CURRICULUM VITAE

PERSONAL DATA

Surname:	Balestra
First Name:	Dario
Gender:	Male
Address:	Via della Piantata 20-F
City:	Pontegradella (Ferrara)
Postcode:	44123
Country :	Italy
Telephone:	+039 3395706249
Email:	blsdra1983@gmail.com
Nationality:	Italian
Date of birth:	14th March 1983
Military service:	exempt

PROFESSIONAL EXPERIENCE

Period:	1st May 2013 – 30st April 2014
Position:	Postdoctoral fellow, research grant founded by Emilia Romagna region, Italy (competitive call).
Institution:	Laboratory of Molecular Biology and Hemostasis, Department of Molecular Biology, University of Ferrara, Italy.
Research field:	New strategies for the identification in the coagulation field of biomarkers related to rehabilitative therapies.
Supervisor:	Prof. Mirko Pinotti.

Period:	1st May 2012 – 30st April 2013
Position:	Postdoctoral fellow, research grant founded by Telethon Foundation, Research and Cure for Inherited Genetic Diseases, Italy (competitive call).
Institution:	Laboratory of Molecular Biology and Hemostasis, Department of Molecular Biology, University of Ferrara, Italy.
Research field:	New therapeutic strategies for human inherited genetic diseases caused by splicing mutations.
Supervisor:	Prof. Mirko Pinotti.

Period:	1st January 2009 – 31st December 2011
Position:	PhD student, Research doctorate in Biochemistry, Molecular Biology and Biotechnologies, founded by Ministerial grant for Doctorates (Italy), competitive call.
Institution:	Laboratory of Molecular Biology and Hemostasis, Department of Molecular Biology, University of Ferrara, Italy.

Research field: -Molecular characterization of splicing mutations occurring in genes for coagulation factors.
-Development of engineered small RNAs (U1snRNA) as innovative therapeutic tool for inherited diseases caused by splicing mutations
Supervisor: Prof. Francesco Bernardi.
Award: Award as Best PhD Thesis of Research doctorate in Biochemistry, Molecular Biology and Biotechnologies

Period: 28th October 2010 – 22nd March 2011
Position: Research Scholar, founded by Institute for Higher Studies (IUSS Ferrara) of University of Ferrara, Italy, competitive call.
Institution: The Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, USA.
Research field: In vivo evaluation of efficacy of the engineered small RNA (U1snRNA) in the treatment of coagulation FVII deficiency associated to the splicing mutation FVII-9726+5g/a mutation.
Supervisor: Prof. Valder R. Arruda.

Period: 4th October 2009 – 6th February 2010
Position: Research Technician.
Institution: Department of Molecular Biology, University of Ferrara, Italy.
Research field: assistant technician for the laboratories held during the course of Molecular Biology, University of Ferrara, Italy.
Supervisor: Prof. Francesco Bernardi.

Period: 23th February 2009 – 22nd May 2009
Position: Research Technician.
Institution: Department of Molecular Biology, University of Ferrara, Italy.
Research field: assistant technician for the laboratories held during the course of Recombinant Technologies, University of Ferrara, Italy.
Supervisor: Prof. Mirko Pinotti.

Period: 3th December 2007 – 3th December 2008
Position: Research Fellow, founded by University of Ferrara, Italy.
Institution: Laboratory of Molecular Biology and Hemostasis, Department of Molecular Biology, University of Ferrara, Italy
Research field: Approaches to correction of splicing mutation in the gene of coagulation factor VII
Supervisor: Prof. Francesco Bernardi

EDUCATION

Period:	1st January 2009 – 31st December 2011
Description:	PhD. Research doctorate in Biochemistry, Molecular Biology and Biotechnologies, founded by Ministerial grant for Doctorates (Italy), competitive call.
Institution:	Laboratory of Molecular Biology and Hemostasis, Department of Molecular Biology, University of Ferrara, Italy.
Research field:	-Molecular characterization of splicing mutations occurring in genes for coagulation factors. -Development of engineered small RNAs (U1snRNA) as innovative therapeutic tool for inherited diseases caused by splicing mutations
Supervisor:	Prof. Francesco Bernardi.
Award:	Award as Best PhD Thesis of Research doctorate in Biochemistry, Molecular Biology and Biotechnologies

