TMS Day of Science 2020 Programme

Prizes sponsored by S.I.A.L.

8.50-9.00	Welcome and introduction: Professor Anna Teti, Fabianna Tennant, Marco Ponzetti, Antonio Maurizi
Session 1: Physics	Chair: Francesco Salamida
9.00-9.30	Expert lecture by Professor Aldo Ianni from the Gran Sasso National Laboratories: "Direct detection of dark matter".
9.30-9.45	*Oral Presentation 1* Valerio D'Andrea from UNIVAQ & INFN: "Beyond Standard Model Physics with the Neutrinoless double beta decay".
9.45-10.00	*Oral Presentation 2* Riccardo Biondi from INFN: "First Direct Experimental Evidence of CNO neutrinos".
10.00-10.02	Session closing remarks and introduction to next session chairs.
Session 2: Chemistry	Chair: Luisa Giansanti
10.05-10.35	Expert lecture by Professor Avi Schroeder from the Israel Institute of Technology, who will talk about novel drug delivery systems.
10.35-10.45	*Oral Presentation 1* Elena Allegratti from UNIVAQ: "Liposomes as a potential drug delivery system for levodopa".
10.45-10.55	*Oral Presentation 2* Vincenzo Marsicano from UNIVAQ: "Gold-catalyzed regioselective functionalization of phthalimido-protected propargylamines: an easy way to access valuable chemicals".
10.55-11.05	*Oral Presentation 3* Valeria Nori from UNIVAQ and Cardiff University: "Triarylborane catalyzed amination reaction: a novel metal-free approach".
11.05-11.20	Session closing remarks and coffee break.
Session 3: Biology	Chairs: Anna Teti
11.20-11.50	Expert lecture by Professor Karl Kadler from the University of Manchester: "The circadian clock, collagen and the search for anti-fibrotics".
11.50-12.00	*Oral Presentation 1* Fabianna Tennant from the University of Manchester: "The Role of Extracellular Vesicles in Osteosarcoma Progression".
12.00-12.10	*Oral Presentation 2* Sara Ponziani from UNIVAQ: "ADC's in Anti-Tumour Therapy".
12.10-12.20	*Oral Presentation 3* Lucia di Nardo from the Catholic University of the Sacred Heart, Rome: "Next-generation sequencing analysis to identify molecular alterations in different basal cell carcinoma subtypes".

12.20-12.22	Session closing remarks
Award Ceremony	Best Presentation (Biology)
	Best Poster (Biology)
	Best Presentation (Physics)
	Best Poster (Physics)
	Best Presentation (Chemistry)
	Best Poster (Chemistry)
	Curiosity Award
13.00	Closing Remarks

Oral Presentation Abstracts

1. The Role of Extracellular Vesicles in Osteosarcoma Progression

Alfredo Cappariello[2], Argia Ucci[1], **Fabianna Tennant**[1], Alexander Loftus[1], Christopher George[1], Kirsty Shefferd[1], Alice Green[1], Simona Delle Monache[1], Maurizio Muraca[2,3], Anna Teti[1], Nadia Rucci[1].
[1]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy; [2]Oncohematology Department, IRCCS Bambino Gesù Children's Hospital Research Laboratories, Rome, Italy; [3]Department of Women's and Children's Health, University of Padua, Padua, Italy

Extracellular Vesicles (EVs) are becoming increasingly recognized as integral signalling vehicles in a range of cancers, including bone malignancies. However, the specific mechanisms by which EVs fuel osteosarcoma progression remain to be fully uncovered. We evaluated the effects of EVs derived from the human osteosarcoma cell line MNNG-HOS (HOS-EVs) on bone resident cells and *vice versa*, finding that HOS-EVs are internalized by osteoblasts and osteoclasts *in vitro,* with potent inhibitory effects on osteoblast metabolic activity, number and alkaline phosphatase (Alp) activity. HOS-EVs reduced the expression of cell cycle and pro-osteoblastogenic genes, whilst transcription of pro-osteoclastogenic factors was increased. Consistently, ELISA analysis demonstrated that HOS-EVs stimulated the release of pro-osteoclastogenic cytokines from osteoblasts such as RankL, Il1b, Il6 and Lcn2. Conversely, HOS-EVs failed to induce osteoclastogenesis, yet treatment with HOS-EVs elicited a reduction in calvarial bone volume and promoted *in vitro* angiogenesis. HOS-EVs were also found to dramatically increase Serpin b2 expression in osteoblasts. To evaluate the significance of this finding we subjected osteoblasts to a forced over-expression of Serpin b2, finding that while Serpin b2 over-expression appeared to have no effect on osteoblast differentiation and viability, pro-osteoclastogenic mRNAs were up regulated. Finally, HOS cells treated with osteoblast-derived EVs (OB-EVs) exhibited a striking decrease in metabolic activity, which was accompanied by reductions in HOS cell motility and invasion following pre-treatment with OB-EVs. Overall, we shed light on a complex EV-mediated crosstalk between osteosarcoma cells and bone resident cells, which appears to influence osteosarcoma progression.

