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The potential of immunomodulatory kits to generate leukaemia-derived dendritic cells competent to (re)activate the immune system against leukaemic blasts in acute myeloid leukaemia

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München, 20.12.2023 Lara Kristina Klauer

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AML	Acute myeloid leukaemia
BCL-2	B-cell lymphoma 2
BiTE	Bispecific T-cell engager
Ca-Iono	Calcium ionophore
CAR	Chimeric antigen receptor
CIK cells	Cytokine-induced killer cells
CLL-1	C-type lectin-like molecule 1
CSA	Cytokine secretion assay
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CTX	Cytotoxicity fluorolysis assay
DC	Dendritic cells
DC_{leu}	Leukaemia-derived dendritic cells
DNA	Desoxyribonucleic acid
FLT-3	FMS-like tyrosin kinase 3
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good manufacturing practice
HSCT	Haematopoietic stem cell transplantation
ICA	Intracellular cytokine assay
IDH-1	Isocitrate dehydrogenase 1
IDH-2	Isocitrate dehydrogenase 2
IFNy	Interferon gamma
iNKT cells	Invariant natural killer cells
LAA	Leukaemia-associated antigens
MHC	Major histocompatibility complex
MNC	Peripheral blood mononuclear cells
moDC	Monocyte-derived dendritic cells
mRNA	Messenger ribonucleic acid
NK cells	Natural killer cells
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death 1 ligand 1
PGE_1	Prostaglandin E_1
PGE_2	Prostaglandin E_2
Picibanil	OK-432
PRAME	Preferentially expressed antigen in melanoma
RHAMM	Receptor for hyaluronan-mediated motility
TCR	T cell receptor
TNFa	Tumour necrosis factor alpha
WB	Whole blood
WT-1	Wilms tumor protein 1

Publikationsverzeichnis *Index of publications*

Folgende Publikationen sind gemäß den Richtlinien der Medizinischen Fakultät der Ludwig-Maximilians-Universität München Bestandteil der kumulativen Dissertation von Lara Kristina Klauer:

Publikation I: Generation of Leukaemia-Derived Dendritic Cells (DC_{leu}) to Improve Anti-Leukaemic Activity in AML: Selection of the Most Efficient Response Modifier Combinations

Christoph Schwepcke *, Lara Kristina Klauer *, Diana Deen, Daniel Christoph Amberger, Zuzana Fischer, Fatemeh Doraneh-Gard, Carina Gunsilius, Annika Hirn-Lopez, Tanja Kroell, Johanna Tischer, Melanie Weinmann, Jan-Ole Werner, Andreas Rank, Christoph Schmid, Helga Maria Schmetzer

* C.S. and L.K.K. contributed equally

Schwepcke C, Klauer LK*, Deen D, et al. Generation of Leukaemia-Derived Dendritic Cells (DCleu) to Improve Anti-Leukaemic Activity in AML: Selection of the Most Efficient Response Modifier Combinations. Int J Mol Sci. 2022;23(15):8333. doi:10.3390/ijms23158333 [* C.S. and L.K.K. contributed equally]*

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Publikation II: Immunomodulatory kits generating leukaemia derived dendritic cells do not induce blast proliferation ex vivo: IPO-38 as a novel marker to quantify proliferating blasts in acute myeloid leukaemia

Caroline Plett *, Lara Kristina Klauer *, Daniel Christoph Amberger, Selda Ugur, Alexander Rabe, Zuzana Fischer, Diana Deen, Annika Hirn-Lopez, Carina Gunsilius, Jan-Ole Werner, Jörg Schmohl, Doris Krämer, Andreas Rank, Christoph Schmid, Helga Maria Schmetzer

* C.P. and L.K.K. contributed equally

Plett C, Klauer LK*, Amberger DC, et al. Immunomodulatory kits generating leukaemia derived dendritic cells do not induce blast proliferation ex vivo: IPO-38 as a novel marker to quantify proliferating blasts in acute myeloid leukaemia. Clin Immunol. 2022;242:109083. doi:10.1016/j.clim.2022.109083 [*C.P. and L.K.K. contributed equally]*

Impact Factor: 10.190 (2021)

Publikation III: Interferon Gamma Secretion of Adaptive and Innate Immune Cells as a Parameter to Describe Leukaemia-Derived Dendritic-Cell-Mediated Immune Responses in Acute Myeloid Leukaemia in vitro

