

**VENUE:** Università degli Studi di Genova - Aula E - Dipartimento di Medicina Interna e Specialità Biomediche (DIMI)  
Viale Benedetto XV, 6 - 16132 Genoa, Italy

### Wednesday, January 15<sup>th</sup>

**09.00-10.45 Overview of NAD synthesis pathways, cancer metabolism, cellular and *in vivo* models to study cancer metabolism**

Alessio Nencioni - Università degli Studi di Genova, Italy

**10.45-11.15** *Coffee break*

**11.15-12.45 Methodologies to evaluate the modulation of NAD synthesis pathways (evaluation of intracellular NAD(H) content, NAD synthetizing activity in cells and with recombinant proteins)**

Santina Bruzzone - Università degli Studi di Genova, Italy

**14.00-15.00 Discussion of specific approaches in ESRs' projects on NAD synthesis**

Alessio Nencioni - Università degli Studi di Genova, Italy

Santina Bruzzone - Università degli Studi di Genova, Italy

**15.00-17.00 Lab training: cancer cell cultures for cell viability assays and measurement of intracellular NAD content** [collect and count cells, seed cells in 96-well plates

(for cell viability assay) and in 12-well plate (for NAD-content determination); treatment of cells with inhibitors of NAD synthesis]

Francesco Piacente - Università degli Studi di Genova, Italy

Irene Caffa - Università degli Studi di Genova, Italy

(with the help of ESR1 and ESR2).

### Thursday, January 16<sup>th</sup>

**09.00-10.45 Overview of NAD<sup>+</sup>-signalling pathways (Sirtuins/PARP/CD73/CD38/TRPM2)**

Antonio De Flora - Università degli Studi di Genova, Italy

**10.45-11.15** *Coffee break*

**11.15-12.45 Methodologies to determine sirtuins/CD73/CD38 enzymatic activities. Determination of IC<sub>50</sub> and of different mechanism of inhibition**

Santina Bruzzone - Università degli studi di Genova, Italy

**14.00-14.30 Discussion of specific approaches in ESRs' projects on NAD signalling**

Alessio Nencioni - Università degli Studi di Genova, Italy

Santina Bruzzone - Università degli Studi di Genova, Italy

**14.30-17.30 Lab training: cell viability assay; collection of samples for determination of NAD content and protein determination assay**

(with the help of Francesco Piacente - Università degli Studi di Genova, Italy  
ESR1 and ESR2)

**Friday, January 17<sup>th</sup>****09.00-10.00 Overview of studies targeting NAD biology in cancer**

Alessio Nencioni - Università degli Studi di Genova, Italy

**10.00-10.15** *Coffee break***10.15-13.00 Lab training: determination of NAD content on collected samples with a cycling enzymatic assay**Santina Bruzzone - Università degli Studi di Genova, Italy  
(with the help of ESR1 and ESR2)**14.00-15.00 Concluding remarks and end of the meeting**

Alessio Nencioni - Università degli Studi di Genova, Italy

**Training Activity overview:**

The course will introduce the ESR to the key biochemical assays for measuring intracellular nucleotides and to the cellular models for studying NAD biosynthetic and NAD/nucleotide-signaling enzymes and their inhibitors in cancer. Fellows will have the opportunity to understand the advantages and drawbacks of the different methods and techniques (i.e. different types of enzymatic assays, different methods for detecting cell viability). The goal is to learn how and why to use one approach rather than another.

Fellows will be allowed to bring specific cases of interest. ESRs 1 and 2 will be involved in the organisation of the course.

Duration: 3 days.

Format: theoretical with practical demonstration.

**Expected outcome:**

1. Understanding the need and the ways of measuring the activity of NAD-producing enzymes or of nucleotide-processing enzymes in order to identify inhibitors and define their IC50 and mode of inhibition (i.e. competitive vs. non-competitive).
2. Understanding the different cellular models that are useful for studying NAD-lowering drugs and the pros and cons of the different types of viability/cell proliferation assays.
3. Ability to critically evaluate the application and use of such methods, their advantages and their limitations.