9th Waddensymposium

on

"Precision medicine: towards personalized therapies"

This symposium is organized in collaboration with:

`

NRC

National Reference Center

www.waddensymposium.eu

Location: Fletcher Hotel Duinzicht
Oud Nieuwlandseweg 13
Ouddorp (Zeeland), the Netherlands
www.fletcherhotelduinzicht.nl

9th Waddensymposium
"Precision medicine: towards personalized therapies"

June 25 - 27, 2017

Sunday June 25

18.00 Arrival Fletcher Hotel Duinzicht, Ouddorp (Zeeland), the Netherlands

20.00 Dinner

Monday June 26

07.30 - 08.15 Breakfast
08.15 - 08.30 Welcome, symposium in perspective: Willem Fibbe, Leiden [the Netherlands]

Genomics

Chair: **Frank Staal**

08.30 - 09.00	Willem Ouwehand *"Lessons learned from the 100 000 Genomes Project (100KGP)"*	[United Kingdom]
09.00 - 09.30	Luigi Naldini *"Novel gene therapy strategies"*	[Italy]
09.30 - 10.00	Jean-Charles Boisset *"Mapping the physical network of cellular interactions identifies new niches in the mouse bone marrow"*	[the Netherlands]
10.00 - 10.30	Cisca Wijmenga *"Interplay between genetics and the microbiome"*	[the Netherlands]

10.30 - 11.00 Coffee break

Chair: **Jaap Jan Zwaginga**

11.00 - 11.30	Radek Skoda *"The mutational landscape of myeloproliferative neoplasms"*	[Switzerland]

11.30 - 12.30 **Short abstracts - 15 min.**

Karin Pike-Overzet [the Netherlands] *"Mutation in VPS4B found in a T-B+NK+ severe combined immunodeficiency patient with atypical presentation"*

Laura Claessens [the Netherlands] *"Characterizing disease heterogeneity in Type 1 Diabetes patients by combined genetic, clinical and immunological profiling"*

Vincent van Unen [the Netherlands] *"Interactive Visual Analysis of Mass Cytometry Data by Hierarchical Stochastic Neighbor Embedding Reveals Rare Cell Types"*

Monday June 26

12.30 - 13.15 **Lunch**

13.30 **transfer**

14.00 - 19.00 **Informal discussion between young talented scientists / invited speakers**

19.00 **Diner at restaurant "Het Visserhuis" Burgh-Haamstede**

Tuesday June 27

07.30 - 08.15 **Breakfast**

Organoids & Reprogramming

Chair: **Willem Fibbe**

08.15 - 08.45 Robert Vries [the Netherlands]
"Organoids, personalizing medicine from drug development to treatment"

08.45 - 09.15 Jeffrey Beekman: [the Netherlands]
"Intestinal organoids for personalized medicine in cystic fibrosis"

09.15 - 09.45 Niels Geijsen [the Netherlands]
"Pluripotent stem cells in development and disease"

09.45 - 10.15 **Coffee break**

Immunotherapies & Regulation

Chair: **Frans Claas**

10.15 - 10.45 Sofie Lucas: [Belgium]
"Targeting CARP on Treg's: a novel approach for cancer Immunotherapy"

10.45 - 11.15 Hermann Einsele: [Germany]
"Adoptive Cellular Therapy and Redirecting T Cell Strategies for personalized Treatment in Cancer and Infections"

11.15 - 11.45 Elmar Jaeckel [Germany]
"Downregulation of the alloimmune response by CAR Tregs"

Tuesday June 27

Chair: **Frits Koning**

11.45 - 13.00 **Short abstracts - 15 min.**

Natasja de Vries [the Netherlands] *"Unravelling local and systemic immune profiles in pancreatic and colorectal cancer using mass Cytometry"*

Tatjana Nikolic [the Netherlands] *"Immune intervention with tolerogenic dendritic cells in type 1 diabetes - D-SENSE trial"*

Marieke Fransen [the Netherlands] *"Immune-modulating antibody therapy for the treatment of cancer"*

Koen Schepers [the Netherlands] *"The role of product and patient heterogeneity in determining treatment efficacy of mesenchymal stromal cell-mediated immunomodulation therapy"*

14.00 **Lunch at "Strandpaviljoen paal 10" Ouddorp**

16.00 **END OF MEETING**

Lessons learned from the 100 000 Genomes Project (100KGP)

Willem H Ouwehand (on behalf of the NIHR BioResource – Rare Diseases)

