

## Giorgio Gribaudo – Curriculum

Actual position: Full Professor of Microbiology, Department of Life Sciences and Systems Biology, University of Torino.

### Education:

Doctor in Biology, 1982, University of Torino.

Ph.D in Microbiology, 1990, University of Pisa.

### Research experience:

1982-1984 - Research Fellow, Institute of Microbiology, University of Torino.

1985-1987 and 1989 - Post Doctoral Associate, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, USA.

1987-1990 - Ph. D Program in Microbiology, engaged in research activities at the Institute of Microbiology, University of Torino.

1990-1993 - C.N.R. Research Associate, Institute of Microbiology, University of Torino.

1993-1996 – Research Associate, Department of Public Health and Microbiology, University of Torino.

1996-1998 - Assistant Professor, Department of Public Health and Microbiology, University of Torino.

1999-2006 - Associate Professor of General Microbiology, Faculty of Sciences. Group Leader, Laboratory of Molecular Virology, Department of Public Health and Microbiology, University of Torino.

2007-2012 - Full Professor of General Microbiology, Faculty of Sciences. Group Leader, Laboratory of Molecular Virology, Department of Public Health and Microbiology, University of Torino.

Since 2013 - Full Professor of General Microbiology, Head, Laboratory of Microbiology and Virology, Department of Life Sciences and Systems Biology, University of Torino.

### Scientific Societies:

Società Italiana di Microbiologia Generale e Biotecnologie Microbiche (SIMGBM).

Società Italiana di Virologia - Italian Society for Virology (SIV-ISV).

American Society for Microbiology (ASM).

### Fields of investigation and areas of interest:

Giorgio Gribaudo is an expert of virus-host cell interactions, and research and development of antiviral agents. Since 1984, he has focused his research activities on three main areas of interest:

A. Characterization of the mechanisms of host intrinsic and innate antiviral defenses; molecular mechanisms of the antiviral activity of Interferons (IFN) and of IFN-inducible genes;

B. Analysis of the molecular mechanisms that regulate virus-host cell interactions in experimental models of Herpesviruses;

C. Identification, characterization, and validation of new antiviral compounds against human viruses.

A. Characterization of the mechanisms of host intrinsic and innate antiviral defenses; molecular mechanisms of the antiviral activity of Interferons (IFN) and of IFN-inducible genes. In this area, the following studies were developed:

1) Characterization of the biochemical and functional properties of murine IFN-gamma. Generation and characterization of monoclonal antibodies to murine IFN-gamma.

2) Molecular cloning and functional characterization of transcriptional enhancers of IFN-activatable genes (murine IFI200 gene family); analysis of the molecular mechanisms that regulate the expression of IFN-inducible genes, such as MHC Class I, IFI200, 2'-5' Oligoadenylate Synthetase; characterization of IFN-activatable transcription factors (STATs factors).

3) Characterization of the molecular mechanisms of the antiviral activity of IFNs against RNA viruses; role of the IFN-inducible enzyme 2'-5' Oligoadenylate Synthetase in the inhibition of Picornavirus gene expression.

4) Study of mechanisms of the antiviral activity of IFNs on the replication of Herpesviruses. Identification of the role of the cellular transcription factor NF- $\kappa$ B in the IFN-mediated inhibition of Murine Cytomegalovirus (MCMV) immediate-early (IE) gene expression.

5) Molecular cloning of new IFN-inducible proteins and functional characterization of their activities (IFI 200 proteins, p203 and p204).

6) Characterization of the IFN-inducible human protein IFI16. Study of its effects on the regulation of inflammatory gene expression in endothelial cells. Generation and validation of adenoviral vectors for IFI16 expression in primary cells. Study of IFI16 antiviral activity and characterization of its role as a potent restriction factor of the replication of Human Cytomegalovirus.

B) Analysis of the molecular mechanisms that regulate virus-host cell interactions in experimental models of Herpesviruses. In this area of investigation, he has focused his attention primarily on the Human Cytomegalovirus (HCMV), the most complex member of the human Herpesviruses family. In particular, by

using molecular genetics, molecular biology, biochemistry, cell biology, and bioinformatic approaches, he has investigated how some HCMV proteins: 1) determine the virus's ability to infect a wide variety of target cell-types; 2) dysregulate host-cell gene expression to make a cellular environment conducive for replication; and 3) counteract intrinsic and innate antiviral responses. In particular, the following studies were developed:

1) Analysis of HCMV replication strategies in post mitotic cells. Elucidation of HCMV-mediated mechanisms of induction of cellular enzymes coopted to sustain replication of viral DNA (i.e. enzymes of de novo deoxyribonucleotides synthesis). In this study, he identified some enzymes of this pathway as new targets for antiviral intervention (i.e. thymidylate synthetase, ribonucleotide reductase, deoxycitidylate deaminase, and folilpolyglutammate syntetase), since their pharmacological inhibition led to inhibition of HCMV replication.

