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Studies on Chemokine and Cytokine Release in Akute Myeloid Leukemia and Myelodysplastic Syndromes

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München, 23.07.2022 Marion Merle

Contents

1 Abbreviations

AML	Acute Myeloid Leukaemia
ALL	Acute Lymphoblastic Leukaemia
APC	Antigen presenting Cells
BCL-2	B-Cell Lymphoma 2
BiTE	Bispecific T-Cell Engager
CAR-T	Chimeric Antigen Receptor T-cell Therapy
CCL	CC-Chemokine Ligand
CD	Cluster of Differentiation
CIK	Cytokine-induced Killer Cells
CML	Chronic Myelogenous Leukaemia
CR	Complete Remission
CTL	Cytotoxic T-Lymphocyte
CTLA-4	Cytotoxic T-Lymphocyte-Associated Protein 4
CTX	Cytotoxicity Assay
CXCL	CXC-Chemokine Ligand
DART	Dual-affinity Retargeting Antibody
DC	Dendritic Cells
DCleu	Leukemia-Derived Dendritic Cells
DLI	Donor-Lymphocyte Infusion
FDA	Food and Drug Administration
FLT3	Fms Like Tyrosinekinase 3
FLT3-L/FL	FLT3-Ligand
GM-CSF	Granulocyte Macrophage Colony-Stimulating Factor
GMP	Good Manufacturing Practice
GVHD	Graft Versus Host Disease
GVL	Graft Versus Leukemia
HLA	Human Leukocyte Antigen
IDH1/2	Isocitrat-Dehydrogenase 1/2
IFN-γ	Interferon-γ
IL	Interleukin
LAA	Leukemia-Associated Antigens
LSA	Leukemia Specific Antigens
MDS	Myelodysplastic Syndromes
MHC	Major Histocompatibility Complex
MLC	Mixed Lymphocyte Culture
MNC	Mononuclear Cells
mo-DC	Monocytoid DC
MRD	Minimal Residual Disease
NK	Natural-Killer Cells
NPM1	Nucleophosmin 1
PB	Peripheral Blood
PD1	Programmed Cell Death Protein 1
PD-L1	Programmed-Death Ligand 1
PGE	Prostaglandin E

PR1	HLA-A2 Nonameric Peptide Derived From Neutrophil Elastase and Proteinase 3
SCF	Stem Cell Factor
SCT	Stem Cell Transplantation
TH	T-Helper Cell
TIM-3	T-Cell Immunoglobulin and Mucin Domain-Containing Protein 3
TNF-α	Tumor Necrosis Factor-α
T_{reg}	Regulatory T-cell
WT1	Wilms Tumor Suppressor Gene1

2 Publications

2.1 Publications Included in this Thesis

The following publications were summarized for this cumulative medical thesis in accordance with the examination rules of the medical faculty of the LMU Munich:

Publication I:
Cytokine Release Patterns in Mixed Lymphocyte Culture (MLC) of T-Cells with Dendritic Cells (DC) Generated from AML Blasts Contribute to Predict anti-Leukaemic T-Cell Reactions and Patients' Response to Immunotherapy
D. Fischbacher, **M. Merle**, A. Liepert, C. Grabrucker, T. Kroell, A. Kremser, J. Dreyßig, M. Freudenreich, F. Schuster, A. Borkhardt, D. Kraemer, C.-H. Koehne, H.J. Kolb, C. Schmid & H.M. Schmetzer
Cell Communication & Adhesion 2016, 22:2-6, 49-65, DOI: 10.1080/15419061.2016.1223634

Publication II:
Serum Chemokine-release Profiles in AML-patients Might Contribute to Predict the Clinical Course of the Disease
M. Merle, D. Fischbacher, A. Liepert, C. Grabrucker, T. Kroell, A. Kremser, J. Dreyssig, M. Freudenreich, F. Schuster, A. Borkhardt, D. Kraemer, C.-H. Koehne, H. J. Kolb, C. Schmid & H. M. Schmetzer
Immunological Investigations 2019, DOI: 10.1080/08820139.2019.1661429

Publication III:
Conversion of AML-blasts to leukemia-derived dendritic cells (DCleu) in 'DC-culture-media' shifts correlations of released chemokines with antileukemic T-cell reactions.
M. Merle, D. Fischbacher, A. Liepert, C. Grabrucker, T. Kroell, A. Kremser, J. Dreyssig, M. Freudenreich, F. Schuster, A. Borkhardt, D. Kraemer, C.-H. Koehne, H. J. Kolb, C. Schmid & H. M. Schmetzer
Immunobiology. 2021 Mar 20;226(3):152088. doi: 10.1016/j.imbio.2021.152088.

2.2 Other Publications (Contributions to international Congresses)

H.M. Schmetzer, A. Liepert, C. Grabrucker, D. Fischbacher, M. Freudenreich, **M. Merle**, R. Reibke, F. Schuster, S. Reuther, A. Kremser, J. Loibl, C. Schmid, T. Kroell, A. Borkhardt, HJ Kolb
Leukemia-derived DC for T-cell therapy against AML progenitors
Blood 112, 11, abstract 5442, (2008)

H.M. Schmetzer, A. Liepert, C. Grabrucker, D. Fischbacher, **M. Merle**, M. Freudenreich, F. Schuster, S. Reuther, A. Kremser, J. Loibl, R. Reibke, C. Schmid, T. Kroell, H.J. Kolb
The composition and quality of leukemia-derived dendritic cells, T-cells and cellular microenvironment is predictive for the antileukemic T-cell cytotoxic reactions of DC-primed T-cells and the response to therapy
Bone Marrow Transplantation 43, suppl. 1, abstract 901, 263(2009)

D. Fischbacher, **M. Merle**, A. Liepert, C. Grabrucker, A. Kremser, J. Loibl, C. Schmid, T. Kroell, HJ Kolb, H.M. Schmetzer
The composition and quality of leukemia-derived dendritic cells, T-cells and cellular microenvironment is predictive for the antileukemic T-cell cytotoxic reactions of DC-primed T-cells and the response to therapy
Bone Marrow Transplantation 43, suppl. 1, 265, abstract 905, (2009)

H.M. Schmetzer, A. Liepert, C. Grabrucker, D. Fischbacher, **M. Merle**, M. Freudenreich, F. Schuster, S. Reuther, A. Kremser, J. Loibl, R. Reibke, C. Schmid, T. Kroell, H.J. Kolb
The composition and quality of leukemia-derived leukemia-derived DC, T-cells and the cellular microenvironment is predictive for the antileukemic T-.cell cytotoxic reactions of DC-primed T-cells and the response to therapy
XI. Wissenschaftliches Symposium der Medizinischen Klinik III, Herrsching, Highlight-Vortrag, 61 (2009)

M. Merle, D. Fischbacher, A. Liepert, C. Grabrucker, A. Kremser, J. Loibl, C. Schmid, T. Kroell, H.J. Kolb, H.M. Schmetzer
CXCL8 and CCL2 secretion by dendritic cells generated from AML blasts (DCleu) as well as IFN gor IL6 release in mixed lymphocyte cultures (MLC) from T-cells after DC-priming are predictive for antileukemic T-cell reactions
XI. Wissenschaftliches Symposium der Medizinischen Klinik III, Herrsching, 50 (2009)

H.M Schmetzer , A. Liepert, C. Grabrucker, D. Fischbacher, **M. Merle**, R. Reibke, J. Tischer, T. Kroell, C. Schmid, H.J. Kolb
Clinical relevance of in vitro generated dendritic cells of leukemic origin (Dcleu) in patients with AML Bone Marrow Transplantation 45, suppl. 2, abstract 960, 300 (2010)

M. Merle, D. Fischbacher, A. Liepert, C.Grabrucker, T.Kroell, A.Kremser, J.Dreyssig, M.Freudenreich, F.Schuster, A. Borkhardt, D. Kraemer, C. Koehne, H. J. Kolb, C. Schmid, H. M. Schmetzer

Conversion of aml-blasts to leukemia-derived Dendritic Cells (DCleu) in 'DC-culture-media' shifts the (serum) chemokine-release profile to a more 'inflammatory' (in culture) going along with improved antileukemic T-cell-reactivity
European journal of Cancer, vol 110, Supp 1, P5.01, (2019)

M. Merle, D. Fischbacher, A. Liepert, C.Grabrucker, T.Kroell, A.Kremser, J.Dreyssig, M.Freudenreich, F. Schuster, A. Borkhardt, D.Kraemer, C.H. Koehne, H.J. Kolb, C. Schmid, H.M. Schmetzer
AML-blast conversion to leukemia-derived dendritic cells (DCLEU) in 'DC-culture-media' shifts the (SERUM) chemokine-release to a more 'inflammatory' (in culture) going along with improved antileukemic T-cell-reactivity
EBMT, Bone Marrow Transplantation 54, suppl. 1, P069 (2019)

3 Co-Authors’ confirmation

All co-authors signed a confirmation document, that Marion Merle has the permission to use the publications for his medical thesis. Furthermore, they confirmed that the publications are not part of another doctoral theses. The Documents were submitted separately with this thesis.

4 Introduction

4.1 AML/MDS

Abnormal cell-differentiation and clonal proliferation of myeloid precursor-cells (blasts) are characteristics of Acute myeloid leukaemia (AML) and Myelodysplastic Syndromes (MDS). Accumulation of blasts results in an ineffective haematopoiesis leading to symptoms of neutropenia, anaemia and thrombocytopenia like fever and infections, inappetence and degradation of performance status as well as haemorrhages. Moreover, infiltration of extramedullary tissues like liver, spleen and skin can be seen [1, 2]. Due to high proliferation rates of leukemic blasts, symptoms mostly develop rapidly and may lead to death within weeks after diagnosis [3]. Therefore, immediate diagnostic assessment and evaluation of treatment options are essential. The prognosis can be estimated using the 2017 European LeukemiaNet risk stratification, which allows a distinction of three risk groups (favourable, intermediate and adverse), based on clinical features like age, primary/secondary AML, comorbidities and certain cytogenetic or molecular aspects [4].

4.2 Standard Treatment

The goal of most treatments is achievement of complete and long-lasting remission, which is defined as presence of less than 5% blasts in the bone marrow and none in the peripheral blood, no evidence of extramedullary manifestations and recovery of peripheral blood parameters. Even in complete remission (CR) 50% of patients show a minimal residual disease (MRD), which means persistence of leukemic blasts in the bone marrow that may lead to relapse [5, 6].
If a patient is estimated fit for intensive chemotherapy, therapy comprises several phases.
In induction chemotherapy mostly anthracycline plus cytarabine-based regimen are applied (e.g., 7+3) [7].
Induction is followed by consolidation therapy in order to stabilize remission or eliminate MRD. Depending on the patient's stratified risk and availability of a suitable stem cell donor allogeneic stem cell transplantation (SCT) or more cycles of a cytarabine-based chemotherapy are applied. Allogeneic SCT may achieve cure in 50 – 60% of cases and is therefore considered the therapy with the strongest antileukemic effect [8 - 10]. It is also a chance of cure for patients who relapse after chemotherapy. Due to the significant mortality from graft-versus-host disease (GVHD) and opportunistic infections caused by immunosuppression application of SCT is limited for younger patients (< 60 years) and patients with higher risk and no significant comorbidities. In case of relapse after SCT, application of Donor lymphocyte infusions (DLI) can reinstall remission, what demonstrates the important role of T-cells in antileukemic reactions [11-14].
There is no maintenance therapy which can be considered standard in the treatment of AML and its role in reducing the risk of relapse in patients who didn't receive SCT remains controversial [15]. Nevertheless, an improvement of survival was described through maintenance with CC-486, an oral form of Azacytidine, following standard induction and cytarabine consolidation therapy [16].

