica nella popolazione in territorio a rischio.*

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# ZnONWs-Based Electrical Biosensor for Chronic Lymphatic Leukemia Diagnosis

T. Crisci,[a] G. Chianese,[a] M. Casalino,[a] M. Gioffrè,[a] L. De Stefano,[a] I. Rea,[a,*] M. Terracciano,[b] N. Borbone,[b] G. Oliviero,[c] G. Piccialli[b]

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The semiconductors currently used in microelectronic industry, such as silicon and its related materials (silicon oxide, silicon nitrides and porous silicon) have been extensively exploited in biosensors fabrication and hundreds of papers and reviews can be found in recent literature [1-5]. On the other hand, the nanostructured metal oxides showed great potential for the detection of biomolecules, owing to several characteristics such as the controllable size/shape, large specific surface area, biocompatibility, catalytic and optical properties, chemical stability [6]. Among them, nanostructured Zinc Oxide (ZnO) is considered an excellent candidate material for electrical transduction due to both the ability to modulate its electrical conductivity and its fast electron transfer kinetics. Different methods for fabrication of nanostructured ZnO-based devices are available, such as Vapor-Liquid-Solid growth (VLS), Metal Organic Chemical Vapor Deposition (MOCVD), High-Pressure Pulsed Laser Deposition (HP-PLD) [7, 8]. Recently, the hydrothermal synthesis was proposed as an advantageous alternative approach because it requires mild experimental conditions, simple equipment, low-cost reagents and it can be indifferently used on several surfaces [9].

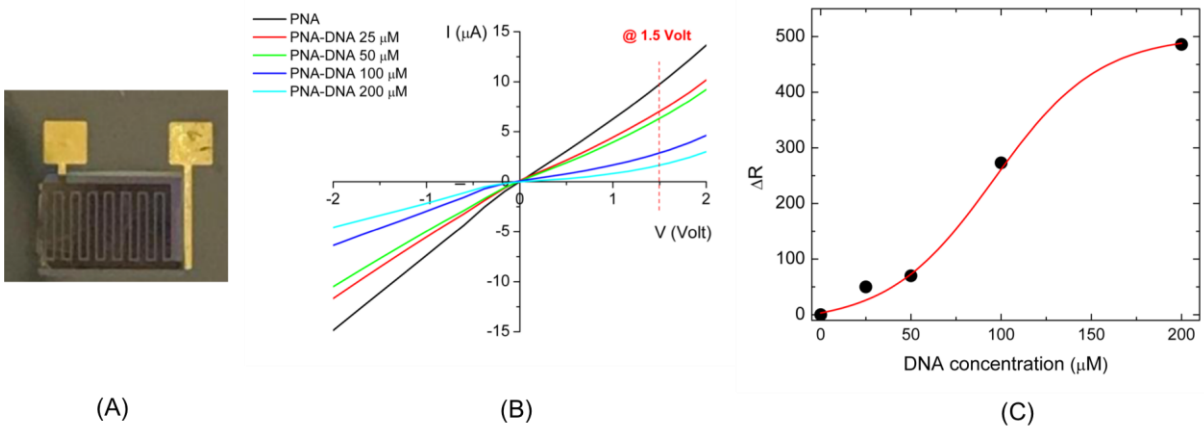


**Figure 1.** (A) A photograph of the ZnONWs-based electrical sensor; (B) I-V characteristics of ZnONWs sensor functionalized with PNA probe, before and after interaction with increasing concentrations of complementary DNA; (C) Dose-response curve of ZnONWs biosensor.

One of the key issues in the developing of biosensors is the functionalization strategy used to correctly immobilize the bioprobes on the material surfaces. From this point of view, nanostructured ZnO allows to covalently bind the biomolecules on its surface by using a

soft chemical strategy, preserving the specific functionalities of the biological compounds and controlling their orientation.
In this work, a ZnO nanowires (ZnONWs)-based electrical biosensor (Figure 1 A) was realized for Chronic Lymphatic Leukemia (CLL) diagnosis. The biosensor was obtained covalently immobilizing a PNA probe, complementary to a DNA sequence codifying for a surface glycoprotein (CD5) overexpressed in CLL cells, on the surface of ZnONWs. Figure 1 (B) shows the I-V curves of the electrical biosensor after interaction with DNA target at different concentrations. Calculating the resistance of device at 1.5 V and plotting it as function of DNA concentration, a linear behavior was observed between 50 and 150 μM (Figure 1 C).

