LET'S BE A SCIENTIST!

2024

AMGEN® Biotech Experience
Scientific Discovery for the Classroom
Italy

7 students, **10 days**, many **experiments**, lots of **scientific discussions** and a generous amount of fun! These are the ingredients of "Let's be a Scientist", a project of **Amgen Biotech Experience Italy** in collaboration with TIGEM (Telethon Institute of Genetics and Medicine) and SSM (Scuola Superiore Meridionale).

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Amedeo Iorillo

LS Aeclanum di Mirabella - Eclano (AV)

As a high school student with a passion for biology, I was extremely thrilled to be selected for an internship at the Telethon Institute of Genetics and Medicine (TIGEM).

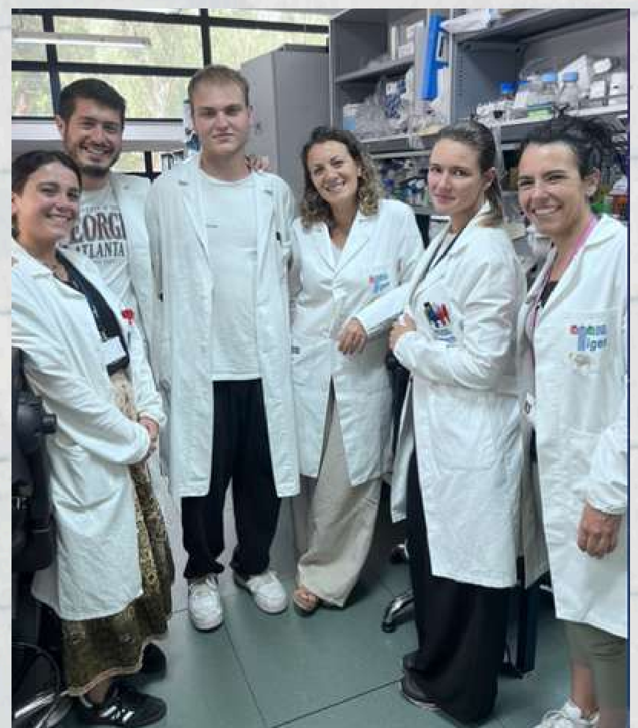
This opportunity **allowed me to work alongside esteemed young scientists in the field of gene therapy**, and thanks to them I had the chance not only to gain a lot of knowledge on the specific subject, but also to learn how to fit in a real laboratory habitat.

During my internship, I had the privilege to be involved in several key activities and projects under the mentorship of experienced scientists. My responsibilities included:

1. **Literature Review**: I conducted extensive reviews of scientific literature to understand the current advancements in gene therapy for retinal diseases. This helped me grasp the theoretical foundations of the research being conducted at TIGEM.
2. **Laboratory Work**: I participated in various laboratory activities, including:
   - **Gene Editing Techniques**: Learning CRISPR-Cas9 technology to edit genes associated with retinal diseases.
   - **Cell Culture**: Culturing retinal cells and maintaining them under sterile conditions for experimentation.
   - **Molecular Cloning**: Cloning specific gene sequences into vectors for further analysis and manipulation.
   - **Data Analysis**: I assisted in analyzing experimental data using bioinformatics tools. This involved interpreting gene expression profiles and identifying potential therapeutic targets.
   - **Team Meetings**: Attending regular team meetings and seminars, where I had the opportunity to present my findings and receive feedback from senior researchers.

My group (Trapani's team) is currently working on retinal diseases like Stargardt disease and retinitis pigmentosa. This hereditary disorder is caused by mutations in the ABCA4 gene, leading to the accumulation of toxic substances in the retina and resulting in progressive vision loss. This group of genetic disorders affects the photoreceptor cells in the retina, causing progressive peripheral vision loss and night blindness. It can result from mutations in various genes, including RHO, RPGR, and USH2A.

I had the opportunity to increment my lab skills and techniques by working with my group: PCR, western blot, trasfections, electrophoresis, etc... were daily routine in the lab. I truly understood how real lab's life is, and the skills I gained will surely be fundamental for my future.
Another aspect of lab's life is represented by public relationships and collaboration. So, groups' meetings and seminars are really important here at TIGEM because by dialoguing and discussing with people, or just by listening at someone who knows more than you do, you can learn a lot and go further your limits, and that's what here at TIGEM is expected.



