

BIOGRAPHICAL SKETCH

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NAME: Lugli, Enrico

POSITION TITLE: Senior Group Leader, Laboratory of Translational Immunology; Head, Flow Cytometry Core, Fondazione Humanitas per la Ricerca, Rozzano, Milan, Italy

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Univ of Modena and Reggio Emilia, Modena, Italy	M.Sc.	07/2004	Medical Biotechnology
Univ of Modena and Reggio Emilia, Modena, Italy	Ph.D.	12/2007	Immunology
National Institutes of Health, Vaccine Research Center, Bethesda, MD, USA	Postdoctoral	12/2012	T Cell Immunology, Cancer Immunotherapy, Immune Reconstitution

A. Personal Statement

I am an expert of human T cell biology, and I study the complexity of the human T cell compartment to define the cellular and molecular mechanisms leading to the development of long-lived, potent memory T cells in cancer immunotherapy. More recently, my laboratory has been interested in the mechanisms of immunosuppression in the human tumor microenvironment. During my career, I made substantial contribution to the field by pioneering the concept of stem-like memory T cell differentiation and its application to adoptive cell therapy (ACT). I have identified memory T cells with stem-like characteristics (Gattinoni*, Lugli* et al., **Nat Med**, 2011; Lugli et al, **J Clin Invest.**, 2013), I translated IL-15 therapy in patients with cancer (Conlon*, Lugli* et al., **J Clin Oncol**, 2015), and I have defined for the first time that a hierarchy of differentiation exists among PD-1+ exhausted T cells in cancer (Brummelman et al., **J Exp Med**, 2018) and in physiology (Galletti et al., **Nature Immunol**, 2020). Recently, we have shown that NaCl supplementation prevents exhaustion and enhances anti-tumor immune responses (Scirgolea et al., **Nat Immunol**, 2024). My discoveries inspired a multitude of translational and clinical studies aiming at defining correlates of successful response to ACT and immune checkpoint blockade, or at employing stem-like T cells to improve the efficacy of ACT. The lab, involving 17 people with expertise in cellular and molecular biology, technology development and bioinformatics has active collaborations with several basic science and clinical groups worldwide on cancer immunology and immunotherapy. The lab is currently funded by highly competitive national and international research grants as well as by industries/pharmaceutical companies. In addition, I am a recognized expert of high-dimensional flow cytometry and related computational analyses.

Selected publications (last 5 years). Total citations: 23,968. H-index: 54 (Google Scholar).

***First, last or corresponding**

1. Scirgolea, C., Sottile, R., De Luca, M., [...]. and **Lugli, E***. NaCl enhances CD8+ T-cell effector functions in cancer immunotherapy. **Nat Immunol**. 2024; 25(10):1845-1857.
2. Swatler J, De Luca M, Rotella I, [...] and **Lugli E***. CD4+ Regulatory T Cells in Human Cancer: Subsets, Origin, and Molecular Regulation. **Cancer Immunol Res**. 2024;12(4):393-9.