Lara Kristina Klauer, Olga Schutti, Selda Ugur, Fatemeh Doraneh-Gard, Daniel Christoph Amberger, Nicole Rogers, Doris Krämer, Andreas Rank, Christoph Schmid, Britta Eiz-Vesper, Helga Maria Schmetzer

Klauer LK, Schutti O, Ugur S, et al. Interferon Gamma Secretion of Adaptive and Innate Immune Cells as a Parameter to Describe Leukaemia-Derived Dendritic-Cell-Mediated Immune Responses in Acute Myeloid Leukaemia in vitro. Transfus Med Hemother. 2021;49(1):44-61. doi:10.1159/000516886

Impact Factor: 4.040 (2021)

Publikationsbeiträge *Contributions to publications*

Lara Kristina Klauer war für folgende Punkte in Publikation I, II und III verantwortlich:

Publikation I

- Durchführung von Teilen der Experimente
- Auswertung und Interpretation der Daten zusammen mit C.S.
- Ausarbeitung des Manuskripts inkl. Abstrakt, Einleitung, Material u. Methoden, Ergebnisse, Diskussion, Zusammenfassung, Literaturrecherche, Abbildungen, Tabellen zusammen mit C.S.
- Einreichung des Manuskripts zur Publikation sowie
  Einarbeitung der Kritik der Reviewer zur Finalisierung des Manuskripts zur Publikation

Publikation II

- Durchführung von Teilen der Experimente
- Auswertung und Interpretation der Daten zusammen mit C.P.
- Ausarbeitung des Manuskripts inkl. Abstrakt, Einleitung, Material u. Methoden, Ergebnisse, Diskussion, Zusammenfassung, Literaturrecherche, Abbildungen, Tabellen zusammen mit C.P.
- Einreichung des Manuskripts zur Publikation sowie
  Einarbeitung der Kritik der Reviewer zur Finalisierung des Manuskripts zur Publikation

Publikation III

- Durchführung der Experimente
- Erhebung, Auswertung und Interpretation der Daten
- Ausarbeitung des Manuskripts inkl. Abstrakt, Einleitung, Material u. Methoden, Ergebnisse, Diskussion, Zusammenfassung, Literaturrecherche, Abbildungen, Tabellen
- Koordination der Ko-Autor-Kommunikation
- Einreichung des Manuskripts zur Publikation sowie
  Einarbeitung der Kritik der Reviewer zur Finalisierung des Manuskripts zur Publikation

Eine schriftliche Bestätigung aller Ko-Autoren über 1) deren Beitrag zu angeführten Publikationen, 2) deren Einverständnis zur Verwendung der angeführten Publikation als Teil der kumulativen Dissertation von Lara Kristina Klauer, und 3) die Tatsache, dass angeführte Publikationen nicht Teil einer anderen kumulativen Dissertation sind, liegt separat vor.

1. Einleitung *Introduction*

Acute myeloid leukaemia

Acute myeloid leukaemia (AML) is a malignant disorder of the hematopoietic system that is characterised by the uncontrolled clonal proliferation of abnormally differentiated myeloid blasts. Accumulating in the bone marrow, blasts displace the physiological haematopoiesis, resulting in erythrocytopenia, thrombocytopenia and leukocytopenia [1]. The diagnosis of AML is based on morphological, immunophenotypic and genomic (cytogenetic and molecular) abnormalities of blasts, which lead to further subtype classifications and risk stratifications decisive for therapeutic procedures [1-3]. AML is the predominant form of acute leukaemia in adults, with a median age of 68 years at diagnosis [4]. Untreated AML is a uniformly fatal disease.