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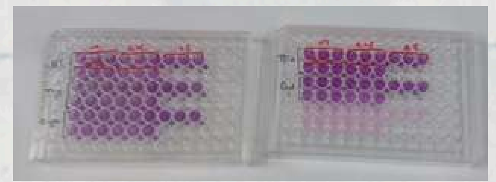
Annalisa Priore
LS N. Sensale - Nocera Inf. (SA)

For ten days, from the 15th to the 26th of July, me and other six high school students had the opportunity to work at the "Telethon Institute of Genetics and Medicine" (Tigem) and see with our eyes what happens inside a lab.
This project, who is called "Let's be a scientist", is organized by AMGEN and taught me a lot. **I understood that being a research fellow is a mix of different things: hard work, discipline, respect and, last but not least, passion.**

During these days at Tigem, I have been working in the blue area in the Polishchuk's lab under the supervision of my tutor Raffaella Petruzzelli. The TIGEM, indeed, is divided into 3 different areas recognisable by the colour of the floor. There is the green, the red and, like said before, the blue area and every colour is associated with the type of studies that are taken there: molecular therapy, genomic medicine and cell biology respectively.

My tutor, Raffaella, in the research team where I have been working, is focused on the study of Wilson Disease (WD) and, in particular, on the discovery of novel therapeutic strategies for patients suffering from this disorder. This pathology is an autosomal recessive disorder characterized by impaired copper excretion caused by defects in the copper transporter protein ATB7B. The clinical features of WD result from toxic accumulation of copper first in the liver and then in the brain. Treatments for WD are focused on two aspects: copper excretion, that must be increased with the use of copper chelants, and copper absorption, that must be reduced through the diet. In the lab, there are different tools to study WD: hepatic cell lines that present loss of function in ATP7B gene (ATP7B KO HepG2 cells) and animal models that recapitulate the same features of WD phenotypes. One of the animal models I got the opportunity to see in this lab is Caenorhabditis Elegance. This is a tiny worm (1mm) and is very useful in WD research studies as the Cua1 gene, the orthologous of ATP7B in humans, is deleted.

Therefore, it is widely used in the lab to test new drugs and, thanks to its anatomy is much easier to understand the neurological abnormalities present in WD.



At Tigem, I have been involved in different experimental procedure and I got the chance to watch and learn several research techniques:
Immunofluorescence: This experiment has been done to look at the over-expression of a Prion protein in the ATP7B KO HepG2 cells because this protein can have implications in WD conditions. Following fixation of ATP7B KO Hepatic cells we incubate them with a primary mouse antibody against Prion and, after several washing, the cells were incubated again with the secondary mouse antibody that has to be conjugated with the fluorochrome. We then analyzed the slides under Confocal Microscopy and the results of this experiment confirmed that the presence of the prion protein in the cells increase cell mortality upon copper treatment.

MTT: This assay is helpful to measures cell viability and proliferation. Metabolically active cells reduce yellow MTT to purple formazan crystals, which are quantified via spectrophotometry. Higher absorbance indicates more viable cells. We used it to understand if the vitality of the cells was affected by the presence of Cu. In particular we used 3 types of cells (wild type, ko for ATP7b and double ko for ATP7B and Prion protein) and different concentrations of copper. The results were not as expected probably because of the overcrowding of the cells.

Real time qPCR: this experiment quantifies the amount of RNA in samples by amplifying target sequences and measuring fluorescence emitted during each cycle. This allows for precise quantification of nucleic acids in real-time. We used this technique to verify the presence of the transmembrane protein CD44, that promotes copper uptake, in different cell lines. This experiment demonstrated that this gene is higher expressed in Hela cells (ovarian cell lines) but not in the hepatic cells ko for ATP7B used in the current research project held by Raffaella in the lab.