The 100KGP aims to achieve analysis by whole genome sequencing (WGS) of the DNA samples of 100 000 NHS patients by 2018. The key objective is to establish WGS as standard care in the domains of infection, cancer and rare diseases in the NHS. So far just over 36,000 rare disease samples have been analysed by WGS and ~3,000 samples are for Bleeding, Thrombotic and Platelet Disorders (BPD, n=~1,300), Primary Immune Disorders (PID, n=~1,400) and Stem Cell and Myeloid Disorders (SMD, n=~300). Clinical and laboratory phenotype data have been captured using Human Phenotype Ontology (HPO) terms. There are 264 known genes for BPD, PID and SMD and in the first round of analysis the known genes are reviewed for pathogenic and likely pathogenic variants by a multi-disciplinary team (MDT). Clinicians are asked to only enroll patients with unexplained rare disorders and hence causal variants in known genes were identified in <15% of cases. Samples from patients without causal variants in known genes are entered in a second round of analysis using newly developed statistical methods. Such methods can be applied to automatically cluster patients based on phenotypic similarities and also exploits the richness of information from the Mouse Genome Informatics and the Online Mendelian Inheritance in Man databases. These approaches have shown to be effective in identifying variants in novel genes with a high likelihood of being causal. The sequencing of a large number of samples in a single programme has also been helpful in replicating several recent gene discoveries by other groups, thereby reducing the risk of erroneous discoveries. Historically the cost of Sanger sequencing has prevented the comprehensive genetic analysis of DNA samples from patients with an assumed inherited disorder. High throughput sequencing (HTS)-based gene panel tests provide an opportunity to analyse DNA samples for causal variants in known genes at an affordable cost. Such panel tests will aid the molecular diagnosis of patients with causal variants in known genes. It is predicted however that by 2022 a WGS test will cost <~€200 rendering HTS gene panel tests obsolete in the near future.

In conclusion the 100KGP project commenced in 2013 and so far 36,000 DNA samples have been analysed. WGS is now an accredited service and genomic medicine centres have been established at 11 academic hospitals, where the sequencing results are reviewed by MDTs with input from clinicians, clinical geneticists and bioinformaticians. The 100KGP has laid the blueprint for the delivery of genomics services in the NHS.

Novel gene therapy strategies

Luigi Naldini

Current results from ongoing trials of Hematopoietic Stem Cell (HSC) gene therapy performed with lentiviral vectors for the treatment of some primary immunodeficiencies (like Wiskott Aldrich syndrome, WAS) and storage diseases (like metachromatic leukodystrophy, MLD) show stable and extensive genetic engineering of hematopoiesis with polyclonal reconstitution by gene modified HSC with substantial therapeutic benefit. These findings provide a new therapeutic perspective for patients affected by these diseases and, conceivably, several other ones. Autologous HSC gene therapy may eventually become a first treatment option for those patients candidate to allogenic HSC transplant (HSCT) who lack a fully matched normal donor. Autologous HSC gene therapy would not only be available to virtually any patient but may also substantially reduce the morbidity of the treatment as compared to allogenic HSCT, because there is no risk of graft-vs-host disease and therapeutic benefit may often be achieved by partial chimerism with transduced HSC, thus relieving the need for fully myeloablative and immunosuppressive preparatory conditioning. In some diseases, such as MLD, genetic engineering of HSC may even surpass the benefit of conventional HSCT, because it may engage novel therapeutic mechanisms, such as increased dosage and biodistribution of the replaced gene product over what can be achieved by transplanting normal HSC. These clinical results also prove the feasibility to manipulate HSC ex vivo without hampering their long-term repopulation potential and open the way to design improved gene therapy strategies. To further enhance the safety and efficacy of gene transfer, we have devised novel strategies to target gene expression to selected lineages by transcriptional and post-transcriptional, micro-RNA mediated regulation and to precisely edit the genome by artificial endonucleases. These strategies are now being translated into new therapeutic strategies for treating more common diseases, such as cancer. More precise genetic engineering can be achieved by correcting disease-causing mutations *in situ*, thus restoring both the function of the gene and its physiological expression control. Targeted gene editing, however, is constrained in HSC by quiescence and low expression of the DNA repair machinery. We could overcome these barriers and provide evidence of correction of SCID-X1 causing mutations in the *IL2RG* gene. We have validated this approach in an *ad hoc* humanized SCID-X1 mouse model to support the scientific rationale and safety of the proposed treatment, and identify the conditioning regimen and degree of chimerism with edited cells required to correct the disease. As the first ex vivo gene therapies have now progressed to the market, constant engagement of the drug companies and regulatory agencies becomes essential to define appropriate quality standards for manufacturing and release and to build suitable pipelines for supplying these personalized and expensive therapies. Concomitantly, the scientific community has engaged in a debate open to all society stakeholders to address the ethical implications raised by the prospective application of genome editing to a growing range of uses, including the possibility to modify the human germline.

Mapping the physical network of cellular interactions identifies new niches in the mouse bone marrow

Jean-Charles Boisset

The environment or niche, in which a cell resides, influences its function. For example, numerous studies have described the many cell types that comprise the niche for hematopoietic stem cells (HSC) in the bone marrow (BM). However, studying the composition of specific niches usually requires *a priori* assumptions about the cell of interest and the cell types present in the niche. We show that building a cellular network, based on physical cell interaction and single-cell mRNA sequencing, allows the systematic discovery of new niches in the mouse bone marrow, without previous information on the different cell types present. This was achieved by manually picking doublets (or other structures composed of a few cells that can be easily distinguished) from mildly dissociated BM. These physically interacting cells were further micro-dissected into single-cells, and their individual transcriptome were sequenced, retaining the information on which cells interacted with which. Based on the single-cell transcriptomes and clustering algorithm, we were able to identify the different cell types present in the BM, and notably to describe the differentiation path of the neutrophil lineage at the single cell level. We then reconstructed a cellular network by analyzing the frequency of interaction in between the different cell types. We found that megakaryocytes specifically interact with neutrophils, and plasma cells with myeloblasts/promyelocytes (precursors of the neutrophils). We confirmed these interactions *in situ* in the BM. This strategy can be utilized to discover new niches in a variety of organs.

