Understanding response to rituximab treatment in rheumatoid arthritis through immune fingerprinting of T and B cells

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English Summary
Nederlandse Samenvatting
List of Publications
Author Contributions
PhD Portfolio
Curriculum Vitae
Acknowledgments

ENGLISH SUMMARY

The cells of our immune system are tasked to defend our body from the billions of different pathogens which may attack us during our lifetime. The ability to recognize virtually any invading agent stems from special receptors on the cells of the adaptive immune system, i.e. T cells and the antibody producing B cells. These receptors, termed T cell receptors (TCRs) and B cell receptor (BCRs) contain a constant domain, which as the name suggests is similar among all receptors, and a variable domain, which is unique to each TCR and BCR. The latter is the area dedicated to the recognition of invading pathogens. TCRs and BCRs are uniquely formed during the development of each T or B cell; therefore, each T or B cell carries a unique TCR or BCR with a unique specificity against a given antigen, i.e. the part of pathogens which gets recognized. The binding of a TCR or BCR with a matching antigen may trigger so-called "clonal expansion": receptor-carrying T or B cells undergo several rounds of proliferation which results in the formation of an "army" of clones all carrying an identical TCR or BCR able to recognize and attack the invading pathogen. Therefore, knowing the ensemble of all the TCRs and BCRs present in an individual at a given timepoint, i.e. its TCR or BCR repertoire, can tell us something about the ongoing immune responses within that individual.

In immunological research this is achieved using next-generation sequencing (NGS), by which thousands and thousands of single TCRs and BCRs from any given patient sample can be sequenced at once. Through the analysis of these BCR or TCR repertoires researchers can identify and quantify all the different BCRs and TCRs in the sample, and spot which have undergone clonal expansion, indicating that they might be involved in an ongoing immune response. In the past years, we applied this technique to study autoimmune responses, i.e. erroneous immune responses elicited by antigens which are present within our own body, or autoantigens. In particular, we focused on rheumatoid arthritis (RA), a chronic autoimmune disease characterized by inflammation in the peripheral joints, which if untreated, can lead to tissue damage and synovial joint bone destruction. In patients suffering from RA, autoantibodies, i.e. antibodies targeting autoantigens, can be detected in peripheral blood years before the onset of clinical symptoms. In addition, in a subset of patients, large B cell infiltration can be detected at the site of inflammation, i.e. the synovium. This indicate that B cells play an important role not only in disease pathogenesis but also in the perpetuation of clinical symptoms. In fact, a therapeutic approach which targets these cells proved to be very effective in reducing disease activity. An example is rituximab, a therapeutic monoclonal antibody directed against the molecule CD20 which is expressed on B cells. Patients treated with rituximab experience > 98% depletion of the B cells in peripheral blood lasting for at least 4-5 months and clinical response is achieved in 50-65% of the cases. Yet, a proportion of patients does not respond to treatment. Understanding the mechanism behind this

incomplete response might not only lead to an improvement in treatment strategies but might also shed more light on the cellular and molecular components which drive clinical response and are involved in disease pathogenesis.

PART I: BCR repertoire analysis for the evaluation of clinical response to rituximab

In the first part of this thesis, we focused on evaluating clinical response to rituximab by analysis of the BCR repertoire at different timepoints and in different tissues in patients undergoing treatment with rituximab. First, we analyzed the changes induced by the therapy in the BCR repertoire at 4 and 16/24 weeks after treatment, both in blood and in synovial tissue in the joint (**Chapter 2**). We observed drastic changes in the peripheral blood BCR repertoire which at 4 weeks post-treatment was composed of fewer but more expanded and mutated BCR clones. These changes were also observed at the second follow up timepoint. On the contrary, no major differences in the synovial tissue repertoire were observed apart from an increase in the amount of mutated BCR clones at 16/24 weeks after treatment. These results raise the concern to what extend rituximab is able to reach and target B cells residing in the site of active inflammation, i.e. the synovium. Yet, when focusing on the peripheral blood BCR repertoire, a higher clonal overlap between the pre-treatment and the 4 weeks post-treatment repertoire was observed in non-responders, indicating that clinical response might be linked to complete disruption of the (pre-treatment) peripheral blood repertoire.