PCR: The PCR (Polymerase Chain Reaction) amplifies specific DNA segments, creating millions of copies from a small initial sample. It involves repeated cycles of denaturation, annealing, and extension using a DNA polymerase enzyme, a mix of primers that anneals specifically to the target sequence, and a mix of nucleotides. We performed a PCR on cDNA samples isolated from RNAs of different human cell lines to confirm the amplification of CD44. After running the samples on a 3% agarose gel through electrophoresis the results were almost the same as the real time, so the experiment was successful.
Western blot: Western blot is a laboratory technique used to detect specific proteins in a sample. It involves gel electrophoresis to separate proteins by size, transferring them to a membrane, and using antibodies to visualize the target protein.

Apart from experiments we had to do also drugs treatments to the cells and cells passaging (or splitting). This consists in transferring a small number of cells from a growing culture to fresh growth medium to prevent overcrowding. In this way it is possible to maintain cell health and continuous growth. What I have learned in tissue culture, is that sterility and precision are essential to ensure consistent experimental conditions and long-term maintenance of cell lines.
I also prepared some solutions like HEPES that is a buffering agent used in biological and cell culture studies to maintain physiological ph. To prepare it I had to dissolve HEPES powder in distilled water, adjust the pH with NaOH or HCl, and bring to the desired volume. In addition, I made the agarose gel by mixing TAE 1x with agarose, heat it, add a safe DNA gel stain (syber safe) and then pouring the gel with a comb in the appropriate mold.
At the end I want to thank my tutor Raffaella but also the other members of Polishuck’s lab Federico, Roberta and Domenico for being so patient with me and Lena for the “magic solution”.

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Giuseppe Caporaso

LS Rummo - Benevento

From 15th to 28th of July I had the opportunity to attend the TIGEM's (Telethon Institute of Genetics and Medicine, in Pozzuoli (NA)) research labs thanks to the internship program "Let's be a scientist" organized by Amgen Biotech Experience Italy. I was one of the seven lucky high school students that had the possibility to live, for ten days, an extract of the researchers' lives.

At the beginning of our adventure each student was assigned to a tutor, a researcher each belonging to different research groups, to observe the activities carried out by the team and to do experiments under professional supervision. Having had different experiences we felt the need to share what we've learnt in order to achieve a more complete understanding of what being a scientist and a researcher mean.

During this period I had the opportunity to work with the scientists of Brunella Franco's research group, in particular with Francesco M. and Nunziana, two PhD students, Alessandro, an undergraduate student, Roberta and Francesco C. In particular Francesco was studying how mutations found in the HNF1B gene affected patients causing kidney cysts and diabetes (comprehending MODY5 (Maturity Onset Diabetes of the Young), a rare form of diabetes caused by an abnormal functioning of pancreatic beta cells leading to an insufficient production of insulin).

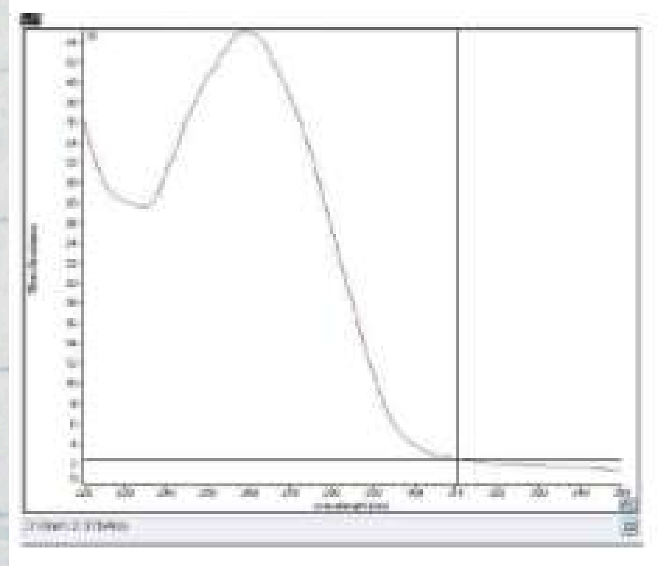
Since the first day I was involved in the research process as Francesco and Alessandro explained to me what they're working on, introduced me to the other members of the group, explained to me some of the techniques we've been using in the following days and made me observe the cells that Francesco was cultivating.