3. Whiteside SK, Grant FM, Alvisi G, [...], **Lugli E**, and Roychoudhuri R. Acquisition of suppressive function by conventional T cells limits antitumor immunity upon T(reg) depletion. **Science Immunology**. 2023;8(90):eabo5558.
4. Wischniewski V, Maas RR, Aruffo PG, [...], **Lugli E**, and Joyce JA. Phenotypic diversity of T cells in human primary and metastatic brain tumors revealed by multiomic interrogation. **Nat Cancer**. 2023;10.1038/s43018-023-00566-3.
5. Zehn D*, Thimme R*, **Lugli E***, de Almeida GP, and Oxenius A*. 'Stem-like' precursors are the fount to sustain persistent CD8(+) T cell responses. **Nat Immunol**. 2022;23(6):836-47.
6. Alvisi G, Termanini A, Soldani C, [...], **Lugli E***, and Lleo A. Multimodal single-cell profiling of intrahepatic cholangiocarcinoma defines hyperactivated Tregs as a potential therapeutic target. **J Hepatol**. 2022;77(5):1359-1372.
7. Bonnal, R. J. P.*, Rossetti, G.*, **Lugli, E***, [...] and Pagani, M. Clonally expanded EOMES(+) Tr1-like cells in primary and metastatic tumors are associated with disease progression. **Nat. Immunol**. **22**, 735-745, doi:10.1038/s41590-021-00930-4 (2021).
8. De Biasi S, Gibellini L, Lo Tartaro D, [...], **Lugli E***, and Cossarizza A*. Circulating mucosal-associated invariant T cells identify patients responding to anti-PD-1 therapy. **Nature communications**. 2021;12(1):166910.1038/s41467-021-21928-4.
9. Galletti G, De Simone G, Mazza EMC, [...] and **Lugli E***. Two subsets of stem-like CD8(+) memory T cell progenitors with distinct fate commitments in humans. **Nat Immunol**. 2020. *Accompanying commentary by Chu et al "Two parallel worlds of memory T cells"*.
10. **Lugli E***, Galletti G, Boi SK, and Youngblood BA. Stem, Effector, and Hybrid States of Memory CD8(+) T Cells. **Trends Immunol**. 2020;41(1):17-28. *Cover paper. Top downloaded paper*.
11. Kared H, Tan SW, Lau MC, Chevrier M, Tan C, How W, Wong G, Strickland M, Malleret B, Amoah A, Pilipow K, Zanon V, Govern NM, Lum J, Chen JM, Lee B, Florian MC, Geiger H, Ginhoux F, Ruiz-Mateos E, Fulop T, Rajasuriar R, Kamarulzaman A, Ng TP, **Lugli E**, and Larbi A. Immunological history governs human stem cell memory CD4 heterogeneity via the Wnt signaling pathway. **Nat Commun**. 2020;11(1):821.
12. Alvisi G, Brummelman J, Puccio S, [...] and **Lugli E***. IRF4 instructs effector Treg differentiation and immune suppression in human cancer. **J Clin Invest**. 2020;130(6):3137-50.
13. De Simone G, Mazza EMC, Cassotta A, [...] and **Lugli E***. CXCR3 Identifies Human Naive CD8(+) T Cells with Enhanced Effector Differentiation Potential. **J Immunol**. 2019;203(12):3179-89. *Cover paper. Top downloaded paper*.
14. Brummelman J, Haftmann C, Nunez NG, Alvisi G, Mazza EMC, Becher B, and **Lugli E***. Development, application and computational analysis of high-dimensional fluorescent antibody panels for single-cell flow cytometry. **Nat Protoc**. 2019;14(7):1946-69.
15. Blank CU*, Haining WN*, Held W*, Hogan PG*, Kallies A*, **Lugli E***, [...] and Zehn D*. Defining 'T cell exhaustion'. **Nat Rev Immunol**. 2019;19(11):665-74. *Top downloaded paper*.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

- 2015/09-present Principal Investigator (tenured 2022), Laboratory of Translational Immunology and Head, Humanitas Flow Cytometry Core, IRCCS Humanitas Research Hospital, Milan, IT
- 2013/01-2015/09 Project Leader (supervisor: Dr. D. Mavilio), IRCCS Humanitas Research Hospital, Milan, IT

Other Experience and Professional Memberships

- 2013-present Member, Italian Association of Immunology (SIICA)
- 2012-present Member, International Society for the Advancement of Cytometry (ISAC)

Honors

2023 Italian Ministry of Health, PNRR Research Grant
2023 Elected member, Henry Kunkel Society, New York, USA
2023 Elected member, Editorial Board, European Journal of Immunology
2022 Italian Association for Cancer Research (AIRC), Investigator Grant
2021 US Cancer Research Institute Lloyd J. Old STAR Award
2017 Italian Association for Cancer Research (AIRC), Investigator Grant
2014 European Research Council (ERC) Starting Grant
2014 Research Award, Fondazione Lorini, IT
2012-2016 Marie Curie Career Integration Grant (CIG)
2012 SEMM/SIPOD fellow declined, incompatible with Marie Curie CIG
2012 TRAINOncology fellow declined, incompatible with Marie Curie CIG
2012-2016 International Society for the Advancement of Cytometry (ISAC) scholar
2012 FOCIS conference travel award, June 20-23, Vancouver, BC

Complete List of Published Work in MyBibliography:

https://pubmed.ncbi.nlm.nih.gov/?term=lugli+e+not+lugli+eb&show_snippets=off&sort=date&size=200

Invited speaker at international meetings (selection)