Standard treatment of AML

The standard treatment of AML generally comprises a high-dose induction chemotherapy (e.g. Anthracycline + Cytarabine), resulting in an initial remission in about 40 - 80% of cases. Depending on the patient's risk stratification, treatment is continued by a high-to-medium-dose consolidation chemotherapy (e.g. Cytarabine) and/or complemented by an allogeneic haematopoietic stem cell transplantation (HSCT) [2, 3]. HSCT hereby constitutes one of the most potent treatment options: through the induction of the graft-vs-leukaemia effect, mediated by allogeneic T and potentially other immunoreactive cells, it has the potential to eradicate residual leukaemic blasts, promising stable remission or even cure. The outcome though is often restrained by the development of significant treatment-related mortalities like infections or graft-vs-host disease [5-7]. Over the past years, treatment options have diversified due to the heterogeneity of AML and novel agents have been incorporated into the latest treatment recommendations of AML. Depending on the underlying genomic abnormalities and the associated risk stratification, the addition of Midostaurin or Gilteritinib (a tyrosine kinase inhibitor for patients with FMS-like tyrosine kinase 3, FLT-3, mutations), Enasidenib or Ivosidenib (an isocitrate dehydrogenase inhibitor for patients with isocitrate dehydrogenase 1 or 2, IDH-1 or -2, mutations) or Gemtuzumab ozogamicin (an antibody-drug conjugate of anti-CD33 and the desoxyribonucleic-acid-DNA-damaging toxin calicheamicin for patients with CD33-positive leukaemia) has been shown to prolong survival [2, 3, 8-14]. Moreover, the hypomethylating agents Azacitidine and Decitabine and the B-cell lymphoma 2 (BCL-2) inhibitor Venetoclax have been shown to improve survival, especially in patients unfit for intensive chemotherapy [2, 3, 8, 15]. With an overall 5-year-survival-rate of 30.5%

and only 9.4% for patients over the age of 65 years [4], the outcome though remains dissatisfactory, highlighting the urgent need for novel therapeutic options.

Immunotherapy of AML

Over the past decade, increased attention has been focused on uncovering the interrelation between leukaemia and the immune system. The immune system is set up with various ingenious surveillance mechanisms to efficiently detect and eliminate abnormally differentiated (precancerous and cancerous) cells. Yet, tumour cells have developed strategies to escape the immune surveillance even in the presence of a functioning immune system, a core attribute of cancer and, in particular, leukaemia [16-19].

Various strategies have been developed to redirect the immune system to overcome the leukaemic immune escape and (re)gain control over AML. In order to surmount the immunosuppressive leukaemic environment created through the upregulation of checkpoint molecules on T (and other immunoreactive) cells, monoclonal antibodies targeting checkpoint receptors or ligands are being contrived to unleash (pre-existing) anti-leukaemic immune responses. Antibodies directed against programmed cell death protein 1 (PD-1) have shown promising results in solid tumours and hodgkin lymphoma and are now being studied, next to antibodies directed against i.a. programmed cell death 1 ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), in AML [18, 20]. To stimulate an efficient anti-leukaemic immune response, a combination with treatment strategies more explicitly engaging leukaemic cells though may be necessary. Concepts specifically targeting leukaemic blasts based on bispecific antibodies and gene-modified T cells expressing novel T cell receptors (TCR) or chimeric antigen receptors (CAR) are under investigation and have yet shown promising results. Bispecific antibodies are being designed to engage T and other immunoreactive cells with target-antigen-expressing leukaemic blasts. Following the success of Blinatumomab, a bispecific antibody engaging $CD3^+$ T cells and $CD19^+$ leukaemic blasts (bispecific T-cell engager, BiTE) in lymphatic leukaemia, variations of BiTEs targeting i.a. CD33, CD123 or C-type lectin-like molecule 1 (CLL-1) are being explored in AML [18, 21]. Furthermore, T cells are being genetically modified to express engineered CARs targeting specific antigens on leukaemic blasts. The CAR combines extracellular antibody binding and intracellular signalling, resulting in a major-histocompatibility-complex-(MHC)-independent activation of T cell effector function. Motivated by the compelling results of anti-CD19 CAR T cells in lymphoid malignancies, CAR T cells targeting i.a. CD33 and CD123 are getting investigated in AML [18, 22, 23]. In addition, T cells are being genetically modified to express TCRs targeting specific intra- and extracellular antigens on leukaemic blasts. Here, TCRs specific for i.a. wilms tumor protein 1 (WT-1) and receptor for hyaluronan-mediated motility (RHAMM) are being

studied [18, 24, 25]. Overall, the success of targeted immunotherapy, however, is highly dependent on the use of a competent target antigen (or molecule in case of checkpoint inhibition) to ensure on-tumour efficacy but prevent or limit off-tumour toxicity. Yet, the selection of a target antigen proves to be complex due to the extensive heterogeneity of AML and an expression pattern overlapping with healthy haematopoietic and non-haematopoietic tissue [26, 27].

Dendritic-cell-based immunotherapy of AML

Searching for new immunomodulatory strategies to gain control over AML, dendritic cells (DC) have attracted attention. DC are antigen-presenting immune cells that have the exceptional competence to bridge the innate and adaptive immune system and consequently initiate and regulate antigen-specific immune responses [28, 29]. Manipulating DC to present tumour or tumour-associated antigens imposes to be an auspicious approach to mediating tumour-specific and anti-tumour immunity.

