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How CD4+ T-cell help shapes functional and dysfunctional CD8+ T-cell differentiation

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Curriculum Vitae

Julia Laura Charlotte Busselaar was born on 10th January 1994 in Amsterdam, the Netherlands. She completed her pre-university education cum laude at the Vossius Gymnasium in Amsterdam in 2012. In the same year, Julia started her bachelor studies Biomedical Sciences at the University of Amsterdam, which she completed in 2015. In 2016, she took a gap year in which she worked for the university as supervisor for 1st and 2nd year bachelor students during their practical courses. Subsequently she enrolled in the master's Biomedical Sciences at the University of Amsterdam, with a specialization in oncology. During this time she performed her first internship in the group of Jannie Borst at the Netherlands Cancer Institute. Under supervision of Sander de Kivit, she developed a new *in vitro* platform for delivering deliberate costimulatory and coinhibitory signals to human T cells, which was later published. Julia performed her second internship at the VUmc Cancer Center Amsterdam in the group of Victor van Beusechem. Under supervision of Maxime Blijlevens, she studied the relationship between p53 transcriptional activity and cisplatin sensitivity in human lung cancer. This was followed by completing the Article 9 course for working with laboratory animals at the University of Amsterdam, and writing her master thesis in the group of Yvette van Kooyk at the VUmc, about therapeutic vaccinations in infection and cancer. After obtaining her Master's degree cum laude in 2018, Julia re-joined the group of Jannie Borst at the NKI as a PhD candidate. She continued her PhD research at the LUMC in Leiden, when the Borst lab moved there in 2019. The results of her studies are presented in this thesis. Currently, Julia works as a postdoctoral researcher in the group of Linde Meyaard at UMC Utrecht, where she investigates novel ways of blocking immune inhibitory receptors.

Portfolio

Factual narrative of the PhD trajectory

In September 2018, Julia joined the lab of professor Jannie Borst at the Netherlands Cancer Institute (NKI) as PhD candidate. She was appointed on a project on the molecular mechanisms of $CD4^+$ T-cell help to $CD8^+$ T cells, which was initiated by a previous PhD student, Tomasz Ahrends. Her first task was to analyse an RNA sequencing dataset generated by Tomasz just before he left the lab, and incorporate this data into the revision of his final paper. Unexpectedly, the results from this additional dataset shifted the interpretation of Tomasz's data and total concept of the paper. Julia generated and adapted figures, reinterpreted the data and wrote the revised version of the manuscript based on this new concept (Chapter 2 of this thesis), which was crucial for its acceptance for publication.

In May 2019, 8 months after Julia started, the Borst group moved from the NKI to the Leiden University Medical Center (LUMC). At that time Julia was the only group member that would be performing mouse experiments at the new institute, which meant that she also took care of all the paperwork, ethical approval of experimental protocols and the transfer of genetically modified mice to the LUMC. Together with Douwe Bosma, who joined the lab as a PhD student in October 2019, she re-established the murine *in vivo* DNA tattoo vaccination model, testing various strategies to optimize the induced T cell responses.

The process of moving to the LUMC took several months, which Julia used to dive deep into the literature on T cell biology. During this time she developed a new theory about T cell differentiation, namely that $CD4^+$ T-cell help shifts the differentiation spectrum of $CD8^+$ T cells, and that 'exhausted' $CD8^+$ T cells in cancer are formed from incompletely differentiated 'helpless' cells. Julia enthusiastically presented her theory to her promotor Jannie, who was initially reluctant to divert Julia's project but agreed to let her write a perspective article on it during the first Covid-19 lockdown in spring 2020. This resulted in a publication (Chapter 3 of this thesis), which also convinced Jannie of the new theory and its importance. Despite the highly competitive nature of the T-cell exhaustion field, Jannie agreed to let Julia divert her project and investigate her differentiation model experimentally.

Over the next few years, together with Douwe, Julia designed and performed experiments to test the new hypothesis. From the same *in vivo* mouse experiments, they both performed data-analysis individually, whereby Julia focused on the $CD8^+$ T cells and Douwe on the $CD4^+$ T cells (resulting in Chapter 4 and 5 of this thesis). When the research group generated a large single cell RNA and TCR sequencing dataset of $CD4^+$ and $CD8^+$ T cells but had no bioinformatician to analyse it, Julia taught herself R programming language so that she could perform the analyses independently. These data were used both in her own project and in the work of Douwe.

She also closely collaborated with PhD students Mo Staal and Elselien Frijlink, together forming the 'mouse team' of the Borst group. Julia followed several PhD courses and attended many scientific conferences, where she also presented her own work.