2. ADCs in Anti-Tumour Therapy

Sara Ponziani

ADCs (Antibody-Drug-Conjugates) are immunoconjugates used in drug delivery to transport the drug in the organism safely and selectively, without dispersing the payload in non-target sites. In particular the treatment of tumours with the classical chemotherapy determines widespread toxicity, responsible of severe side effects that can be mitigated by the use of

ADCs. This project is related to the use as a target of the galectin-3 binding protein (Gal-3BP), a secreted protein associated with cancer and metastasis, over-expressed by most cancers. The study involves the use of a non-internalizing immunoconjugate created in our labs, with a humanized and engineered antibody (1959sss) directed to Gal-3BP and a maytansinoid derivative (tubulin inhibitor) as a payload. Engineering the antibody is a very important step, because it allows to make a conjugate with a controlled DAR (Drug-Antibody-Ratio), producing a conjugation product extremely homogeneous. This construct, called 1959sss-DM3, let us to cure A375m melanoma-carrying mice: after in vitro tests to control the specificity of the construct addressed to its target site, the ADC was tested in vivo on A375m xenograft on CD1 mice with a schedule administration of a dose of 10mg / kg twice a week which lead to a complete remission of the tumour. This conjugate thus may represent an innovative product to develop for precision cancer therapy.

3. Next-generation sequencing analysis to identify molecular alterations in different basal cell carcinoma subtypes

L. Di Nardo[1], C. Pellegrini[2], M.G. Maturo[2], F Ricci [3], A. Di Stefani [1], L. Del Regno [1], B. Fossati [1], T. Rocco[2], M.C. Fargnoli[2], K.
Peris[1] [1]Institute of Dermatology, Catholic University-Fondazione Policlinico A.Gemelli-IRCCS, Rome, Italy;[2]Department of Dermatology, Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy; [3]Istituto Dermopatico dell'Immacolata (IDI), Rome

Basal cell carcinoma (BCC) is the most common cutaneous neoplasia in fair-skinned individuals characterized by a great variability in histological features ranging from superficial to nodular common subtypes until the morpheaform and infiltrative rare patterns. Although the aberrant activation of the Sonic Hedgehog pathway (Hh) is known to drive the initiation of BCC, the occurrence of resistance during long-term Hh inhibitors highlights the need for further investigations aimed at elucidating the complex behaviour of BCC subtypes. We performed target sequencing of 57 BCCs (26 superficial and 31 nodular) and matched blood samples on Ion PGM™ System platform of a cancer panel of 12 genes (*CSMD1, CSMD2, DPH3* promote*r, PTCH1, SMO, GLI1, NOTCH1, NOTCH2, TP53, ITIH2, DPP10, STEAP4*), while *TERT* promoter was analysed by direct Sanger sequencing. We confirmed the central role of *PTCH1* and *TP53* that harboured mutations in 72% and 46% of BCCs, respectively. Additionally, we found a high mutation rate in *CSMD1* (63%), *NOTCH1* (44%) and *DPP10* (35%) and frequent non-coding mutations in *TERT* promoter (58%) and *DPH3* promoter (49%). A significant association between *PTCH1* (p=0.008376) and *NOTCH1* (p=0.01764) mutations with the superficial subtype was observed, while the Principal Component Analysis (PCA) confirmed the *PTCH1* mutational profile as the characterizing variable of the superficial BCCs. Moreover, *CSMD1* mutations significantly co-occurred with *TP53* changes (p=0.0002), which may be linked to treatment-related heterogeneity. These findings offer detailed knowledge about the molecular alterations underlying the tumorigenic mechanism of BCCs with a view towards the development of novel rationale-based therapies.