For elderly patients or patients estimated unfit for intensive chemotherapy low-dose cytarabine or hypomethylating agents like Azacytidine especially in combination with Venetoclax are treatment options for at least achieving a cytoreduction and prolonging survival [17, 18].

4.3 Alternative treatment options

Treatment of AML by chemotherapy is leading to CR in 70 – 80% of patients under 60 years, in older patients, however, remission rates are lower [19, 20]. Patients who profit from allogeneic SCT show a disease-free survival of 50 – 60%, but only represent 20% of all patients. With the standard chemotherapeutic regimen mentioned above and SCT treatment options for AML were stagnating for some time. Considering the heterogeneity of AML, fortunately, in the past decade treatment options have diversified through the development of various targeted therapies.

Those alternative, targeted therapies have improved CR rates of chemotherapy but relapse rates remain high, with over 60% in higher risk patients and an overall median disease-free survival of less than 1 year [21, 22].

Various non-immunotherapeutic and immunotherapeutic strategies have been studied in AML-therapy, which specifically reduce the tumour load and may influence immune responses on a therapeutic way.

Non-immunotherapeutic strategies include antileukemic protein kinase inhibitors (e.g., the FLT3-inhibitors Midostaurin and Gilteritinib), mitochondrial inhibitors (e.g., the IDH2 mutant inhibitor Enasidenib and IDH1 mutant inhibitor Ivosidenib), pro-apoptotic agents (e.g., the BCL2-inthibitor Venetoclax), cell cycle inhibitors (e.g., the Aurora A kinase inhibitor Alisertib) and epigenetic regulators (e.g., the hypomethylating agent Acacytidine) [23].

In the years 2017 – 2019 the FDA approved Midostaurin, Gilteritinib, Venetoclax, Glasdegib, Enasidenib and Ivosidenib as a treatment for adult patients in different settings [24 – 29].

Immunotherapeutic strategies include transfer of specific antibodies, blockade of checkpoint inhibitors, adoptive cellular therapies and Dendritic cell (DC) -based vaccination, whose success relies on the recognition of suitable tumour antigens by immune effector cells and thus activation of immune cell responses.

Antibodies have been used with or without modification by conjugation with toxins or radioactive isotopes to increase their antileukemic efficacy [30].

Mono- or bispecific antibody-based approaches target membrane-expressed (CD33, CD123, FLT3) or leukaemia associated or specific antigens (LAA, LSA, e.g.: WT-1, PR1, NPM1) [31 – 33, 23]. In 2017 the FDA approved Gemtuzumab Ozogamizin – a anti-CD33 monoclonal antibody – for newly diagnosed CD33-positive AML as a supplemental treatment to standard induction chemotherapy [34].

Bispecific antibodies recognize two different epitopes or antigens which may be constructed to attract cells of the immune system like T-cells and NK-cells and to recognize multiple surface antigens on leukemic stem cells [35]. Bispecific antibodies which bind to anti-CD3 are being studied as bispecific T-cell engagers (BiTes) or as dual-affinity retargeting antibodies (DARTS) [36, 37]. Among others the CD22/CD3-bispecific drug AG330 is being tested in clinical trials in AML-patients [36].

Moreover, several radiolabelled antibodies have been applied in AML therapy, which however led to major hematologic toxicity with serious cytopenia [37].
Another immunotherapeutic approach is checkpoint inhibition: Its goal is to activate antitumor immunity mediated by T-cells through blockade of PD1, PD-L1, CTL4 and TIM3 pathway among others [23, 32, 40].
Currently PD-L1 and PD1 inhibitors are being tested in clinical trials in combination with hypomethylating agents (like Azacytidine) which may increase their expression on leukemic cells [41].
In summary, depending on certain cytogenetic- or molecular genetic findings (e.g., CD33+, FLT3 mutation) specific drugs have been applied additionally to standard treatment in the last decade, with the result of a prolonged overall survival [6, 18, 42].
Cellular therapy includes chimeric antigen receptor T-cell (CAR-T) therapy. After being recently approved for paediatric ALL and aggressive B-cell lymphoma it is now being studied in AML therapy targeting CD33, CD123, CD44v6, CLEC12A, CD38, and FLT3 in preclinical and clinical trials [43 - 45]. CARs can be designed against any LAA and thus the genetically modified T-cells expressing CAR are redirected against leukemic cells [43]. Most CAR-T therapies are still in the process of preclinical studies with encouraging results or have only recently entered phase I trials in AML.
Other adoptive cellular therapies utilize the innate immune system. They are based on enhancement of the clinical efficacy of allogeneic natural killer-cells (NK) by several approaches and transfer of non-specific immune effector cells like Cytokine-induced Killer cells (CIK) or γδ T lymphocytes [46, 47].
One approach is expansion of allogeneic NKs in vitro and application together with SCT in order to exert cytotoxic effects on leukemic cells and to amplify GVL activity [48]. The above-mentioned CIK cells can be developed from blood lymphocytes in the presence of IL-1, IL-2 or IL-15 and are expressing T-cell- as well as NK-markers [49].
Human γδ T lymphocytes are immune cells of the peripheral blood which are characterized by T-cell receptor expression. A possible infiltration of various tumour sites, cytokine production and exertion of cytotoxic activity against malignant targets in an HLA-unrestricted way are some of their most important properties [50].
It is known for a long time that the anti-leukemic potential of NK and T-cells can be enhanced by administration of IL-2 in vivo. In combination with histamine promising results were shown in studies with AML-patients in CR. Thus, an approval by the European Medicines Agency of the drug combination as maintenance immunotherapy followed in 2008 [51].
Altogether results of these alternative AML-therapies are promising, but also limited by multiple mechanisms of resistance and heterogeneous antigen expression of AML blast populations. Additionally, side effects like toxicities to normal hematopoietic stem cells including prolonged cytopenia and adverse events like toxicity against other targets for example neurotoxicity and cytokine release syndromes may be caused by most of these therapies [50, 52, 32]. Moreover, antibody-based strategies and adoptive cellular therapies represent passive immunotherapies, which means they aren’t capable of establishing an immunological memory.
To generate or boost leukaemia-specific immune responses peptide and DC-based vaccines against LAAs have been studied [40].

4.4 Dendritic Cell-based therapy

One of the most promising approaches of active specific immunotherapy are DC-based strategies [53 - 55]. DC are professional antigen presenting cells (APC) which stimulate various immune cells (e.g., naïve or CD4 T- and B-lymphocytes, CD8 cytotoxic T-lymphocytes (CTL)) by processing antigens and presenting their fragments through major histocompatibility complex (MHC) [56 - 58].
Ex-vivo generation of DC from CD14+ monocytes (mo-DC) is possible using several strategies of loading with LAA like mRNA transfection, dendritic/AML-cell fusion or pulsion with leukemic cell lysates followed by adoptive retransfer of these manipulated DC to the patient by an intradermal or intravenous vaccination [59].
Another approach is generation of leukaemia-derived DC (DCleu) from leukemic blasts using different DC-generating protocols. Mononuclear cells (MNC) from the patients' peripheral blood (PB) are put into culture with combinations of response modifiers, which leads to generation of DCs expressing antigens characteristic for DCs (e.g., CD80, CD86, CD206) and blasts (e.g., CD34, CD65, CD117) simultaneously [60]. The proportion of generated DCleu from blasts can be evaluated using a special flow cytometric gating protocol [60].
Our group and others have shown that conversion to DCleu is possible in vitro from every given AML-patient selecting the most effective out of several previously tested DC-generating methods [61 - 64, 58]. In a mixed lymphocyte culture (MLC) T-cells are stimulated by DC/DCleu and activated against leukemic blasts and the antileukemic capacity is tested afterwards in a cytotoxicity assay (CTX).
In summary ex-vivo generation of mo-DC and DC/DCleu and vaccination is challenging, as the whole process is expensive, consuming a lot of time and good manufacturing practice (GMP) conditions are obligatory. Except that DCleu trigger the adaptive and innate immune system against blasts and induce an immunologic memory, their special property is the presentation of the individual patients' whole set of antigens. Furthermore, conversion of patients' blasts to DCleu could possibly be induced in vivo using certain compositions of modifiers which may stimulate hematopoietic cells and induce DC differentiation and maturation.

4.5 Cytokines and Chemokines

As soluble factors in the microenvironment of cells, cytokines and chemokines might influence interactions of immune cells with malignant cells [65]. Among others, cytokines are expressed by immunocompetent and stromal cells. Their properties are mediation of immune responses and immune escape of malignant cells. The function of effector cells is determined by T helper cells (TH) and can be either a pro-inflammatory or anti-tumour response depending on cytokines like Interleukin (IL) -2, Interferon (IFN) -γ and Lymphotoxin-α which mediate a TH1 response or IL-3, IL-4, IL-5, IL-9, IL-10 and IL-13 which mediate a TH2 response and T-cell anergy [66, 67]. T-cell reactions after DC- or MNC-stimulation might correlate with dominating TH1 or TH2 cytokine levels.
Chemokines are chemotactic cytokines which are released by various cells, e.g. fibroblasts, keratinocytes, lymphocytes, monocytes and also tumour-cells. In multiple inflammatory and immune responses, they act as chemoattractants mediating cell migration and activation of specific leukocyte subtypes [68, 69]. In malignant diseases chemokines represent growth- or survival factors. They are involved in the regulation of haematopoiesis, angiogenesis,

metastasis and tumour infiltration by leukocytes [70, 71]. Source cells, target cells and known functions of the analysed cytokines (IL-2, IL-4, IL-6, IFN-γ and Tumour-necrosis-factor (TNF) - α) and chemokines (CCL2, CCL5, CXCL8, CXCL9, CXCL10 and IL-12) are given in the table below (modified after Merle et al 2019 (Publication II) and Merle et al 2021 (Publication III)).