During this time **I saw so many different experiments made by the researchers using different techniques**; surely I gained a lot from this experience: I learnt how to analyze protein expression in cells with Western Blot; how to amplify the nucleotide sequence in DNA with PCR; how to introduce mutations in genes by CRISPR/Car9; how to read technical graphs produced to summarize what it has been discovered after an experiment. In addition, **I learnt how to search and correctly understand a scientific publication and how earning new information about what it has been studied is important**. This experience let me also understand the role of technology in scientific research as programs and AI can help scientists make hypotheses and collect information from the previous studies of other people. Last but not least I learnt how beneficial it is working in groups as you have people you can count on.

[KO] = 41,9 ng/µL
260/280 (contaminazione da proteine) = 1,99
230/260 (contaminazione da fenoli) = 2,47

[WT] = 302 ng/µL
260/280 = 1,94
230/260 = 1,71

[OE] = 376 ng/µL
260/280 = 1,97
230/260 = 1,76



In these 10 days I had the pleasure to execute many experiments by my own hand in parallel with my supervisors. The one I was more involved in was the analysis of the sex of DNA extracted by cellular pellets. This experience lasted two days as it was needed for the PK (proteinase K) one night to work properly. The first day I collected different cellular pellets each one with cells with different expressions of the protein OFD1 (Wild Type, Knock Out, OverExpressing). I induced cellular lysis and degradation of proteins adding a buffer with enzymes (in which there was also PK) that affect proteins and cellular structures despite DNA.

This buffer had to work overnight at a temperature of 55 ºC in order to have optimal results. The day after we had to inactivate the enzymes raising the temperature of the samples to 75 ºC. We added Ethanol 100% concentrated in order to purify the samples. We collected the DNA filaments that were floating in the ethanol solutions and washed them with a solution of ethanol 70% concentrated. We analyzed the samples with a Spectrophotometer to evaluate how pure our samples were in order to determine if it was necessary to do other purifying processes or if it was ready for the next phase. Luckily the samples were pure enough to be processed in the PCR. The PCR (Polymerase chain reaction) is a technique used to amplify (make numerous copies of) specific regions of the Nucleotide sequence. The samples for the PCR need to have different components: DNA; Primers selected in order to pair with a specific region of DNA (one Fw and one Rv to amplify both the filaments of DNA); DNA polymerase; ions that are cofactors of the DNA polymerase. In order to achieve the best result possible in PCR the thermal cycler has to be setted with the right program (each program has phases with different temperatures and different timings). Selecting the right program we charged the samples and waited until it had done. The last phase of our experiment was electrophoresis, a technique used to separate particles based on their size and on their charge. These particles flow through a gel made of agarose thanks to electric current. The gel for our experiment had a concentration of agarose of 2% and we charged our samples in duplicate. After time our DNA flowed through the gel, we took our gel from the cell and put it in Gel Doc to see if our DNA was amplified correctly and how the primers interacted with it in order to understand, in our case, the sex of the cells that composed our pellets. It comes out, after this experiment, that our cells were female as we expected.

In the end, **it has been an experience that I never could have lived without this project. Working for ten days with so many people all sharing the same interest in science let me learn a lot of things, first of all how to work in groups and how talking with each other sharing everybody his own ideas it's important in order to find solutions to any kind of issue.** It's definitely an experience I would like to repeat as every day's a school day.

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Elena Occhione

LS E. Fermi - Ragusa

During the time I had the chance to spend at Tigem's Laboratories I had the opportunity to follow the experiments carried out by the Post-Doc researcher Simona Pellecchia. She was diving into some experiments involving a Tet-On Cas9 inducible system aimed at knocking out the BRCA1 gene in triple-negative breast cancer (TNBC) cells. This research is particularly important, as it seeks to understand how knocking out BRCA1 might influence the sensitivity of these cancer cells to PARP inhibitors.