2024 ImmunOctoberfest, Munich, DE
2024 CAR T cell days, Lille, FR
2024 Portuguese Society of Immunology meeting, Porto, PT
2024 CIMT winter school, Obergurgl, AT
2023 3rd Human and Translational Immunology Conference (a Nature Immunology meeting), Rhodes, GR
2023 Karolinska Institutet Immunology Retreat, Oct 4-7, Stockholm, SE
2023 CICON 2024, Sept 20-23, Milan, Italy (talk selected from submitted abstract)
2023 Forbeck forum on immunotherapy and radiotherapy combinations", Apr 27-30, 2023, Como, Italy
2022 ImmunOctoberfest, 19-23 Sept 2022, Munich, Germany
2022 Viral hepatitis and beyond: from basic science to cure: 30-31 May 2022, Freiburg, Germany
2020 4th French International Symposium on CAR T Cells, Lille, FR
2019 Italian Society of Experimental Hematology annual meeting, Bari, IT
2019 Society for the Immunotherapy of Cancer (SITC) annual meeting, Washington DC, USA
2019 European Association for Nuclear Medicine annual meeting, Barcelona, ES
2019 Miltenyi Immuno-Oncology Day "Mobilizing specific T cells against cancer", Paris, FR
2019 2nd Human and Translational Immunology Conference (a Nature Immunology meeting), Kos, GR

Invited seminars (selection)

2024. Stanford University, Palo Alto, CA; Bristol Myers Squibb, Redwood City, CA; Genentech, South San Francisco, CA; Roche Glycart, Zurich, CH; INGM, Milan, IT
2023. Univ of Tübingen, DE; Universitäts Klinikum Hamburg, DE; Univ of Cambridge, UK; UCL, UK; Nature Campus, London, UK; CEMM, Vienna, AT
2022. UPenn, Philadelphia, USA; Human Technopole, Milan, IT; Miltenyi Biotech, Bologna, IT; Institute for Oncology Research, Bellinzona, CH; Institut Curie, Paris, FR
2020. Institute for Research in Biomedicine, Bellinzona, CH; University of Padova, IT; 10X Genomics Italian user meeting; 10X Genomics Italian-Israeli user meeting
2019. Lund University, SE; Université de Lausanne (twice), CH; Salk Institute, USA; Fred Hutchinson Cancer Research Center, USA; Bristol Myers Squibb, Redwood City, USA; Radboud University, Nijmegen, NL; University of Zurich, CH; Roche Glycart AG, CH; Technical University of Munich, DE.

Fellowships/grants of lab members (under my supervision)

EMBO: J. Swatler, 2023-24, € **120.000**; Italian Association for Cancer Research (AIRC): J. Swatler, 2025-2027, € **105.000**; Giorgia Contarini, 2025-2027, € **75.000**; B. Cianciotti and I. Malenica, 2023-25, € **210.000**; C. Scirgolea, 2022; € **25.000**; G. Galletti and S. Puccio, 2019-2021, € **180.000**; G.

De Simone, 2018-2020, € **75.000**; J. Brummelman, 2017-2019, € **90.000**; E. Scamardella, 2017-2019, € **75.000**. Fondazione Veronesi fellowships; E. Mazza, 2017; EUR **27.000**; K. Pilipow, 2018-2020; EUR **85.000**.

Advisor. BD Biosciences, Biolegend, Swarm Therapeutics, Menarini, Amgen, Pfizer

Ad hoc reviewer for:

Journals: Cell, Nature, Nature Immunol, Nature Reviews Immunol, Nature Biotech, Nat Biomed Eng, Nat Nanotech, Immunity, Cancer Cell, J Exp Med, J Clin Invest, Science Translational Med, Nat Commun, Science Immunol, Nature Protoc, Blood, Bioinformatics, J Immunol, Frontiers Immunol, Leukemia, Eur J Immunol, Cancer Immunol Res, J Immunotherapy Cancer, Cancer Immunol Immunother, Cytometry A

Funding agencies: ERC Starting/Consolidator Grant, MRC UK, Wellcome Trust, CRUK, KWF and NWO (Netherlands), FWO (Belgium), Swiss Cancer League, Swiss National Science Fund, Israel Science Foundation, Czech Science Foundation, Italian Ministry of Health.