Chemokine/ Cytokine	**Source**	**Target Cells**	**Known Functions**	**Reference**
'anti-tumour response related chemokines':				
CXCL9	Macrophages, DC, lymphocytes	Neutrophils, $CD8^+$ T-cells	Inhibition of angiogenesis and tumour growth; Promotion of T-cell-infiltration in inflammatory disorders	[72 - 74]
CXCL10	leukocytes, epithelial and endothelial cells, stromal cells	Neutrophils, $CD8^+$ T-cells	Inhibition of angiogenesis and tumour growth; autoimmune reactions	[74 - 77]
IL-12	DC, B-lymphocytes, macrophages	NK-cells, CTL	Inhibition of angiogenesis and tumour growth; immunomodulation; Promotion of differentiation of naïve T-cells into TH1	[78]
'Tumour growth promoting/ inflammatory chemokines':				
CXCL8	tumour cells, phagocytes, DC, T_{regs}, fibroblasts, epithelial cells, mast cells	Neutrophils, T-cells, endothelial cells	Promotion of tumour proliferation, survival, angiogenesis and metastasis; proinflammatory	[79 - 82, 70, 71]
CCL5	fibroblasts, epithelial cells, DC, monocytes, macrophages, platelets, tumour cells, T-cells	MNC, $CD8^+$ T-cells, T_{regs}, macrophages	Promotion of tumour progression and metastasis; enhancement of tumour-associated macrophages and $T_{regs;}$ chronic inflammatory disorders	[83 – 85, 70, 74]
CCL2	stromal cells, leukocytes, DC, endothelial cells, tumour cells	MNC, macrophages	Promotion of tumour-initiation, progression and angiogenesis; proinflammatory	[86 - 89]
Cytokines:				
IL-2	TH1, CTL	T-cells	Promotion of T-cell proliferation, CTL and suppressor T-cell differentiation; activation of NK, B-cells and macrophages	[66]

IL-4	TH2, eosinophils, basophils, possibly mast cells	T-cells	Promotion of DCleu differentiation, TH1 /2-differentiation and proliferation of $CD4^+$ T-cells	[90, 66]
IL-6	Mononuclear phagocytic cells, T and B lymphocytes, fibroblasts, endothelial cells, keratinocytes, hepatocytes, bone marrow cells	T and B lymphocytes	Promotion of haematopoiesis, T-cell activation/differentiation and CTL-differentiation (in the presence of IL-2); enhancement of CTL-functions; plasma cell-maturation	[91, 66]
IL-10	Activated T-cells, mainly TH2, B-cells, monocytes, macrophages, NKs	T-cells	Immunosuppression; blockade of T-cell proliferation and induction of anergy; promotion of suppressor type cells; inhibition of APC functions of DC; inhibition of IFN-γ and IL-2 production by TH1 and IL-4 and -5 by TH2	[92, 66]
IFN- γ	TH, CTL, NKs, monocytes, Macrophages, DC	T-cells	Promotion of TH1-differentiation, immunogenicity of tumour cells; and monocyte effector functions; direct antiproliferative effect on TH2; inhibition of IL-4 production by naive T-cells	[93, 66]
TNF-α	macrophages, monocytes, NKs, T-cells	Almost all nucleated cells, tumour cells	Proinflammatory; promotion of IL-10 release. cell apoptosis and resistance to infection and cancers	[94, 95, 66]

4.6 Outline of this doctoral Thesis

This project is based on the ex-vivo generation of DC/DCleu with five standard methods applying different combinations of immune modifiers. In MCM-Mimic MNC were incubated in a medium containing granulocyte-macrophage-colony-stimulating factor (GM-CSF), IL-4, FLT3-Ligand (FL), IL-6, IL-1β, prostaglandin E2 (PGE2) and TNF-α [96, 97].
In Calcium Ionophore DC were generated by incubation of MNC in the presence of A23187 and IL-4, whereas in the medium Picibanil OK-432, a lysis product of Streptococcus pyogenes, GM-CSF, IL-4 and PGE2 were added [97 – 99]. In the medium Cytokines MNC were added to culture with GM-CSF, TNF-α, IL-3, IL-4, stem-cell-factor (SCF) and FL [97, 100]. In the fifth medium Poly (I:C) double-stranded RNA was used as a modulating agent together with GM-CSF, IL-4 and incubated with the MNC [97, 101].
Afterwards DC and DCleu were characterized and quantified by Flow-cytometry according to a gating-strategy described by former members of our group [97, 60, 102].
In an MLC T-cells from AML patients, stem cell donors or patients' T-cells obtained after SCT were incubated as effector cells with DC/DCleu or blast-containing MNC and after this stimulation their lytic potential against leukemic blasts was tested in a cytotoxicity assay

(CTX). The antileukemic capacity of T-cells after DC/DCleu- and MNC-stimulation could be evaluated by measuring fractions of blast targets before and after effector-cell contact.
Cytokine- and chemokine-levels were quantified in serum and DC-culture supernatants by Cytometric-Bead-Array (CBA) - cytokine-levels additionally in supernatants of MLC – and correlated with antileukemic T-cell reactivity following MLC and the clinical course of the disease. In this context Cut-off values were defined to predict ex-vivo antileukemic reactions and the patients' response to immunotherapy or occurrence of GVHD.
CBA is a well-established method in the evaluation and quantification of cytokines and chemokines. Using the Becton Dickinson (BD) CBA Human Chemokine-Kit I, the BD CBA Human-IL12 Kit and the TH1/TH2-CBA-Kit II a preconfigured set of cytokines or chemokines could be measured simultaneously by Flow cytometry, after incubation of serum or supernatant probes with the antibody-coated capture beads.
Since IL-2, IL-4, IL-6, IFN-γ,TNF-α, CCL2, CCL5, CXCL8, CXCL9, CXCL10 and IL-12 are significant mediators of immune-reactions, the goal of this trial was to define release profiles in serum of AML-/MDS-patients in correlation with disease subtypes and the clinical course as well as in DC-culture supernatants and MLC in correlation with the antileukemic functionality of DC-stimulated T-cells.

4.7 Publications Included in this Thesis and Contributions

The ex-vivo generation of DC/DCleu using various methods and the analysis of the cytokine and chemokine release in patients' serum, DC-culture supernatants, MLC are the main targets of the three presented publications.

4.7.1 Publication I, Fischbacher et al 2016

Title: Cytokine Release Patterns in Mixed Lymphocyte Culture (MLC) of T-Cells with Dendritic Cells (DC) Generated from AML Blasts Contribute to Predict anti-leukemic T-Cell Reactions and Patients' Response to Immunotherapy
Authors: D. Fischbacher, M. Merle, A. Liepert, C. Grabrucker, T. Kroell, A. Kremser, J. Dreyßig, M. Freudenreich, F. Schuster, A. Borkhardt, D. Kraemer, C.-H. Koehne, H.J. Kolb, C. Schmid & H.M. Schmetzer

Journal: Cell Communication & Adhesion, 22:2-6, 49-65, DOI: 10.1080/15419061.2016.1223634

In a first step DC/DCleu were generated ex-vivo using three standard methods: 1. 'MCM-Mimic' 2. 'Picibanil' 3. 'Ca-ionophore'. In an MLC the obtained DC/DCleu or MNC were co-cultured with thawed T-cells (autologous, allogenic or after SCT) and afterwards the composition of T-cells was analysed by flow cytometry. In the following CTX the antileukemic capacity of the stimulated T-cells was assessed. By CBA concentrations of IL -2, -4, -6, -10, TNF-α and IFN-γ in supernatants of MLC and AML-/MDS-patients' serum were evaluated.
Results were correlated with the lytic activity of T-cells in the CTX and clinical parameters: Increased cytokine-levels were detected in MLC culture-medium after DC-/MNC-stimulation

of T-cells compared to serum. No significant differences in cytokine release between cases with and without lytic activity of stimulated T-cells, response to immunotherapy (SCT or DLI) and GVHD were detected. Nevertheless, predictive cut-off values for response to immunotherapy, GVHD and the antileukemic activity of T-cells could be determined. Cases with higher detectable amounts of TH2 cytokines (IL-4 and IL-10) showed a lower probability of a response to immunotherapy or GVHD. A higher probability of a lytic T-cell activity was observed in cases with a higher release of inflammatory cytokines (TNF-α, IL-6) or cytokines mediating a TH1 response (IL-2, IFN- γ) and less of a TH2 response (IL-4, -10).
Altogether, assessment of cytokine levels can help to predict patients' response to immunotherapy, occurrence of GVHD and the lytic functionality of T-cells but without further functional tests they are not sufficient to predict antileukemic T-cell activity.

Contribution: Marion Merle carried out parts of the experimental work, contributed to data acquisition, analysis and statistical work. She discussed results especially together with Dr. Fischbacher and Prof. Dr. Helga Maria Schmetzer

4.7.2 Publication II, Merle et al. 2019

Title: Serum Chemokine-release Profiles in AML-patients Might Contribute to Predict the Clinical Course of the Disease
Authors: M. Merle, D. Fischbacher, A. Liepert, C. Grabrucker, T. Kroell, A. Kremser, J. Dreyßig, M. Freudenreich, F. Schuster, A. Borkhardt, D. Kraemer, C.-H. Koehne, H. J. Kolb, C. Schmid & H. M. Schmetzer

Journal: Immunological Investigations, DOI: 10.1080/08820139.2019.1661429

In this trial five standard methods ('MCM-Mimic', 'Ca-Ionophore', 'Picibanil', 'Poly (I:C)', 'Cytokines') were applied for ex-vivo generation of DC/DCleu from AML-/MDS-patients' serum in a first step. Like in publication I the obtained DC/DCleu or MNC were then co-cultured with patients' or allogeneic T-cells in an MLC. Afterwards the antileukemic capacity of the stimulated T-cells compared to MNC-stimulation was evaluated in a CTX. Levels of CXCL8, -9, -10, CCL2, -5 and IL-12 were assessed in serum of AML-/MDS-patients and correlated with clinical data and the course of the disease. Serum chemokine-levels distinguished only slightly between various groups like age groups, FAB-types, blast proportions, cytogenetic-risk-groups, but higher levels of CXCL8, -9, -10 and lower levels of CCL2 and –5 showed a tendential correlation with more favourable subtypes (<50 years of age, <80% blasts in the PB). Moreover, in persisting disease higher CCL5-levels and at relapse higher CCL2-levels in comparison to first diagnosis were detected, which might be a hint for a changing 'disease activity' on a chemokine basis. Furthermore, patients' response to immunotherapy correlated with higher levels of CXCL8, −9, −10 and CCL2 and lower levels of CCL5; for an occurrence of GVHD reversed correlations were seen. Considering all results, we conclude that in serum of AML/MDS-patients higher levels of CXCL8, −9, −10 and lower levels of CCL5 and in part of CCL2 correlate with more favourable subtypes and an enhanced

antitumor'-reactive function. This knowledge might contribute to refine immune-modifying approaches that promote antileukemic adaptive immune responses.
Contribution: Marion Merle carried out the majority of the experimental work, was responsible for data acquisition, assessment and interpretation including all statistical work and drafted the manuscript together with Prof. Dr. Schmetzer.

