

BIOGRAPHICAL SKETCH

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NAME: Chiarle, Roberto

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POSITION TITLE: Professor of Pathology

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Torino, Torino	M.D.	11/1993	Medicine and Surgery
University of Torino, Torino	Residency	11/1997	Pathology
New York University, New York, NY	Postdoctoral Fellow	09/2001	Molecular Pathology
Harvard University, Boston, MA	Other training	04/2010	Visiting Professor Immunology

A. Personal Statement

Dr. Chiarle's laboratory research work is focused on the biology and therapy of tumors driven by genetic alterations of the Anaplastic Lymphoma Kinase (ALK) gene, which is involved in the pathogenesis of neuroblastoma, Anaplastic Large Cell Lymphoma (ALCL), Non-Small Cell Lung Cancer (NSCLC), and other tumors (Chiarle et al. *Nat Medicine*, 2005, Chiarle et al. *Nat Rev Cancer*, 2008, Menotti et al., *Nat Medicine* 2019, Mastini et al *Science* TM 2023, Voena et al, *Nat Rev Cancer*, 2025). In this context, one main area of research aims at discovering novel immunotherapies for ALK-driven tumors, including the development of an ALK vaccine and ALK CAR T cells. To study these immunotherapies, we have developed mouse models for ALK-rearranged lymphoma, ALK-rearranged lung carcinoma and ALK-mutated neuroblastoma. The Chiarle lab has recently developed CAR T cells that specifically recognize the ALK receptor expressed on the surface of neuroblastoma cells (Bergaggio et al, *Cancer Cell* 2023), and Dr. Chiarle has submitted to the FDA an IND proposal for a Phase I clinical trial to test ALK.CAR T cells in the treatment of children with relapsed/refractory neuroblastoma. The trial is now open for enrollment with Dr. Chiarle being the IND holder and Sponsor of the trial (NCT06803875). Leveraging on these models, we recently developed an ALK vaccine that primes the immune system to recognize and eradicate ALK-rearranged lymphoma and lung cancer cells (Chiarle et al. *Nat Medicine*, 2008, Voena et al. *Cancer Immunology Research* 2015, Cheong et al, *Nat Commun* 2024). We also demonstrated that patients with ALK-rearranged NSCLC or ALCL spontaneously develop an immune response against ALK (Awad et al. *Oncotarget* 2017, Stadler et al, *Cancer Immunol Res* 2025). We patented the concept of the ALK vaccine and are now aiming to move toward its clinical. Preliminary data generated in Dr. Chiarle lab demonstrate the potent therapeutic efficacy of the peptide-based ALK vaccine in mouse models (Mota et al, *Nature Cancer* 2023) and were key to secure financial support for the clinical trial and instrumental for its design.

B. Positions, Scientific Appointments and Honors

2024- Scientific Advisory Board, American-Italian Cancer Foundation (AICF)
 2024- Scientific Consultant for ALKEMIST Bio Srl, Italy
 2022- Director of Hematopathology, European Institute of Oncology, Milan, Italy
 2022 - Scientific Review Committee, Leukemia&Lymphoma Society of America (LLS)

2021 -	Reviewer, SciLab grants, Karolinska Institut, Sweden
2020 -	Reviewer, Little Princess Trust Project Grants, UK
2020 -	Professor of Pathology, Harvard University, Boston, MA
2019 -	Affiliate Member, The Broad Institute, The Broad Institute of MIT and Harvard
2018 -	Editorial Board, Cells (Basel)
2018 -	Editorial Board, Cancers (Basel)
2014 - 2020	Board, Clinical and Translational Research Committee (CTREC), BCH, Boston, MA
2014 -	Medical Staff, Department of Pathology, Boston Children's Hospital, Boston, MA
2014 -	Full Professor in Pathology, University of Torino, Italy
2013 -	Elected Member, American Society of Haematology (ASH)

Honors

2022	Boston Investment Conference 2022 Awardee
2020	Pappendick Award – BCH
2017	TIS Career Development Award
2015	Grant for Oncology Innovation (GOI), Merck-Serono
2013	“Francesco De Luca” International prize, Italian National Academy of Sciences “Lincei”
2013	PoC Award, European Research Council (ERC)
2011	AICR Award, Association for International Cancer Research
2010	Outstanding Research in Oncology, Italian National prize “Carlo Chianello” Foundation
2009	Starting Grant Investigator Award, European Research Council (ERC)
2007	“Premio G. Costa” for Basic Research, University of Torino
2006	Junior Investigator Award, Italian National Prize “Premio Sapio” for Outstanding Research
2001	Young investigator award, University of Torino
1997	Summa cum laude, University of Torino Residency program
1993	Summa cum laude, University of Torino