2) Molecular characterization of the UL72 protein of HCMV. This study was developed through the expression and characterization of recombinant pUL72, as well as by the production of mutant viruses with functional inactivations of the UL72 gene (BAC recombineering technology). The combination of these approaches allowed the functional analysis of UL72 importance during HCMV infection.

3) Then, by using the BAC-recombineering, he studied the role of NF- $\kappa$ B in the context of HCMV replicative cycle in cells of different origin and in different physiologic conditions. These studies demonstrated that the cellular kinase IKK2, an upstream activator of NF- $\kappa$ B, is activated by HCMV infection. Pharmacological or genetic inhibition of IKK2 activity was shown to prevent HCMV replication. This result supported the conclusion of the importance of the IKK2/NF- $\kappa$ B axis for HCMV IE gene expression and progression of the replicative cycle

4) Elucidation of the role of cellular transcription factors Elk-1 and SRF in regulating the activity of the Major IE promoter (MIEP) of HCMV. Both factors were demonstrated to be required for optimal MIEP activity and IE gene expression in nonproliferating cells. Binding of Elk-1 and SRF to the MIEP was found to compensate for the lack of HCMV-induced NF- $\kappa$ B activity even in growing cells. Altogether, findings of points 3 and 4 highlight the importance of the combination of different MIEP binding sites (NF- $\kappa$ B, Elk-1, and SRF) to optimize IE gene expression in cells in different physiological states.

5) Analysis of the molecular basis of pro-angiogenic activity of HCMV during infection of lymphatic (LEC) and blood-derived (BEC) endothelial cells. In this study, a global proteomic approach allowed to define the capability of HCMV to induce haemangiogenesis and lymphangiogenesis through an indirect mechanism, that relies on the stimulation of IL-6 and GM-CSF secretion from infected EC cells.

6) Functional characterization of the US12 gene family of HCMV. BAC-recombineering was applied to analyze expression and functions of US12, US13, US15, US16; US19, US20, and US21 genes. To date, this study has led to the identification of new HCMV tropism factors, such as those encoded by the US16 and US20 genes required for HCMV infection of endothelial, epithelial and dendritic cells, and the first herpesvirus-encoded viroporin as the US21 protein.

C) Identification, characterization, and validation of new antiviral compounds against human viruses. In this area of activity, the following studies were developed:

1) Exploitation of the activities of cellular enzymes induced by HCMV proteins as targets to design innovative strategies for anti-HCMV treatment.

2) Generation and validation of fully human monoclonal antibodies able to neutralize HCMV infection and suitable for the development of new antiviral tools for prophylactic and therapeutic intervention.

3) Generation and validation of innovative cell-based assays (engineered indicator cell lines) for detection of HCMV infectious particles and for searching of new antiviral molecules against IE protein activities.

4) Identification and characterization of the antiviral activity of new peptide-derivatized dendrimers and oligodeoxynucleotides that block attachment and/or entry of HCMV, Herpes simplex virus type 1 and type 2.

5) Identification and characterization of new small-molecule inhibitors targeting the activities of the transcription factor IE2 of HCMV.

6) Identification and characterization of the antiviral activity of plant extracts against HSV-1/2 and Influenza A/B viruses, coronaviruses and Respiratory Syncytial Virus (RSV) for development of innovative microbicides.

7) Characterization of the antiviral activity of new DHODH inhibitors against HSV-1/2, Influenza A/B, RSV, hCoV-229E, hCoV-OC43 and SARS-CoV-2 viruses.

8) Characterization of the broad-spectrum antiviral activity of new inhibitors of the M<sup>pro</sup> enzyme of human coronaviruses.

9) Characterization of the broad-spectrum antiviral activity of new inhibitors of the Pin1 and PDIA3 enzymes.

Overall, the research activity has produced 130 articles in peer-reviewed international journals, and more than 50 communications at national and international congresses.

Inventor of six patents on antiviral agents (five international PCT patents).

Metrics: H-index 33, total citations 3354 (Scopus).

Recipient of the following research grants:

1999-2001: As Scientific Leader of Research Unit, C.N.R. Biotechnology Program

2000-2001: As Scientific Leader of Research Unit, PRIN 2000

2003-2004: As Scientific Leader of Research Unit, PRIN 2003  
2003, 2004, 2006-2009: As Leader of Research Unit, "Ricerca Sanitaria Finalizzata", Piedmont Region  
2006-2007: As Scientific Leader of Research Unit, "Ricerca Scientifica Applicata", Piedmont Region  
2007-2009: As Scientific Leader of Research Unit, PRIN 2007  
2013-2015: As Scientific Leader of Research Unit, PRIN 2010-11  
2021: As Scientific Leader of Research Unit, FIRB COVID 2020  
2021-2023: As Scientific Leader of Research Unit, INFRA-P2, Piedmont Region  
2022-2024: As Scientific Leader of Research Unit, NATO SPS  
2001-2022: As Scientific Leader of Research Unit, University of Torino

### **Selected publications (2017-2023)**

Luganini A., Cavaletto N., Raimondo S., Geuna S., Gribaudo G. Loss of the human cytomegalovirus US16 protein abrogates virus entry into endothelial and epithelial cells by reducing the virion content of the pentamer. *J. Virol.*, pii: e00205-17, 2017. doi: 10.1128/JVI.00205-17.