4.7.3 Publication III, Merle et al. 2021

Title: Conversion of AML-blasts to leukaemia-derived dendritic cells (DCleu) in 'DC-culture-media' shifts correlations of released chemokines with antileukemic T-cell reactions
Authors: M. Merle, D. Fischbacher, A. Liepert, C. Grabrucker, T. Kroell, A. Kremser, J. Dreyßig, M. Freudenreich, F. Schuster, A. Borkhardt, D. Kraemer, C.-H. Koehne, H. J. Kolb, C. Schmid & H. M. Schmetzer

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In analogy to publication II DC/DCleu were generated ex-vivo from serum of AML-/MDS-patients with 5 standard methods. Afterwards those DC/DCleu and MNC were co-cultured with T-cells (autologous, allogeneic or after SCT) in an MLC. T-cell-composition was analysed by flow cytometry. In a CTX the blast-lytic capacity of DC- or MNC-stimulated T-cells was assessed. By CBA the release of CXCL8, -9, -10, CCL2, -5 and IL-12 was quantified in supernatants from DC-generating media. Results were correlated with the patients' serum values, the antileukemic activity of T-cells following MLC (in comparison to MNC-stimulation) and the clinical course of the disease. Higher chemokine-levels in supernatants of DC-cultures in general correlated with an improved antileukemic activity of DC-stimulated T-cells compared to MNC-stimulation, whereas in serum higher levels of CXCL8, -9 and –10 and lower levels of CCL2 and –5 correlated with an improved antileukemic T-cell activity after DC-stimulation. This might indicate a changing functionality of CCL2 and –5 in the context of DC-stimulation compared to serum from a rather 'inflammatory' or 'tumour-promoting' to a rather 'antitumour'-reactive functionality. This might help to refine immune-modifying approaches which are based on antileukemic (adaptive) immune-responses.
Contribution: Marion Merle carried out the majority of experiments, was responsible for data acquisition, assessment and interpretation including all statistical work and drafted the manuscript together with Prof. Dr. Schmetzer.

4.7.4 Conclusion

In our project we were able to show that an ex-vivo generation of DC/DCleu from MNC of every AML-/MDS-patient is possible with at least one of various methods. Compared to MNC-stimulation our results also indicate a more effective blast-lytic capacity of T-cells following DC-stimulation, although not in every case.
An analysis of the cytokine-release revealed that DC- or MNC-stimulation of T-cells resulted in increased cytokine- (IL-2, -4, -6, -10 and TNF-α, IFN -γ) -levels in MLC compared to serum.

In serum minimal differences in chemokine-levels subdivided into several groups (e.g., age groups, FAB-types, blast proportions, cytogenetic-risk-groups) could be detected.
As higher levels of all studied chemokines (CXCL8, -9, -10, CCL2, -5, IL-12) in DC-culture supernatants but higher values of CXCL8-, -9-, and -10- and lower values of CCL2- and -5 in serum correlated with improved antileukemic activity of T-cells after DC-stimulation (compared to MNC-stimulation) a change of functionality of CCL2 and –5 from a more 'inflammatory' or 'tumour-promoting' to a more 'antitumour'-reactive functionality can be suspected.
Predictive cytokine- and chemokine-cut-off-values for antileukemic T-cell-activity, response to immunotherapy and occurrence of GVHD could be determined. Thus, cytokine- and chemokine-profiles can help to indicate the lytic T-cell-activity, patients' response to immunotherapy and occurrence of GVHD. Without functional assays they are not sufficient to predict an antileukemic T-cell-activity but at least they might contribute to develop immune-modifying strategies that promote antileukemic (adaptive) immune-responses.

5 Summary/Zusammenfassung

5.1 Summary

This thesis includes three publications based on a potential treatment strategy for AML-/MDS-patients which utilizes DC and DCleu. These DC/DCleu are known as potent activators of multiple immunoreactive cells. The special feature of DCleu is, that they are representing the individual patient's whole set of leukemic antigens and can be converted from leukemic blasts. In this trial five standard DC/DCleu-generating methods (MCM-Mimic, Calcium-Ionophore, Picibanil, Poly (I:C), Cytokines), containing different combinations of immune-modifiers (e.g., Interleukin 2 and 4, GM-CSF, TNF-α, Prostaglandin E2, OK-432) were applied. As our group has shown before DCleu can be generated ex-vivo from any patient using at least one of three DC/DCleu-generating methods (MCM-Mimic, Calcium-Ionophore, Picibanil) which have been proven most successful earlier. In a Mixed-lymphocyte-culture (MLC) the generated DC/DCleu or mononuclear cells (MNC) were afterwards incubated as effector-cells with autologous or allogeneic T-cells. In a last step the antileukemic capacity of the (DC- or MNC-) stimulated T-cells was assessed in a cytotoxicity assay (CTX) by analysing the proportions of lysed blasts. Thereby, an improved antileukemic functionality of DC/DCleu-stimulated T-cells in contrast to MNC-stimulation was observed. Nevertheless, there were cases in which DC/DCleu-stimulation of T-cells against leukemic targets was not successful.
In order to explain an antileukemic activity of DC/DCleu and T-cells in those reactions a possible role of soluble factors like cytokines (Publication I) and chemokines (Publication II and III) in serum (Publication I and II) and supernatants of MLC (Publication I) and DC-generating methods (Publication III) was evaluated.
In Publication I concentrations of the cytokines Interleukin-2, -4, -6, -10, tumour-necrosis-factor-α and interferon-γ were analysed in supernatants of MLC and in AML-/MDS-patients' serum by cytometric-bead-array (CBA). Cytokine levels were correlated with the lytic activity of T-cells stimulated by MNC or DC in a CTX and clinical data.

Compared to serum higher cytokine-levels in MLC culture-medium were detected. Despite differences of cytokine-release between cases with and without lytic T-cell-activity, response to immunotherapy or graft-versus-host-disease (GVHD) were not significant, predictive cut-off-values could be defined. Cases with higher detectable amounts of the TH2 cytokines IL-4 and IL-10 showed a lower probability of a response to immunotherapy or occurrence of GVHD. A higher probability of a lytic T-cell activity was observed in cases with a higher release of the inflammatory cytokines TNF-α and IL-6 or TH1-mediating cytokines IL-2 and IFN- γ and a lower release of TH2-mediating cytokines IL-4 and IL-10.

In Publication II levels of the chemokines CCL2, CCL5, CXCL8, CXCL9, CXCL10 and IL-12 were analysed by CBA in patients' serum. Comparing several groups (e.g., age groups, FAB-types, blast proportions, cytogenetic-risk-groups) only minimal differences regarding chemokine release could be detected. Rather favourable subtypes (<50 years of age, <80% blasts in the peripheral blood) tendentially correlated with higher levels of CXCL8, −9, −10 and lower levels of CCL2 and −5. Furthermore, higher CCL5-levels in persisting disease and higher CCL2-levels at relapse compared to first diagnosis might indicate a changing 'disease activity' on a chemokine basis.

We also observed correlations of serum chemokine-levels with response to immunotherapy and occurrence of GVHD in the course of the disease. A higher CXCL8-, -9-, -10- and CCL2-release and a lower CCL5-release correlated with response to immunotherapy. Opposite correlations with an occurrence of GVHD were seen.

From those findings it may be concluded, that higher levels of CXCL8, −9, −10 in AML-/MDS-patients' serum and lower levels of CCL5 and partly of CCL2 correlate with more favourable subtypes and enhanced antitumor-reactive functionality.

Publication III is based on the assessment of chemokine-release in supernatants of five standard DC/DCleu-generating media by CBA. Results were compared to the patients' serum values, antileukemic T-cell-activity following MLC and the clinical course.

Despite wide variations between the patients, correlations of chemokine-release with improved antileukemic T-cell activity after stimulation with DC in an MLC could be observed. A higher release of chemokines in general correlated with improved antileukemic activity of DC-stimulated T-cells (compared to stimulation with blast-containing MNC). In serum however, higher values of CXCL8-, -9-, and -10- and lower values of CCL5 and -2 showed correlations with an improved antileukemic capacity of T-cells stimulated by DC (compared to MNC-stimulation).

Thus, comparing chemokine release in DC-culture supernatants versus serum, this might point to a changing functionality of CCL2 and –5 in a context of 'DC'-stimulation from a rather 'inflammatory' or 'tumour-promoting' to a more 'antitumour'-reactive functionality.

In summary, DC/DCleu are promising targets for immunotherapeutic strategies as a potential treatment of AML or MDS. Ex-vivo trials have proven the potent antileukemic capacity of DC-stimulated T-cells and to refine those strategies is the goal of ongoing trials.

Altogether, by correlating cytokine- or chemokine-levels with ex-vivo functional tests conclusions on the antileukemic functionality of DC-stimulated T-cells or patients' response to immunotherapy and GVHD can be gained. Cytokine- or chemokine-levels alone, however, cannot completely be considered as useful tools, as only few results correlated with clinical features – although CBA-assays are sensitive and reliable tools to specifically detect and quantify soluble cytokines and chemokines.

For a better understanding of the functionality of soluble factors like cytokines and chemokines in antileukemic reactions further measurements during the whole process of MLC and cytotoxicity assay are necessary.
Nevertheless, in the future in the context of an immunotherapy cytokine and chemokine profiling might contribute to anticipate cellular activities of T-cells and DC and the patients' response to the initiated therapy.

5.2 Zusammenfassung

Die Publikationen, die in dieser Dissertation enthalten sind, basieren auf einer potentiellen Behandlungsstrategie für AML-/MDS-Patienten, welche dendritische Zellen (DC) und DC leukämischen Ursprungs (DCleu) verwendet. Diese DC/DCleu sind bekanntermaßen potente Stimulatoren verschiedenster immunreaktiver Zellen. Die Besonderheit von DCleu ist, dass diese das vollständige leukämische Antigenrepertoire jedes einzelnen Patienten präsentieren und dass sie aus leukämischen Blasten umgewandelt werden können. In dieser Versuchsreihe kamen fünf Standard DC/DCleu-Generierungsmethoden (MCM-Mimic, Calcium-Ionophore, Picibanil, Poly (I:C), Cytokines), welche unterschiedliche Kombinationen von immunmodifizierenden Agentien beinhalten (z.B. Interleukin 2 und 4, GM-CSF, TNF-α, Prostaglandin E2, OK-432), zur Anwendung. Wie unsere Arbeitsgruppe zuvor gezeigt hat, können von jedem Patienten DCleu generiert werden unter Verwendung von mindestens einer von drei DC/DCleu-Generierungsmethoden (MCM-Mimic, Calium-Ionophore, Picibanil), welche sich vorab als effektivste Methoden erwiesen haben. In einer gemischten Lymphozytenkultur (MLC) wurden die generierten DC/DCleu oder mononukleären Zellen (MNC) als Effektorzellen im Anschluss mit (autologen und allogenen) T-Zellen inkubiert. In einem letzten Schritt wurde dann die antileukämische Kapazität der stimulierten T-Zellen in einem Zytotoxizitätstest (CTX) durch Analyse des lysierten Blastenanteils evaluiert. Hierbei wurde eine verbesserte antileukämische Funktionalität DC/DCleu-stimulierter T-Zellen im Vergleich zu einer MNC-Stimulation beobachtet. Nichtsdestotrotz, gab es Fälle, in denen eine DC/DCleu-Stimulation von T-Zellen gegen leukämische Blasten nicht erfolgreich war.
Um die antileukämische Kapazität von DC/DCleu und T-Zellen in diesen Reaktionen zu erklären wurde eine mögliche Rolle löslicher Faktoren wie Zytokine (Publikation I) und Chemokine (Publikation II und III) im Serum (Publikation I und II) und Zellkulturüberständen von MLC (Publikation I) und DC-Generierungsmethoden (Publikation III) untersucht.
In der Publikation I wurden Konzentration der Zytokine Interleukin-2, -4, -6, -10, Tumor-Nekrose-Faktor- α und Interferon-γ in Überständen von MLC und Serum von Patienten mit AML/MDS mit Hilfe eines Immunassays zur Detektion von Zytokinen/Chemokinen (Cytometric-bead-array (CBA)) analysiert. Die Zytokinkonzentrationen wurden mit der lytischen Aktivität von MNC- oder DC-stimulierten T-Zellen in einem Zytotoxizitätsversuch (CTX) und klinischen Daten korreliert.
Im Vergleich zum Serum wurden höhere Zytokinwerte im MLC-Kulturmedium gefunden. Obwohl keine signifikanten Unterschiede der Zytokinfreisetzung zwischen Fällen mit und ohne lytischer T-Zellaktivität, Ansprechen auf eine Immuntherapie oder Graft-versus-host-disease (GVHD) gefunden wurden, konnten prädiktive Cut-off Werte definiert werden. Fälle in denen größere Mengen der TH2-Zytokine IL-4 und IL-10 detektiert wurden, zeigten eine geringere Wahrscheinlichkeit für ein Ansprechen auf eine Immuntherapie oder Entwicklung