Period:	27th September 2005 – 11th July 2007
Description:	Master Degree in Biomolecular and Cellular Sciences
Training:	Laboratory of Molecular Biology and Hemostasis, Department of Molecular Biology, University of Ferrara, Italy
Research field:	Coagulation Factor VII deficiency: splicing mutations characterization and rescue by modified U1snRNA
Diploma thesis:	U1snRNP-mediated rescue of mRNA in severe factor VII deficiency
Final degree mark:	110/110 magna cum laude.
Average score of the University studies:	29.4 / 30
Weighted average of the University studies:	29.45 / 30

Period:	9th September 2002 - 19th October 2005
Description:	Bachelor Degree in Biomolecular and Cellular Sciences
Training:	Laboratory of Molecular Biology and Hemostasis, Department of Molecular Biology, University of Ferrara, Italy
Research field:	Creation of minigenes to study
Diploma thesis:	Construction of Coagulation Factor VII minigenes to study intronic mutations
Final degree mark:	110/110 magna cum laude.
Average score of the University studies:	28.91 / 30
Weighted average of the University studies:	29.05 / 30

Period:
Description: High School Diploma
Institution: High School L.Ariosto, Scientific course. Ferrara, Italy
Final degree mark: 80/100.

PUBLICATIONS

Journal of Thrombosis and Haemostasis. Under revision

An engineered U1 small nuclear RNA rescues splicing-defective coagulation *F7* gene expression in mice

Dario Balestra*, Armida Faella†, Paris Margaritis†,‡ , Nicola Cavallari*, Franco Pagani§, Francesco Bernardi*, Valder R. Arruda†,‡ and Mirko Pinotti*.

** Department of Life Sciences and Biotechnology, and LTTA, University of Ferrara, Ferrara, Italy;*
† Department of Pediatrics, The Children's Hospital of Philadelphia, Philadelphia, PA, USA;
‡ University of Pennsylvania, Perelman School of Medicine, Philadelphia, PA, USA;
§ International Centre for Genetic Engineering and Biotechnology, Trieste, Italy.

Biochim Biophys Acta. 2012 Jul;1822(7):1109-13. Epub 2012 Mar 9. PMID: 22426302.

Activation of a cryptic splice site in a potentially lethal coagulation defect accounts for a functional protein variant

Nicola Cavallari[a], **Dario Balestra[a]**, Alessio Branchini[a], Iva Maestri[b], Ampaiwan Chuamsunrit[c], Werasak Sasanakul[c], Guglielmo Mariani[d], Franco Pagani[e], Francesco Bernardi[a], Mirko Pinotti[a],

[a] Department of Biochemistry and Molecular Biology, and LTTA, University of Ferrara, Italy
[b] Experimental and Diagnostic Medicine, Section of Anatomic Pathology, University of Ferrara, Italy
[c] Department of Pediatrics, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[d] Department of Internal Medicine and Public Health, University of L'Aquila, Italy
[e] International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Hum Mol Genet. 2012 Jun 1;21(11):2389-98. Epub 2012 Feb 23. PMCID: PMC3349419.

An exon-specific U1 small nuclear RNA (snRNA) strategy to correct splicing defects.

Eugenio Fernandez Alanis,[1,†] Mirko Pinotti,[2,†] Andrea Dal Mas,[1,†] **Dario Balestra,[2]** Nicola Cavallari,[2] Malgorzata E. Rogalska,[1] Francesco Bernardi,[2] and Franco Pagani[1]

[1]Human Molecular Genetics, International Centre for Genetic Engineering and Biotechnology, Trieste, Italy, and [2]Department of Biochemistry and Molecular Biology, University of Ferrara, Italy.
[†]The authors wish to be known that, in their opinion, the first three authors should be regarded as join First Authors.