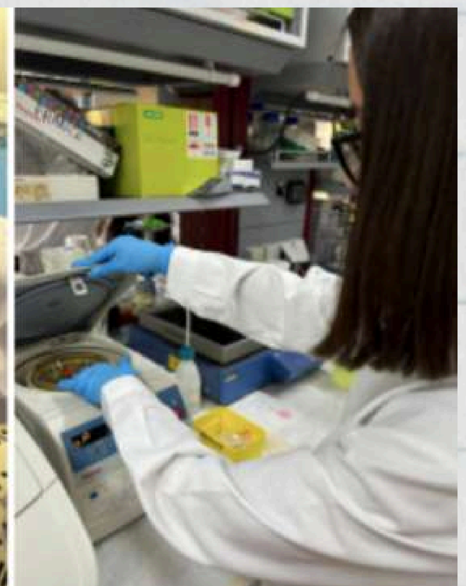
TNBC is quite notorious: it's a highly aggressive form of breast cancer that lacks oestrogen receptors, progesterone receptors, and HER2. Because of this unique profile, treatment options for TNBC are often quite limited, and many standard therapies can be ineffective: **that's why innovative strategies are crucial in finding new ways to treat this challenging subtype of cancer**. One promising direction in this regard is the exploration of PARP inhibitors, especially for tumours that have deficiencies in DNA repair mechanisms, like those associated with BRCA1 mutations.

What makes this research particularly exciting is the combination of cutting-edge CRISPR/Cas9 technology with tetracycline-controlled transcriptional activation (Tet-On) systems. This powerful match allows researchers to induce specific gene knockouts in a controlled way, enabling them to elucidate the role of genes like BRCA1 in cancer biology. In our experiments, we utilized engineered non-tumorigenic cell lines, specifically MCF12A, as our model to knockout the BRCA1 gene.

One of the most stimulating moments for me was observing the system's effectiveness just 24 hours after we introduced doxycycline. Indeed, we were able to monitor the expression of the blue fluorescent reporter fused to Cas9 protein using a fluorescent microscope. **Seeing the results under the microscope really highlighted the impact of our work: it was priceless seeing all those little cells shining on the computer's monitor, letting us know the experiment was successful!**

I had a hands-on role in the research process, particularly in the cloning of single guide RNA (sgRNA) for our CRISPR applications.

This involved several intricate steps, including the phosphorylation and annealing of oligonucleotides, ligation of the annealed sgRNA oligonucleotides into a linearised plasmid vector and transformation of competent E. coli Top10 cells with the ligation reaction using a heat-shock method. The transformed cells were plated on LB agar containing the ampicillin selection and incubated overnight at 37°C. In the end, Colonies were screened using colony PCR to confirm the insertion of the sgRNA sequence by using specific primers for the sgRNA insert for amplification.



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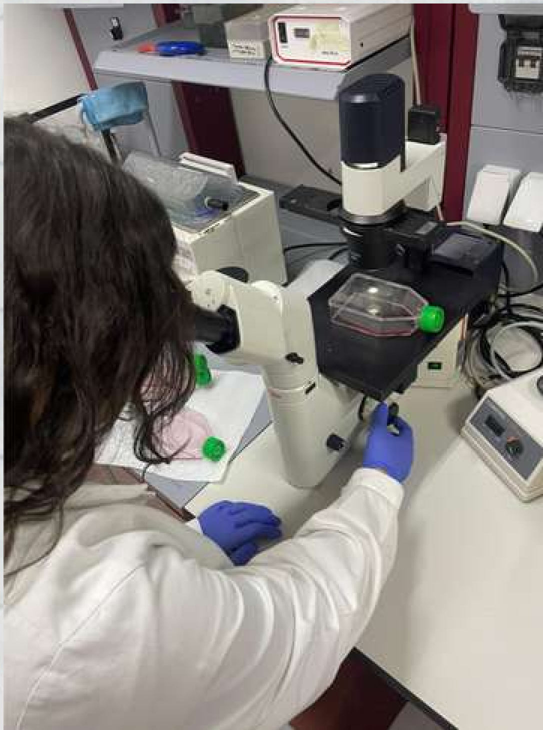
Rossella D'Arienzo
IISS Marconi Margherita Hack - Bari

Amgen Biotech Experience (ABE) is an innovative science education program that introduces students to the exciting world of scientific research and builds bridges between school and real-life science. Thanks to this project, I had the opportunity to join the Telethon Institute of Genetics and Medicine (TIGEM) for 10 days together with other six students. TIGEM is a multidisciplinary research institute dedicated to the study of rare genetic diseases with the aim to develop innovative gene therapies.