There are various methods for generating antigen-specific DC. They can be generated from monocytes (moDC) in vitro and modified to present leukaemic-antigens by pulsing them with leukaemic peptides or cell lysates, fusing them with leukaemic blasts or electroporating them with leukaemia-associated-antigen-(LAA)-encoding messenger ribonucleic acid (mRNA) [30-34]. Administered to the patient as vaccine, these antigen-specific moDC have shown their aptitude to initiate anti-leukaemic activity; yet again, their effectiveness is dependent and constricted to the use of an adequate target antigen.

In contrast, DC can also be generated from leukaemic blasts (DC_{leu}) in vitro and in vivo. These DC_{leu} are characterised by the simultaneous expression of dendritic- (e.g. CD80, CD206) and leukaemic-antigens (e.g. CD34, CD117), thereby presenting the individual's whole leukaemic antigen repertoire [35-38]. The generation of (DC and) DC_{leu} (DC/DC_{leu}) can be achieved following DC/DC_{leu}-generating protocols comprising different response modifier combinations (e.g. granulocyte-macrophage colony-stimulating factor, GM-CSF, prostaglandin E_1, PGE_1, prostaglandin E_2, PGE_2, picibanil, OK-432) inducing haematopoietic differentiation, dendritic maturation and activation [39-41]. The great potential of DC/DC_{leu} to (re)activate the immune system against leukaemia has been demonstrated before [36, 39, 41-44].

Overall, DC/DC_{leu}-based immunotherapy imposes to be one of the most auspicious immunomodulatory strategies to treat AML. It not only overcomes the difficulty of finding an adequate target antigen, initiating an anti-leukaemic immune response based on the individual's whole leukaemic

antigen repertoire, but also circumvents cumbersome cell engineering under good manufacturing practice (GMP) conditions by generating DC/DC_{leu} in vivo.

Outline and overview of this doctoral thesis

The treatment regimen for AML has not been changed significantly in years. Fewer than 30% of patients experience stable remission or disease [4], indicating the need for novel therapeutic options. Immunotherapy, with its potential to support or activate the immune system to (re)gain control over AML and its potential to build an immunological memory concomitantly, carries the promise of providing long-term disease control. Especially DC/DC_{leu}-based immunotherapy constitutes a sophisticated way to prompt a leukaemia-specific and anti-leukaemic immunity based on the individual's whole leukaemic antigen repertoire.

This doctoral thesis discusses I) strategies to generate DC/DC_{leu} from myeloid leukaemic blast in a whole-blood-(WB)-environment using distinct combinations of response modifiers, II) the safeness of DC/DC_{leu}-generating protocols regarding the alteration of blast cell proliferation, and III) the effects of DC/DC_{leu} on the leukaemia-specific and anti-leukaemic activity of innate and adaptive immune cells, in vitro.

Overview Publication I - *Generation of Leukaemia-Derived Dendritic Cells (DC_{leu}) to Improve Anti-Leukaemic Activity in AML: Selection of the Most Efficient Response Modifier Combinations*

Schwepcke C, Klauer LK*, Deen D, et al. Generation of Leukaemia-Derived Dendritic Cells (DCleu) to Improve Anti-Leukaemic Activity in AML: Selection of the Most Efficient Response Modifier Combinations. Int J Mol Sci. 2022;23(15):8333. doi:10.3390/ijms23158333 [* C.S. and L.K.K. contributed equally]*

The objective of publication I was to develop and evaluate new DC/DC_{leu}-generating protocols specifically designed for the generation of DC/DC_{leu} from myeloid leukaemic blasts in a WB-environment. Although there are established DC/DC_{leu}-generating protocols that have shown their potential to generate DC/DC_{leu} in a peripheral-blood-mononuclear-cell-(MNC)-environment, it is important to respect that an MNC-environment cannot represent the complete spectrum of soluble and cellular components that are involved in an immune response and define the patient- and tumour-specific environment like a WB-environment does. In consideration of future clinical applications, it though is essential to factor in all components that can influence the generation of DC/DC_{leu} and the mediation of DC/DC_{leu}-based anti-leukaemic immunity, making a progression to DC/DC_{leu}-generation in a WB-environment inevitable.