The same analysis was repeated in a new cohort of patients starting on rituximab treatment and applying a novel NGS approach that we develop to assess TCR and BCR repertoires more sensitively and quantitatively (**Chapter 3**). Patients were also followed up for a longer time and peripheral blood samples were obtained at one, three, six and twelve months after treatment. We confirmed that at one month post-treatment the peripheral blood BCR repertoire is less diverse, more expanded and more mutated. These effects started to revert at six months post-treatment, indicating a re-establishment of the pre-treatment condition possibly due to the cessation of drug efficacy and consequent repopulation of the B cell compartment. To assess the correlation between the timing of B cell depletion and repopulation and clinical responses, we defined in each patients a timepoint of achieved depletion and achieved repopulation based on the percentage of unmutated BCR clones in the peripheral blood repertoire, which we hypothesized be a good proxy for the fraction of naïve B cells in peripheral blood. Early depletion (= timepoint of depletion within one month of treatment) or early repopulation (= timepoint of repopulation within six month of treatment) did not correlated with clinical response at six or twelve months post-treatment. However, repopulation within the first six months did significantly correlate with decreased disease activity in the subsequent period (i.e. 6-12 months post-treatment), suggesting it is repopulation rather than

depletion that influences response to rituximab.
Overall, the results reported in the first part of this thesis show how prediction of clinical response to rituximab therapy remains challenging mostly because the underlying mechanisms behind rituximab efficacy are still incompletely understood.

PART II: Developing TCR repertoire analysis for the assesment of immunogenicity

In the second part of this thesis clinical response to rituximab was evaluated in respect to a phenomenon which is known to influence efficacy of all biologicals in general, i.e. drug immunogenicity. Immunogenic responses against biologicals manifest with the presence of antibodies directed against the drug, therefore termed anti-drug antibodies or ADAs. These ADAs are known to impair availability of the drug and can therefore lead to reduced clinical response. The development of ADAs implies the present of both drug specific B and T cells, which get activated upon recognition of the biological agent, and start building an immune response against it. Therefore, TCR and BCR repertoire analysis could be applied to study also these immunogenic responses. We developed a novel methodology to identify the antigen specific TCR clones in the total repertoires by combining TCR sequencing with an *in vitro* stimulation assay (**Chapter 4**). We evaluated the dynamics of clonal expansion in single TCR clones at different timepoints after antigen stimulation. We adopted an already validated algorithm normally used in transcriptomic data analysis to detect TCR clones with a differentially increased frequency in the post-stimulation repertoire, and appointed them as the antigen-responsive ones. We additionally show the versatility of this methodology in obtaining more information about functional phenotype or specificity of the selected antigen-responsive TCR clones, highlighting its potential as valuable immunological tool for antigen specific immune-monitoring in clinical settings of large patient cohorts and complex and variable antigens of interest.
The application of this methodology allowed us to study immunogenic T cell responses against rituximab (**Chapter 5**). Peripheral blood samples obtained from patients treated with rituximab which developed ADAs at one year post-treatment were stimulated *in vitro* with a pool of rituximab-derived peptides. Rituximab-specific T cells were shown to produce IFN-γ, IL-5 and IL-10, the latter two being important cytokines mediating maturation and differentiation of antibody-producing B cells. Rituximab-responsive TCR clones were selected as differentially over-expanded clones in the post-stimulation TCR repertoire as described in chapter four and were shown to be polyclonal, differing between patients. The *in-vitro* identified rituximab-responsive TCR clones were then traced back in the *ex-vivo* peripheral blood TCR repertoire obtained prior to and at one, three, six and twelve months post-treatment. Most of the rituximab-responsive TCR clones appeared in blood within the first month of treatment preceding the serological detection of ADAs. These clonal responses could still be detected in samples obtained

at one year after treatment and in some cases even in the pre-treatment sample. These results indicate that immunogenic T cell responses to rituximab develop very early after treatment and might be present even before treatment initiation, building a rationale for pre-treatment assessment in candidate patients. However, since immunogenic responses against biologicals can be considered physiological immune responses against a novel antigen, they should be expected in every treated patient. Future treatment strategies should therefore be adjusted to prevent or at least to minimize the development of clinically relevant ADA-development.