Interplay between genetics and the microbiome

Cisca Wijmenga

The human gut microbiome have been linked to many common diseases and traits, including lipid metabolism, immune diseases and cancer. Multiple factors, such as age, gender, BMI, diet, medications and lifestyle influence gut microbiome. The role of genetic factors in microbiome has been suggested by substantial heritability of selected bacteria in twin studies, however was not yet investigated in a big scale.

In this work we aimed to identify genetic variants that influence gut microbiome composition in healthy population. For the discovery we used the population cohort of LifeLines-DEEP (LLD) that includes 1200 individuals from Groningen, the Netherlands. For replication we used 500 samples from the Functional Genomics project (500FG) from Nijmegen, the Netherlands. For both cohorts extensive information on diet, diseases and lifestyle was also available. Gut microbiome composition was analysed by paired-end metagenomic shotgun sequencing and SNPs were associated to singel bacteria and pathways.

Overall, we observed moderate effect of genetic variants on microbiome composition. Amongst others, we observed strong effect of multiple SNPs in the LCT locus on *Bifidobacteria*. Interestingly, the bacterial abundance of Bifidobacteria was dependent on both LCT genotypes and the amount and frequency of consumption of milk and sourmilk products.

Overall, our results indicate that genetic variants influence gut microbiota composition in combination with dietary factors and that genetic variation in C-type lectin molecules are important in shaping the gut microbiome.

The mutational landscape of myeloid proliferative neoplasms

Radek C. Skoda, University Hospital Basel, Experimental Hematology, Department of Biomedicine

Myeloproliferative neoplasms (MPN) are clonal disorders of the hematopoietic stem cell. Somatic gain of function mutations in the genes for *JAK2*, *CALR* or *MPL* can be found in about 90% of patients with MPN and are thought to be the disease driving mutations. In addition, accumulation of mutations in genes encoding epigenetic regulators, including *TET2*, *ASXL1*, *DNMT3A* and *EZH2*, transcription factors and signaling components modify the course of the disease and can contribute to disease initiation and/or progression. Next generation sequencing allows detecting somatic mutations in blood of patients with MPN and establishing the clonal architecture. With our current knowledge we find mutations in approximately 90% of patients with MPN and only 10% remain without a clonal marker. The presence of 2 or more somatic mutations in the same patient significantly reduced overall survival and increased the risk of transformation into acute myeloid leukemia (AML). We followed and quantitated somatic mutations in serial samples from MPN patients and found that the number of mutations between early and late patient samples did not significantly change, suggesting that the mutation rate in MPN is rather low. Functionally, mutations in JAK2, CALR and MPL genes have been shown to cause MPN phenotype when expressed in mouse models, while mutations in other genes primarily affect disease initiation and/or progression. The prognostic value of individual gene mutations is currently being assessed by multiple studies. Interestingly, some of the gene mutations frequent in MPN, in particular *TET2, DNMT3A and JAK2,* are also often detected in normal individuals with clonal hematopoiesis.

Mutation in VPS4B found in a T-B+NK+ severe combined immunodeficiency patient with atypical presentation

Anna-Sophia Wiekmeijer, Lieke van Roon, Gertjan Driessen, Martijn H. Brugman, Gijs W.E. Santen, Sandra A. Vloemans, Wibowo Arindrarto, Jasper J. Saris, Renske Oegema, Robbert G.M. Bredius, Mirjam van der Burg, Frank J.T. Staal, **Karin Pike-Overzet**

Severe combined immunodeficiency (SCID) is a set of rare genetic disorders characterized by a lack of T cells in peripheral blood (PB). The deficiency in T cells can be accompanied by defects in either B or NK cells or both, depending on the underlying genetic disorder. Currently, 17 genes are officially acknowledged to be involved in SCID, with a further 3 genes recently reported in case studies or small patient cohorts. However, between 7 to 33% of patients remain without molecular diagnosis.

Here, we present data on a female patient with an atypical presentation of SCID. Genetic and metabolic analysis for ADA and PNP deficiency was initiated, but this showed no abnormalities. Also in the RAG1 and RAG2 genes no pathogenic mutations could be detected. In addition, IL2RG analysis was normal.

By transplanting patient derived hematopoietic stem and progenitor cells into the NOD/Scid-Il2rg-/- (NSG) mouse model, we could demonstrate that this patient had a hematopoietic cell-intrinsic defect resulting in a lack of T cells in the peripheral blood, confirming the suspicion of SCID. Both the B- and the NK-cell lineages were unaffected. Phenotypic analysis of the thymocytes of these mice revealed a block in T-cell development at the CD3- to CD3+ CD4+CD8+ double positive transition. The subsequent CD4+ and CD8+ single positive populations were almost completely lacking.