Therefore, ten new DC/DC_{leu}-generating protocols (kits) were contrived for the utilisation in a WB-environment using distinct combinations of response modifiers, including calcium ionophore (Ca-Iono), GM-CSF, tumour necrosis factor alpha (TNFa), PGE_1, PGE_2 and/or OK-432. The potential of kits was assessed with respect to their performance in generating DC/DC_{leu} from leukaemic WB (in vitro) using a unique classification and ranking system; further, the potential of generated DC/DC_{leu} was assessed with respect to their performance in stimulating immunoreactive cells to an improved anti-leukaemic activity using a cytotoxicity fluorolysis assay (CTX). Overall, all new kits showed proficient DC/DC_{leu}-generation. Kit-I (GM-CSF + OK-432), -K (GM-CSF + PGE_2) and -M (GM-CSF + PGE_1) though evinced the highest efficiency in generating (mature) DC/DC_{leu}, further proficient in stimulating immunoreactive cells to an improved anti-leukaemic activity. This very generation of functional DC/DC_{leu} from leukaemic WB constitutes an essential step for the translation of DC/DC_{leu}-based immunotherapy into clinical application.

Overview Publication II - *Immunomodulatory kits generating leukaemia derived dendritic cells do not induce blast proliferation ex vivo: IPO-38 as a novel marker to quantify proliferating blasts in acute myeloid leukaemia*

Plett C, Klauer LK*, Amberger DC, et al. Immunomodulatory kits generating leukaemia derived dendritic cells do not induce blast proliferation ex vivo: IPO-38 as a novel marker to quantify proliferating blasts in acute myeloid leukaemia. Clin Immunol. 2022;242:109083. doi:10.1016/j.clim.2022.109083 [* C.P. and L.K.K. contributed equally]*

The objective of publication II was to assess the effect of DC/DC_{leu}-generating protocols on the alteration of blast cell proliferation using the cell proliferation markers CD71 and Ki-67 and the novel marker IPO-38. The great performance of DC/DC_{leu}-generating-protocols in stimulating DC/DC_{leu}-mediated anti-leukaemic activity has been shown in multiple instances. Its overall potential though is closely linked to its safeness and thus not only depends on the presence of anti-leukaemic but also on the absence of pro-leukaemic effects. Particularly with respect to future clinical applications, potentially adverse effects initiated by DC/DC_{leu}-generating protocols, especially the induction of blast proliferation, have to be precluded.

Therefore, blast proliferation was quantified before and after treatment with the DC/DC_{leu}-generating protocols Kit-D, -I, -K, -M in a WB-environment (in vitro) using the selected proliferation markers Ki-67, CD71 and IPO-38. Overall, kits were found to not or only insignificantly (<5%) induce blast proliferation in the majority of cases while successfully generating functional DC/DC_{leu}. Although single cases experienced an increase in blast proliferation, it could be compensated by DC/DC_{leu}-stimulated anti-leukaemic activity (as assessed by CTX) most of the time. Altogether, kit treatment appeared to be safe in vivo. Despite the different operating principles of the selected proliferation

markers, all three markers considered were capable of detecting and quantifying proliferating blasts in (un)cultured leukaemic WB. At this, IPO-38 transpired to be the marker with the highest sensitivity; a combination with CD71, a marker rather depicting cell proliferative capacity than cell proliferation, may be of value in the assessment of proliferation dynamics.

Overview Publication III - *Interferon Gamma Secretion of Adaptive and Innate Immune Cells as a Parameter to Describe Leukaemia-Derived Dendritic-Cell-Mediated Immune Responses in Acute Myeloid Leukaemia in vitro*

Klauer LK, Schutti O, Ugur S, et al. Interferon Gamma Secretion of Adaptive and Innate Immune Cells as a Parameter to Describe Leukaemia-Derived Dendritic-Cell-Mediated Immune Responses in Acute Myeloid Leukaemia in vitro. Transfus Med Hemother. 2021;49(1):44-61. doi:10.1159/000516886

The objective of publication III was to display and further specify DC/DC_{leu}-mediated leukaemia-specific immune responses by scrutinising the interferon-gamma-(IFNy)-secretion of immunoreactive cells. IFNy has a central function in the homeostasis of the immune system: it is not only an integral element of regular immunity, promoting innate and adaptive immune responses, but also significantly associated with anti-tumour immunity, promoting tumour surveillance. Secreted by innate and adaptive immunoreactive cells to mediate the different stages of an immune response, IFNy mirrors the state of immune activation.