einer GVHD. Eine höhere Wahrscheinlichkeit für eine lytische T-Zellaktivität wurde in Fällen mit einer höheren Freisetzung der inflammatorischen Zytokine TNF-α und IL-6 oder der TH1-vermittelnden Zytokine IL-2 und IFN-γ und eine geringere Freisetzung der TH2-vermittelnden Zytokine IL-4 und IL-10 beobachtet.
In der Publikation II wurden die Konzentrationen der Chemokine CCL2, CCL5, CXCL8, CXCL9, CXCL10 und IL-12 mittels CBA im Serum von AML-/MDS-Patienten analysiert. Im Vergleich zwischen verschiedenen Gruppen (z.B. Altersgruppen, FAB-Typen, Blasten-Anteil, zytogenetisches Risiko) wurden nur minimale Unterschiede festgestellt, eine höhere Freisetzung von CXCL8, -9, -10 und eine niedrigere Freisetzung von CCL2 und –5 korrelierte tendenziell mit günstigeren Subtypen (<50 Jahre, <80% Blasten im peripheren Blut). Darüber hinaus wurde eine höhere CCL5-Freisetzung bei Krankeitspersistenz und eine signifikant erhöhte CCL2-Freisetzung im Rezidiv im Vergleich zur Erstdiagnose gefunden, was auf eine Veränderung der Krankheitsaktivität auf Chemokin-Niveau hindeuten mag.
Wir konnten auch Korrelationen von Serum-Chemokin-Konzentrationen mit einem Ansprechen auf eine Immuntherapie und das Auftreten einer GVHD beobachten. Höhere Werte für CXCL8, -9, -10 und CCL2 und niedrigere CCL5-Werte korrelierten mit dem Erreichen eines Ansprechens auf eine Immuntherapie. Darüber hinaus korrelierten höhere Werte für CCL2 und –5 und niedrigere Werte für CXCL8, -9 und –10 mit dem Auftreten einer GVHD.
Aus diesen Befunden kann geschlossen werden, dass im Serum von AML-Patienten höhere Konzentrationen von CXCL8, -9, -10 und niedrigere Konzentrationen von CCL5 und teils CCL2 mit günstigeren Subtypen und verbesserter antitumor-reaktiver Funktion korrelieren könnten.
Die Publikation III basiert auf der Analyse der Chemokinfreisetzung in Überständen von fünf Standard DC/DCleu-Generierungsmethoden mittels CBA. Die Ergebnisse wurden mit den Serumkonzentrationen der Patienten, antileukämischen T-Zellreaktionen nach MLC und dem klinischen Verlauf der Patienten verglichen.
Obwohl die Ergebnisse große Unterschiede in den Chemokinkonzentrationen zwischen den Patienten ergaben, konnten Korrelationen mit einer verbesserten antileukämischen T-Zell Aktivität nach Stimulationen von T-Zellen mit DC in einer MLC beobachtet werden. Eine höhere Chemokinfreisetzung korrelierte generell mit einer verbesserten antileukämischen Aktivität von T-Zellen nach DC-Stimulation (im Vergleich zu einer Stimulation mit Blasten-enthaltenden MNC). Im Serum jedoch korrelierten höhere Konzentrationen von CXCL8, -9 und –10 und niedrigere Konzentrationen von CCL2 und –5 mit einer verbesserten antileukämischen Aktivität von DC-stimulierten (im Vergleich zu MNC-stimulierten) T-Zellen. Im Kontext einer DC-Stimulation mag die Chemokinfreisetzung in DC-Kultur-Überständen im Vergleich zum Serum auf eine Veränderung der (CCL-5 und –2 assoziierten) Funktionalität von einer eher “inflammatorischen” oder “tumorunterstützenden” Funktionalität (im Serum) zu einer eher “tumorunterdrückenden” Funktionalität (in DC-Kultur-Überständen) hindeuten.
Zusammenfassend sind DC/DCleu vielversprechende Ziele immuntherapeutischer Strategien zur Behandlung von AML/MDS. Versuche haben ex-vivo die potente antileukämische Kapazität DC-stimulierter T-Zellen nachweisen können und diese Strategien zu verfeinern ist Gegenstand weiterer Studien.
Insgesamt könnten die Analyse von Zytokin- und Chemokinprofilen und die Korrelation mit ex-vivo funktionalen Tests dazu beitragen, zumal sie Hinweise auf die antileukämische lytische T-Zell-Aktivität nach DC-Stimulation, ein Ansprechen der Patienten auf eine Immuntherapie und Entwicklung einer GVHD liefern können.

Zytokin- oder Chemokinwerte alleine jedoch, können nicht als sinnvolles Instrument erachtet werden, zumal nur wenige Ergebnisse mit klinischen Merkmalen korrelierten – dennoch sind CBA-Messungen ein sensitives und verlässliches Werkzeug um lösliche Zytokine und Chemokine nachzuweisen und zu quantifizieren. Für ein besseres Verständnis der Funktionalität löslicher Faktoren wie Zytokine und Chemokine in antileukämischen Reaktionen, sind weitere Messungen während dem ganzen Verlauf von MLC und Zytotoxizitätsversuch notwendig. Nichtsdestotrotz könnte die Analyse von Zytokin- und Chemokinprofilen in einem Kontext einer Immuntherapie zukünftig dazu beitragen die zellulären Aktivitäten von T-Zellen und DC und die Antwort der Patienten auf die begonnene Therapie vorherzusagen.

6 References

1. **Lowenberg B**, Downing JR, Burnett A., Acute myeloid leukemia. N Engl J Med 1999, 341, (14), 1051-62.
2. **Giles FJ**, Keating A, Goldstone AH, Avivi I, Willman CL, Kantarjian HM. Acute myeloid leukemia. .Hematology Am Soc Hematol Educ Program. 2002:73-110. doi: 10.1182/asheducation-2002.1.73.PMID: 12446420
3. **Wandt H**, Schäkel U, Kroschinsky F, Prange-Krex G, Mohr B, Thiede C, Pascheberg U, Soucek S, Schaich M, Ehninger G. MLD according to the WHO classification in AML has no correlation with age and no independent prognostic relevance as analyzed in 1766 patients. Blood. 2008 Feb 15;111(4):1855-61. doi: 10.1182/blood-2007-08-101162. Epub 2007 Dec 4. PMID: 18056840.
4. **Döhner H**, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017; 129:424–47. doi:10.1182/blood-2016-08-733196.
5. **Haferlach T**, Kohlmann A, Kern W, Hiddemann W, Schnittger S, Schoch C. Gene expression profiling as a tool for the diagnosis of acute leukemias. Semin Hematol. 2003 Oct;40(4):281-95. doi: 10.1016/s0037-1963(03)00193-8. PMID: 14582079.
6. **Short NJ**, Zhou S, Fu C, Berry DA, Walter RB, Freeman SD, Hourigan CS, Huang X, Nogueras Gonzalez G, Hwang H, Qi X, Kantarjian H, Ravandi F. Association of Measurable Residual Disease With Survival Outcomes in Patients With Acute Myeloid Leukemia: A Systematic Review and Meta-analysis. JAMA Oncol. 2020 Dec 1;6(12):1890-1899. doi: 10.1001/jamaoncol.2020.4600. PMID: 33030517; PMCID: PMC7545346.
7. **Döhner H**, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D, Larson RA, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz MA, Sierra J, Tallman MS, Löwenberg B, Bloomfield CD; European LeukemiaNet. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010 Jan 21;115(3):453-74. doi: 10.1182/blood-2009-07-235358. Epub 2009 Oct 30. PMID: 19880497.
8. **Giralt S**, Ballen K, Rizzo D, Bacigalupo A, Horowitz M, Pasquini M, Sandmaier B. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. Biol Blood Marrow Transplant. 2009 Mar;15(3):367-9. doi: 10.1016/j.bbmt.2008.12.497. PMID: 19203728; PMCID: PMC3110087.
9. **Vyas P**, Appelbaum FR, Craddock C. Allogeneic hematopoietic cell transplantation for acute myeloid leukemia. Biol Blood Marrow Transplant. 2015 Jan;21(1):8-15. doi: 10.1016/j.bbmt.2014.10.026. Epub 2014 Nov 13. PMID: 25452033.
10. **Sweeney C**, Vyas P. The Graft-Versus-Leukemia Effect in AML. Front Oncol. 2019 Nov 19; 9:1217. doi: 10.3389/fonc.2019.01217. PMID: 31803612; PMCID: PMC6877747.