C. Contribution to Science (*denotes Corresponding author)

1. Oncogenic ALK signaling in tumor. Chromosomal translocations involving the Anaplastic Lymphoma Kinase (ALK) gene were first discovered in 1994 in a subset of T cell lymphoma, named Anaplastic Large Cell Lymphoma (ALCL). More recently, various structural and genetic alterations of the ALK gene including chromosomal translocations, point mutations, amplifications and alternative transcription start sites have been described in several solid tumors such as non-small cell lung cancer (NSCLC), neuroblastoma, melanoma, inflammatory myofibroblastic tumor, breast cancer, colon cancer and others. We have been studying for almost 20 years the oncogenic signaling initiated by ALK in different tumor types. To this end, we developed genetically engineered mouse models (GEMM) for ALK-rearranged lymphoma and lung cancer. We discovered several mediators of oncogenic ALK activity, including Stat3, Shp2, and the Rho Family GTPases, in particular Cdc42. Also, we described a novel role for NPM-ALK in regulating the expression of TCR-related molecules in T-cells by means of a Stat3-DNMT1 mediated genetic silencing. Very recently, we described a link between NPM-ALK signaling and angiogenesis and metastasis formation in ALCL and NSCLC mediated by the HIF factors. We also discovered two phosphatases (PTPN1 and PTPN2) that regulate the phosphorylation and activation of ALK in lymphoma, and together with the SHP2/PTPN11 phosphatase contribute to resistance to ALK TKIs in lymphoma.

- a. **Chiarle R**, Simmons WJ, Cai H, Dhall G, Zamo A, Raz R, Karras JG, Levy DE, Inghirami G. Stat3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. **Nat Medicine**. 2005 Jun;11(6):623-9. PMID: 15895073.
- b. Choudhari R, Minero VG, Menotti M, Pulito R, Brakebusch C, Compagno M, Voena C, Ambrogio C, **Chiarle R***. Redundant and nonredundant roles for Cdc42 and Rac1 in lymphomas developed in NPM-ALK transgenic mice. **Blood**. 2016 Mar 10;127(10):1297-306. PMID: 26786839.
- c. Karaca Atabay E, Mecca C, Wang Q, Ambrogio C, Mota I, Prokoph N, Mura G, Martinengo C, Patrucco E, Leonardi G, Hossa J, Pich A, Mologni L, Gambacorti-Passerini C, Brugieres L,

Geoerger B, Turner SD, Voena C, Cheong TC*, **Chiarle R***. Tyrosine phosphatases regulate resistance to ALK inhibitors in ALK+ anaplastic large cell lymphoma. *Blood*. 2022 Feb 3;139(5):717-731 PMID: PMC8814675.

- d. Mastini C, Campisi M, Patrucco E, Mura G, Ferreira A, Costa C, Ambrogio C, Germina G, Martinengo C, Peola S, Mota I, Vissio E, Molinaro L, Arigoni M, Olivero M, Calogero R, Prokoph N, Tabbò F, Shoji B, Brugieres L, Geoerger B, Turner SD, Cuesta-Mateos C, D'Aliberti D, Mologni L, Piazza R, Gambacorti-Passerini C, Inghirami GG, Chiono V, Kamm RD, Hirsch E, Koch R, Weinstock DM, Aster JC, Voena C, **Chiarle R***. Targeting CCR7-PI3Ky overcomes resistance to tyrosine kinase inhibitors in ALK-rearranged lymphoma. *Science Transl Med*. 2023 Jun 28;15(702) PMID: PMC10804420.

2. Immunotherapy of ALK positive cancers. The ALK protein has many features of an ideal tumor onco-antigen: it is specifically expressed by tumor cells with very low and limited expression in normal tissues, it is naturally immunogenic in humans and it is required for tumor survival and growth, which greatly decreases the chances of ALK negative clones to escape the immune system. ALK positive lymphoma patients mount a natural immune response against ALK. Antibodies against ALK are detectable in patients with ALK-translocated ALCL and NSCLC, and CD8+ and CD4+ T effector (Teff) responses to ALK peptides are present specifically in ALCL patients. ALK-rearranged lymphoma patients spontaneously develop an immune response against ALK that correlates with decreased risk of disease dissemination and relapse. To exploit these unique features, we recently developed the concept of using ALK as a shared antigen for a cancer vaccine. In preclinical GEMM, the ALK vaccine showed high efficiency in prophylactic and therapeutic treatment of ALK-rearranged lymphoma and lung cancer. We patented and licensed our ALK vaccine to a company. Our current effort is to develop a peptide formulation of the ALK vaccine for clinical use and a Phase I clinical trial will be started soon in NSCLC patients in collaboration with the Dana-Farber-Cancer Institute. We have received an approval from the FDA to start in October 2024 a Phase I clinical trial to test ALK-specific CAR T cells in patients with refractory/relapsed neuroblastoma.