***Acknowledgments****: This work was supported by: POR FESR 2014-2020. PROGETTI TRASFERIMENTO TECNOLOGICO E DI PRIMA INDUSTRIALIZZAZIONE PER LE IMPRESE INNOVATIVE AD ALTO POTENZIALE PER LA LOTTA ALLE PATOLOGIE ONCOLOGICHE. CAMPANIA TERRA DEL BUONO. Biochip per la diagnosi rapida e il follow-up della leucemia linfatica cronica nella popolazione in territorio a rischio.*

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# Cloud-Based Measurement System for the Management of Data from Chronic Lymphatic Leukemia Diagnosis Biosensor

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The biosensor for Chronic Lymphatic Leukemia diagnosis consists of an electrochemical system that converts its chemical characteristic into electrical signals. Therefore, it is possible to convert its characteristic parameters into digital signals and collect data into a Cloud for analysis. The sensing mechanism uses the variation of the electrical conductivity of the NF-ZnO after the cross-breeding process on the bio-probe fixed on the mRNA material. In order to measure these physical quantities and convert it into digital signals its I-V characteristic has been considered related to the mRNA concentration. In particular exciting the biochip with a known current and varying the mRNA concentration, it goes against the electrons transferring from an electrode to the other. Due to its characteristic, it can be compared with a typical resistor [1]. Depending of its electrical size several methods can be considered to measure the resistance of such a material.[2] Experimental results have shown that its typical resistance is about 166kΩ so it can be classified as a resistor of a medium value and the better method to measure it is the two-terminal measurement. A suitable system composed by a constant current generator, and an analogue to digital acquisition system has been designed in order to perform the required measurement. In the **Errore. L'origine riferimento non è stata trovata.** a block scheme of the system is shown.

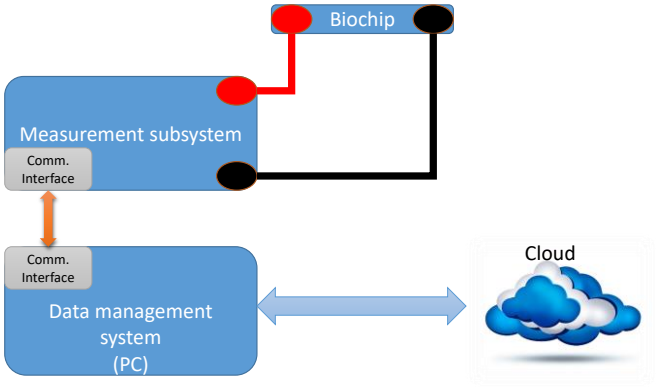


**Figure 1.** Measurement bank block scheme.

In order to collect, manage and analyse measurement results without the presence of the measurement bank a cloud system has been used. In addition, the cloud system allows to integrate the result performed by several measurement banks and to perform statistical analysis on the data gathering information useful to the research development.

***Acknowledgments****: This work was supported by: POR FESR 2014-2020. PROGETTI TRASFERIMENTO TECNOLOGICO E DI PRIMA INDUSTRIALIZZAZIONE PER LE IMPRESE INNOVATIVE AD ALTO POTENZIALE PER LA LOTTA ALLE PATOLOGIE ONCOLOGICHE. CAMPANIA TERRA DEL BUONO. Biochip per la diagnosi rapida e il follow-up della leucemia linfatica cronica nella popolazione in territorio a rischio.*

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**OC-05**

# Targeting cancer cells with nucleic acid aptamers

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Aptamers are short oligonucleotides able to bind with high affinity and specificity their target by acquiring a structured folding. They hold great promise as antagonist of cancer-associated proteins as well as delivery carriers of secondary reagents to target cells [1, 2]. Indeed, aptamers against cell surface receptors, may inhibit the receptor signalling and be internalized into the cell cytoplasm in a receptor-mediated manner. The last function permits their successful application as delivery vehicles of different therapeutic cargoes, including anti-cancer drugs, toxins, and siRNA or miRNA molecules [3]. This allows the cargos' action to be restricted to receptor-expressing target tissues with a consequent reduction of unwanted off-target effects. Here we describe the functional characterization of an aptamer-siRNA conjugate (AsiC), named Gint4.T-STAT3, containing an aptamer that binds to and antagonizes the oncogenic receptor tyrosine kinase PDGFRβ (Gint4.T) and a siRNA targeting the signal transducer and activator of transcription-3 (STAT3) reported as key regulator of highly aggressive tumours including GBM. The AsiC allows the efficient delivery and silencing of STAT3 in PDGFRβ expressing GBM cells reducing cell migration and viability and preventing patient-derived glioma stem-like cell (GSC) function and expansion. Moreover, we show that combining STAT3 AsiC with aptamer-miR-10b antagonist treatments further enhances the inhibition of GSCs sphere formation. Results highlighted the potential of Gint4.T-STAT3 as a high promising inhibitor of GBM tumour dissemination.