11. **Kolb HJ**. Graft-versus-leukemia effects of transplantation and donor lymphocytes. Blood. 2008 Dec 1;112(12):4371-83. doi: 10.1182/blood-2008-03-077974. PMID: 19029455.
12. **Jedlickova Z**, Schmid C, Koenecke C, Hertenstein B, Baurmann H, Schwerdtfeger R, Tischer J, Kolb HJ, Schleuning M. Long-term results of adjuvant donor lymphocyte transfusion in AML after allogeneic stem cell transplantation. Bone Marrow Transplant. 2016 May;51(5):663-7. doi: 10.1038/bmt.2015.234. Epub 2015 Oct 5. PMID: 26437060.
13. **Schmid C**, Labopin M, Schaap N, Veelken H, Schleuning M, Stadler M, Finke J, Hurst E, Baron F, Ringden O, Bug G, Blaise D, Tischer J, Bloor A, Esteve J, Giebel S, Savani B, Gorin NC, Ciceri F, Mohty M, Nagler A; EBMT Acute Leukaemia Working Party. Prophylactic donor lymphocyte infusion after allogeneic stem cell transplantation in acute leukaemia - a matched pair analysis by the Acute Leukaemia Working Party of EBMT. Br J Haematol. 2019 Mar;184(5):782-787. doi: 10.1111/bjh.15691. Epub 2018 Nov 22. PMID: 30467839.
14. **Ghimire S**, Weber D, Mavin E, Wang XN, Dickinson AM, Holler E. Pathophysiology of GvHD and Other HSCT-Related Major Complications. Front Immunol. 2017 Mar 20; 8:79. doi: 10.3389/fimmu.2017.00079. PMID: 28373870; PMCID: PMC5357769.
15. **Wei AH**, Döhner H, Pocock C, Montesinos P, Afanasyev B, Dombret H, Ravandi F, Sayar H, Jang JH, Porkka K, Selleslag D, Sandhu I, Turgut M, Giai V, Ofran Y, Kizil Çakar M, Botelho de Sousa A, Rybka J, Frairia C, Borin L, Beltrami G, Čermák J, Ossenkoppele GJ, La Torre I, Skikne B, Kumar K, Dong Q, Beach CL, Roboz GJ; QUAZAR AML-001 Trial Investigators. Oral Azacitidine Maintenance Therapy for Acute Myeloid Leukemia in First Remission. N Engl J Med. 2020 Dec 24;383(26):2526-2537. doi: 10.1056/NEJMoa2004444. PMID: 33369355.
16. **Roboz GJ**, Montesinos P, Selleslag D, Wei A, Jang JH, Falantes J, Voso MT, Sayar H, Porkka K, Marlton P, Almeida A, Mohan S, Ravandi F, Garcia-Manero G, Skikne B, Kantarjian H. Design of the randomized, Phase III, QUAZAR AML Maintenance trial of CC-486 (oral azacitidine) maintenance therapy in acute myeloid leukemia. Future Oncol. 2016 Feb;12(3):293-302. doi: 10.2217/fon.15.326. Epub 2016 Jan 19. PMID: 26785287; PMCID: PMC5684733.
17. **Burnett AK**. Treatment of Older Patients With Newly Diagnosed AML Unfit for Traditional Therapy. Clin Lymphoma Myeloma Leuk. 2018 Sep;18(9):553-557. doi: 10.1016/j.clml.2018.06.027. Epub 2018 Jun 30. PMID: 30007570.
18. **Estey EH**. Acute myeloid leukemia: 2019 update on risk-stratification and management. Am J Hematol. 2018; 93:1267–91. doi:10.1002/ajh.25214.
19. **Burnett A**, Wetzler M, Löwenberg B. Therapeutic advances in acute myeloid leukemia. J Clin Oncol. 2011 Feb 10;29(5):487-94. doi: 10.1200/JCO.2010.30.1820. Epub 2011 Jan 10. Erratum in: J Clin Oncol. 2011 Jun 1;29(16):2293. PMID: 21220605.
20. **Tallman MS**, Gilliland DG, Rowe JM. Drug therapy for acute myeloid leukemia. Blood. 2005 Aug 15;106(4):1154-63. doi: 10.1182/blood-2005-01-0178. Epub 2005 May 3. Erratum in: Blood. 2005 Oct 1;106(7):2243. PMID: 15870183.
21. **Almeida AM**, Ramos F. Acute myeloid leukemia in the older adults. Leuk Res Rep. 2016 Jun 16; 6:1-7. doi: 10.1016/j.lrr.2016.06.001. PMID: 27408788; PMCID: PMC4927655.

22. **Rashidi A**, Weisdorf DJ, Bejanyan N. Treatment of relapsed/refractory acute myeloid leukaemia in adults. Br J Haematol. 2018 Apr;181(1):27-37. doi: 10.1111/bjh.15077. Epub 2018 Jan 9. PMID: 29318584.
23. **Winer ES**, Stone RM. Novel therapy in Acute myeloid leukemia (AML): moving toward targeted approaches. Ther Adv Hematol. 2019 Jul 10; 10:2040620719860645. doi: 10.1177/2040620719860645. PMID: 31321011; PMCID: PMC6624910.
24. **Kim ES**. Midostaurin: First Global Approval. Drugs. 2017 Jul;77(11):1251-1259. doi: 10.1007/s40265-017-0779-0. PMID: 28612232.
25. **Kim ES**. Enasidenib: First Global Approval. Drugs. 2017 Oct;77(15):1705-1711. doi: 10.1007/s40265-017-0813-2. PMID: 28879540.
26. **Dhillon S**. Ivosidenib: First Global Approval. Drugs. 2018 Sep;78(14):1509-1516. doi: 10.1007/s40265-018-0978-3. Erratum in: Drugs. 2019 Jan;79(1):107. PMID: 30209701; PMCID: PMC6315051.
27. **Dhillon S**. Gilteritinib: First Global Approval. Drugs. 2019 Feb;79(3):331-339. doi: 10.1007/s40265-019-1062-3. PMID: 30721452.
28. **DiNardo CD**, Pratz K, Pullarkat V, Jonas BA, Arellano M, Becker PS, Frankfurt O, Konopleva M, Wei AH, Kantarjian HM, Xu T, Hong WJ, Chyla B, Potluri J, Pollyea DA, Letai A. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood. 2019 Jan 3;133(1):7-17. doi: 10.1182/blood-2018-08-868752. Epub 2018 Oct 25. PMID: 30361262; PMCID: PMC6318429.
29. **Hoy SM**. Glasdegib: First Global Approval. Drugs. 2019 Feb;79(2):207-213. doi: 10.1007/s40265-018-1047-7. PMID: 30666593.
30. **Williams BA**, Law A, Hunyadkurti J, Desilets S, Leyton JV, Keating A. Antibody Therapies for Acute Myeloid Leukemia: Unconjugated, Toxin-Conjugated, Radio-Conjugated and Multivalent Formats. J Clin Med. 2019 Aug 20;8(8):1261. doi: 10.3390/jcm8081261. PMID: 31434267; PMCID: PMC6723634.
31. **Lichtenegger FS**, Krupka C, Haubner S, Köhnke T, Subklewe M. Recent developments in immunotherapy of acute myeloid leukemia. J Hematol Oncol. 2017; 10:142. doi:10.1186/s13045-017-0505-0
32. **Valent P**, Sadovnik I, Eisenwort G, Bauer K, Herrmann H, Gleixner KV, Schulenburg A, Rabitsch W, Sperr WR, Wolf D. Immunotherapy-Based Targeting and Elimination of Leukemic Stem Cells in AML and CML. Int J Mol Sci. 2019 Aug 29;20(17):4233. doi: 10.3390/ijms20174233. PMID: 31470642; PMCID: PMC6747233.
33. **Sallman DA**, Lancet JE. What are the most promising new agents in acute myeloid leukemia? Curr Opin Hematol. 2017 Mar;24(2):99-107. doi: 10.1097/MOH.0000000000000319. PMID: 28030373.
34. **Jen EY**, Ko CW, Lee JE, Del Valle PL, Aydanian A, Jewell C, Norsworthy KJ, Przepiorka D, Nie L, Liu J, Sheth CM, Shapiro M, Farrell AT, Pazdur R. FDA Approval: Gemtuzumab Ozogamicin for the Treatment of Adults with Newly Diagnosed CD33-Positive Acute Myeloid Leukemia. Clin Cancer Res. 2018 Jul 15;24(14):3242-3246. doi: 10.1158/1078-0432.CCR-17-3179. Epub 2018 Feb 23. PMID: 29476018.
35. **Guy DG**, Uy GL. Bispecific Antibodies for the Treatment of Acute Myeloid Leukemia. Curr Hematol Malig Rep. 2018 Dec;13(6):417-425. doi: 10.1007/s11899-018-0472-8. PMID: 30280288; PMCID: PMC6295344.

36. **Krupka C**, Kufer P, Kischel R, Zugmaier G, Lichtenegger FS, Köhnke T, Vick B, Jeremias I, Metzeler KH, Altmann T, Schneider S, Fiegl M, Spiekermann K, Bauerle PA, Hiddemann W, Riethmüller G, Subklewe M. Blockade of the PD-1/PD-L1 axis augments lysis of AML cells by the CD33/CD3 BiTE antibody construct AMG 330: reversing a T-cell-induced immune escape mechanism. Leukemia. 2016 Feb;30(2):484-91. doi: 10.1038/leu.2015.214. Epub 2015 Aug 4. PMID: 26239198.
37. **Al-Hussaini M**, Rettig MP, Ritchey JK, Karpova D, Uy GL, Eissenberg LG, Gao F, Eades WC, Bonvini E, Chichili GR, Moore PA, Johnson S, Collins L, DiPersio JF. Targeting CD123 in acute myeloid leukemia using a T-cell-directed dual-affinity retargeting platform. Blood. 2016 Jan 7;127(1):122-31. doi: 10.1182/blood-2014-05-575704. Epub 2015 Nov 3. PMID: 26531164; PMCID: PMC4705603.
38. **Krupka C**, Kufer P, Kischel R, Zugmaier G, Bögeholz J, Köhnke T, Lichtenegger FS, Schneider S, Metzeler KH, Fiegl M, Spiekermann K, Baeuerle PA, Hiddemann W, Riethmüller G, Subklewe M. CD33 target validation and sustained depletion of AML blasts in long-term cultures by the bispecific T-cell-engaging antibody AMG 330. Blood. 2014 Jan 16;123(3):356-65. doi: 10.1182/blood-2013-08-523548. Epub 2013 Dec 3. PMID: 24300852.
39. **Bodet-Milin C**, Kraeber-Bodéré F, Eugène T, Guérard F, Gaschet J, Bailly C, Mougin M, Bourgeois M, Faivre-Chauvet A, Chérel M, Chevallier P. Radioimmunotherapy for Treatment of Acute Leukemia. Semin Nucl Med. 2016 Mar;46(2):135-46. doi: 10.1053/j.semnuclmed.2015.10.007. PMID: 26897718.
40. **Vago L**, Gojo I. Immune escape and immunotherapy of acute myeloid leukemia. J Clin Invest. 2020 Apr 1;130(4):1552-1564. doi: 10.1172/JCI129204. PMID: 32235097; PMCID: PMC7108895.
41. **Giannopoulos K**. Targeting Immune Signaling Checkpoints in Acute Myeloid Leukemia. J Clin Med. 2019 Feb 12;8(2):236. doi: 10.3390/jcm8020236. PMID: 30759726; PMCID: PMC6406869.
42. **Short NJ**, Konopleva M, Kadia TM, Borthakur G, Ravandi F, DiNardo CD, Daver N. Advances in the Treatment of Acute Myeloid Leukemia: New Drugs and New Challenges. Cancer Discov. 2020 Apr;10(4):506-525. doi: 10.1158/2159-8290.CD-19-1011. Epub 2020 Feb 3. PMID: 32014868.
43. **Hofmann S**, Schubert ML, Wang L, He B, Neuber B, Dreger P, Müller-Tidow C, Schmitt M. Chimeric Antigen Receptor (CAR) T Cell Therapy in Acute Myeloid Leukemia (AML). J Clin Med. 2019 Feb 6;8(2):200. doi: 10.3390/jcm8020200. PMID: 30736352; PMCID: PMC6406805.
44. **He X**, Feng Z, Ma J, Ling S, Cao Y, Gurung B, et al. Bispecific and split CAR T cells targeting CD13 and TIM3 eradicate acute myeloid leukemia. Blood. 2020; 135:713–23. doi.org/10.1182/blood.2019002779.
45. **Liu F**, Cao Y, Pinz K, Ma Y, Wada M, Chen K, et al. First-in-human CLL1-CD33 compound CAR T cell therapy induces complete remission in patients with refractory acute myeloid leukemia: update on phase 1 clinical trial. Blood. 2018;132 Supplement 1:901. doi.org/10.1182/blood-2018-99-110579.
46. **Handgretinger R**, Schilbach K. The potential role of γδ T cells after allogeneic HCT for leukemia. Blood. 2018 Mar 8;131(10):1063-1072. doi: 10.1182/blood-2017-08-752162. Epub 2018 Jan 22. PMID: 29358176.