Using whole exome sequencing, we identified a *de novo* heterozygous missense mutation in the VPS4B gene that is predicated to act as dominant negative molecule. Interestingly, another dominant negative mutation in VPS4B has been shown to impair scission of microvesicles from the T-cell plasma membrane, decreasing the T cell's capacity for extracellular communication. We are currently doing functional experiments to see if the patient-specific mutation could affect T cell activation, which could imply its involvement in T cell development, resulting in SCID.

Characterizing disease heterogeneity in Type 1 Diabetes patients by combined genetic, clinical and immunological profiling

Laura Claessens[1], Menno van Lummel[1], Joris Wesselius[1], Bobby Koeleman[2], Bart Roep[1,3].
[1] Department of Immunohematology and Blood Transfusion, Leiden University Medical center, Leiden, the Netherlands.
[2] Department of Medical Genetics, University Medical Center Utrecht, Utrecht, the Netherlands.
[3] Department of Diabetes Immunology, City of Hope, Duarte, California, USA.

Introduction. Type 1 Diabetes (T1D) patients display heterogeneity in disease pathology, disease progression and treatment response. Currently, over 50 genomic regions have been identified that contribute to T1D susceptibility, with HLA being the most important locus. However, the contribution of non-HLA regions to susceptibility remains unclear and, together with environmental triggers and regulators, they may contribute to the heterogeneity observed among patients. Our aim was to map disease heterogeneity by creating genetic, clinical and immunological profiles for a cohort of 157 T1D patients and define which parameters are best in explaining variation in disease pathogenesis.

Methods. Immunological profiling consisted of a lymphocyte stimulation test to measure T lymphocyte proliferation in response to islet-autoantigens GAD65, IA-2, PPI and DRiP. Additionally, we performed ELISPOT to quantify IL-10 and IFN-γ production by T lymphocytes in response to islet-epitopes. For genetic profiling, patients were HLA-genotyped to determine HLA-DR-DQ risk and SNP-genotyped using the Infinium® ImmunoArray-24 v2.0 BeadChip to generate a non-HLA genetic risk score (GRS) based on 95 non-HLA SNPs that have a known association with T1D. So far, our clinical profile includes the parameters disease duration and age at disease onset.

Results. We found higher T lymphocyte proliferation responses against GAD65, IA-2, PPI and DRiP in T1D patients compared to individuals with celiac disease as controls. Among T1D patients, 82% responded positively to one or more islet-autoantigens. These patients responded to either IA-2 alone or in combination with one or more of the other islet-autoantigens, suggesting an important role for IA-2 in early stages of disease. Patients responding positively to all four islet-autoantigens had a longer disease duration than patients responding to fewer or no islet-autoantigens, an indication of epitope spreading. Based on the multitude and amplitude of the proliferation responses, patients were hierarchically clustered into non-, low-, intermediate- and high-responders. Correlation with genetic and clinical profiles demonstrated that high-responders have a longer disease duration compared to other responders. They also had a significantly higher non-HLA GRS ($p<0.005$), whereas HLA DR-DQ risk was not significantly correlated. In a multivariate regression model, 26.4% of variation in T lymphocyte proliferation could be explained by disease duration, age at disease onset, HLA DR-DQ risk and non-HLA GRS combined, with the latter being the most important, explaining 18.7% of variation. ELISPOT demonstrated that T lymphocytes in individual patients almost exclusively produced either IFN-γ or IL-10 in response to stimulation with islet-epitopes. Patients with an IFN-γ response had an earlier age at disease onset than patients with an IL-10 response. HLA-DR-DQ risk, non-HLA GRS and disease duration were not significantly associated with the type of response.

Discussion. These data provide new insights into T1D disease heterogeneity and highlight the importance of patient stratification in the development of effective treatment strategies. We are currently collecting additional clinical data, such as autoantibody status and HbA1c, to further map heterogeneity.

Interactive Visual Analysis of Mass Cytometry Data by Hierarchical Stochastic Neighbor Embedding Reveals Rare Cell Types

Vincent van Unen[1*], Thomas Höllt[1,2*], Nicola Pezzotti[2*], Na Li[1], Marcel J.T. Reinders[1,2], Elmar Eisemann[2], Frits Koning[1], Anna Vilanova[2], Boudewijn P. F. Lelieveldt[1,2]

[1]Leiden University Medical Center, Leiden, the Netherlands;
[2]Delft University of Technology, Delft, the Netherlands;

Mass cytometry allows high-resolution dissection of the cellular composition of the immune system. However, the high-dimensionality, large size, and non-linear structure of the data poses considerable challenges for data analysis. Here, we introduce Hierarchical Stochastic Neighbor Embedding (HSNE), a computational approach that constructs a hierarchy of non-linear similarities. We integrated HSNE into the Cytosplore^{+HSNE} framework to facilitate interactive exploration and analysis of the hierarchy by a set of corresponding two-dimensional plots with a stepwise increase in detail up to the single-cell level. We validated its discovery potential by re-analyzing a study on gastrointestinal disorders and two other publicly available mass cytometry datasets. We found that Cytosplore^{+HSNE} efficiently identifies rare cell populations, missed in a previous analysis, without a need for downsampling and in a very short time span. Thus, Cytosplore^{+HSNE} offers single-cell resolution while exploring mass cytometry datasets on tens of millions of cells on a standard computer.