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**OC-06**

# Metabolic rewiring connectivity drives enhanced growth drug response by versatile glucose and glutamine utilization in K-Ras tumors.

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Cancer cells are characterized by a flexible metabolic rewiring which use glucose and glutamine to sustain enhanced and unrestricted growth. [1]
Here we show, by systems metabolomics approach, how a detailed metabolic connectivity analysis of *K-ras*-driven lung and colon cancer cells, is useful to investigate the versatile metabolic response of glucose and glutamine utilization to drugs. We rationalize the fact that targeting only one metabolic pathway is not effective, but the combined inhibition of glucose and glutamine utilization is essential to induce a crash in metabolic fluxes, redox balance and mitochondrial activity. Finally we show that oncogenic *K-ras* is able to coordinate glucose and glutamine utilization activating multiple metabolic pathways to adapt to metabolic demands, to sustain enhanced cell proliferation and to survive under drugs treatment. Therefore, it could be envisaged that the identification of metabolic profiling and connectivity signature may be helpful for cancer precision medicine.

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**OC-07**

# In search of New Anticancer Lead Compounds: Combined Use of Molecular Networking and Spectroscopic Techniques for the Fast Detection of New Skeletons

Alessia Caso,[a,*] Germana Esposito,[a] Gerardo Della Sala,[b] Joseph R. Pawlik,[c] Roberta Teta,[a] Alfonso Mangoni[a] and Valeria Costantino[a]

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Caribbean sponges are a prolific source of bioactive secondary metabolites. In particular, sponges of the genus *Smenospongia* have proven to be very rich in chlorinated compounds [1,2,3]. Smenamide A and B showed an interesting antiproliferative activity at nanomolar concentrations, against Calu-1 lung cancer cell line. Therefore, a total synthesis was planned to obtain larger amounts of the compounds to be used in further biological assays. The synthetic project allowed to the synthesis of two analogues of smenamide A, *ent*-smenamide A and 16-*epi*-smenamide A [4]. Recently, the family of smenamide compounds was further expanded with the isolation of smenamides C, D, and E [5].
This study report on the use of Molecular Networking as a potent dereplication strategy to reveal the presence in the organic extract of *S. aurea* of two new members of the smenamide family, smenamide F and G [6].