47. **Romee R**, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Schappe T, Leong JW, Abdel-Latif S, Schneider SE, Willey S, Neal CC, Yu L, Oh ST, Lee YS, Mulder A, Claas F, Cooper MA, Fehniger TA. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. Sci Transl Med. 2016 Sep 21;8(357):357ra123. doi: 10.1126/scitranslmed. aaf2341. PMID: 27655849; PMCID: PMC5436500.
48. **Björklund AT**, Clancy T, Goodridge JP, Béziat V, Schaffer M, Hovig E, Ljunggren HG, Ljungman PT, Malmberg KJ. Naive Donor NK Cell Repertoires Associated with Less Leukemia Relapse after Allogeneic Hematopoietic Stem Cell Transplantation. J Immunol. 2016 Feb 1;196(3):1400-11. doi: 10.4049/jimmunol.1501434. Epub 2016 Jan 8. PMID: 26746188.
49. **Guo Y**, Han W. Cytokine-induced killer (CIK) cells: from basic research to clinical translation. Chin J Cancer. 2015 Mar 5;34(3):99-107. doi: 10.1186/s40880-015-0002-1. PMID: 25962508; PMCID: PMC4593361.
50. **Przespolewski A**, Szeles A, Wang ES. Advances in immunotherapy for acute myeloid leukemia. Future Oncol. 2018; 14:963–78. doi:10.2217/fon-2017-0459.
51. **Romero AI**, Thorén FB, Aurelius J, Askarieh G, Brune M, Hellstrand K. Post-consolidation immunotherapy with histamine dihydrochloride and interleukin-2 in AML. Scand J Immunol. 2009 Sep;70(3):194-205. doi: 10.1111/j.1365-3083.2009.02303.x. PMID: 19703009.
52. **Cummins KD**, Gill S. Anti-CD123 chimeric antigen receptor T-cells (CART): an evolving treatment strategy for hematological malignancies, and a potential ace-in-the-hole against antigen-negative relapse. Leuk Lymphoma. 2018 Jul;59(7):1539-1553. doi: 10.1080/10428194.2017.1375107. Epub 2017 Sep 13. PMID: 28901790.
53. **Claxton D**, Kao SF. Autologous immunotherapy for human leukemias. Blood Cells Mol Dis. 2003 Jul-Aug;31(1):121-4. doi: 10.1016/s1079-9796(03)00120-7. PMID: 12850495.
54. **Roddie H**, Klammer M, Thomas C, Thomson R, Atkinson A, Sproul A, Waterfall M, Samuel K, Yin J, Johnson P, Turner M. Phase I/II study of vaccination with dendritic-like leukaemia cells for the immunotherapy of acute myeloid leukaemia. Br J Haematol. 2006 Apr;133(2):152-7. doi: 10.1111/j.1365-2141.2006.05997.x. PMID: 16611305.
55. **Amberger DC**, Schmetzer HM. Dendritic Cells of Leukemic Origin: Specialized Antigen-Presenting Cells as Potential Treatment Tools for Patients with Myeloid Leukemia. Transfus Med Hemother. 2020 Dec;47(6):432-443. doi: 10.1159/000512452. Epub 2020 Nov 5. PMID: 33442338; PMCID: PMC7768130.
56. **Brossart P**. Dendritic cells in vaccination therapies of malignant diseases. Transfus Apher Sci. 2002 Oct;27(2):183-6. doi: 10.1016/s1473-0502(02)00041-1. Erratum in: Transfus Apheresis Sci. 2003 Feb;28(1):103. Brossart M D, Peter [corrected to Brossart, Peter]. PMID: 12350054.
57. **Galea-Lauri J**, Darling D, Mufti G, Harrison P, Farzaneh F. Eliciting cytotoxic T lymphocytes against acute myeloid leukemia-derived antigens: evaluation of dendritic cell-leukemia cell hybrids and other antigen-loading strategies for dendritic cell-based vaccination. Cancer Immunol Immunother. 2002 Aug;51(6):299-310. doi: 10.1007/s00262-002-0284-4. Epub 2002 Jun 14. PMID: 12111118.

58. **Schmetzer HM**, Schmid C. The T-Cells' Role in Antileukemic Reactions - Perspectives for Future Therapies. In: Visentainer J, Sell A, editors. Importance of Non-HLA Gene Polymorphisms in Hematopoietic Stem Cell Transplantation: INTECH Open Access Publisher; 2012. p. 59–82. doi:10.5772/25891.
59. **Van Acker HH**, Versteven M, Lichtenegger FS, Roex G, Campillo-Davo D, Lion E, Subklewe M, Van Tendeloo VF, Berneman ZN, Anguille S. Dendritic Cell-Based Immunotherapy of Acute Myeloid Leukemia. J Clin Med. 2019 Apr 27;8(5):579. doi: 10.3390/jcm8050579. PMID: 31035598; PMCID: PMC6572115.
60. **Schmetzer HM,** Kremser A, Loibl J, Kroell T, Kolb HJ. Quantification of ex vivo generated dendritic cells (DC) and leukemia-derived DC contributes to estimate the quality of DC, to detect optimal DC-generating methods or to optimize DCmediated T-cell-activation-procedures ex vivo or in vivo. Leukemia 2007, 21, (6), 1338-41.
61. **Choudhury BA**, Liang JC, Thomas EK, Flores-Romo L, Xie QS, Agusala K, Sutaria S, Sinha I, Champlin RE, Claxton DF. Dendritic cells derived in vitro from acute myelogenous leukemia cells stimulate autologous, antileukemic T-cell responses. Blood. 1999 Feb 1;93(3):780-6. PMID: 9920826.
62. **Stripecke R**, Levine AM, Pullarkat V, Cardoso AA. Immunotherapy with acute leukemia cells modified into antigen-presenting cells: ex vivo culture and gene transfer methods. Leukemia. 2002 Oct;16(10):1974-83. doi: 10.1038/sj.leu.2402701. PMID: 12357348.
63. **Kremser A**, Dressig J, Grabrucker C, Liepert A, Kroell T, Scholl N, et al. Dendritic cells (DCs) can be successfully generated from leukemic blasts in individual patients with AML or MDS: An evaluation of different methods. J Immunother. 2010; 33:185–99. doi:10.1097/CJI.0b013e3181b8f4ce.
64. **Dreyssig J**, Kremser A, Liepert A, Grabrucker C, Freudenreich M, Schmid C, Kroell T, Scholl N, Tischer J, Kufner S, Salih H, Kolb HJ, Schmetzer HM. Various 'dendritic cell antigens' are already expressed on uncultured blasts in acute myeloid leukemia and myelodysplastic syndromes. Immunotherapy. 2011 Sep;3(9):1113-24. doi: 10.2217/imt.11.108. PMID: 21913833.
65. **Franciszkiewicz K**, Boissonnas A, Boutet M, Combadière C, Mami-Chouaib F. Role of chemokines and chemokine receptors in shaping the effector phase of the antitumor immune response. Cancer Res. 2012 Dec 15;72(24):6325-32. doi: 10.1158/0008-5472.CAN-12-2027. Epub 2012 Dec 7. PMID: 23222302.
66. **Borish LC**, Steinke JW. 2. Cytokines and chemokines. J Allergy Clin Immunol. 2003 Feb;111(2 Suppl):S460-75. doi: 10.1067/mai.2003.108. PMID: 12592293.
67. **Dinarello CA.** Historical insights into cytokines. Eur J Immunol. 2007 Nov;37 Suppl 1(Suppl 1):S34-45. doi: 10.1002/eji.200737772. PMID: 17972343; PMCID: PMC3140102.
68. **Palomino DC**, Marti LC. Chemokines and immunity. Einstein (Sao Paulo). 2015 Jul-Sep;13(3):469-73. doi: 10.1590/S1679-45082015RB3438. PMID: 26466066; PMCID: PMC4943798.
69. **Hughes CE**, Nibbs RJB. A guide to chemokines and their receptors. FEBS J. 2018 Aug;285(16):2944-2971. doi: 10.1111/febs.14466. Epub 2018 Apr 24. PMID: 29637711; PMCID: PMC6120486.
70. **Piqueras B**, Connolly J, Freitas H, Palucka AK, Banchereau J. Upon viral exposure, myeloid and plasmacytoid dendritic cells produce 3 waves of distinct chemokines to

recruit immune effectors. Blood. 2006 Apr 1;107(7):2613-8. doi: 10.1182/blood-2005-07-2965. Epub 2005 Nov 29. PMID: 16317096; PMCID: PMC1895384.

71. Levina V, Su Y, Nolen B, Liu X, Gordin Y, Lee M, Lokshin A, Gorelik E. Chemotherapeutic drugs and human tumor cells cytokine network. Int J Cancer. 2008 Nov 1;123(9):2031-40. doi: 10.1002/ijc.23732. PMID: 18697197; PMCID: PMC2574811.

72. Padovan E, Spagnoli GC, Ferrantini M, Heberer M. IFN-alpha2a induces IP-10/CXCL10 and MIG/CXCL9 production in monocyte-derived dendritic cells and enhances their capacity to attract and stimulate CD8+ effector T cells. J Leukoc Biol. 2002 Apr;71(4):669-76. PMID: 11927654.

73. Kitko CL, Levine JE, Storer BE, Chai X, Fox DA, Braun TM, Couriel DR, Martin PJ, Flowers ME, Hansen JA, Chang L, Conlon M, Fiema BJ, Morgan R, Pongtornpipat P, Lamiman K, Ferrara JL, Lee SJ, Paczesny S. Plasma CXCL9 elevations correlate with chronic GVHD diagnosis. Blood. 2014 Jan 30;123(5):786-93. doi: 10.1182/blood-2013-08-520072. Epub 2013 Dec 20. PMID: 24363401; PMCID: PMC3907763.

74. **Wong JL**, Berk E, Edwards RP, Kalinski P. 2013. IL-18-primed helper NK cells collaborate with dendritic cells to promote recruitment of effector CD8+ T cells to the tumor microenvironment. Cancer Res. 73(15):4653–62.

75. **Liu M**, Guo S, Stiles JK. The emerging role of CXCL10 in cancer (Review). Oncol Lett. 2011 Jul;2(4):583-589. doi: 10.3892/ol.2011.300. Epub 2011 May 9. PMID: 22848232; PMCID: PMC3406435.

76. **Pardanani A**, Tefferi A. 2012. Cytogenetic risk stratification in myelodysplastic syndromes: are we there yet? J Clin Oncol. 30(21):2703–04.

77. **Carvalheiro T**, Horta S, JAG VR, Santiago M, Salvador MJ, Trindade H, Radstake TRDJ, JAP DS, Paiva A. 2018. Increased frequencies of circulating CXCL10-, CXCL8- and CCL4-producing monocytes and Siglec-3-expressing myeloid dendritic cells in systemic sclerosis patients. Inflammation Res. 67:169–77.