In the meantime, Julia kept up with all the literature in the exhaustion field, and noticed that many of other people's findings could be explained beautifully by the new differentiation model. In 2021, she contributed as a second author to an invited review written by her promotor Jannie, highlighting the importance of $CD4^+$ T-cell help for explaining the biological mechanisms of PD-1 blockade therapy (Chapter 7 of this thesis).

In 2022, Julia contributed substantially to the application for a new CCD licence for *in vivo* animal studies, which provided valuable insights into ethical and regulatory processes. In 2023, she supervised the writing assignment of Merel Sijbranda, a master student Infection and Immunity at Utrecht University. Julia later adapted this writing assignment into a review about the importance of Type-I interferon signaling in $CD4^+$ T-cell help delivery (Chapter 6 of this thesis), which was published in 2024.

In December 2024, Julia concluded her PhD at the LUMC. In 2025 she became a mother and focused on her new family, while also writing the revision of her final paper. This work, which provided substantial experimental evidence for the new T cell differentiation model (Chapter 4 of this thesis), was eventually published in 2026. Also the main work of Douwe, to which Julia contributed (Chapter 5 of this thesis), was published in 2026.

CRediT Table

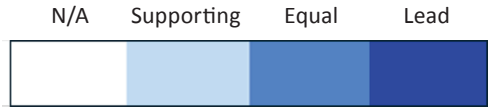
CRediT table for the thesis of Julia Busselaar

Ch.	Type*	Short Title	Conceptualization	Data Curation	Formal Analysis	Funding Acquisition	Investigation	Methodology	Project Administration	Resources	Software	Supervision	Validation	Visualization	Writing – Original Draft	Writing – Review & Editing	Preregistered	Preprinted	Published with Peer Review
1	Introduction	Chapter 1. Introduction															☐	☐	☐
2	PhD project chapter	Chapter 2. Memory															☐	☐	☑
3	PhD project chapter	Chapter 3. Helpless dysfunction															☐	☐	☑
4	PhD project chapter	Chapter 4. CD8 differentiation															☐	☐	☑
5	PhD project chapter	Chapter 5. CD4 differentiation															☐	☐	☑
6	PhD project chapter	Chapter 6. IFN															☐	☐	☑
7	PhD project chapter	Chapter 7. PD-1															☐	☐	☑
8	Discussion	Chapter 8. Discussion															☐	☐	☐

**PhD project chapters are the direct result of the PhD project of the PhD candidate.*
Some theses also include Collaboration Chapters, to which the PhD candidate has contributed but fall outside the PhD project.

Left to right colored:
Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original draft, Writing – Review and editing.

Color scale:



Left to right ticked:
Preregistered, Preprinted, Published with Peer Review

For more info, see: https://osf.io/download/6845fa0e26e4486cf1d539468/

List of Publications

1. **CD4⁺ T cell help creates memory CD8⁺ T cells with innate and help-independent recall capacities**
   Ahrends T, Busselaar J, Severson T, Babala N, de Vries E, Bovens A, Wessels L, van Leeuwen F, Borst J.
   Nature Communications 2019; 10(1): 553. doi: 10.1038/s41467-019-13438-1

2. **Helpless priming sends CD8⁺ T cells on the road to exhaustion**
   Busselaar J, Tian S, van Eenennaam H, Borst J.
   Frontiers in Immunology 2020; 11: 592569; doi: 10.3389/fimmu.2020.592569

3. **Mechanism of action of PD-1 receptor/ligand targeted cancer immunotherapy**
   Borst J, Busselaar J, Bosma DMT, Ossendorp F.
   European Journal of Immunology 2021; 51(8): 1911-1920. doi: 10.1002/eji.202048994

4. **The importance of type I interferon in orchestrating the cytotoxic T-cell response to cancer**
   Busselaar J*, Sijbranda M*, Borst J.
   Immunology Letters 2024; 270: 106938; doi: 10.1016/j.imlet.2024.106938

5. **PD-1 or CTLA-4 blockade promotes CD86-driven Treg responses upon radiotherapy of lymphocyte-depleted cancer in mice**
   Frijlink E, Bosma DMT, Busselaar J, Battaglia TW, Staal MD, Verbrugge I, Borst J.
   Journal of Clinical Investigation 2024; 134(6): e171154. doi: 10.1172/JCI171154.

6. **CD4⁺ T-cell help delivery to monocyte-derived dendritic cells promotes CD4⁺ effector differentiation of helper and cytotoxic T cells**
   Bosma DMT, Busselaar J, Staal M, Frijlink E, Mack M, Salerno F, Borst J.
   Immunology Letters 2025; 273: 107022. doi: 10.1016/j.imlet.2025.107022

7. **CD27 costimulation supports metabolic fitness of CD4⁺ T cells by enhancing de novo nucleotide and protein synthesis**
   Ngoc Minh TT*, Verleng LJ*, Schrama E, Busselaar J, de Vries E, Borst J, Berkers CR, Zaal EA, de Kivit S.
   The Journal of Immunology 2025; vkaf075. doi: 10.1093/jimmun/vkaf075

8. **Phagocytosis of necroptotic cells optimizes type 1 conventional dendritic cells for induction of a cytotoxic T-cell response**
   Staal MD, Wang Z, Bosma DMT, Busselaar J, Schrama E, de Vries E, Xiao Y, Borst J.
   Cell Death and Differentiation 2026; doi: 10.1038/s41418-026-01689-7.

