

BIOGRAPHICAL SKETCH

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NAME: Tomasoni, Susanna

eRA COMMONS USER NAME (credential, e.g., agency login): Not available

POSITION TITLE: Head Laboratory of Gene Therapy and Cellular Reprogramming

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.*)

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Università degli Studi di Milano, Italy	Biol. Sci.	02/1991	Biological Sciences
Università di Bologna, Italy	D. Ph.D	12/1995	Physiology

A. Personal Statement

The main objective of the proposed project is to develop an in vitro model of Autosomal Dominant Polycystic Kidney Disease (ADPKD) for drug screening starting from induced pluripotent stem cells (iPSCs) derived from genetically characterized patients affected by polycystic kidney diseases. As part of this team, I have gained the skills required to carry out the activities here proposed and particularly the studies involving the differentiation of induced pluripotent stem cells (iPSCs) into renal progenitor cells.

After completing the PhD in 1995, I entered the Istituto di Ricerche Farmacologiche "Mario Negri" IRCCS, where I gained my expertise in the field of gene therapy, focusing my interest in the construction of viral vectors, and in the development of in vitro and in vivo gene transfer techniques. Since my appointment as Head of Gene Therapy Unit in 2000, I have been engaged in studies on molecular mechanisms involved in the progression of kidney diseases, and on characterization of the morphological-structural lesions associated with them. The main purpose of my research is identification of promising pharmacological, gene and/or cell therapies, which could be effective not only in halting but also in regressing the progression of renal disease.

The experimental approaches used in my research span from molecular to cellular biology, including the generation of induced pluripotent stem cells (iPSCs) and the development of protocols to differentiate them in other different cell types. One of main areas of expertise of my laboratory is the generation of iPSCs by reprogramming human adult cells (skin fibroblasts or peripheral blood mononuclear cells -PBMC- from healthy subjects and patients) with different reprogramming systems using both integrative (Lentivirus) and non-integrative (Sendai virus) vectors. Notably, we recently obtained iPSCs from PBMC of patients suffering from a familiar steroid-resistant nephrotic syndrome with a mutation in the PAX2 gene. We developed protocols for obtaining populations of renal progenitors and functional podocytes in vitro from human induced pluripotent stem cells. PAX2 mutant iPSCs had been instrumental in generating a three-dimensional system aimed to build ureteric bud-like tubules to investigate ureteric bud development. This system allowed us to discover budding defects in ureteric bud-like tubules derived from a patient with a mutation in the PAX2 gene. This system was also used to model polycystic kidney disease using tubular cell lines of human and non-human origin allowing to establish a platform useful for drug screening.

1. Ciampi O, Romano E, Benigni A, **Tomasoni S**. Generation of two isogenic iPS cell lines (IRFMNi002-A and IRFMNi002-B) from a patient affected by Focal Segmental Glomerulosclerosis carrying a heterozygous c.565G>A mutation in PAX2 gene. *Stem Cell Res.* 2018 Oct 29;33:175-179.
2. Benedetti V, Brizi V, Guida P, **Tomasoni S**, Ciampi O, Angeli E, Valbusa U, Benigni A, Remuzzi G, Xinari C. Engineered Kidney Tubules for Modeling Patient-Specific Diseases and Drug Discovery. *EBioMedicine.* 2018 Jul;33:253-268.
3. Imberti B*, **Tomasoni S***, Ciampi O, Pezzotta A, Derosas M, Xinari C, Rizzo P, Papadimou E, Novelli R, Benigni A et al: Renal progenitors derived from human iPSCs engraft and restore function in a mouse model of acute kidney injury. *Sci Rep* 2015, 5:8826 (*Contributed equally).
4. Ciampi O, Iacone R, Longaretti L, Benedetti V, Graf M, Magnone MC, Patsch C, Xinari C, Remuzzi G, Benigni A, **Tomasoni S**. Generation of functional podocytes from human induced pluripotent stem cells. *Stem Cell Res.* 2016, Jul;17(1):130-9.

B. Positions, Scientific Appointments, and Honors

2022 – now: Head, Molecular Medicine Department

2012 – 2022: Head, Laboratory of Gene Therapy and Cellular Reprogramming, IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri”, Bergamo, Italy

2010 –2012: Head, Laboratory of Cell and Gene Therapy, Department of Molecular Medicine, IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri”, Bergamo, Italy

2000 - 2010: Head, Unit of Gene Therapy, Department of Molecular Medicine, IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri”, Bergamo, Italy

1998 - 2000: Scientist at the IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri”, Bergamo, Italy

1995 - 1998: Post-doctoral Fellow at the IRCCS-Istituto di Ricerche Farmacologiche “Mario Negri”, Bergamo, Italy

1994: Research Fellow at the Renal Division, Brigham & Women’s Hospital, Harvard Medical School, Boston, USA

1991 - 1994: Ph.D student at the University of Milan, Milan, Italy as part of a collaboration program between the University of Milan and the University of Bologna

C. Contributions to Science

One of the major objectives of my research is focused on finding new strategies for modulating the immune response to prevent acute and chronic allograft rejection through gene therapy by the means of adenoviral and adeno-associated viral vectors.