78. **Schulz EG**, Mariani L, Radbruch A, Höfer T. Sequential polarization and imprinting of type 1 T helper lymphocytes by interferon-gamma and interleukin-12. Immunity. 2009 May;30(5):673-83. doi: 10.1016/j.immuni.2009.03.013. Epub 2009 Apr 30. PMID: 19409816.

79. **Rollins BJ**. Inflammatory chemokines in cancer growth and progression. Eur J Cancer. 2006 Apr;42(6):760-7. doi: 10.1016/j.ejca.2006.01.002. Epub 2006 Feb 28. PMID: 16510278.

80. **Zhu YM**, Woll PJ. Mitogenic effects of interleukin-8/CXCL8 on cancer cells. Future Oncol. 2005 Oct;1(5):699-704. doi: 10.2217/14796694.1.5.699. PMID: 16556047.

81. **Kornblau SM**, McCue D, Singh N, Chen W, Estrov Z, Coombes KR. 2010. Recurrent expression signatures of cytokines and chemokines are present and are independently prognostic in acute myelogenous leukemia and myelodysplasia. Blood. 116(20):4251–61.

82. **Alfaro C**, Teijeira A, Oñate C, Pérez G, Sanmamed MF, Andueza MP, Alignani D, Labiano S, Azpilikueta A, Rodriguez-Paulete A, et al. 2016. Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of Neutrophil Extracellular Traps (NETs). Clinical Cancer Res. 22(15):3924–36.

83. **Aldinucci D**, Colombatti A. 2014. The inflammatory chemokine CCL5 and cancer progression. Mediators Inflamm. 2014:292376.

84. **Tan MC**, Goedegebuure PS, Belt BA, Flaherty B, Sankpal N, Gillanders WE, Eberlein TJ, Hsieh CS, Linehan DC. 2009. Disruption of CCR5-dependent homing of regulatory T cells inhibits tumor growth in a murine model of pancreatic cancer. J Immunol. 182(3):1746–55.
85. **Chang LY**, Lin YC, Mahalingam J, Huang CT, Chen TW, Kang CW, Peng HM, Chu YY, Chiang JM, Dutta A, et al. 2012. Tumor-derived chemokine CCL5 enhances TGF-β-mediated killing of CD8(+) T cells in colon cancer by T-regulatory cells. Cancer Res. 72 (5):1092–102.
86. **Yoshimura T**, Liu M, Chen X, Li L, Wang JM. Crosstalk between Tumor Cells and Macrophages in Stroma Renders Tumor Cells as the Primary Source of MCP-1/CCL2 in Lewis Lung Carcinoma. Front Immunol. 2015 Jun 26; 6:332. doi: 10.3389/fimmu.2015.00332. PMID: 26167165; PMCID: PMC4481164.
87. **Yoshimura T,** 2018. The chemokine MCP-1 (CCL2) in the host interaction with cancer: a foe or ally? Cell Mol Immunol. 15(4):335–45.
88. **Salcedo R**, Ponce ML, Young HA, Wasserman K, Ward JM, Kleinman HK, Oppenheim JJ, Murphy WJ. Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. Blood. 2000 Jul 1;96(1):34-40. PMID: 10891427.
89. **Ferreira AM**, Rollins BJ, Faunce DE, Burns AL, Zhu X, Dipietro LA. The effect of MCP-1 depletion on chemokine and chemokine-related gene expression: evidence for a complex network in acute inflammation. Cytokine. 2005 Apr 21;30(2):64-71. doi: 10.1016/j.cyto.2004.12.006. PMID: 15804597.
90. **King C**, Mueller Hoenger R, Malo Cleary M, Murali-Krishna K, Ahmed R, King E, Sarvetnick N. Interleukin-4 acts at the locus of the antigen-presenting dendritic cell to counter-regulate cytotoxic CD8+ T-cell responses. Nat Med. 2001 Feb;7(2):206-14. doi: 10.1038/84659. PMID: 11175852.
91. **Kishimoto T**. IL-6: from its discovery to clinical applications. Int Immunol. 2010 May;22(5):347-52. doi: 10.1093/intimm/dxq030. Epub 2010 Apr 21. PMID: 20410258.
92. **Mazzolini G**, Atorrasagasti C, Rizzo M, Murillo O, Dubrot J, Tirapu J, Arina A, Alfaro C, Azpilicueta A, Berasain C, et al. Immunotherapy and immunoescape in colorectal cancer. World J Gastroenterol. 2007. 13:5822–5831.
93. **Yang YG**, Wang H, Asavaroengchai W, Dey BR. Role of interferon-gamma in GVHD and GVL. Cell Mol Immunol. 2005. 2:323–329.
94. **van Horssen R**, Ten Hagen TL, Eggermont AM. TNF-alpha in cancer treatment: molecular insights, antitumor effects, and clinical utility. Oncologist. 2006 Apr;11(4):397-408. doi: 10.1634/theoncologist.11-4-397. PMID: 16614236.
95. **Horiuchi T**, Mitoma H, Harashima S, Tsukamoto H, Shimoda T. Transmembrane TNF-alpha: structure, function and interaction with anti-TNF agents. Rheumatology (Oxford). 2010 Jul;49(7):1215-28. doi: 10.1093/rheumatology/keq031. Epub 2010 Mar 1. PMID: 20194223; PMCID: PMC2886310.
96. **Lee AW**, Truong T, Bickham K, Fonteneau JF, Larsson M, Da Silva I, Somersan S, Thomas EK, Bhardwaj N. A clinical grade cocktail of cytokines and PGE2 results in uniform maturation of human monocyte-derived dendritic cells: implications for immunotherapy. Vaccine. 2002 Dec 19;20 Suppl 4:A8-A22. doi: 10.1016/s0264-410x(02)00382-1. PMID: 12477423.

97. **Kremser A**, Dressig J, Grabrucker C, Liepert A, Kroell T, Scholl N, Schmid C, Tischer J, Kufner S, Salih H, Kolb HJ, Schmetzer H. Dendritic cells (DCs) can be successfully generated from leukemic blasts in individual patients with AML or MDS: an evaluation of different methods. J Immunother. 2010 Feb-Mar;33(2):185-99. doi: 10.1097/CJI.0b013e3181b8f4ce. PMID: 20139775.
98. **Houtenbos I**, Westers TM, Stam AG, de Gruijl TD, Scheper RJ, Ossenkoppele GJ, van de Loosdrecht AA. Serum-free generation of antigen presenting cells from acute myeloid leukaemic blasts for active specific immunisation. Cancer Immunol Immunother. 2003 Jul;52(7):455-62. doi: 10.1007/s00262-003-0389-4. Epub 2003 Apr 11. PMID: 12690520.
99. **Sato M**, Takayama T, Tanaka H, Konishi J, Suzuki T, Kaiga T, Tahara H. Generation of mature dendritic cells fully capable of T helper type 1 polarization using OK-432 combined with prostaglandin E(2). Cancer Sci. 2003 Dec;94(12):1091-8. doi: 10.1111/j.1349-7006.2003.tb01405.x. PMID: 14662025.
100. **Westers TM**, Stam AG, Scheper RJ, Regelink JC, Nieuwint AW, Schuurhuis GJ, van de Loosdrecht AA, Ossenkoppele GJ. Rapid generation of antigen-presenting cells from leukaemic blasts in acute myeloid leukaemia. Cancer Immunol Immunother. 2003 Jan;52(1):17-27. doi: 10.1007/s00262-002-0316-0. Epub 2002 Dec 7. Erratum in: Cancer Immunol Immunother. 2003 Aug;52(8):523. PMID: 12536236.
101. **Rouas R**, Lewalle P, El Ouriaghli F, Nowak B, Duvillier H, Martiat P. Poly(I:C) used for human dendritic cell maturation preserves their ability to secondarily secrete bioactive IL-12. Int Immunol. 2004 May;16(5):767-73. doi: 10.1093/intimm/dxh077. Epub 2004 Mar 29. PMID: 15096480.
102. **Grabrucker C**, Liepert A, Dreyig J, Kremser A, Kroell T, Freudenreich M, Schmid C, Schweiger C, Tischer J, Kolb HJ, Schmetzer H. The quality and quantity of leukemia-derived dendritic cells from patients with acute myeloid leukemia and myelodysplastic syndrome are a predictive factor for the lytic potential of dendritic cells-primed leukemia-specific T cells. J Immunother. 2010 Jun;33(5):523-37. doi: 10.1097/CJI.0b013e3181d87ffd. PMID: 20463595.
103. **Ruffini PA**. The CXCL8-CXCR1/2 Axis as a Therapeutic Target in Breast Cancer Stem-Like Cells. Front Oncol. 2019 Feb 6; 9:40. doi: 10.3389/fonc.2019.00040. PMID: 30788286; PMCID: PMC6373429.
104. **van Attekum MH**, Eldering E, Kater AP. Chronic lymphocytic leukemia cells are active participants in microenvironmental cross-talk. Haematologica. 2017 Sep;102(9):1469-1476. doi: 10.3324/haematol.2016.142679. Epub 2017 Aug 3. PMID: 28775118; PMCID: PMC5685246.
105. **Aldinucci D**, Colombatti A. The inflammatory chemokine CCL5 and cancer progression. Mediators Inflamm. 2014; 2014:292376. doi: 10.1155/2014/292376. Epub 2014 Jan 2. PMID: 24523569; PMCID: PMC3910068.
106. **Naschberger E**, Croner RS, Merkel S, Dimmler A, Tripal P, Amann KU, Kremmer E, Brueckl WM, Papadopoulos T, Hohenadl C, Hohenberger W, Stürzl M. Angiostatic immune reaction in colorectal carcinoma: Impact on survival and perspectives for antiangiogenic therapy. Int J Cancer. 2008 Nov 1;123(9):2120-9. doi: 10.1002/ijc.23764. PMID: 18697200.

107. **Chow MT**, Luster AD. Chemokines in cancer. Cancer Immunol Res. 2014 Dec;2(12):1125-31. doi: 10.1158/2326-6066.CIR-14-0160. PMID: 25480554; PMCID: PMC4258879.
108. **Klinke DJ 2nd**. Enhancing the discovery and development of immunotherapies for cancer using quantitative and systems pharmacology: Interleukin-12 as a case study. J Immunother Cancer. 2015 Jun 16; 3:27. doi: 10.1186/s40425-015-0069-x. PMID: 26082838; PMCID: PMC4468964.
109. **King C**, Mueller Hoenger R, Malo Cleary M, Murali-Krishna K, Ahmed R, King E, Sarvetnick N. Interleukin-4 acts at the locus of the antigen-presenting dendritic cell to counter-regulate cytotoxic CD8+ T-cell responses. Nat Med. 2001 Feb;7(2):206-14. doi: 10.1038/84659. PMID: 11175852.
110. **Kishimoto T**. IL-6: from its discovery to clinical applications. Int Immunol. 2010 May;22(5):347-52. doi: 10.1093/intimm/dxq030. Epub 2010 Apr 21. PMID: 20410258.

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