Pluripotent stem cells in development and disease

Niels Geijsen

Modulation of protein function is used to intervene in cellular processes but is often done indirectly by means of introducing DNA or mRNA encoding the effector protein. Direct intracellular delivery of proteins has remained challenging. Our lab developed a method termed iTOP, for induced transduction by osmocytosis and propanebetaine, in which a combination of NaCl hypertonicity-induced macropinocytosis and a transduction compound (propane-betaine) induces the highly efficient transduction of proteins into a wide variety of primary cells[1]. We demonstrate that iTOP is a useful tool in systems in which transient cell manipulation drives permanent cellular changes, such as is the case with for example CRISPR/Cas9 ene editing. iTOP-mediated delivery of recombinant Cas9 protein and short guide RNA, enables highly efficient gene targeting in a non-integrative manner. We will discuss both *in vitro* and *in vivo* applications of this technology.

Targeting GARP on Tregs: a novel approach for cancer Immunotherapy

Stéphanie Liénart[1], Julia Cuende[1], Bas van der Woning[2], Hans De Haard[2], Michael Saunders[2], Pierre G. Coulie[1] and **Sophie Lucas[1]**

[1] de Duve Institute, Université catholique de Louvain (UCL), Brussels, Belgium
[2] argen-x bvba, Ghent, Belgium

Regulatory T lymphocytes (Tregs) are essential to prevent auto-immunity, but excessive Treg function contributes to cancer progression by inhibiting anti-tumor immune responses. Tregs exert contact-dependent inhibition of immune cells through the production of active TGF-β1. On the Treg cell surface, TGF- β 1 is in an inactive form bound to membrane protein GARP and then activated by an unknown mechanism. We demonstrate that GARP is involved in this activation mechanism. Two anti-human GARP monoclonal antibodies were generated that block the production of active TGF- β 1 by human Tregs. These antibodies recognize a conformational epitope that requires amino-acids $GARP_{137\text{-}139}$ within GARP/TGF- β 1 complexes. A variety of antibodies recognizing other GARP epitopes did not block active TGF- β 1 production by human Tregs. In a model of xenogeneic graft-versus-host disease in NSG mice, the blocking antibodies inhibited the immunosuppressive activity of human Tregs. We also derived two anti-murine GARP antibodies that block active TGF- β 1 production by mouse Tregs. These antibodies inhibit the growth of P815 mastocytoma in DBA/2 mice. Altogether, our data show that anti-GARP antibodies may serve as therapeutic tools to boost immune responses to infection or cancer, via a mechanism of action distinct from that of currently available immuno-modulatory antibodies. Used alone or in combination with tumor vaccines or antibodies targeting the CTLA4 or PD1/PD-L1 pathways, blocking anti-GARP antibodies may improve the efficiency of cancer Immunotherapy.

Adoptive Cellular Therapy and Redirecting T Cell Strategies for personalized Treatment in Cancer and Infections

Hermann Einsele

Adoptive cell therapy in the setting of allogeneic HSCT is the only curative treatment for high-risk hematological malignancies (high-risk leukemia, myelodysplastic syndromes, advanced myeloproliferative disorders, high-risk lymphomas and multiple myeloma), and is currently applied in approximately 15,000 patients per year in Europe and is the most expensive treatment available in clinical medicine. If the main complications of allogeneic HSCT (infections, relapse of the underlying hematological malignancy and GVHD) could be reduced or even eliminated, this curative treatment modality could be offered at a much lower risk and cost to a much larger patient population in Europe and worldwide.

Over the decades both autologous and allogeneic cellular Immunotherapy have evolved to a standard treatment option for several malignant and non-malignant disorders offering long-term disease control or even cure in the majority of cases. However, infectious complications as a result of an immunosuppressive environment or missing or dysfunctional cellular effectors leave infectious complications as one of the most important challenges. I will present the underlying mechanisms leading to these complications and discuss novel cellular strategies to overcome in particular infectious complications of viral (CMV, EBV, HSV, HHV6) and fungal (Candida, Aspergillus) origin by harnessing the effects of adoptive Immunotherapy. Taking both technical aspects of selection and culturing methods as well as functional aspects of the different cellular compartments such as central or effector memory or gamma-delta T lymphocytes into account we provide an overview of where adoptive Immunotherapy stands to today in light of the most recent pharmaceutical developments.

In addition novel strategies of trispecific antibodies and gene-modified T cells will be discussed.

