European School of Molecular Medicine

2026 Summer call PhD selections

AVAILABLE POSITIONS

Principal Investigator	Jens Geginat
Institute of Affiliation	INGM, UNIMI

PROJECT INFO	
Title of the proposed project:	Role of regulatory T-cells in immunotherapy of melanoma
Short description of the project	Tumors generate a tolerogenic microenvironment to escape immune responses. Immunotherapy unleashes anti-tumor CTL responses and is used to treat immunogenic tumors like melanoma. We showed that EOMES$^+$Tr1-like cells, a regulatory T-cell subsets that we have previously identified and characterized, are consistently enriched among TILs and are associated with tumor stage and poor patients survival. Moreover, EOMES$^+$Tr1-like cells were associated with responsiveness to anti-PD1 immunotherapy in melanoma patients. In melanoma-bearing mice we showed that IL-10 produced by Tr1-cells inhibited CTL response, promoted tumor growth and was key for responsiveness to Immunotherapy. Moreover, EOMES$^+$Tr1-like cells were efficiently activated by tumor antigens to produce IL-10 in melanoma patients. Finally, preliminary data suggested that immunotherapy may induce a switch from tumor-reactive Tr1-cells to CD4$^+$CTL. Consistently, trajectory analysis and TCR clonotype sharing suggested that EOMES$^+$Tr1-like cells may contain precursors of CD4$^+$CTL. **Hypothesis:** EOMES$^+$Tr1-like cells in melanoma patients inhibit CD8$^+$CTL responses, promote tumor immune escape and are targeted by immunotherapy. Successful targeting leads to the differentiation of EOMES$^+$Tr1-like cells to CD4$^+$CTL that do not suppress CD8$^+$CTL but kill MHC class 2 tumor cells, potentially leading to tumor eradication. **Aims:** 1. Identifying genes expressed in tumor-reactive and IL-10 producing T-cell clonotypes in primary tumors of melanoma patients that may regulate the transition to CD4$^+$CTL. 2. Longitudinal analysis of T-cell clonotypes upon immunotherapy (anti-PD1 monotherapy or combination with anti-LAG3) to investigate if EOMES$^+$Tr1-cells could differentiate to CD4$^+$CTL. 3. Identifying factors that promote the generation of CD4$^+$CTL *in vitro* and in humanised mice. **Experimental Design:** 1. Tumor-reactive and IL-10 secreting T-cells in the blood of melanoma patients will be cloned and TCRs sequenced to track tumor-reactive clonotypes among TILs. TILs and circulating T-cells will be analysed by single-cell RNA and TCR sequencing to identify genes characteristic for tumor-infiltrating Tr1-cells and their circulating precursors. Extensive Bioinformatic analysis. 2. With the same experimental strategy tumor-reactive T-cell clonotypes will be tracked in the blood before and after immunotherapy with anti-PD1 to identify the precursors of

	$CD4^+$CTL. Moreover, anti-PD1 monotherapy will be compared to combination therapy with anti-PD1 and anti-LAG3 to investigate the mechanism of action of anti-LAG3. 3. TCR signal strength and cytokine conditions will be altered to promote the differentiation of Tr1-cells to $CD4^+$CTL *in vitro*. We will investigate the relevance of genes that are predicted to drive this transition according to bioinformatic analysis by overexpression or silencing. Neutralising antibodies to identified candidates and/or checkpoint receptors will be tested in humanised NSG mice if they shift the balance from Tr1-cells to $CD4^+$CTL. Finally, the impact on the control of autologous melanoma cells in humanised mice will be assessed. **Expected results:** We expect to clarify if tumor-promoting $EOMES^+$Tr1-like cells may differentiate to anti-tumoral $CD4^+$CTL upon immunotherapy. We hope to identify neutralising antibodies that promote $CD4^+$CTL differentiation *in vitro* and in humanised mice *in vivo*. **Impact on cancer:** Not all melanoma patients respond to immunotherapy and it is unknown why. Understanding the mechanisms of immunotherapy adresses therefore a major unmet medical need. Moreover, neutralising antibodies that promote anti-tumoral $CD4^+$CTL may pave the way for new immunotherapeutic strategies.
Main research area for the project	Tumor Immunology
Second research area for the project	
3 key words for the project	Interleukin-10, regulatory T-cells, Immunotherapy

LAB INFO	
Main topic/s of the lab	Immune regulation, T lymphocytes, Interleukin-10, cancer, autoimmunity
Short description of the lab activity	Introduction CD4+ T-cells are central players of adaptive immune responses, because they co-ordinate the activity of several other immune cells such as dendritic cells (DC), B cells and cytotoxic CD8+T-cells. CD4+T-cells can be subdivided into two broad subsets of helper and regulatory cells, which promote and suppress immune responses, respectively. Both regulatory and helper T-cells can produce Interleukin-10 (IL-10), which is a potent anti-inflammatory cytokine but also promotes cytotoxic T-cell responses and B-cell antibody production. Consequently, IL-10 has a dual role in autoimmunity and cancer: On the one hand, it is required to maintain intestinal immune homeostasis and to prevent colitis, but on the other hand it could promote the generation of pathogenic autoantibodies in systemic lupus erythematosus (SLE) or cytotoxic anti-tumor T-cell responses.