Blood. 2009 Jun 18;113(25):6461-4. Epub 2009 Apr 22. PMID: 19387004.

Rescue of coagulation factor VII function by the U1+5A snRNA.

Mirko Pinotti[1], **Dario Balestra[1]**, Lara Rizzotto[1], Iva Maestri[2], Franco Pagani[3], and Francesco Bernardi[1]

Departments of [1]Biochemistry and Molecular Biology and [2]Experimental and Diagnostic Medicine, University of Ferrara, Ferrara; and [3]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

Blood. 2008 Mar 1;111(5):2681-4. Epub 2007 Dec 21. PMID: 18156490

U1-snRNA-mediated rescue of mRNA processing in severe factor VII deficiency.

Mirko Pinotti[1], Lara Rizzotto[1], **Dario Balestra[1]**, Marzena Anna Lewandowska[2], Nicola Cavallari[1], Giovanna Marchetti[1], Francesco Bernardi[1], and Franco Pagani[2]

[1]Department of Biochemistry and Molecular Biology, University of Ferrara, Ferrara; and [2]International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

AWARDS

- Calogero Vullo Award during the 57th National Meeting of the Italian Society of Biochemistry and Molecular Biology (SIB), September 18th-20th 2013, Ferrara (ITA).

- Young Invertigator Award at the XXIV Congress of the International Society on Thrombosis and Haemostasis (ISTH). Amsterdam (NL). June 29th – July 4th 2013

- Awarded by IUSS-Ferrara 1391 (The University Institute for Higher Studies), University of Ferrara, as best PhD thesis 2012 in Biochemistry, Molecular Biology and Biotechnologies.

- "Best of the Best oral comunication" award at the XXII National SISET Congress (Italian Society of Trombosis and Hemostasis). Vicenza (ITA) , 4th – 6th October 2012.

PARTICIPATIONS IN CONFERENCES

LVII National Meeting of the Italian Society of Biochemistry and Molecular Biology (SIB). Ferrara (ITA). September 18th-20th 2013

Aberrant mRNA splicing in coagulation factor deficiencies: from molecular mechanisms to RNA-based therapeutic approaches.

Dario Balestra, Nicola Cavallari, Elena Barbon, Daniela Scalet, Eugenio Fernandez Alanis, Andrea Dal Mas, Malgorzata E. Rogalska, Franco Pagani, Francesco Bernardi and Mirko Pinotti
Lecture and Calogero Vullo Award

XXIV Congress of the International Society on Thrombosis and Haemostasis (ISTH). Amsterdam (NL). June 29th – July 4th 2013

A very rare simultaneous presence of a ring chromosome 13 and a splicing site mutation on Factor X gene .

M. Menegatti, **D. Balestra**, B. Fabrizzi, R. Asselta, M. Pinotti, F. Peyvandi.
Abstract

XXIV Congress of the International Society on Thrombosis and Haemostasis (ISTH). Amsterdam (NL). June 29th – July 4th 2013

Delivery of a modified U1 small nuclear RNA alleviates splicing-defective coagulation Factor VII expression in mouse models.

D. Balestra, A. Faella, N. Cavallari, P. Margaritis, F. Pagani, F. Bernardi, V. R. Arruda and M. Pinotti.
Lecture (Young Invertigator award)

XXIV Congress of the International Society on Thrombosis and Haemostasis (ISTH). Amsterdam (NL). June 29th – July 4th 2013

Restoration of coagulation factor IX function impaired by different splicing mutations by a unique exon-specific U1 small nuclear RNA (snRNA).

D. Balestra, N. Cavallari, E. F. Alanis, A. Dal Mas, E. Rogalska Malgorzata, F. Bernardi, F. Pagani and M. Pinotti.
E-Poster

18th Congress of the European Hematology Association. Stockholm (SVE), 13th-16th June 2013

A very rare simultaneous presence of a ring chromosome 13 and a splicing site mutation on factor X gene.