Downregulation of the alloimmune response by CAR-Tregs

Elmar Jaeckel

CD4+CD25highFOXP3+ regulatory T cells (Tregs) are involved in graft-specific tolerance after solid organ transplantation. However, adoptive transfer of polyspecific Tregs alone is insufficient to prevent graft rejection even in rodent models, indicating that graft-specific Tregs are required. Todo et al. have recently demonstrated that induction of operational tolerance after liver transplantation is feasible, if supported by transfer of graft specific cells. As polyspecific where so far unspecific to control potent allospecific immune responses, graft-specific Tregs will be needed. We therefore developed a highly specific chimeric antigen receptor that recognizes the HLA molecule A*02 (referred to as A2-CAR).

Transduction into nTregs changes the specificity of the nTregs without alteration of their regulatory phenotype and epigenetic stability. Activation of nTregs via the A2-CAR induced proliferation and enhanced the suppressor function of modified nTregs. Compared to nTregs, A2-CAR Tregs exhibited superior control of strong allospecific immune responses in vitro and in humanized mouse models. A2-CAR Tregs completely prevented rejection of allogeneic target cells and tissues in immune reconstituted humanized mice in the absence of any immunosuppression.

Conclusion:
A2-CAR Tregs can induce graft-specific tolerance without perturbation of the general immune competence of the recipient. Therefore, these modified cells have great potential for incorporation into clinical trials of Treg supported weaning after allogeneic liver transplantation.

Unravelling local and systemic immune profiles in pancreatic and colorectal cancer using mass Cytometry

Natasja de Vries

Advances in the understanding of the immunological pathogenesis of cancer have led to new approaches in the treatment of cancer. The rise in targeted immunotherapies such as anti-CTLA-4 and anti-PD-1/PD-L1 antibodies have shown encouraging results in traditionally immunogenic types of cancer, but have not yet had a great impact on the treatment of gastrointestinal cancers, including pancreatic cancer (PC) and colorectal cancer (CRC). For a more in depth understanding of the nature of anti-tumor immunity, a system-wide analysis of the lymphoid and myeloid lineage populations that play a role in the pathogenesis of PC and CRC is pivotal. The global aim of this study is to unravel local and systemic immune profiles in PC and CRC using high-dimensional system-wide analyses by mass cytometry. We designed a mass cytometry panel comprising 39 metal isotope-tagged monoclonal antibodies directed against markers of the lymphoid and myeloid lineages of the innate and adaptive immune system. This mass cytometry panel will be applied to resected pancreatic and colorectal tumor tissue as well as associated lymph node tissue, and peripheral- and portal vein blood samples of the same patients. To identify and visualize the composition of the innate and adaptive immune cell compartments, we will apply the in house developed high-dimensional single-cell data analysis tools Cytosplore and HSNE. In this way, local and systemic immune profiles that are associated with PC and CRC are expected to be determined. These immune profiles will be correlated with clinical follow-up data (i.e. overall survival and disease-free survival), thereby conceptualizing a prognostic immune profile score. We anticipate that this knowledge may pave the way for new approaches to exploit the potential of the immune system to eradicate gastrointestinal cancers.

Immune intervention with tolerogenic dendritic cells in type 1 diabetes - D-SENSE trial

Tatjana Nikolic, Petra Sonneveld, Joris Welthuis, Sandra Laban, Antoinette Joosten, Jaap-Jan Zwaginga, Bart O. Roep

Type 1 diabetes (T1D) is a chronic autoimmune disease, for which insulin replacement remains the only therapeutic option. This therapy is limited to control collateral damage and symptoms, rather than curing the disease. Furthermore, due to poor glucose regulation by administered insulin, patients with T1D have a high risk to develop severe complications such as skin and eye disorders, nephropathy, neuropathy and cardiovascular complications. This warrants development of therapies that aim to regulate autoimmune response and prevent beta-cell destruction. Several clinical studies have been initiated recently aiming to restore immune tolerance. An attractive candidate for this aim are anti-inflammatory dendritic cells (DCs). The use of DCs for tolerance induction is currently under investigation also for clinical allergy, rheumatoid arthritis and multiple sclerosis.

We have generated and extensively investigated tolerogenic DCs (tolDCs) generated from monocytes treated with VitD3 and Dexamethason. These tolDCs show stable semi-mature phenotype and potent immunomodulatory function. TolDCs control autoreactive T cell responses and induce antigen-specific regulatory T cells (Tregs) through which they transfer infectious tolerance. Generation protocol for clinical-grade TolDCs has been developed and validated and we obtained an approval to perform a prospective, single-center, phase I safety study entitled D-Sense.

This trial aims to evaluate safety and feasibility of intradermal administration of pro-insulin peptide-pulsed tolDCs using microneedles. Each patient will receive two injections of autologous tolDCs at one of three test doses (0.5, 1 and 2x10^6 tolDC). For each dose, a group of three patients will be treated, one of which will also serve as a placebo control for intradermal injections by microneedles. The study has been initiated and so far three patients have been successfully treated with two tolDC injections at the lowest dose (0.5x10^6/injection). Currently, tolDCs are being cultured for the next administration dose and the treatments with the highest dose are planned for the end of the year.