4. Beyond Standard Model Physics with the Neutrinoless double beta decay

Valerio D'Andrea

The dominance of the matter over the antimatter in our universe is one of the most interesting aspects of cosmology. One of the favored models to explain this dominance is the leptogenesis, that is based on the violation of the lepton number. In many extensions of the Standard Model, neutrinos are assumed to be their own antiparticles (Majorana particles), explaining the origin of the low neutrino mass and leading to lepton number violating processes. At present, the only feasible experiments having the potential of establishing that the massive neutrinos are Majorana particles are the ones searching for the neutrinoless double beta (0νββ)) decay. One of the most promising techniques to discover 0νββ decay is by operating High-Purity Germanium detectors enriched in 76Ge. The GERDA experiment, located in the Laboratori Nazionali del Gran Sasso is adopting this technique with 40 kg of enriched germanium deployed into ultra-pure cryogenic liquid argon. GERDA is the leading experiment in the field, reporting the highest limit on the half-life of 0νββ decay with 1.8·1026 yr (90% C.L.), the lowest background index with 6·10−4 cts/(keV·kg·yr) and an excellent energy resolution of 0.12% (FWHM). In this talk, I will discuss the experimental setup, the analysis procedures and the

latest results of GERDA, as well as the future plans to continue the search for 0νββ decay of 76Ge.

5. First Direct Experimental Evidence of CNO neutrinos
Riccardo Biondi

We report the direct observation of neutrinos produced in the carbon-nitrogen-oxygen (CNO) fusion cycle in the Sun with the Borexino detector at the Laboratori Nazionali del Gran Sasso in Italy. This is the first experimental evidence of the existence of such reaction sequence in a star. The CNO solar neutrino interaction rate is 7.2+3.0−1.7 counts per day per 100 tonnes of target at 68% C.L., corresponding to a flux of neutrinos on Earth of 7.0+3.0−2.0 × 108 cm−2 s−1. The absence of CNO signal is disfavoured at 5σ.

6. Liposomes as a potential drug delivery system for levodopa
Elena Allegratti

Parkinson's desease is a neurodegenerative disorder that causes the death of dopaminergic neurons and the loss of dopamine, which is a neurotransmitter involved in the body movements. The presence of the blood-brain barrier prevents the delivery of dopamine to the brain while levodopa, a precursor of this molecule, can cross the barrier and it is rapidly transformed into dopamine by DOPA decarboxylase. However, less than 3% of the administered levodopa gets to the brain so high doses are given to patients to reach the minimum therapeutic concentration. Moreover, levodopa is easily oxidized thus becoming inactive. Despite these limitations, levodopa remains the most effective therapy in the treatment of the disease. A suitable delivery system leading to an increase of the concentration of levodopa in the brain could overcome these limitations. Liposomes, sphere-shaped vescicles, could be suitable levodopa carriers because they could vehiculate also antioxidant molecules together with levodopa to prevent its oxidation. Here the results of an investigation focused on the inclusion of levodopa and an antioxidant (L-ascorbic acid or quercetin) in mixed liposomes are reported. The effect of liposomes composition and of the preparation technique on liposomes properties were studied in detail.

7. Gold-catalyzed regioselective functionalization of phthalimido-protected propargylamines: an easy way to access valuable chemicals.
Marsicano V. (1, 2), Arcadi A. (1) and Michelet V. (2)
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Gold was probably one of the first metals to be discovered by mankind and has been always associated with the concepts of power, beauty and wealth. Although gold has been, and still is nowadays, mostly used in jewellery and coinage, the past decades were marked by an increase in the use of this metal as catalyst in chemical transformations thanks to its unique and powerful reactivity. [1] In several type of transformations, gold has demonstrated better performances than other transition metals, especially in terms of robustness, selectivity and mildness of reaction conditions. Moreover, the reactivity of the metal centre can be finely modulated by choosing the right ligand and additives, enabling to access different organic architectures from the same substrate. [2,3] Nitrogen-containing internal alkynes represents interesting building blocks in organic chemistry for accessing nitrogen-containing entities with widespread applications in the synthesis of bioactive structures. [4] Herein, we would like to report the preliminary results obtained on the use of gold catalysis in the regioselective conversion of phthalimido-protected propargylamines into valuable carbonyl compounds as an alternative to the use of much more toxic mercury reagents. [5]