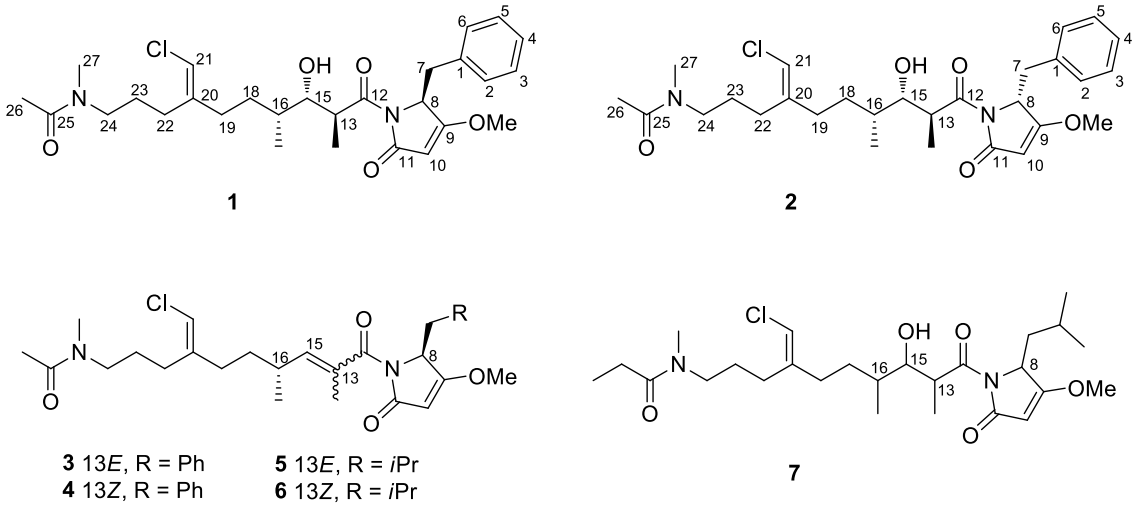


**Figure 1.** Structures of smenamide F (**1**), smenamide G (**2**), and smenamides A–E (**3**–**7**).

The structure of smenamide F (**1**) and G (**2**) was determined by spectroscopic analysis (NMR, MS, ECD). The relative and the absolute configuration at C-13, C-15, and C-16 was determined on the basis of the conformational rigidity of a 1,3-disubstituted alkyl chain system (i.e. the C-12/C-18 segment of smenamide F). Both compounds showed selective moderate antiproliferative activity against MCF-7 and MDA-MB-231 breast cancer cell lines.

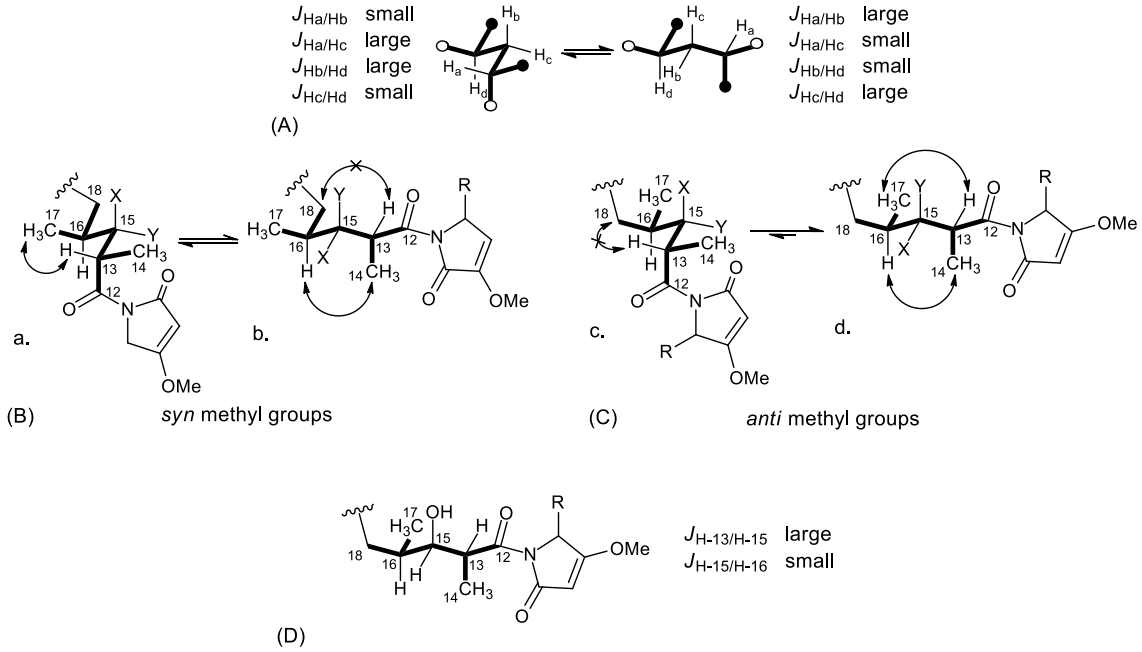


**Figure 2.** (**A**) The only two possible conformations of a 1,3-disubstituted alkyl chain; (**B**) Conformation with syn methyl groups; (**C**) Conformation with anti-methyl groups; (**D**) Configuration of the C-12/C-18 polyketide portion of the molecule.