Next to feasibility and safety endpoints, we are monitoring the effects of the treatment on appropriate biomarkers of disease and immune regulation as well as on metabolic disease control. For these aims, we employ immunomonitoring developed and optimized in our laboratory. We are performing detailed multicolor flow cytometric analysis of full blood to analyse: general leukocyte composition (monocyte/T/B/NK), specific T cell subsets and focusing in particular at changes in regulatory T cell (Treg) compartment. The change in proliferation of beta-cell specific T cells to autoantigens (proinsulin, GAD65, IA-2) alone or in the presence of the vaccine peptide are detected using lymphocyte stimulation assay (LST). Using ELISPOT, we are following changes in IL-10 and IFNg production in response to proinsulin or the vaccine peptide as as a measure of immunomodulation towards induced Treg phenotype. Finally, autoreactive CD8 T cells will be monitored using Diab-Q-kit, to follow up numeric and qualitative changes of autoreactive effector cells in peripheral blood. All parameters will be acquired and analysed on a “per sample/per patient” basis.

This is the first clinical study in the Netherlands testing the safety of tolerogenic DCs to control autoimmunity, which will allow better insight into the capacity to induce immunoregulation in T1D.

Immune-modulating antibody therapy for the treatment of cancer

Marieke F. Fransen, Jan Willem Kleinovink, Thorbald van Hall, Ferry Ossendorp

Cancer immunotherapy has proven to be very promising in recent years. Many new immunotherapeutic strategies are now available, all aiming to improve the patient's own immune response against tumors. We have been investigating the mode of action of immune-modulating antibodies in pre-clinical models; focusing on administration efficacy and target organs and cells. Immune-modulating antibodies, including checkpoint blocking antibodies, can interact with molecules on immune cells and thereby activate or enhance an immune response, against cancer. At present more than 10 different immune-modulating antibodies are in various stages of pre-clinical or clinical research for treatment of cancer patients. In order to be able to choose the right treatment for each patient, it is important to understand more of the mechanisms of these drugs. By gaining more insight into the working mechanisms of these therapies, and the various factors necessary for therapeutic efficacy, we aim to contribute to adequate prediction of which type of therapy is needed for which patient.

In our pre-clinical models of cancer, we have shown that expression of the checkpoint blocking molecule PD-L1 on tumors cells and immune infiltrating cells are both involved in the therapeutic efficacy of PD-L1 and PD-1 blocking antibodies, and therefore, PD-L1 expression within the tumor, but not necessarily on the tumor-cells themselves, can be used as a biomarkers for eligibility for these therapies (Kleinovink et al. Oncoimmunology 2017). We have also shown that local administration of a low dose of antibody is effective for tumor control by antagonistic CTLA-4 and PD-1 blocking antibody and agonist 4-1BB and CD40 antibody therapy, minimizing side effects (refs). Furthermore, we have analyzed the role of the tumor-draining lymph node in the efficacy of immune-modulating antibodies. We observed that anti-CD40 agonist antibody treatment of tumor-bearing mice gave strong anti-tumor effectiveness, even when tumor-draining lymph nodes (TDLN) were resected. Depletion studies revealed that treatment effect was CD8+ T cell dependent. We are currently investigating intratumoral CD8+ T cell activation by anti-CD40 therapy and we are analyzing the role of TDLN in other types of antibody therapy, such as PD-1 or PD-L1 blocking antibodies.

Taken together, our preclinical studies contribute to the understanding of the mechanisms of immune-modulating antibody therapy of cancer and may lead to better dosing, timing, routing and combinations of these potent biological drugs.

The role of product and patient heterogeneity in determining treatment efficacy of mesenchymal stromal cell-mediated immunomodulation therapy

Koen Schepers, Anna-Sophia Wiekmeijer, Ellen Schrama, Estefania Salcedo, Nicole Borggreven, Jennefer Wetzel, Yuichi Sakamoto, Yu Ji, Ridge Droste, Quirijn Dees, Helene Roelofs, Willem E. Fibbe

Mesenchymal stromal cells (MSCs) are increasingly applied in the treatment of a variety of clinical conditions and amongst others can be used to modulate immune responses in conditions related to auto-/allo-immunity, including acute graft-versus-host disease. While pilot data are promising, treatment responses have been highly variable. Preclinical studies indicate that MSCs can produce various immunomodulatory molecules to suppress both innate and adaptive immune cells. These immunomodulatory molecules are not expressed at steady state, but require activation by pro-inflammatory cytokines. Our preliminary data show that stimulating MSCs with different types and concentrations of pro-inflammatory molecules results in differential expression of immunomodulatory molecules by MSCs and an associated differential immunomodulating function. Furthermore, we observed heterogeneity within and between MSC preparations to express immunomodulatory molecules upon pro-inflammatory challenge. Hence, we hypothesize that the effectiveness of MSC-therapy largely depends on the immune milieu encountered in the patient and on the capacity of MSCs to respond to pro-inflammatory stimuli. Further development of MSC-immunomodulation therapy therefore depends on increased insight in the heterogeneity of the clinical MSC products and the heterogeneity in the inflammatory environment in the patients. To further delineate the functional heterogeneity within and between MSC preparations, our laboratory combines flow and mass cytometric analysis with antibody array-based secretome analysis, mRNA sequencing, functional assays and cellular barcoding. To unravel the heterogeneity between patients as wells as mechanisms of action of MSCs in patients, we are combining this with extensive monitoring of the blood of MSC-treated patients. To this end we will use mRNA sequencing, antibody arrays and flow cytometry to elucidate cellular and molecular immune profiles that are associated with outcome of MSC treatment. Together, these analyses will allow a better understanding of the cross-talk between MSCs and the *in vivo* inflammatory milieu, ultimately allowing patient stratification and more effective therapy design.