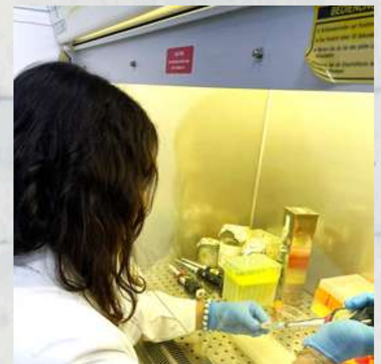
During this period, I had the opportunity to work in Dr. Sandro Banfi's group interacting with different researchers and in particular I have been working with Filomena, Martina and Paola. Their work is focused on finding new therapies and understanding the molecular basis of inherited retinal diseases, such as retinitis pigmentosa. **They give me the chance to collaborate with them, understanding the scientific reasons of their experimental research and performing different experiments.** One of the main experiments I performed involves cell culture using HeLa cells, which are immortalized tumor cells. These cells are widely used in the laboratory because they are easy to grow and manipulate, and they represent a useful model for many types of research. I used a culture medium composed of DMEM, 10% FBS, and 1% antibiotics (to avoid contamination). During my experience, I learned to thaw cells, split them (subculture) to keep cells growing, and freeze them again for long-term storage.
Furthermore, I performed a PCR, for detecting the possible presence of mycoplasm within the Hela cell culture. Mycoplasma is a type of bacterium that can contaminate cell cultures so altering the results of the experiments. It can be easily found on the operator's hands, so it is important to check its absence. For this purpose, we took the medium that had been in contact with the cells and then amplified, via PCR, the possible presence of mycoplasma DNA. After that we analyzed DNA using agarose gel and running the samples through an electrophoretic run.

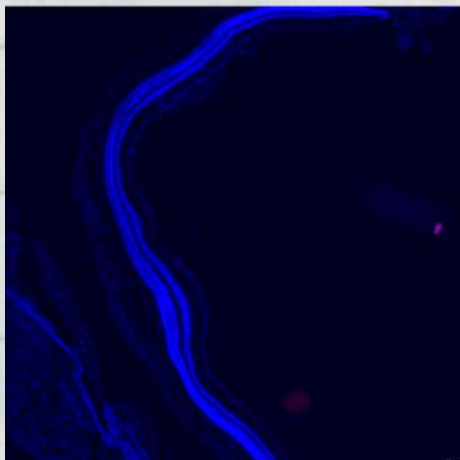


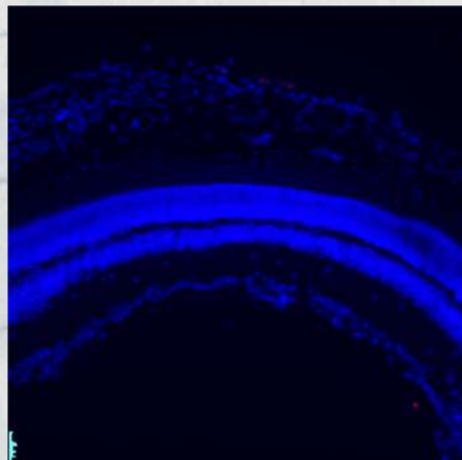
Another activity I performed was extracting RNA from HeLa cells used in culture. Although this experiment had no specific purpose, it served me to practice and improve my skills, having previously seen an extraction of RNA from the retina.

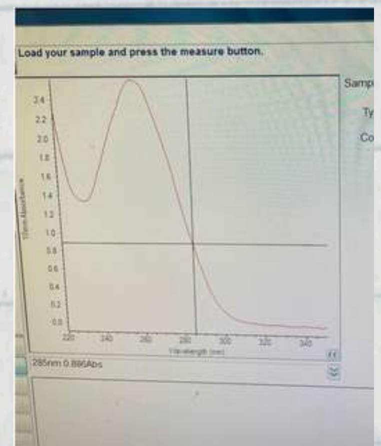
Finally, I performed immunofluorescence with DAPI, a dye that binds DNA and allows cell nuclei to be visualized under a fluorescence microscope. This technique was useful for studying the organization and morphology of cells and visualizing in detail the various layers of the retina. I performed immunofluorescence on retinal sections of two mouse models: wild type and a diseased mouse model (P347S).