Major Collaborations

Rahul Roychoudhuri, T cell differentiation and immunosuppression in cancer, University of Cambridge, UK

Benjamin Youngblood, Methylation profiling in T cells, St. Jude Children's Hospital, Memphis, USA

Evan Newell, High-dimensional analysis of antigen-specific T cells, Fred Hutchinson Cancer Center, USA

Johanna Joyce, T cell profiling of brain tumors, UniL, Lausanne, CH

Axel Kallies, mouse models of Tregs in antitumor immunity, University of Melbourne, Australia

Luca Gattinoni, Adoptive T cell transfer, Regensburg Center of Regenerative Medicine, Germany

C. Contributions to Science

1. Identification of stem-like memory T cells and role in cancer immunotherapy. During my postdoc, I pioneered the concept of stem-like differentiation of memory T cells and its implication in adoptive T cell transfer (ACT) immunotherapy of cancer (**Gattinoni*, Lugli* et al., Nat Med, 2011**). These cells are important to maintain long-lived memory T cell responses (**Lugli, et al., J Clin Invest, 2013**). Since then, thousands of studies acknowledged the importance of our discovery: there is agreement in the scientific community that stem cell memory T cells (Tscm) should be induced/employed in clinical trials to improve the effectiveness of ACT in patients with cancer. My independent lab has shown that Tscm contribute to immune reconstitution following ACT in bone marrow transplanted individuals (**Roberto et al., Blood 2015**), and has identified the molecular signals at the basis of Tscm differentiation (**Kared et al., Nat Commun, 2020**). In this regard, the use of antioxidants during ex vivo expansion promotes stem-like properties and improves anti-tumor immunity of chimeric antigen receptor (CAR)-redirected T cells in mouse models (**Pilipow et al, JCI Insight, 2018**)

We were the first to show that a subset with enhanced functionality and stem-like properties (later renamed Tpex) exists among PD-1+ exhausted T cells in human tumors (**Brummelman et al., J Exp Med, 2018**). Subsequent independent studies have shown the importance of these stem-like T cells in determining the success of immune checkpoint blockade. By using several omics technologies, we have subsequently redefined the model of memory T cell differentiation in humans by reporting the existence of two subsets of stem-like T cell progenitors, the Tstem and Tpex, with parallel differentiation programs and distinct fate commitments (functional vs. dysfunctional, respectively) in response to different antigenic repertoires (**Galletti et al., Nature Immunol, 2020**). Thus, we identified the most suitable stem-like progenitors for ACT application. On the basis of these results, we recently proposed the concept of "hybrid" T cell differentiation according to which multiple functional states can co-exist in T cells and how they can be exploited in cancer immunotherapy (**Lugli et al., Trends Immunol, 2020**). More recently, we have shown that NaCl supplementation can prevent exhaustion and enhance anti-tumor immune responses (Scirgolea et al., **Nat Immunol, 2024**).

2. Immunosuppression in the tumor microenvironment. The landscape of immune cells infiltrating tumors is highly complex, featuring subsets of pro-tumoral and anti-tumoral cells. By

using high content single cell technologies, we recently identified unprecedented cellular players mediating immunosuppression in the human tumor microenvironment. These cell populations are common to several cancers and are capable to inhibit anti-tumor immune responses and drive disease progression. In one study, we discovered that the differentiation and function of a subset of highly suppressive effector regulatory T (Treg) cells is governed by the expression of key transcription factors, interferon regulatory factor 4 (IRF4; **Alvisi et al., J Clin Invest, 2020**) and mesenchyme homeobox-1 (MEOX-1; **Alvisi et al., J Hepatol, 2022**). In a different study, performed in collaboration with Massimiliano Pagani at IFOM, Milan, we reported the presence of human FOXP3- Tregs expressing the transcription factor EOMES and correlating with disease progression (**Bonnal*, Rossetti*, Lugli*, et al., Nature Immunol, 2021**). Nevertheless, these cells are associated with improved response to anti-PD-1 immunotherapy. We have identified several transcription factors acting in concert with either IRF4 or EOMES and that are potentially involved in the specification of these Treg subsets. We are currently using molecular and omics technologies to assess the function of these transcription factors genome-wide and how they can shape anti-tumor immunity.

3. Development of cutting-edge single cell technologies for immune profiling (together with the Humanitas Flow Cytometry Core). In collaboration with, and supported by BD Biosciences, we were among the first to optimize 30-parameter high-dimensional flow cytometry (**Mazza et al., Cytometry, 2018; Brummelman et al., J Exp Med, 2018**). In these papers, we also optimized computational strategies for the analysis of complex flow cytometry data. Developing 30-parameter antibody panels is complex and time-consuming. We recently conceived a detailed protocol to provide the community with instructions in this regard (**Brummelman et al., Nature Protocols, 2019**) and developed simplified computational solutions to simplify the analysis of these complex data (**Puccio et al., Nat Commun, 2023**).