Therefore, DC/DC_{leu} were generated following the DC/DC_{leu}-generating protocols Kit-I, -M in a WB-environment (in vitro) and used to stimulate T cell enriched immunoreactive cells. Stimulated immunoreactive cells did not only show an increase in anti-leukaemic activity (as assessed by CTX), but also an increase in IFNy-secreting innate (natural killer cells, NK cells, cytokine-induced killer cells, CIK cells, invariant natural killer cells, iNKT cells) and adaptive (T cells) immune cells (as assessed by a cytokine secretion assay, CSA; frequencies of IFNy-positive cells validated by an intracellular cytokine assay, ICA), enlightening the induction of leukaemia-specific alongside anti-leukaemic activity through DC/DC_{leu}. Interestingly, stimulation of DC/DC_{leu}-stimulated immunoreactive cells with LAA (WT-1 and preferentially expressed antigen in melanoma, PRAME) did not further enhance IFNy secretion, implying effective activation of leukaemia-specific cells. Remarkably, the IFNy-secretion of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells and $CD3^-CD56^+$ NK cells showed a positive correlation with the comprehensively achieved anti-leukaemic cytotoxicity. Ultimately, IFNy was not only able to disclose the leukaemia-specific activity of innate and adaptive immunoreactive cells, but also, in correlation with the achieved anti-leukaemic activity, to display the (indirect and direct) participation of immunoreactive cells in DC/DC_{leu}-mediated immune responses in vitro and potentially in vivo.

Conclusion

DC/DC_{leu}-based immunotherapy has great potential. By the expression of the individual's whole leukaemic antigen repertoire, DC_{leu} have the extraordinary competence to initiate a comprehensive leukaemia-specific and anti-leukaemic immune response. In the course of the three studies comprised in this doctoral thesis, it was possible to develop new DC/DC_{leu}-generating protocols (kits) that facilitate the generation of (mature) DC/DC_{leu} in a WB-environment, competent to stimulate T and other immunoreactive cells to an improved leukaemia-specific and anti-leukaemic activity, without an induction of blast proliferation, in vitro. Ultimately, studies on DC/DC_{leu}-based immunotherapy need to be expanded from in vitro to in vivo settings and eventually translated into clinical application to further assess the value of DC/DC_{leu} in the treatment of AML.

2. Zusammenfassung *Summary*

Summary

(Leukaemia derived) dendritic cells (DC, DC_{leu}) are potent mediators of antigen-specific innate and adaptive immune responses in AML. Originating from myeloid leukaemic blasts, DC_{leu} are characterised by the simultaneous expression of dendritic- and leukaemic-antigens, giving them the exceptional competency to stimulate leukaemia-specific and anti-leukaemic activity.

The generation of DC and DC_{leu} is based on DC/DC_{leu}-generating protocols comprising diverse sets of response modifiers that induce haematopoietic differentiation, dendritic maturation and activation. Most protocols though are developed for the application in an MNC-environment. In consideration of future clinical applications, progression to DC/DC_{leu}-generation in a WB-environment is essential. Developing new protocols specifically designed for the generation of DC/DC_{leu} in a WB-environment, the protocols Kit-I (GM-CSF + OK-432), -K (GM-CSF + PGE_2) and -M (GM-CSF + PGE_1) showed the greatest efficiency in generating (mature) DC/DC_{leu}, proficient in stimulating anti-leukaemic activity. As the general potential of DC/DC_{leu}-based immunotherapy is also closely linked to its safeness, potentially adverse effects initiated by DC/DC_{leu} generating protocols, especially the induction of leukaemic blast proliferation, have to be precluded. Analysing the proliferation of leukaemic blasts using the cell proliferation markers CD71 and Ki-67 and the novel marker IPO-38, DC/DC_{leu}-generating protocols could be shown to not or only insignificantly induce blast proliferation, suggesting safe in vivo application. At this, IPO-38 transpired to be the marker with the highest sensitivity; a combination with CD71, a marker rather depicting cell proliferative capacity than cell proliferation, may be of value in the assessment of proliferation dynamics. In the interest of displaying and further specifying DC/DC_{leu}-mediated immune responses, DC/DC_{leu}-stimulated innate and adaptive immune cells were further scrutinised regarding their activation status utilising the cytokine IFNy. Analysing the IFNy-secretion-profiles of immunoreactive cells, it could be shown that DC/DC_{leu} not only stimulate anti-leukaemic but also leukaemia-specific activity, specifically in T, NK, CIK and iNKT cells. Stimulation of DC/DC_{leu}-stimulated cells with LAA did not affect IFNy-secretion-profiles, implying effective activation of leukaemia-specific cells through DC/DC_{leu}. Remarkably, the IFNy-secretion-profiles of $CD3^+$ T cells, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells and $CD3^-CD56^+$ NK cells positively correlated with the comprehensively achieved anti-leukaemic activity. IFNy, overall, did not only impose to be an adequate parameter to display (indirectly and directly) participating immunoreactive cells of DC/DC_{leu}-mediated leukaemia-specific and anti-leukaemic immune responses, but also to assess and monitor DC/DC_{leu}-based immunotherapy in vitro and potentially in vivo.