Thanks to this experience, I have acquired valuable practical and theoretical skills in the field of cellular and molecular biology, which will be useful in my future scientific career. I greatly appreciated the support of people with whom I have worked. **They constantly assisted me, encouraging me and helping me to fully understand both the theoretical and practical parts of the laboratory work.**









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Massimo Palmisano

Liceo G. Salvemini - Bari

"Research is the process of going up alleys to see if they are blind". I came across this quote from the Greek philosopher Plutarch last year. It has a pretty strong meaning for me: "research" means you can either find astonishing results or go back home knowing that the path you were following is a dead end. **However, in both cases scientific research comes to a success, even if it seems to be a failure.**

I've always been curious and enthusiastic about the world of scientific research. Thanks to "Let's be a scientist", a project of Amgen Biotech Experience (ABE) Italy in collaboration with SSM (Scuola Superiore Meridionale) and TIGEM (Telethon Institute of Genetics and Medicine) I've had the opportunity, along with 6 other students with whom I shared this experience, to live two weeks as a research fellow.

The project Let's be a scientist of the ABE Italy, allowed me and my colleagues to be part of a 10 days stage in the SSM - TIGEM center of research (Pozzuoli, NA). Here each one of us has been assigned to a distinct research group: this gave us the chance to have different experiences and to discuss them.

The environment in which the research fellows work is uplifting and stimulates everyone who's involved there. The research group I worked with enhanced my curiosity in their work and also made me experience what being a researcher means (including what Plutarch called blind alleys).

Their research is centered around understanding the role of RagGTPases, which are crucial regulators of cellular metabolism and growth. This class of proteins controls the activities of both mTORC1 (mechanistic target of rapamycin complex 1) and TFEB (Transcription factor EB). Specifically, they investigate the implications of self-activating mutations in RagD and how these mutations affect the activity of MiT/TFE transcription factors (TF), impairing it. These TF are essential for the proper functioning of lysosomes and autophagy, processes critical for cellular homeostasis.

The dysfunction of MiT/TFE due to these mutations has been linked to the development of some kinds of cancer, renal tubulopathy (a kidney disorder) and dilated cardiomyopathy (a heart condition). This discovery has significant implications for understanding the molecular basis of these diseases and potentially developing targeted therapies.
The research carried out by the research group I worked with has the goal to find a therapeutic response for the effects of the constitutive activation of RagD, such as reactivation of MiT/TFE function.

Participating in this research allowed me to learn various laboratory techniques, such as cell culture, gel electrophoresis, PCR and immunofluorescence. I was able to learn the use of many laboratory instruments, such as Western blot and Nanodrop and confocal microscope too. Also, I had the opportunity to see the animal facility. I observed firsthand the meticulous process of designing experiments, collecting data, and analyzing results. Each day was filled with new discoveries and challenges that fueled my curiosity.

This experience at SSM - TIGEM was incredibly formative and solidified my decision to pursue a career in scientific research. The dedication and enthusiasm of Dr. Ballabio's team deeply inspired me. I am grateful for the opportunity to be part of such an important discovery and am eager to continue my academic and professional journey with the same spirit of passion.

I want to extend my heartfelt thanks to TIGEM, Dr. Andrea Ballabio's entire team, (in particular the Dr. Irene Sambri, Rossella Pennella and Barbara Rossi) and Mrs. Anna Pascucci, director of ABE Italy, for welcoming and guiding me through this extraordinary scientific adventure.

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Alfonso Grimaldi

LS G. Da Procida - Salerno

Throughout my life, I have always had a strong inclination towards discovery and understanding how the world around us works. Over the years, this curiosity has drawn me closer to science, particularly biology and biotechnology. Thanks to this inclination, I have always wanted to work in research and experience life as a researcher. I had the opportunity to live this vision of science during an internship at the TIGEM research center in Pozzuoli, Naples, through the Amgen Biotech Experience (ABE) program.

This program, promoted by ANISN (Associazione Nazionale Insegnanti di Scienze Naturali) in collaboration with the University Federico II of Naples and the CNR, provides high school students with materials, content, and training to get closer to molecular biology and biotechnology.