M. Menegatti , **D. Balestra**, B. Fabbrizi , R. Asselta , M. Pinotti , F. Peyvandi , A. Bianchi Bonomi
Abstract

54th ASH (American Society of Hematology) Annual Meeting and Exposition. Atlanta (USA), 8th – 11th December 2012.

Delivery of a modified U1 small nuclear RNA alleviates splicing-defective coagulation factor VII expression in mouse models

D. Balestra, A. Faella, N. Cavallari, P. Margaritis, F. Pagani, F. Bernardi, V. R. Arruda and M. Pinotti
Lecture

XXII National SISET Congress (Italian Society of Trombosis and Hemostasis). Vicenza (ITA) , 4th – 6th October 2012.

An exon-specific U1 small nuclear RNA (snRNA) strategy to correct splicing mutations associated to hemophilia B.

D. Balestra, N. Cavallari, E. Fernandez Alanis, A. Dal Mas, M. E. Rogalska, F. Pagani, F. Bernardi and M. Pinotti.

Lecture ("Best of the Best oral comunication" award)

36th FEBS Congress. Torino (ITA), 25th – 30th June 2011

Aberrant splicing reverts a potentially lethal coagulation deficiency caused by a +1g/t splicing mutation.

N. Cavallari, **D. Balestra**, L. Rizzotto, I. Maestri, A. Chamsunri, F. Bernardi and M. Pinotti

Abstract

XVI Telethon congress. Riva del Garda. Trento (ITA), 7th - 9th March 2011.

Rna–based therapeutic approaches for blood coagulation factor deficiencies caused by splicing mutations.

D. Balestra, M. Baroni, E. Bussani, A. Canella, N. Cavallari, A. Dal Mas, E. Fernandez, P. Ferraresi, C. Mattioli, F. Pagani and M. Pinotti.

Poster

XXI National SISET Congress (Italian Society of Trombosis and Hemostasis). Bologna (ITA) , 28th - 31st October 2010.

Rescue of coagulation factor VII mRNA processing and protein function by engineered U1+5A snRNA.

D. Balestra , N. Cavallari , I. Maestri , R. Mari , L. Rizzotto, F. Pagani, F. Bernardi, M. Pinotti

Lecture

XXI National SISET Congress (Italian Society of Trombosis and Hemostasis). Bologna (ITA) , 28th - 31st October 2010.

The complete impairment of factor VII gene expression by the IVS6+1g/t mutation is compatible with a severe but not lethal bleeding disorder.

N. Cavallari, **D. Balestra**, L. Rizzotto, A. Chuamsunrit, G. Mariani, F. Pagani, F. Bernardi and M. Pinotti.

Abstract

XX National SISET Congress (Italian Society of Trombosis and Hemostasis). Firenze (ITA) , 25th -28th September 2008.

U1-snRNA-mediated rescue of mRNA processing in severe factor VII deficiency.

M. Pinotti, **D. Balestra**, L. Rizzotto, N. Cavallari, F. Pagani, F. Bernardi.

Abstract

8th International Winter Meeting on Coagulation. Bormio (ITA) , 6th -12th April 2008

Molecular genetics and biology of congenital hemorrhagic diseases.

F. Bernardi, M. Pinotti, **D. Balestra**, P. Caruso , G. Marchetti.

Abstract

NATIONAL AND INTERNATIONAL COURSES AND CONGRESSES

- 23th National Meeting of PhD Student in Biochemistry. Urbino (Italy) 8th - 11th June 2010.

- Seminar on "Pyrosequencing, a new allied in farmacogenetics and oncogenetics". Ferrara (Italy) 26th November 2009

- High Formation Course "Nano- and biotechnologies for diagnostics and therapy". Urbino (Italy) 10th -11th September 2009.

- Workshop on Alternative Splicing and Disease. Montpellier (France) 20th -25th July 2009

- XV Scientific Convention Telethon. Riva del Garda (Italy) 9th -11th March 2009

- High Formation Course "The contribution of biotechnologies for the development of new therapeutic strategies". Urbino (Italy) 7th -8th July 2008.