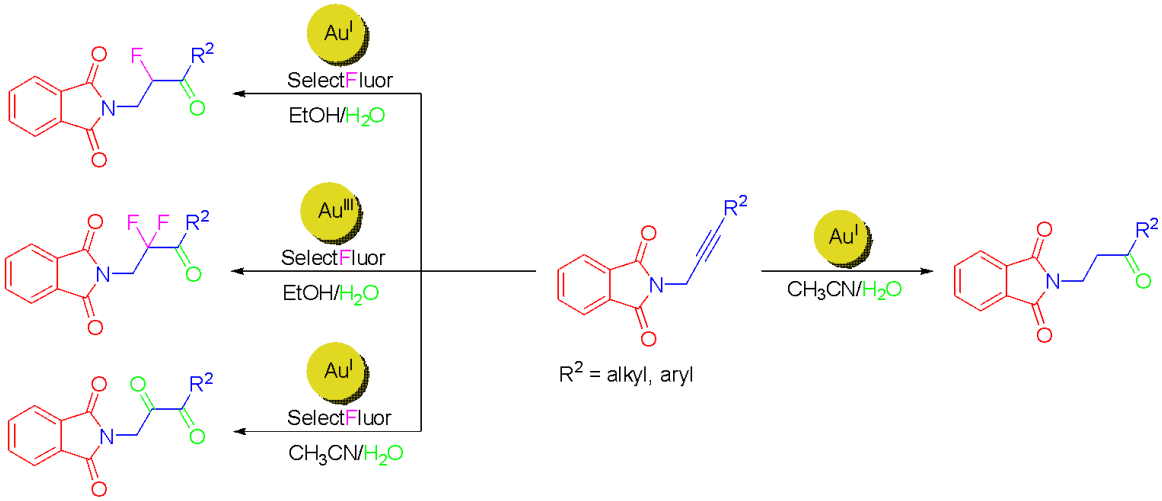

Figure 1. Examples of the reactions studied.

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8. Triarylborane catalyzed amination reaction: a novel metal-free approach

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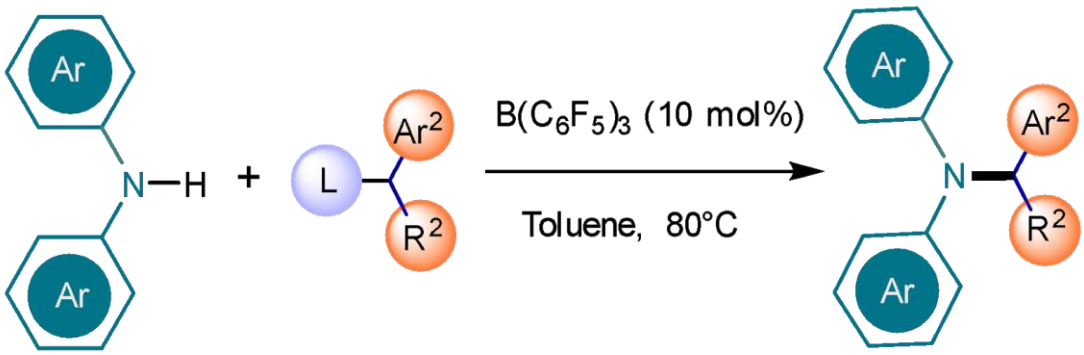
C-N coupled compounds are ubiquitous structural units in natural products, pharmaceuticals, agrochemicals and functional materials.[1] However, forming C–N bonds is still one of the major challenges in the field of cross-coupling chemistry. In the last few decades considerable step forwards have been made in this field using metal catalysis exploiting palladium, copper or nickel as catalysts.[2,3] A metal-free approach for the facile synthesis of C-N coupled products is highly desirable. Tris(pentafluorophenyl)borane has successfully demonstrated its ability to act as a metal-free catalyst for an ever-increasing range of organic transformations.

The ability of halogenated triarylboranes to accept a lone pair of electrons from donor substrates renders them excellent Lewis acids which can be exploited as a powerful tool in organic synthesis.[4] Herein we report the C–N cross coupling reactions of a wide varieties of amine substrates including diarylamines, N-methylphenyl amines, carbazoles, 1H-indoles and 1H-pyrroles with esters using catalytic amounts of $B(C_6F_5)_3$. This mild reaction protocol gives access to C–N cross coupled products (35 examples) in good to excellent yields (up to 95%). The C–N coupling reaction at the propargylic position has also been demonstrated to yield synthetically useful propargyl amines. On the other hand, unprotected 1H-indoles and 1H-pyrroles at the C3/C2 positions afforded exclusively C3 C–C cross coupled products. Extensive DFT studies have been employed to understand the mechanism for this transformation.



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