Finally, in **chapter six** the findings and results presented in this thesis are summarized and discussed in the light of current literature with an outlook to future perspectives.

NEDERLANDSE SAMENVATTING

De cellen van ons immuunsysteem hebben de taak ons lichaam te verdedigen tegen de miljarden verschillende ziekteverwekkers die ons tijdens ons leven kunnen aanvallen. Het vermogen om vrijwel elke binnendringend ziekteverwekker te herkennen is gecodeerd in een speciale receptor op de cellen van het adaptieve immuunsysteem, d.w.z. T-cellen en de antilichaam-producerende B-cellen. Deze receptoren, T-cel receptoren (TCR's) en B-cel receptor (BCR's) genoemd, bevatten een constant domein, dat, zoals de naam doet vermoeden, vergelijkbaar is tussen alle receptoren, en een variabel domein, dat uniek is voor elke TCR en BCR. Dit laatste gebied zorgt voor de herkenning van de binnendringende ziekteverwekker. TCR's en BCR's worden op unieke wijze gevormd tijdens de ontwikkeling van elke B- of T-cel; daarom draagt elke T- of B-cel een unieke TCR of BCR met een unieke specificiteit tegen een bepaald antigeen, d.w.z. het deel van ziekteverwekkers dat herkend wordt. De binding van een TCR of BCR met een passend antigeen veroorzaakt de zogenaamde 'klonale expansie', waarbij de receptor-dragende T- of B-cellen verschillende proliferatierondes ondergaan, wat resulteert in de vorming van een 'leger van klonen' die allemaal de unieke TCR of BCR dragen die de binnendringende ziekteverwekker kan herkennen en aanvallen. Daarom is het kennen van het geheel van alle TCR's en BCR's die op een bepaald tijdstip in een individu aanwezig zijn, d.w.z. zijn TCR- of BCR-repertoire, belangrijk: Het kan ons iets vertellen over de aanhoudende immuunreacties bij dat individu.

Het in kaart brengen van deze receptoren kan worden bereikt met behulp van next-generation sequencing (NGS), waarbij duizenden en duizenden afzonderlijke TCR's en BCR's sequenties in één keer uitgelezen kunnen worden, uitgaande van het monster van een bepaalde patiënt. Door de analyse van deze BCR- of TCR-repertoires kan de onderzoeker alle verschillende BCR's en TCR's in het monster onderscheiden, en onderzoeken welke TCRs en BCRs klonale expansie hebben ondergaan. Dat wijst er immers op dat ze mogelijk betrokken zijn bij een aanhoudende immuunrespons.

In de afgelopen jaren hebben we deze techniek toegepast om auto-immuunreacties te bestuderen. Dergelijke immuunreacties zijn ten onrechte opgewekt zijn door antigenen die in ons eigen lichaam aanwezig zijn, oftewel autoantigenen. In het bijzonder hebben we ons gericht op reumatoïde artritis (RA), een chronische auto-immuunziekte die wordt gekenmerkt door ontstekingen in de perifere gewrichten die, indien onbehandeld, kunnen leiden tot weefselschade en botvernietiging in synoviale gewrichten. Bij patiënten die lijden aan RA kunnen auto-antilichamen, d.w.z. antilichamen die zich richten op autoantigenen jaren vóór het begin van de klinische symptomen in perifeer bloed worden gedetecteerd. Bovendien kan bij een subgroep van patiënten infiltratie van B-cellen worden gedetecteerd op de plaats van de ontsteking, d.w.z. het synovium. Dit geeft aan dat B-cellen niet alleen een belangrijke rol spelen bij de pathogenese