- a. **Chiarle R***, Martinengo C, Mastini C, Ambrogio C, D'Escamard V, Forni G, Inghirami G. The anaplastic lymphoma kinase is an effective oncoantigen for lymphoma vaccination. *Nat Medicine*. 2008 Jun;14(6):676-80. PMID: 18469826.
- b. Mota I, Patrucco E, Mastini C, Mahadevan NR, Thai TC, Bergaggio E, Cheong TC, Leonardi G, Karaca-Atabay E, Campisi M, Poggio T, Menotti M, Ambrogio C, Longo DL, Klaeger S, Keshishian H, Sztupinszki ZM, Szallasi Z, Keskin DB, Duke-Cohan JS, Reinhold B, Carr SA, Wu CJ, Moynihan KD, Irvine DJ, Barbie DA, Reinherz EL, Voena C, Awad MM, Blasco RB, **Chiarle R***. ALK peptide vaccination restores the immunogenicity of ALK-rearranged non-small cell lung cancer. *Nature Cancer*. 2023 Jul;4(7):1016-1035. PMID: 37430060.
- c. Bergaggio E, Tai WT, Aroldi A, Mecca C, Landoni E, Nüesch M, Mota I, Metovic J, Molinaro L, Ma L, Alvarado D, Ambrogio C, Voena C, Blasco RB, Li T, Klein D, Irvine DJ, Papotti M, Savoldo B, Dotti G, **Chiarle R***. ALK inhibitors increase ALK expression and sensitize neuroblastoma cells to ALK.CAR-T cells. *Cancer Cell*. 2023 Dec 11;41(12):2100-2116.e10. doi: 10.1016/j.ccell.2023.11.004. PMID: PMC10793157.
- d. Stadler S, Blasco RB, Singh VK, Damm-Welk C, Ben-Hamza A, Welters C, Hansmann L, **Chiarle R***, Wossmann W. Endogenous CD4 T cells that recognize ALK and the NPM1::ALK fusion protein can be expanded from human peripheral blood. *Cancer Immunol Res*. 2025 Apr 2;13(4):487-495. PMID: 39774774.

3. Mechanisms of chromosomal translocations. Chromosomal translocations are key molecular events that frequently occur in most cancers. The mechanisms of chromosomal translocations formation involve several steps that are initiated by the generation of a DNA double strand breaks (DSBs). Inappropriate repair of DSBs by the classical Non-Homologous End-Joining (c-NHEJ) pathways leads to the formation of a chromosomal translocation. To dissect the molecular mechanisms of translocation on a larger scale, we recently developed a method (HTGTS) to sequence genome-wide translocations that originate from a given DSB. By applying HTGTS to primary B cells, we defined the dominant role of AID in determining chromosomal translocations in lymphocytes. Also, we identified several rules that regulate the frequency and pattern of translocations, including the association with gene transcription, gene density, proximity of DSB and nuclear territories. We recently implemented and adapted this

approach to DSB generated by the CRISPR/Cas9 system and were able to efficiently induce oncogenic chromosomal translocations *in vivo* in mice. The application of this technique to several models will provide further insights on how chromosomal translocation are generated in normal and cancer cells. In this context, we recently demonstrated an unexpected effect of PI3Kdelta inhibitors used to treat lymphoma patients on genomic instability mediated by an up-regulation of AID activity. We are currently studying the role of APOBEC enzymes as inducer of chromosomal translocations in solid tumors.