9. **Stem-like PD-1⁺TCF-1⁺ CD8⁺ T cells result from helpless priming and rely on CD4⁺ T-cell help to complete their cytotoxic effector differentiation**
   Busselaar J*, Bosma DMT*, Staal MD, Lei X, Xiao Y, Borst J.
   The Journal of Immunotherapy of Cancer 2026; 14(4): e014041. doi: 10.1136/jitc-2025-014041

10. **Requirements for development of T-helper1 and T-follicular helper cells from a common precursor**
    Bosma DMT*, Busselaar J*, Staal MD, de Koning M, Reljić M, Lei X, de Wit T, Xiao Y, Borst J, Salerno F.
    Cell Reports 2026; 45(5): 117296. doi: 10.1016/j.celrep.2026.117296

List of submitted manuscripts

1. **Discerning and common features of CD4$^+$ conventional and regulatory T cells that determine human pregnancy success**
   Verleng L J, Busselaar J, Mensink M, Schrama E, Kapsenberg H, van der Keur C, Lei X, Eikmans M, Xiao Y, de Kivit S, Borst J.
   Manuscript submitted to Frontiers in Immunology

2. **CD8$^+$ T cell priming, CD4$^+$ T cell help and implications for cancer immunotherapy**
   Kastenmüller W, Busselaar J, Huang A, Borst J.
   Revised manuscript submitted to Immunity

Overview of completed courses and other training

Mandatory activities

Year	Title	Hours
2018	Laboratory Animal Science (Article 9)	80
2020	Leiden University Onboarding Programme Inform & Connect	10
2020	Workshop Scientific Conduct for PhDs	5
2021	Basic Methods and Reasoning in Biostatistics	42

Scientific courses, workshops, conferences and other training activities

Year	Title	Hours
2018	European Congress of Immunology (Amsterdam)	16
2019	Dutch Society for Immunology (NVVI) Symposium (Noordwijk)	16
2019	PhD Course Mouse Genetic Engineering (Leiden)	50
2019	IHB Science Retreat (Leiden)	8
2019	NVVI Annual Meeting (Lunteren)	16
2020	MACS T cell Exhaustion Meeting (Brussels, Belgium)	8
2020	NKI-LUMC Immunology Meeting (Leiden)	8
2020	IMMU Science Retreat (Leiden)	8
2020	NVVI Annual meeting (online)	16
2021	NVVI Symposium (online)	16
2021	Oncode Annual Meeting (online)	16
2021	Dutch Tumor Immunology Meeting (online)	16
2021	European Congress of Immunology (online)	16
2021	IMMU Science Retreat (Leiden)	8
2022	PhD Course Infection and Immunity (Leiden)	42
2022	NKI-LUMC Immunology meeting (Leiden)	8
2022	NVVI Annual Meeting (Lunteren)	16
2022	VIB Conference Immuno-Oncology (Leuven, Belgium)	16
2022	Oncode & HollandBio Meeting (Amsterdam)	5
2022	IMMU Science Retreat (Leiden)	8
2023	Joint Belgian-Dutch Immunology Meeting (Antwerp, Belgium)	16
2023	16th ENII EFIS EJI Summer School on Advanced Immunology (Alghero, Italy)	21

Presentations

Year	Title
2019-23	**Speaker (occasionally)**: Weekly department of Immunology (IMMU) meeting (Leiden)
2019-23	**Speaker (occasionally)**: Weekly Cancer Immunology meeting (Leiden)
2019	**Speaker:** NVVI Annual meeting (Lunteren)
2020	**Speaker:** IMMU Science Retreat (Leiden)
2021	**Poster:** IMMU Science Retreat (Leiden)
2022	**Poster:** NVVI Annual Meeting (Lunteren)
2023	**Poster:** IMMU Science Retreat (Leiden)
2023	**Poster:** 16th ENII EFIS EJI Summer School on Advanced Immunology (Alghero, Italy)

Teaching

Year	Title	Hours
2023	Supervision of writing assignment by Merel Sijbranda, second year master student Infection and Immunity at Utrecht University	20

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Help is essential, not only for T cells but also for people. During my PhD, I was fortunate enough to receive help, guidance and support from so many people. Without you, this thesis would not have been possible.

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