Altogether, DC/DC_{leu}-based immunotherapy has shown auspicious results. By the expression of the individual's whole leukaemic antigen repertoire, DC_{leu} have the extraordinary competence to initiate a comprehensive leukaemia-specific and anti-leukaemic immune response, without being reliant on a preceding selection of leukaemic target antigens. Ultimately, studies on DC/DC_{leu}-based immunotherapy need to be expanded from in vitro to in vivo settings and eventually translated into clinical application to further assess the value of DC/DC_{leu} in the treatment of AML.

Zusammenfassung

Dendritische Zellen (leukämischen Ursprungs) (DC, DC_{leu}) sind potente Mediatoren von antigenspezifischen angeborenen und erworbenen Immunreaktionen in AML. Durch ihren Ursprung in myeloischen leukämischen Blasten zeichnen sich DC_{leu} durch die gleichzeitige Expression von dendritischen und leukämischen Antigenen aus, was ihnen die Kompetenz zur Stimulation leukämiespezifischer und antileukämischer Aktivität verleiht.

Die Generierung von DC und DC_{leu} erfolgt basierend auf DC/DC_{leu}-generierenden Protokollen. Diese setzen sich aus unterschiedlichen Kombinationen an immunantwort-modifizierenden Substanzen mit der Fähigkeit zur Induktion hämatopoetischer Differenzierung sowie dendritischer Ausreifung und Aktivierung zusammen. Da ein Großteil der Protokolle jedoch für die Anwendung in einer MNC-Umgebung ausgelegt ist, ist es im Hinblick auf einen zukünftigen klinischen Einsatz essenziell, die Generierung von DC/DC_{leu} zunächst in einer WB-Umgebung zu etablieren. Im Rahmen der Entwicklung neuer Protokolle, speziell ausgelegt auf die Generierung von DC/DC_{leu} in einer WB-Umgebung, wiesen die Protokolle Kit-I (GM-CSF + OK-432), -K (GM-CSF + PGE_2) und -M (GM-CSF + PGE_1) die größte Effizient in der Generierung von (ausgereiften) DC/DC_{leu} mit der Fähigkeit zur Stimulation von antileukämischer Aktivität auf. Es gilt dabei jedoch zu beachten, dass das generelle Potential der DC/DC_{leu}-basierten Immuntherapie auch eng mit dessen Anwendungssicherheit verknüpft ist. Ein Ausschluss von durch DC/DC_{leu}-generierenden Protokollen induzierten nachteiligen Effekten, insbesondere der Induktion einer Blastenproliferation, ist daher zwingend notwendig. Durch die Analyse der Proliferation von leukämischen Blasten mittels der Proliferationsmarker CD71, Ki-67 und dem neuen Marker IPO-38 konnte gezeigt werden, dass DC/DC_{leu}-generierende Protokolle eine Blastenproliferation nicht oder nur insignifikant induzieren, was auf eine sichere Anwendbarkeit in vivo hindeutet. Der Proliferationsmarker IPO-38 stellte sich in diesem Zusammenhang als Marker mit der höchsten Sensitivität dar; eine Kombination mit CD71, einem Marker besser geeignet zur Bestimmung der Proliferationskapazität als der Proliferation, kann in der Beurteilung der Proliferationsdynamik beitragen. Im Interesse DC/DC_{leu}-stimulierte Immunreaktion darstellen und weiter spezifizieren zu