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# Signal ON and Signal OFF Paper-Based Electroanalytical Strips for Nucleic Acids Detection: Breast Cancer as Case of Study

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The development of paper-based electroanalytical strips as powerful diagnostic tools has gained a lot of attention within the sensor community [1]. In particular, the detection of nucleic acids in complex matrices represents a trending topic, especially when focused towards the development of emerging technologies, such as liquid biopsy. The DNA-based biosensors have been largely applied in this direction and, currently, there are two main approaches based on target/probe hybridization re-ported in literature, namely Signal ON and Signal OFF. In this study, the two approaches are evaluated in combination with paper-based electrodes, using a single strand DNA relative to H1047R (A3140G) missense mutation in exon 20 in breast cancer as the model target. A detailed comparison among the analytical performances, detection protocol, and cost associated with the two systems is provided, highlighting the advantages and drawbacks depending on the application, Figure 1. The present work is aimed to a wide audience, particularly for those in the field of point-of-care, and it is intended to provide the know-how to manage with the design and development stages, and to optimize the platform for the sensing of nucleic acids using a paper-based detection method. In addition to this, recent achievements about the detection of double stranded DNA (associated to HIV) with the employment of redox-tagged triplex-forming oligonucleotides (TFOs) probe are presented [2], highlighting the opportunity of realizing multiple platforms depending on the needs.

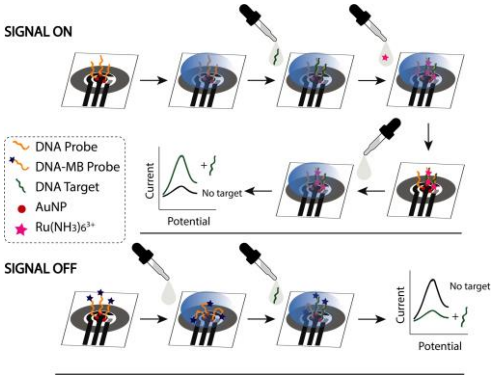


**Figure 1:** Signal ON and Signal OFF platforms for paper-based electrochemical detection of nucleic acids.

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**OC-09**

# Interactions of Porphyrins with Biomolecules, as Potential Anticancer Strategy

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Porphyrins are quite versatile molecules successfully used in many fields: from nanotechnology to biomedicine. These hetero-aromatic macrocycles present remarkable electronic properties which bring to attractive spectroscopic features. The non-covalent interactions of water-soluble achiral porphyrins with biomolecules have been exploited to realize supramolecular switches [1] or to detect and/or stabilize different conformation adopted by proteins and DNA. The interaction of achiral cationic porphyrins with DNA has been extensively studied and utilized as reporters of different sequences of DNA bases [2]. We have reported that a cationic Zn(II)porphyrin (ZnT4) and an anionic Ni(II)porphyrin (NiTPPS) were able to spectroscopically detect the left-handed Z-DNA under highly competitive conditions [3]. Moreover, thanks to its peculiar properties, NiTPPS/Spermine/Z-DNA complex can behave as a reversible AND logic gate, able to reversibly release the chiral information stored in the porphyrin and/or in the DNA helix portion of the supramolecular complex.[3b] To improve the efficiency of our probe we have designed porphyrin spermine derivative able to induce, detect and stabilize the Z form of DNA [4]. Even we have exploited the stabilizing ability of spermine porphyrin derivatives, towards G-quadruplex structures, obtaining some interesting results [5].
Recently we investigated the potency of porphyrins as inhibitor and modulator of proteasome, which is the protein involved in many biological processes. We have found that cationic porphyrins inhibit proteasome peptidase activities, and act as tunable gatekeepers of the 20S, even their positive charge plays a critical role in the inhibition of the CP by shifting the physiological equilibrium between "open" and "closed" conformations [6]. The central role of Ubiquitin Proteasome System in the cell's metabolism and in oncogenes process, explains why the compounds that affect this systems, hold important interest both as biochemical tools to investigate the process controlled by UPS and as novel drugs to treat the diseases stemmed from UPS alteration. The inhibition of proteasome is a promising strategy to cure of tumors.

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**OC-10**

# Biomaterial Design for Regenerative Medicine, Drug Delivery and 3D Microenvironment Mimicry with Potential in Cancer Research

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The combination of biomaterial scaffolds, cells, and bioactive molecules to orchestrate tissue formation and integration within the host environment are at the basis of tissue engineering and regenerative medicine. In the field of tissue engineering and material design, there is a need for tools that can modulate or regulate cell fate, in order to better understand and control the biological response. Different chemoselective conjugation techniques for material surface covalent functionalization with specific glycan structures [1], highlighting how carbohydrate epitopes at the interface between materials and cells may drive their behaviour. For example, neoglucosylated collagen matrices drive F11 neuroblastoma cells to differentiation into active neurons, while different sialylated collagen matrices are able to modulate gene expression toward chondrogenesis or osteogenesis in mMSC. Furthermore, it is now well established that altered glycosylation is a hallmark of malignant transformation [2]. Preliminary results showing that glycosylated scaffolds may be used as models for the study of metastatic niche and regulation mechanisms of cancer stem cell (CSC) will be presented. Finally new chemistries towards 3D scaffolds as drug delivery platforms will be highlited.