My research team, in collaboration with Laboratory of Immunology of Organ Transplantation of our Institute, has demonstrated over time that it is possible to engineer a rat kidney before transplantation by transferring a gene translating into a protein with immunomodulatory activity, which makes the graft able to defend itself against the recipient's immune system without immunosuppressive drugs. In two different studies in rodents, we demonstrated that this approach effectively prevented both acute and chronic rejection of a transplanted kidney without the need for anti-rejection therapy. Very recently, we have demonstrated that it is possible to engineer the kidney of a non-human primate, as well.

Another main research topic of my laboratory has concerned the development of a gene therapy approach to correct a genetic defect in rare diseases. Particularly, the group was focused on studying the Thrombotic Thrombocytopenic Purpura (TTP) that is a disease characterized by anemia, thrombocytopenia and formation of intravascular thrombi. TTP is due to the deficiency of ADAMTS13 protease, a plasma protein able to cleave the Von Willebrand factor secreted from endothelial cells and we demonstrated that it is possible to restore ADAMTS13 production in Adamts13-/- knockout mice by an adenoviral gene delivery approach. We are currently studying technique of gene editing to stably correct a genetic mutation.

1. Tomasoni S, Azzollini N, Casiraghi F, Capogrossi MC, Remuzzi G, Benigni A. CTLA4Ig gene transfer prolongs survival and induces donor-specific tolerance in a rat renal allograft. *J Am Soc Nephrol.* 2000 Apr;11(4):747-52.

2. Benigni A, Tomasoni S, Turka LA, Longaretti L, Zentilin L, Mister M, Pezzotta A, Azzollini N, Noris M, Conti S, Abbate M, Giacca M, Remuzzi G. Adeno-associated virus-mediated CTLA4lg gene transfer protects MHC-mismatched renal allografts from chronic rejection. *J Am Soc Nephrol.* 2006;17(6):1665-1672.
3. Trionfini P, Tomasoni S, Galbusera M, Motto D, Longaretti L, Corna D, Remuzzi G, Benigni A. Adenoviral-mediated gene transfer restores plasma ADAMTS13 antigen and activity in ADAMTS13 knockout mice. *Gene Ther.* 2009;16(11):1373-1379.
4. Tomasoni S, Trionfini P, Azzollini N, Zentilin L, Giacca M, Aiello S, Longaretti L, Cozzi E, Baldan N, Remuzzi G, Benigni A. AAV9-mediated engineering of autotransplanted kidney of non-human primates. *Gene Ther.* 2017 Apr 13.

I have also contributed to clarify the mechanism by which bone marrow-mesenchymal stem cells (BM-MSC) may exert renoprotective effects. Particularly, our studies have shown that administration of BM-MSCs ameliorates renal dysfunction and repairs tubular damage by locally releasing growth factors but also microvesicles and exosomes enriched in mRNAs.

I have also contributed to study the possibility to directly reprogram cells. Indeed, we have demonstrated that bone marrow-mesenchymal stromal cells (BM-MSC) may be directly reprogrammed into functional renal cells by using cell-free extracts of tubular cells.

1. Imberti B, Morigi M, Tomasoni S, Rota C, Corna D, Longaretti L, Rottoli D, Valsecchi F, Benigni A, Wang J, Abbate M, Zoja C, Remuzzi G. Insulin-like growth factor-1 sustains stem cell mediated renal repair. *J Am Soc Nephrol.* 2007;18(11):2921-2928.
2. Tomasoni S, Longaretti L, Rota C, Morigi M, Conti S, Gotti E, Capelli C, Inrona M, Remuzzi G, Benigni A. Transfer of growth factor receptor mRNA via exosomes unravels the regenerative effect of mesenchymal stem cells. *Stem Cells Dev.* 2013 Mar 1;22(5):772-80.
3. Papadimou E, Morigi M, Iatropoulos P, Xinaris C, Tomasoni S, Benedetti V, Longaretti L, Rota C, Todeschini M, Rizzo P et al: Direct reprogramming of human bone marrow stromal cells into functional renal cells using cell-free extracts. *Stem Cell Reports* 2015, 4(4):685-698.

In the last years, I have focused my research in cellular reprogramming, particularly in generating induced pluripotent stem cells and in developing protocols to differentiate them in different cell types. To understand the events underlying kidney development, I have recently set up an easy and efficient protocol to obtain podocytes in vitro starting from human iPSCs. The protocol is divided into three different phases mimicking the stages of kidney development, passing from an intermediate mesoderm stage to a renal progenitor stage to finally obtain mature podocytes that express the main podocyte markers at genetic and protein level. Cells are functional responding to angiotensin II by reducing the gene expression of podocyte markers, and leading to an arrangement of cytoskeletal proteins. Setting up this technology will allow us to generate iPSC from patients affected by genetic disease and to study in vitro how mutations of genes which are important for kidney development can alter the growth or function of podocytes.