können, erfolgte im Weiteren eine Untersuchung von DC/DC_{leu}-stimulierten immunreaktiven Zellen des angeborenen und erworbenen Immunsystems auf ihren Aktivierungsstatus mithilfe des Zytokins IFNy. Durch die Analyse der IFNy-Sekretionsprofile von immunreaktiven Zellen konnte gezeigt werden, dass DC/DC_{leu} nicht nur fähig zur Stimulation von antileukämischer, sondern auch leukämiespezifischer Aktivität sind, insbesondere in T-, NK-, CIK- und iNKT-Zellen. Eine Stimulation von DC/DC_{leu}-stimulierten Zellen mit LAA zeigte keinen Einfluss auf deren IFNy-Sekretionsprofile, was eine effiziente Aktivierung leukämiespezifischer Zellen durch DC/DC_{leu} nahelegt. Bemerkenswerterweise konnte darüber hinaus eine positive Korrelation der IFNy-Sekretionsprofile von $CD3^+$ T-Zellen, $CD3^+CD4^+$ T-Zellen, $CD3^+CD8^+$ T-Zellen und $CD3^-CD56^+$ NK-Zellen mit der gesamt erzielten antileukämischen Aktivität nachgewiesen werden. IFNy imponierte in diesem Zusammenhang nicht nur als ein angemessener Parameter zur Darstellung von (indirekt und direkt) partizipierenden immunreaktiven Zellen an DC/DC_{leu}-vermittelten leukämiespezifischen und antileukämischen Immunreaktionen, sondern auch zur Evaluation und Überwachung von DC/DC_{leu}-Immuntherapie in vitro und potenziell in vivo.

Insgesamt konnte die DC/DC_{leu}-basierte Immuntherapie bereits vielversprechende Ergebnisse vorweisen. Durch die Expression des gesamten individuellen leukämischen Antigenrepertoires besitzen DC_{leu} das außerordentliche Potenzial eine umfangreiche leukämiespezifische und antileukämische Immunreaktion initiieren zu können, ohne dabei auf eine vorherige Selektion von leukämischen Zielantigenen angewiesen zu sein. Um die Bedeutung von DC/DC_{leu} in der Therapie von AML weiter evaluieren zu können, ist es jedoch unabdingbar, DC/DC_{leu}-basierte Studien von in vitro auf in vivo Settings auszuweiten und schließlich auch in die klinische Anwendung zu übersetzen.

3. Publikation I *Publication I*

Generation of Leukaemia-Derived Dendritic Cells (DC_{leu}) to Improve Anti-Leukaemic Activity in AML: Selection of the Most Efficient Response Modifier Combinations

Christoph Schwepcke *, Lara Kristina Klauer *, Diana Deen, Daniel Christoph Amberger, Zuzana Fischer, Fatemeh Doraneh-Gard, Carina Gunsilius, Annika Hirn-Lopez, Tanja Kroell, Johanna Tischer, Melanie Weinmann, Jan-Ole Werner, Andreas Rank, Christoph Schmid, Helga Maria Schmetzer

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4. Publikation II *Publication II*

Immunomodulatory kits generating leukaemia derived dendritic cells do not induce blast proliferation ex vivo: IPO-38 as a novel marker to quantify proliferating blasts in acute myeloid leukaemia

Caroline Plett *, Lara Kristina Klauer *, Daniel Christoph Amberger, Selda Ugur, Alexander Rabe, Zuzana Fischer, Diana Deen, Annika Hirn-Lopez, Carina Gunsilius, Jan-Ole Werner, Jörg Schmohl, Doris Krämer, Andreas Rank, Christoph Schmid, Helga Maria Schmetzer

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5. Publikation III *Publication III*

Interferon Gamma Secretion of Adaptive and Innate Immune Cells as a Parameter to Describe Leukaemia-Derived Dendritic-Cell-Mediated Immune Responses in Acute Myeloid Leukaemia in vitro

Lara Kristina Klauer, Olga Schutti, Selda Ugur, Fatemeh Doraneh-Gard, Daniel Christoph Amberger, Nicole Rogers, Doris Krämer, Andreas Rank, Christoph Schmid, Britta Eiz-Vesper, Helga Maria Schmetzer

Klauer LK, Schutti O, Ugur S, et al. Interferon Gamma Secretion of Adaptive and Innate Immune Cells as a Parameter to Describe Leukaemia-Derived Dendritic-Cell-Mediated Immune Responses in Acute Myeloid Leukaemia in vitro. Transfus Med Hemother. 2021;49(1):44-61. doi:10.1159/000516886

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