TECHNICAL SKILLS AND COMPETENCES

In my research activity I gained experience with the following techniques:

- maintenance of mammalian cellular coltures;
- nucleic acids extraction and purification (DNA,RNA and expression vectors form cells and tissues);
- PCR, RT-PCR, retrotranscription to cDNA, qPCR (real time RT-PCR and gene copy number qPCR)
- Mutagenesis (one site, multi sites, insertion, deletion)
- Cloning of promoter, gene and recombinant cassettes in expression and reporter vectors;
- Endonuclease digestion
- Minigene construction (promoter - cDNA hybrid, cDNA – genomic DNA hybrid)
- Bacterial and Eukaryotic cell cultures (primary and immortalized cell lines)
- Transfection (stable or transient)
- Transformation bacterial cells (chemically competent cells)
- Reporter assay (luciferase and fluorescent protein)
- E.L.I.S.A., Western blot, Immunohystochemistry;
- Enzymatic activity assays
- Sequencing
- Bioinformatics analysis (splice site score prediction, oligonucleotide and probe design, RNA secondary structure, endonuclease digestion)
- Mouse anatomy (tissue and organs explant)
- Laboratory animal care (mouse)
- Animal procedure (blood collection from various sites, euthanasia procedure, suture)

SCIENTIFIC CONTRIBUTION

- Assistant supervisor for various (6) Master and Bachelor degree thesis, during years 2010-2011-2012-2013, for Biology and Biomolecular Sciences classes.
- Various lessons held for Biological Sciences and Bio-molecular Sciences courses.
- Scientific collaborator for the following scientific project:
    - Post-transcriptional and translational mechanisms involved in regulation of gene expression in normal and pathological conditions. Founded by PRIN (ITALY) for 24 months. 2008
    - Study of new innovative therapeutic approaches for inherited coagulation disorders. Founded by Cassa di Risparmio di Ferrara (ITALY) for 24 months. 2008
    - RNA–based therapeutic approaches for blood coagulation factor deficiencies caused by splicing mutations. Founded by Telethon. 2009

LANGUAGE SKILLS

- **English:** Good knowledge (Cambridge English Level B1)
- **French:** Basic Knowledge
- **Italian:** Native speaker

COMPUTER KNOWLEDGE

Operating systems: Excellent
Programming languages : Limited
Word processing: Good
Electronic spreadsheet : Good
Data base: Basic
CAD skills: Basic
Internet skills: Excellent
Data transmission networks: Good
Multimedia: Excellent

NAME AND ADDRESS OF REFEREES:

Referee 1:

Name:	Prof. Mirko Pinotti, Assistant professor
Address:	University of Ferrara, Department of Live Sciences and Biotechnologies, section Molecular Biology Via Fossato di Mortara, 74 44121, Ferrara (FE)
Telephone:	+39 0532 974424
Fax:	+39 0532 974484
Email:	mirko.pinotti@unife.it, pnm@unife.it
Country:	ITALY

Referee 2:

Name:	Prof. Francesco Bernardi, Pro-rector of University of Ferrara
Address:	University of Ferrara, Department of Live Sciences and Biotechnologies, section Molecular Biology Via Fossato di Mortara, 74 44121, Ferrara (FE)
Telephone:	+39 0532 974425
Fax:	+39 0532 974484
Email:	francesco.bernardi@unife.it
Country:	ITALY

Referee 3:

Name:	Prof. Paris Margaritis, Research Assistant Professor
Address:	The Children's Hospital of Philadelphia, Department of Pediatrics, Division Hematology 5056 Colket Translational Research Building 3501 Civic Center Boulevard Philadelphia, PA 19104
Telephone:	+1 267-426-7262
Fax:	+1 215-590-3660
Email:	margaritis@email.chop.edu
Country:	USA

Referee 4:

Name:	Prof. Valder Arruda, Associate Professor of Pediatrics
Address:	The Children's Hospital of Philadelphia, Department of Pediatrics, Division Hematology 5056 Colket Translational Research Building 3501 Civic Center Boulevard Philadelphia, PA 19104
Telephone:	+1 215-590-4907
Fax:	+1 215-590-3660
Email:	arruda@email.chop.edu
Country:	USA

SUMMARY OF POSTDOCTORAL, PhD AND THESIS WORK

Eukaryotic genes are usually fragmented in the genome by the presence of non-coding sequences, the introns. DNA is transcribed to precursor mRNA (pre-mRNA), which subsequently undergoes extensive modifications and in particular the removal of introns (splicing) and junction of exons. This finely orchestrated process implies the correct definition of small exon sequences within large introns and it is catalyzed by a complex of small ribonucleoproteins (snRNPs) and proteins forming the macromolecular complex named spliceosome. The splicing mechanism is essential to guarantee the correct gene expression, the proper protein synthesis and even the diversity of our proteome. Basically, within introns it is possible to recognize some highly conserved sequences with a key role in the splicing process. It is possible identify a donor site (5' end of the intron), a branch site (near the 3' end of the intron) and an acceptor site (3' end of the intron).

The earliest key event in the splicing process is represented by the recognition of the donor splice sites (DSS), in the 5' region of introns, by the U1−snRNP through complementarity with its RNA component (U1-snRNA). Mutations in gene sequences altering the affinity with the U1-snRNA create defective DSS (DDSS), and lead to aberrant splicing events which normally end in loss of gene function. DDSS are a relatively frequent cause (~15%) of clinically severe human disease forms, and the underlying molecular mechanisms are yet poorly understood.

The aim of my work has been to create appropriate cellular models to elucidate the mechanisms underlying aberrant splicing in human inherited diseases and to evaluate the rescue of correct mRNA processing by novel therapeutic approaches.

Consequences of splicing mutations are primarily studied in immortalized cell lines, selected to represent the better genetic environment of the target pathology, mainly due to the impossibility in obtaining samples from patients which are often life-threatening. Moreover, to mimic the pathological condition, synthetic gene constructs called minigenes are exploited, a well-established strategy to dissect the splicing process.

Minigenes are synthetic cassette consisting of the exon affected by the mutation and the surrounding sequences essential for the splicing. Being smaller in dimension (base pairs) than the entire gene, it is easier to be vehicled in cells. If well designed, minigenes are able to exploit the spliceosome of cells and to mimic the processing of the target mRNA *in vitro*, in normal conditions or in the presence of candidate mutations. Comparison of the splicing

profiles, normal and aberrant, usually highlights the genetic elements involved and the sites of action.
Comprehension of aberrant splicing mechanisms has been employed to design and test different mRNA rescue approaches: use of engineered U1-snRNAs and/or antisense oligonucleotides (AON).
Engineered U1s are small plasmids containing the gene expressing the U1snRNA. Changing in this gene the sequence involved in the recognition of the donor splice site, modified U1snRNAs can be designed with an improved (or restored) affinity toward the mutated target sequence, in order to re-direct recognition of DDSS. Modified U1s can bind efficiently DDSS and recruit the other spliceosomal components on the canonical site of interaction, restoring in many cases correct mRNA processing. However the approach based on modified U1snRNAs can be exploited only when the mutation disrupts the consensus sequence of DSS; in fact many alterations do not alter the consensus sequence of DSS, but create new regulatory elements interfering with normal mRNA processing. AON can block the utilization of these new elements by the spliceosome by masking these aberrant sequences.
In summary, in cellular models of disease, created expressing splicing-competent cDNA constructs, the rescue of gene processing, either by engineered or AON, or a combination of both, can be evaluated at mRNA, protein antigen and functional level.
Finally, the best therapeutic approaches, which combine the highest rescue levels and the lower cytotoxicity, are experimented *in vivo* on mouse models of human diseases for long term gene therapy tests.

Data

_02-10-2013_____