van ziekten, maar ook bij het in stand houden van klinische symptomen. In feite bleken therapeutische benaderingen die zich op deze cellen richtten zeer effectief bij het verminderen van ziekteactiviteit. Een voorbeeld is rituximab, een therapeutisch monoklonaal antilichaam dat gericht is tegen het molecuul CD20, dat door B-cellen op het celoppervlak tot expressie wordt gebracht. Bij patiënten die met rituximab worden behandeld, treedt een depletie van > 98% van de B-cellen in het perifere bloed op, die ten minste 4-5 maanden aanhoudt, en in 50-65% van de gevallen wordt een klinische respons bereikt. Toch reageert een deel van de patiënten niet op de behandeling. Het begrijpen van het mechanisme achter deze onvolledige respons zou niet alleen kunnen leiden tot een verbetering van de behandelstrategieën, maar zou ook meer licht kunnen werpen op de cellulaire en moleculaire componenten die de klinische respons aansturen en daarom uiteindelijk betrokken zijn bij de pathogenese van ziekten.

DEEL I: BCR-repertoireanalyse voor de evaluatie van de klinische respons op rituximab

In het eerste deel van dit proefschrift richten we ons op het evalueren van de klinische respons op rituximab door het BCR-repertoire op verschillende tijdstippen en in verschillende weefsels te analyseren bij patiënten die behandeling met rituximab ondergaan. Eerst analyseerden we de door de therapie geïnduceerde veranderingen in het perifere bloed en het BCR-repertoire van het synoviale weefsel 4 en 16/24 weken na de behandeling (**hoofdstuk twee**). We hebben drastische veranderingen waargenomen in het BCR-repertoire van het perifere bloed, dat vier weken na de behandeling bestond uit minder, meer uitgegroeide en meer gemuteerde BCR-klonen. Deze veranderingen werden ook waargenomen op het tweede follow-uptijdstip. Echter, er werden geen grote verschillen in het BCR-repertoire in het synoviale weefsel in het gewricht waargenomen, afgezien van een toename in de hoeveelheid gemuteerde BCR-klonen 16/24 weken na de behandeling. Deze resultaten roepen vragen op over de mate waarin rituximab B-cellen kan bereiken en aanpakken die zich op de plaats van actieve ontsteking bevinden, d.w.z. in het synovium. Patienten die geen respons vertoonden toonden in het bloed een hogere klonale overlap tussen het repertoire vóór de behandeling en het repertoire 4 weken na de behandeling, wat erop wijst dat de klinische respons in verband kan worden gebracht met een volledige verstoring van de (pre-)behandelingsrepertoire.

Dezelfde analyse werd herhaald in een nieuw cohort patiënten die begonnen met behandeling met rituximab. We gebruikten daarbij een nieuwe NGS-aanpak waarmee we TCR- en BCR-repertoires gevoeliger en beter kwantitatief konden onderzoeken (**hoofdstuk drie**). Ook werden patiënten langere tijd gevolgd en werden bloedmonsters één, drie, zes en twaalf maanden na de behandeling afgenomen. We bevestigden dat één maand na de behandeling het BCR-repertoire in het perifere bloed minder divers is, verder uitgegroeide clonen bevat en hun BCRs meer gemuteerd zijn. Deze effecten

begonnen zes maanden na de behandeling weer te verdwijnen, wat wijst op een herstel naar de toestand vóór de behandeling, mogelijk als gevolg van het stopzetten van de werkzaamheid van het geneesmiddel, en de daaruit voortvloeiende herbevolking van het B-celcompartiment. Om de correlatie tussen de timing van de depletie en herbevolking van B-cellen en de klinische reacties te beoordelen, hebben we voor elke patiënt een tijdstip gedefinieerd voor het bereiken van depletie en het bereiken van herbevolking, gebaseerd op het percentage niet-gemuteerde BCR-klonen in het perifere bloedrepertoire, waarvan we veronderstelden dat dit een goede maat is voor de fractie naïeve B-cellen in perifeer bloed. Vroege depletie (= tijdstip van depletie binnen één maand na behandeling) of vroege herbevolking (= tijdstip van herbevolking binnen zes maanden behandeling) correleerde niet met de klinische respons die zes of twaalf maanden na de behandeling werd geëvalueerd. Herbevolking binnen de eerste zes maanden correleerde echter significant met verminderde ziekteactiviteit in de daaropvolgende periode (d.w.z. 6-12 maanden na de behandeling), wat erop wijst dat het eerder herbevolking dan depletie is die de respons op rituximab beïnvloedt.
Samenvattend laten de resultaten gerapporteerd in het eerste deel van dit proefschrift zien hoe het voorspellen van de klinische respons op behandeling met rituximab een uitdaging blijft, vooral vanwege het feit dat het onderliggende mechanisme achter de werkzaamheid van rituximab nog steeds niet volledig wordt begrepen.