The project "Let's Become Scientists!" allowed my colleagues and me to undertake a 10-day internship at TIGEM, where biologists, biotechnologists, engineers, and doctors work together to transform basic research into practical medical treatments to improve the health of people affected by genetic diseases.

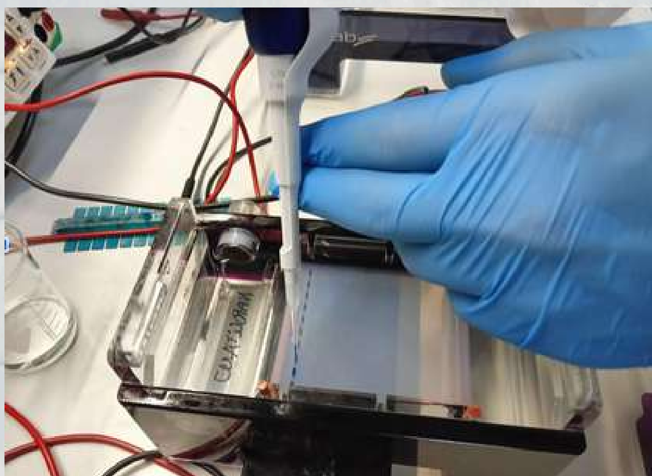
Our research group primarily focuses on discovering new possible cures or treatments for hereditary genetic diseases that cause tumors and neurodegenerative diseases. A key objective is to find new approaches to identify the genetic mutations responsible for these diseases using advanced sequencing techniques. The biological questions we seek to answer include how these mutations influence the development of diseases and how we can intervene to prevent or treat them effectively.

My tutor, Alessandra Esposito, focuses on the metabolic pathway of mTORC1, a key protein that regulates cell growth and proliferation. Our work aims to understand how the non-canonical pathway of mTORC1 can lead to tumor formation, with the goal of developing new targeted therapies.

The methodologies employed in our work include PCR, which allows us to amplify specific segments of DNA, facilitating their detailed analysis. We use agarose gel electrophoresis to separate and visualize amplified DNA fragments. DNA sequencing is a crucial step for identifying mutations present in genes of interest. Finally, western blotting enables us to transfer and analyze specific proteins, providing information on their presence and quantity in biological samples.

During my experiment, I prepared a buffer for protein quantification, which is essential for determining protein concentration in samples. Subsequently, I loaded the amplified DNA via PCR into an agarose gel and performed an electrophoretic run to separate the DNA fragments. This process was followed by a detailed analysis of the results obtained.

The results of my experiment were positive: I was able to confirm that DNA amplification occurred correctly in several patient samples, with only two samples not responding to PCR. This confirms the protocol's effectiveness in most cases and provides a solid basis for further analysis and studies.



Thanks to the research conducted by our group, new prospects are opening up for developing potential cures or therapies against tumors such as follicular lymphoma. Our research aims not only to treat these diseases but also to better understand how cells respond to their environment. This understanding can lead to more targeted and personalized therapeutic interventions, thus improving treatment efficacy and patients' quality of life.
Before I was allowed to follow and collaborate in the researchers' activities, I was introduced to the laboratory work environment. I learned to use specific technologies, such as micropipettes and various devices, and received a clear explanation of the processes I would be performing. This allowed me to gain a theoretical understanding of techniques and procedures such as electrophoretic runs, western blots, and PCR. Additionally, I was provided with all necessary precautions to work safely, especially when handling hazardous reagents like DNA intercalants (such as ethidium bromide) or methanol.
In conclusion, I want to emphasize that not all experiments conducted were successful on the first try. However, this is absolutely normal in a researcher's work. A proactive and confident attitude, supported by solid knowledge of the principles, has always ensured the success of subsequent efforts.
There are not enough words to express my gratitude to Ms. Anna Pascucci for allowing us to experience such an incredible opportunity, my tutor Alessandra Esposito and the entire group of researchers who guided me during my activities.

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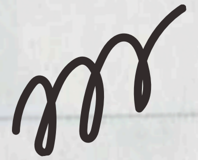

Would you like to live an immersive experience in science? Apply to "Let's be a Scientist"! The next one, it might be you!