Charpak-Amikam Y., Kubsch T., Seidel E., Oiknine-Dijan E., Cavaletto N., Yamin R., Schmiedel D., Wolf D., Gribaudo G., Messerle M., Cicin-Sain L., Mandelboim O. Human cytomegalovirus escapes immune recognition by NK cells through the downregulation of B7-H6 by the viral genes US18 and US20. *Scientific Reports*, 7:8661, 2017. doi: 10.1038/s41598-017-08866-2.

Mercorelli B., Luganini A., Celegato M., Palù G., Gribaudo G., Loregian A. Repurposing the clinically approved calcium antagonist manidipine dihydrochloride as a new early inhibitor of human cytomegalovirus targeting the Immediate-Early 2 (IE2) protein. *Antiviral Res.*, 150: 130-136, 2018.

Luganini A., Terlizzi M.E., Catucci G., Gilardi G., Maffei M., Gribaudo G. The cranberry extract Oximacro® exerts *in vitro* virucidal activity against influenza virus by interfering with hemagglutinin. *Front. Microbiol.* 9: 1826, 2018. doi: 10.3389/fmicb.2018.01826.

Luganini A., Di Nardo G., Munaron L., Gilardi G., Fiorio Pla A., Gribaudo G. Human cytomegalovirus US21 protein is a viroporin that modulates calcium homeostasis and protects cells against apoptosis. *Proc. Natl. Acad. Sci. USA*, 115: E12370-E12377, 2018. doi: 10.1073/pnas.1813183115

Luganini A., Mercorelli B., Messa L., Palù G., Gribaudo G., Loregian A. The isoquinoline alkaloid berberine inhibits human cytomegalovirus replication by interfering with the viral Immediate Early-2 (IE2) protein transactivating activity. *Antiviral Res.* 164:52-60, 2019. doi: 10.1016/j.antiviral.2019.02.006

Simon LM., Morandi E., Luganini A., Gribaudo G., Martinez-Sobrido L., Turner D.G., Oliviero S., Incarnato D. In vivo analysis of the influenza A mRNA structurome identifies critical regulatory motifs. *Nucleic Acids Res.*, 47:7003-7017, 2019, doi: 10.1093/nar/gkz318

Mercorelli B., Luganini A., Celegato M., Palù G., Gribaudo G., Lepesheva G., Loregian A. The clinically approved antifungal drug posaconazole inhibits human cytomegalovirus replication. *Antimicrob. Agents Chemother.*, 2020, 64: e00056-20, doi:10.1128/AAC.00056-20.

Bozzano F., Dalla Chiesa M., Pelosi, A., Antonini F., Ascierio M.L., Del Zotto G., Moretta F., Muccio L., Luganini A., Gribaudo G., Cenderello G., Dentone C., Nicolini L., Moretta A., Moretta L., De Maria A. HCMV-controlling NKG2C+ NK cells originate from novel circulating inflammatory precursors. *J. Allergy. Clin. Immunol.*, 2021, S0091-6749(21)00087-7. doi: 10.1016/j.jaci.2020.12.648.

Luganini A., Sibille G., Mognetti B., Sainas S., Pippione A.C., Giorgis M., Boschi D., Lolli M.L., Gribaudo G. Effective deploying of a novel DHODH inhibitor against herpes simplex type 1 and type 2 replication. *Antiviral Res.*, 189:105057, 2021, doi: 10.1016/j.antiviral.2021.105057

Calistri A., Luganini A., Mognetti B., Elder, E., Sibille G., Conciatori V., Del Vecchio C., Sainas S., Boschi D., Monserrat N., Mirazimi A., Lolli M.L., Gribaudo G., Parolin C. The new generation hDHODH inhibitor MEDS433 hinders the *in vitro* replication of SARS CoV-2 and other human coronaviruses. *Microorganisms*, 9: 1731, 2021. doi: 10.3390/microorganisms9081731.

Sibille G., Luganini A., Sainas S., Boschi D., Lolli M.L., Gribaudo G. The novel hDHODH inhibitor MEDS433 prevents influenza virus replication by blocking pyrimidine biosynthesis. *Viruses*, 14, 2281, 2022. doi.org/10.3390/v14102281

Luganini A., Serra V., Scarpellino G, Bhat SM, Munaron L, Fiorio Pla A, Gribaudo G. The US21 viroporin of human cytomegalovirus stimulates cell migration and adhesion. *mBio*. 2023 Jul 21:e0074923. doi: 10.1128/mbio.00749-23.