	Role of IL-.10 producing regulatory T-cells in human immune-mediated diseases Regulatory T-cells can be sub-divided into the well-defined CD25+Tregs, which express the lineage-defining transcription factor FOXP3, and the enigmatic Tr1-cells, which produce high levels of IL-10. It is debated whether Tr1-cells represent an independent differentiation lineage or a transient and unstable activation stage. We identified a population of cytotoxic Tr1-like cells in human tissues that produce high levels of IL-10 and are generated upon chronic antigenic activation. They stably express the lineage-defining transcription factor EOMES and are probably derived from pro-inflammatory EOMES+Th1-cells. EOMES+Tr1-like-cells are involved in several immune-mediated diseases. Thus, EOMES+Tr1-like cells, but not FOXP3+Tregs, were functionally impaired in systemic lupus erythematosus and in inflammatory bowel diseases (IBDs). In multiple sclerosis they were enriched and clonally expended in the cerebrospinal fluid, and they produced IL-10 in brain lesions. However, they were activated by EBV, but failed to respond to myelin-derived self-antigens, possibly explaining why they fail to prevent CNS damage. Furthermore, they were enriched and clonally expanded in different types of human tumors. Tumour-infiltrating Tr1-like cells were associated with poor prognosis, but also with response to immunotherapy in melanoma. Unpublished evidence indicates a key role for Tr1-derived IL-10 in tumor growth and immunotherapy responsiveness in melanoma patients.
Recent bibliography	1. The Human Bone Marrow May Offer an IL-15-Dependent Survival Niche for EOMES+ Tr1-Like Cells. Pulvirenti Nadia, Vasco Chiara, Righetti Camilla, Dadova Petra, Boffa Giacomo, Laroni Alice, Vigo Tiziana, Raiola Anna Maria, Crosti Maria Cristina, Maglie Stefano, Valenti Luca, Prati Daniele, Abrigani Sergio, Uccelli Antonio, Geginat Jens Eur J Immunol 2025 May; 55: e202451644 2. Characterization of human CD4+EOMES+GzmK+ T-cell subsets unveils an uncoupling of suppressive functions from IL-10-producing capacities. Pulvirenti Nadia, Silvetri Ylenia, Clemente Francesca, Bosotti Roberto, Carelli Elena, Moschetti Giorgia, Gruarin Paola, Vasco Chiara, Crosti Maria Cristina, Sarnicola Maria Lucia, Valenti Luca, Prati Daniele, Abrignani Sergio, Geginat Jens Eur J Immunol 2024 Apr; 54: e2350675 3. Eomesodermin-expressing type 1 regulatory (EOMES+ Tr1)-like T cells: Basic biology and role in immune-mediated diseases. Geginat Jens, Vasco Chiara, Gruarin Paola, Bonnal Raoul, Rossetti Grazisa, Silvestri Ylenia, Carelli Elena, Pulvirenti Nadia, Scantamburlo Morena, Moschetti Giorgia, Clemente Francesca, Grassi Fabio, Monticelli Silvia, Pagani Massimiliano, Abrignani Sergio Eur J Immunol 2023 May; 53: e2149775

	4. Clonally expanded EOMES+ Tr1-like cells in primary and metastatic tumors are associated with disease progression. Bonnal Raoul J P, Rossetti Grazisa, Lugli Enrico, De Simone Marco, Gruarin Paola, Brummelman Jolanda, Drufuca Lorenzo, Passaro Marco, Bason Ramona, Gervasoni Federica, Della Chiara Giulia, D'Oria Claudia, Martinovic Martina, Curti Serena, Ranzani Valeria, Cordiglieri Chiara, Alvisi Giorgia, Mazza Emilia Maria Cristina, Oliveto Stefania, Silvestri Ylenia, Carelli Elena, Mazzara Saveria, Bosotti Roberto, Sarnicola Maria Lucia, Godano Chiara, Bevilacqua Valeria, Lorenzo Mariangela, Siena Salvatore, Bonoldi Emanuela, Sartore-Bianchi Andrea, Amatu Alessio, Veronesi Giulia, Novellis Pierluigi, Alloisio Marco, Giani Alessandro, Zucchini Nicola, Opocher Enrico, Ceretti Andrea Pisani, Mariani Nicolò, Biffo Stefano, Prati Daniele, Bardelli Alberto, Geginat Jens, Lanzavecchia Antonio, Abrignani Sergio, Pagani Massimiliano Nat Immunol 2021 Jun; 22: 735 5. Ex vivo microRNA and gene expression profiling of human Tr1-like cells suggests a role for miR-92a and -125a in the regulation of EOMES and IL-10R. De Simone Marco, Chirichella Michele, Emming Stefan, Mazzara Saveria, Ranzani Valeria, Gruarin Paola, Moschetti Giorgia, Pulvirenti Nadia, Maglie Stefano, Vasco Chiara, Crosti Maria Cristina, Rossetti Grazisa, Pagani Massimiliano, Abrignani Sergio, Monticelli Silvia, Geginat Jens Eur J Immunol 2021 Dec; 51: 3243
Group composition	PI (1) Staff Scientist (1) Post-doc (3) Ph.D students (3) Graduate Students (1) Undergraduate students (1)
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