We also developed a protocol to reprogram iPSCs into endothelial cells that have been characterized in vitro for gene and protein expression of their typical markers, CD144 and CD31. In our laboratories, we demonstrated that murine embryonic kidney cells can self-organize and re-aggregate as a result of dissociation into single cells by generating kidney 'organoids' in vitro. By exploiting this inquiry system, we found that iPSC-derived endothelial cells have the ability to integrate into acellular rat organoids, forming vessel-like structures.

Together with my research team, we are currently using the CRISPR/Cas9 gene editing technique to correct gene mutations in iPSC from patients with genetic diseases or to knock out a gene in healthy iPSC for loss of function studies or to mimic genetic disorders in vitro. In this context, we have generated CRISPR-Cas9 edited hiPSC knockout for PKD1 and for PKD2, the two genes involved in the onset of ADPKD and that are responsible on average for 85 and 15% of ADPKD cases, respectively. Moreover, we generated hiPSC from ADPKD patients with mutation in PKD1 gene. In addition, using the CRISPR-Cas9 system we have also modified healthy iPSC cells to make them hypoimmunogenic and universal that are no longer patient-specific and therefore usable as possible cell therapy for all patients.

Thanks to the installation of a new Zebrafish laboratory, new transgenic and mutant lines of Zebrafish will be generated by means of the CRISPR/Cas9 technique, which will make it possible to obtain experimental models for studying rare diseases.

1. Ciampi O, Iacone R, Longaretti L, Benedetti V, Graf M, Magnone MC, Patsch C, Xinaris C, Remuzzi G, Benigni A, Tomasoni S. Generation of functional podocytes from human induced pluripotent stem cells. *Stem Cell Res.* 2016 Jul;17(1):130-9.
2. Xinaris C, Benedetti V, Novelli R, Abbate M, Rizzo P, Conti S, Tomasoni S, Corna D, Pozzobon M, Cavallotti D, Yokoo T, Morigi M, Benigni A, Remuzzi G. Functional Human Podocytes Generated in Organoids from Amniotic Fluid Stem Cells. *J Am Soc Nephrol.* 2016 May;27(5):1400-11.
3. Ciampi O, Romano E, Benigni A, Tomasoni S. Generation of two isogenic iPS cell lines (IRFMNi002-A and IRFMNi002-B) from a patient affected by Focal Segmental Glomerulosclerosis carrying a heterozygous c.565G>A mutation in PAX2 gene. *Stem Cell Res.* 2018 Oct 29;33:175-179.
4. Ciampi O, Bonandrini B, Derosas M, Conti S, Rizzo P, Benedetti V, Figliuzzi M, Remuzzi A, Benigni A, Remuzzi G, Tomasoni S. Engineering the vasculature of decellularized rat kidney scaffolds using human induced pluripotent stem cell-derived endothelial cells, *Sci. Rep.* 2019 May 29;9(1):8001.
5. Romano E, Trionfini P, Ciampi O, Benigni A, Tomasoni S. Generation of PKD1 mono-allelic and bi-allelic knockout iPS cell lines using CRISPR-Cas9 system. *Stem Cell Res.* 2020 Jun 19;47:101881.
6. Trionfini P, Ciampi O, Romano E, Benigni A, Tomasoni S. Generation of two isogenic knockout PKD2 iPS cell lines, IRFMNi003-A-1 and IRFMNi003-A-2, using CRISPR/Cas9 technology. *Stem Cell Res.* 2020 Jan;42:101667.
7. Romano E, Trionfini P, Giampietro R, Benigni A, Tomasoni S. Generation of a homozygous CIITA knockout iPS cell line using the CRISPR-Cas9 system. *Stem Cell Res.* 2021 Oct 18;57:102580.
8. Trionfini P, Romano E, Varinelli M, Longaretti L, Rizzo P, Giampietro R, Caroli A, Aiello S, Todeschini M, Casiraghi F, Remuzzi G, Benigni A, Tomasoni S. Hypoimmunogenic Human Pluripotent Stem Cells as a Powerful Tool for Liver Regenerative Medicine, *Int. J. Mol. Sci.* 24 (2023) 11810.

I have also contributed to the characterization of the morphological and structural lesions associated with kidney diseases, with the aim to evaluate the efficacy of pharmacological treatments, with a particular focus on the ultrastructure and function of the glomerular filter. Specifically, using a specific scanning electron microscopy application, our laboratory has documented how glomerular filtration pores are really organized and has assessed a digital morphometrical method for quantifying filtration pore dimensions, a crucial tool for evaluating glomerular filter integrity.

1. A. Remuzzi, S. Conti, B. Ene-Iordache, S. Tomasoni, P. Rizzo, A. Benigni, G. Remuzzi, Role of ultrastructural determinants of glomerular permeability in ultrafiltration function loss, *JCI Insight.* 5 (2020). <https://doi.org/10.1172/jci.insight.137249>.
2. S. Conti, G. Remuzzi, A. Benigni, S. Tomasoni, Imaging the Kidney with an Unconventional Scanning Electron Microscopy Technique: Analysis of the Subpodocyte Space in Diabetic Mice, *Int. J. Mol. Sci.* 23 (2022) 1699.