- a. **Chiarle R**, Zhang Y, Frock RL, Lewis SM, Molinie B, Ho YJ, Myers DR, Choi VW, Compagno M, Malkin DJ, Neuberg D, Monti S, Giallourakis CC, Gostissa M, Alt FW. Genome-wide translocation sequencing reveals mechanisms of chromosome breaks and rearrangements in B cells. **Cell**. 2011 Sep 30;147(1):107-19. PMID: PMC3186939.
- b. Compagno M, Wang Q, Pighi C, Cheong T-C, Meng F-L, Poggio T, Yeap L-S, Karaca E, Blasco RB, Langellotto F, Ambrogio C, Voena C, Wiestner A, Kasar SN, Brown JR, Sun J, Wu CJ, Gostissa M, Alt FW and **Chiarle R***. Phosphatidylinositol 3-Kinase (PI3K) gamma blockade increases genomic instability in B cells. **Nature**. 2017 Feb 23;542(7642):489-493. PMID: PMC5382874.
- c. Tao J, Wang Q, Mendez-Dorantes C, Burns KH and **Chiarle R***. Frequency and mechanisms of LINE-1 retrotransposon insertions at CRISPR/Cas9 sites. **Nature Commun**. 2022 Jun 27;13(1):3685. PMID: PMC9237045.
- d. Cheong TC, Jang A, Wang Q, Leonardi GC, Ricciuti B, Alessi JV, Di Federico A, Awad MM, Lehtinen MK, Harris MH, **Chiarle R***. Mechanistic patterns and clinical implications of oncogenic tyrosine kinase fusions in human cancers. **Nature Commun**. 2024 Jun 14;15(1):5110. doi: 10.1038/s41467-024-49499-0. PMID: 38877018; PMID: PMC11178778.

4. Pathogenesis of human lymphoma. As a pathologist, I have always been interested in the molecular mechanisms that promote tumor formation, in particular lymphoma. B and T cell lymphoma originate from several oncogenetic events that include chromosomal translocation, somatic point mutations, amplifications and alteration of key pathways related to the B-cell receptor (BCR) or T-cell receptor (TCR) signaling. In B cell lymphoma, we have demonstrated that regulation of p27 by Skp2 is a key event in cell transformation of mantle cell lymphoma. We have discovered FBXO11 as a novel oncosuppressor frequently inactivated in diffuse large B cell lymphoma (DLBCL) and Burkitt lymphoma, where it regulates BCL6 stability. We contributed to establish a role for competing endogenous RNA (ceRNA) in lymphoma onset in mouse models. In T cell lymphoma, we have elucidated the role of the cell cytoskeleton and other signaling pathways in promoting lymphoma progression and dissemination. In Burkitt lymphoma, we identified frequent FBXO11 loss-of-function mutations that stabilize BCL6 and developed the concept of a combined targeted therapy against MYC and BCL6.

- a. Ambrogio C, Voena C, Manazza AD, Martinengo C, Costa C, Kirchhausen T, Hirsch E, Inghirami G, **Chiarle R***. The anaplastic lymphoma kinase controls cell shape and growth of anaplastic large cell lymphoma through Cdc42 activation. **Cancer Res**. 2008 Nov 1;68(21):8899-907. PMID: PMC2596920.
- b. Duan S, Cermak L, Pagan JK, Rossi M, Martinengo C, di Celle PF, Chapuy B, Shipp M, **Chiarle R***, Pagano M*. FBXO11 targets BCL6 for degradation and is inactivated in diffuse large B-cell lymphomas. **Nature**. 2012 Jan 5;481(7379):90-3. PMID: PMC3344385.
- c. Menotti M, Ambrogio C, Cheong TC, Pighi C, Mota I, Cassel SH, Compagno M, Wang Q, Dall'Olio R, Minero V, Poggio T, Sharma GG, Patrucco E, Mastini C, Choudhari R, Pich A, Zamo' A, Piva R, Giliani S, Mologni L, Collings CK, Kadoch C, Gambacorti-Passerini C, Notarangelo LD, Anton IM, Voena C and **Chiarle R***. Wiskott-Aldrich syndrome protein (WASP) is a tumor suppressor in T cell lymphoma. **Nat Medicine** 2019 Jan;25(1):130-140. PMID: PMC6556382
- d. Hao Q, Zhan C, Lian C, Luo S, Cao W, Wang B, Xie X, Ye X, Gui T, Voena C, Pighi C, Wang Y, Tian Y, Wang X, Dai P, Cai Y, Liu X, Ouyang S, Sun S, Hu Q, Liu J, Ye Y, Zhao J, Lu A, Wang JY, Huang C, Su B, Meng FL, **Chiarle R***, Pan-Hammarström Q*, Yeap LS*. DNA repair mechanisms that promote insertion-deletion events during immunoglobulin gene diversification. **Science Immunol**. 2023 Mar 31;8(81):eade1167. PMID: PMC10351598

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