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**OC-11**

# Supramolecular Nanostructures for the Selective Delivery of Anticancer Drugs and/or Contrast Agents

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Nanomedicine formulations aim to improve the biodistribution and the target site accumulation of systemically administered (chemo)therapeutic agents. Many different types of nanomedicines have been evaluated over the years, including liposomes, polymers, micelles and hydrogels, and a significant amount of evidence has been obtained showing that these submicrometer-sized carrier materials are able to improve the balance between the efficacy and the toxicity of therapeutic interventions. Besides for therapeutic purposes, nanomedicine formulations have in recent years also been increasingly employed for imaging applications. In this scenario multifunctional nanosystems that combine diagnostic and therapeutic modalities using one unified material have been developed and designated as theranostics [1]. These theranostics nanosystems contain both a drug (small organic molecules, nucleic acids such as RNA and DNA, metal complexes or peptides) and an imaging agent within a single formulation, and they can be used for various different purposes. However, all supramolecular systems currently on the market are not selectively vehicled, and their accumulation on tumor cells is only due to the enhanced permeability and retention (EPR) effect, based on the higher permeability of the vessels around tumor sites. In order to accumulate the drug only on target organs and inside malignant cells, new delivery systems externally modified with bioactive ligands, such as peptides or antibodies have been recently proposed [2]. A considerable number of molecular targets for peptides are either exclusively expressed or overexpressed on both cancer vasculature and cancer cells. They can be classified into three wide categories: integrins; growth factor receptors (GFRs); and G-protein coupled receptors (GPCRs) [3]. These theranostic nanosystems could provide the chance to develop individually designed therapies against various diseases to accomplish personalized medicine [4]. Moreover, the presence of a diagnostic agent in the supramolecular aggregate could also allow a real-time feedback on the efficacy of targeted therapeutic interventions, thus facilitating preclinical efficacy analysis and evaluation of the optimal clinical treatments for each patient.

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**OC-12**

# 3D bioluminescent cancer models

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Bioluminescent (BL) cell-based assays based on two-dimensional (2D) monolayer cell cultures represent well-established bioanalytical tools for preclinical screening of drugs. However, cells in 2D cultures do not often reflect the morphology and functionality of living organisms, thus limiting the predictive value of 2D cell-based assays. Conversely, 3D cell models have the capability to generate the extracellular matrix and restore cell-to-cell communications; thus, they are the most suitable model to mimic in vivo physiology. We developed a nondestructive real-time BL imaging assay of spheroids for longitudinal studies on 3D cell models.
A high-throughput BL 3D cell-based assay in micropatterned 96-well plate format is reported. The assay performance was assessed using the transcriptional regulation of nuclear factor K beta response element. We compared concentration–response curves for tumor necrosis factor-α with those obtained using conventional 2D cell cultures. One of the main advantages of this approach is the nonlysing nature of the assay, which allows for repetitive measurements on the same sample. The assay can be implemented in any laboratory equipped with basic cell culture facilities and paves the way to the development of new 3D bioluminescent cancer models.

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# Diagnostics and Cancer Therapy with Aptamer Nanotechnology

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In the presentation I describe a general approach, which combines development of anti-tumor aptamers, identification of tumor biomarkers, aptamer-assisted fluorescence-guided surgery (AptaFGS) and detection of circulating tumor cells. In two examples, I show how we selected DNA aptamers binding specifically to postoperative human glioblastoma and adenocarcinoma primary tumors, but not to healthy brain and lung cells; determined three-dimensional structure of our lead aptamers; isolated and identified protein targets with biological mass spectrometry. We formulated a tumor specific aptamer spray and applied it during surgery. Main benefits are that the procedure does not require systematic administration of the drug into the circulatory system and involves a few simple steps with no waiting time after the spray application. These bring significant help for a surgeon who needs to distinguish tumor and healthy tissues during surgery. Largely, my study demonstrates the power of synthetic affinity ligands targeting tumors and could supply new opportunities for imaging and eradication of different types of cancer. These findings are of interest to the broad audience in chemical biology, bioanalytical chemistry, cancer biology and medicine.

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