DEEL II: Ontwikkeling van TCR-repertoireanalyse voor de beoordeling van immunogeniciteit

In het tweede deel van dit proefschrift werd de klinische respons op rituximab geëvalueerd met betrekking tot een fenomeen waarvan bekend is dat het de werkzaamheid van alle biologische geneesmiddelen in het algemeen beïnvloedt, namelijk: immunogeniciteit van geneesmiddelen. De immunogene respons tegen biologische geneesmiddelen manifesteert zich in de aanwezigheid van antilichamen die gericht zijn tegen het geneesmiddel, afgekort als “ADA’s”.’ Deze ADA’s verstoren de beschikbaarheid van het geneesmiddel, hetgeen daarom kan leiden tot een verminderde klinische respons op het geneesmiddel. De ontwikkeling van ADA’s impliceert de aanwezigheid van geneesmiddel-specifieke B- en T-cellen, die geactiveerd worden bij herkenning van het geneesmiddel en starten met het opbouwen van een immuunrespons tegen dit geneesmiddel. Daarom zou TCR- en BCR-repertoireanalyse kunnen worden toegepast om ook deze immunogene reacties te bestuderen.
In **hoofdstuk vier** hebben we een nieuwe methodologie ontwikkeld om de antigeen-specifieke TCR-klonen in de repertoires te identificeren door TCR-sequencing met *in vitro* stimulatietesten te combineren. We evalueerden de dynamiek van klonale expansie individueel van alle TCR-klonen op verschillende tijdstippen na antigeen-stimulatie. We gebruikten een reeds gevalideerd algoritme dat normaal wordt gebruikt bij analyse

van genexpressie om TCR-klonen met een aanzienlijk verhoogde frequentie in het post-stimulatierepertoire te detecteren en deze te benoemen als de antigeen-responsief. We lieten de veelzijdigheid van deze methodologie zien bij het verkrijgen van meer informatie over het functionele fenotype of de specificiteit van de geselecteerde antigeen-responsieve TCR-klonen. De methode vormt een waardevol immunologisch hulpmiddel voor monitoring van antigeen-specifieke responsen, zelfs in grote patiëntencohorten en wanneer het gaat om complexe en variabele antigenen.
De toepassing van deze methodologie maakte mogelijk om immunogene T-celreacties tegen rituximab te bestuderen (**hoofdstuk vijf**). Perifere bloedmonsters verkregen van patiënten behandeld met rituximab die één jaar na de behandeling ADA's ontwikkelden, werden in vitro gestimuleerd met een verzameling van rituximab-afgeleide peptiden. Er werd aangetoond dat rituximab-specifieke T-cellen IFN-y, IL-5 en IL-10 produceren, waarbij de laatste twee belangrijke cytokinen zijn die de rijping en differentiatie van antilichaam-producerende B-cellen stimuleren. Rituximab-responsieve TCR-klonen werden geselecteerd als de overmatig uitgegroeide klonen in het post-stimulatie TCR-repertoire zoals beschreven in hoofdstuk vier. De anti-rituximab respons bleek polyklonaal in het individu, en verschilde van patiënt tot patiënt. De rituximab-responsieve TCR-klonen die *in vitro* werden geidentificeerd in de stimulatie testen werden vervolgens ook teruggevonden in het ex-vivo TCR-repertoire in het bloed dat werd verkregen voorafgaand aan behandeling en één, drie, zes en twaalf maanden na de behandeling. De meeste op rituximab reagerende TCR-klonen verschenen in het bloed binnen de eerste maand van de behandeling, ruim voorafgaand aan het ontstaan van ADA's in het bloed. Deze klonale TCR responsen konden nog steeds worden gedetecteerd in monsters die een jaar na de behandeling waren verkregen, en in sommige gevallen zelfs in het monster vóór de behandeling. Deze resultaten geven aan dat immunogene T-celreacties op rituximab zich zeer vroeg na de behandeling ontwikkelen, en zelfs al vóór de start van de behandeling aanwezig kunnen zijn. Dit kan een argument zijn om te onderzoeken of het zinvol is deze TCR responsiviteit in de toekomst te analyseren voordat patiënten met dit middel worden behandeld. Omdat immunogene reacties tegen biologische stoffen kunnen worden beschouwd als fysiologische immuunreacties tegen een nieuw antigeen, kunnen ze bij elke behandelde patiënt worden verwacht. Toekomstige behandelstrategieën zouden daarom beter dienen te worden aangepast om de ontwikkeling van klinisch relevante immunogeniciteit te voorkomen of op zijn minst te minimaliseren.
Tenslotte worden in **hoofdstuk zes** de bevindingen en resultaten gepresenteerd in dit proefschrift samengevat en besproken in het licht van de huidige literatuur en worden perspectieven voor de toekomst besproken.