ORGANIZING COMMITTEE

Prof. Frans Claas
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
F.H.J.Claas@lumc.nl

Prof. Frits Koning
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
F.Koning@lumc.nl

Prof. Sjaak Neefjes
Leiden University Medical Center
Dept. of Chemical Immunology
Einthovenweg 20
2333 ZC Leiden
The Netherlands
j.j.c.neefjes@lumc.nl

Prof. Jaap Jan Zwaginga
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
J.J.Zwaginga@lumc.nl

Prof. Willem Fibbe
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
W.E.Fibbe@lumc.nl

Ms. Amber Gunthardt
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
A.N.Gunthardt@lumc.nl

Prof. Hendrik Veelken
Leiden University Medical Center
Dept. of Hematology
Albinusdreef 2, C2-R
2333 ZA Leiden
The Netherlands
J.H.Veelken@lumc.nl

PARTICIPANTS

Dr. Ramon Arens
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
R.Arens@lumc.nl

Dr. Magda Berkowska
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
M.A.Berkowska@lumc.nl

Laura Claessens BSc
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
L.A.Claessens@lumc.nl

Prof. Hermann Einsele
University Hospital Wuerzburg
Oberduerrbacher Str. 6
97080 Wuerzburg
Germany
Einsele_h@ukw.de

Dr. Marieke Fransen
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
M.F.Herbert-Fransen@lumc.nl

Prof. Masja de Haas
CCTR, Sanquin Research
Plesmanlaan 125
1066 CX Amsterdam
The Netherlands
m.dehaas@sanquin.nl

Dr. Jeffrey Beekman
Academic Medical Center Utrecht
Wilhelmina Children's Hospital (WKZ)
Lundlaan 6
3584 EA Utrecht
The Netherlands
jbeekman@umcutrecht.nl

Dr. Jean-Charles Boisset
Hubrecht Institute
Uppsalalaan 8
3584 CT Utrecht
The Netherlands
j.boisset@hubrecht.eu

Prof. Jacques van Dongen
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
J.J.van_Dongen@lumc.nl

Prof. Peter van den Elsen
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
P.J.van_den_Elsen@lumc.nl

Prof. Niels Geijsen
Hubrecht Institute
Uppsalalaan 8
3584 CT Utrecht
The Netherlands
n.geijsen@hubrecht.eu

Dr. Sebastiaan Heidt
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
s.heidt@lumc.nl

Dr. Elmar Jaeckel
Hannover Medical School
Dept. of Gastroenterology, Hepatology & Endocrinology
Carl-Neuberg-Straße 1
30625 Hannover
Germany
Jaeckel.elmar@mh-Hannover.de

Prof. Luigi Naldini
San Raffaele Telethon Institute for Gene Therapy
via Olgettina 60
20132 Milan
Italy
naldini.luigi@hsr.it

Prof. Willem Ouwehand
Department of Haematology
University of Cambridge
NHS Blood and Transplant Building
Cambridge Biomedical Campus
Cambridge CB2 0PT
United Kingdom
who1000@cam.ac.uk

Dr. Karin Pike-Overzet
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
K.Pike-Overzet@lumc.nl

Dr. Koen Schepers
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
K.Schepers@lumc.nl

Prof. Frank Staal
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
F.J.T.Staal@lumc.nl

Prof. Sophie Lucas
de Duve Institute
Avenue Hippocrate 74
1200 Brussels
Belgium
sophie.lucas@uclouvain.be

Dr. Tanja Nikolic
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
T.Nikolic@lumc.nl

Prof. Paul Parren
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
P.W.H.I.Parren@lumc.nl

Prof. Jon van Rood
Matchis
Plesmanlaan 1b
2333 BZ Leiden
The Netherlands
vanrood@europdonor.nl

Prof. Radek Skoda
University Hospital Basel
Department of Biomedicine
Hebelstrasse 20
4031 Basel
Switzerland
radek.skoda@unibas.ch

Dr. Cristina Teodosio
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
C.I.Teodosio@lumc.nl

Vincent van Unen MSc
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
V.van_Unen@lumc.nl

Natasja de Vries MSc
Leiden University Medical Center
Immunohematology and Blood Transfusion
Albinusdreef 2, E3-Q
2333 ZA Leiden
The Netherlands
N.L.de_Vries@lumc.nl

Dr. Robert Vries
Hubrecht Organoid Technology (HUB)
Yalelaan 62
3584 CM Utrecht
The Netherlands
r.vries@hub4organoids.nl

Prof. Cisca Wijmenga
University Medical Center Groningen
Department of Genetics
Hanzeplein 1
9713 GZ Groningen
cisca.wijmenga@med.umcg.nl