LIST OF PUBLICATIONS

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Chemin K, **Pollastro S**, James E, Ge C, Albrecht I, Herrath J, Gerstner C, Tandre K, Sampaio Rizzi T, Rönnblom L, Catrina A, Holmdahl R, Klareskog L, de Vries N and Malmström V. *A Novel HLA-DRB1*10:01-restricted T cell epitope from citrullinated type II collagen relevant to rheumatoid arthritis*. *Arthritis* Rheumatol. 68:1124-35 (2016).

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AUTHOR CONTRIBUTIONS

Chapter 2

Non-response to rituximab therapy in rheumatoid arthritis is associated with incomplete disruption of the B cell receptor repertoire.

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Chapter 3

Non-response to rituximab therapy in rheumatoid arthritis is associated with incomplete disruption of the B cell receptor repertoire.

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[†]These authors contributed equally to this work

AM, SP, NdV contributed to design of the study. AM, SP, GB, IN, NdV, contributed to the cohort design and acquisition and processing of patients' material. SP, AM, BvS, AvK and NdV contributed to data analysis and interpretation. SP, AM, GB, IN, BvS, SH, CV, MP, AvK, XM and NdV contributed to the manuscript preparation and read and approved the final manuscript.

Chapter 4

Characterization and monitoring of antigen-responsive T cell clones using T cell receptor gene expression analysis.

Sabrina Pollastro[1,2†], Marie de Bourayne[3†], Giulia Balzaretti[1,2†], Aldo Jongejan[4], Barbera D. C. van Schaik[4], Ilse T. G. Niewold[1], Antoine H. C. van Kampen[4], Bernard Maillère[3] and Niek de Vries[1,2]

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NV, BM, SP, and MB contributed to design of the study. SP, MB, GB, and IN contributed to the acquisition and processing of samples. SP, MB, GB, AJ, BS, AK, BM, and NV contributed to data analysis and interpretation. All authors contributed to the article and approved the submitted version.

Chapter 5

T cells clonal responses against rituximab are detected in blood prior to development of anti-rituximab antibodies and can be present before treatment initiation

Sabrina Pollastro[1,2†], Marie de Bourayne[3†], Aldo Jongejan[4], Giulia Balzaretti[1,2], Barbera D.C. van Schaik[4], Ilse T.G. Niewold[1], Antoine H.C. van Kampen[4], Bernard Maillère[3], Niek de Vries[1,2], on behalf of ABIRISK consortium

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[†]These authors contributed equally to this work

NV, BM, SP, and MB contributed to design of the study. SP, MB, GB, and IN contributed to the acquisition and processing of samples. SP, MB, AJ, BS, AK, BM, and NV contributed to data analysis and interpretation. All authors contributed to the article and approved the submitted version.

PHD PORTFOLIO

PhD student: Sabrina Pollastro
PhD period: February 2014 – January 2024
PhD supervisor: Prof. Dr. N. de Vries
PhD co-supervisors: Prof. Dr. A.H.C. van Kampen and Dr. B. Maillère

Courses	**Year**	**ECTS**
FOCIS Advanced Course in Basic & Clinical Immunology	2014	0.75
Basic Laboratory Safety	2014	0.8
Bioinformatics Sequence Analysis	2014	1.1
Practical Biostatistics (e-learning)	2014	1.4
The AMC World of Science	2014	0.7
Advance Immunology	2015	2.9

Seminars, workshops and master classes	**Year**	**ECTS**
Weekly EXIM meetings		
▪ Oral presentation (3x)	2015-2017	1.5
Weekly KIR meetings		
▪ Oral presentation (7x)	2014-2018	3.5
EXIM retreat		
▪ Oral presentation	2015	0.5
▪ Visiting	2016	0.25
PhD retreat Triple I		
▪ Visiting	2014	0.5
Amsterdam Infection Inflammation and Immunity Annual meeting		
▪ Visiting	2017	0.25

(Inter)national conferences and presentations	**Year**	**ECTS**
NVVI Anniversary Congress, NL		
▪ Visiting	2014	0.75
ABIRISK General meeting, Brussels, BE		
▪ Oral presentation	2015	0.5
ABIRISK General meeting, Innsbruck, AT		
▪ Oral presentation	2016	0.5
EPGS symposium, NL		
▪ Visiting	2016	0.25

ABIRISK General meeting, Florence, IT		
▪ Oral presentation	2017	0.5
ABIRISK General meeting, Lisbon, PT		
▪ Visiting	2017	0.5
NVVI annual meeting, NL		
▪ Oral presentation	2017	0.5
ABIRISK General meeting, Basel, CH		
▪ Oral presentation	2018	0.5
European League Against Rheumatism, NL		
▪ Poster	2018	0.5
European Congress of Immunology, NL		
▪ Poster (2x)	2018	1

Others	**Year**	**ECTS**
Invited presentation at CEA, Paris, FR	2017	0.5
LS@W Venture Challenge Spring Edition, NL	2018	1

CURRICULUM VITAE

Sabrina Pollastro was born in Galliate, Italy, on the 2nd of July 1988. In 2007 she started her undergraduate studies in Biotechnology at the University of Eastern Piedmont "Amedeo Avogadro". In 2010 she obtained her bachelor's degree cum laude and was awarded "Best Graduated student in Biotechnology". The same year, she started her master's degree in Molecular Biotechnology and Bioinformatics at the University of Milan. In 2011 she joined the ERASMUS program and moved to Utrecht, The Netherlands. Here she attended a 10 months internship in the Laboratory of Plant Molecular Genetics at Utrecht University. After coming back to Italy, in 2013 she graduated cum laude and was then offered two doctorate positions at the University of Milan but decided to further continue her academic education abroad. In 2014 she started her PhD at the Department of Clinical Immunology and Rheumatology at the Academic Medical Center in Amsterdam, under the supervision of Dr. Niek de Vries. During her PhD she specialized in the analysis of T- and B-cell receptor repertoires applied to both clinical and experimental research. In the last years of her doctorate, she became more and more interested in the bioinformatic aspects of her research and slowly moved out of the wet-lab. After the end of her PhD contract in 2018, she decided to continue her career in science as bioinformatician. In March 2019 she started as a post-doc at Sanquin, in the Immunopathology department led by prof. dr. Marieke van Ham, with focus on antigen-specific B and T cells immune responses. At Sanquin, she is now applying her expertise in T- and B-cell receptor repertoire analysis and further extending her bioinformatic skills to single cell sequencing technologies. She is also involved in the training of students and in lecturing for the University of Amsterdam (Faculty of Science).

In 2019 she got married and she is now mother of two kids, Maya Angelina born in 2020 and Axel Joe, born in 2023.

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