Aus dem Institut für Medizinische Immunologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

The human CXCR3 chemokine system in early differentiated
CD8+ T cell function and solid tumors

Das humane CXCR3 Chemokinsystem in der früh differenzierten
CD8+ T-Zellfunktion und in soliden Tumoren

zur Erlangung des akademischen Grades

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Für meine Eltern.

This MD/PhD thesis presents immunological and clinical literature, the applied methods, the gained results and putative clinical applications (diagnostics and therapeutics) based on the publication "**The intra-tumoral CXCR3 chemokine system is predictive of chemotherapy response in human bladder cancer**" published in *Science Translational Medicine* (2021). In addition, first establishment steps for an adoptive T cell therapy approach were developed, which exploit early differentiated CD8^{+} T cell characteristics studied in this thesis.

1 Abstracts

1.1 Zusammenfassung (Deutsch)

Migration von T-Zellen in den Tumor und langfristiges Überleben der T-Zellen im Tumor sind Attribute für robuste Immunantworten gegen den Tumor. Das CXCR3 Chemokinsystem ermöglicht es, dass CD4+ und CD8+ T-Zellen mithilfe der Interaktion des CXCR3-Rezeptors und seiner Liganden CXCL9/10/11 ins Gewebe zu migrieren. Humane CD8+ T-Zellen mit Stammzelleigenschaft (T$_{SCM}$) bilden einen früh differenzierten T-Zell-Subtyp mit hoher *ex-vivo* CXCR3-Expression. CD8+ T$_{SCM}$ sind in antigen-spezifischen Antworten involviert und behalten dabei ihr proliferatives Potential *in-vivo*. Die Entschlüsselung, wie das CXCR3 Chemokinsystem die Funktion von CD8+ T$_{SCM}$ beeinflusst, könnte zum Aufdecken von Charakteristika erfolgreicher anti-Tumor Immunantworten beitragen. T-Zell-Infiltration wurde als eigenständiger, positiver Prognosefaktor bei Malignomen beschrieben, unter anderem beim urothelialen Blasenkarzinom. Blasentumore besitzen ein immunreiches Milieu mit zytotoxischen CD4+ und dysfunktionalen CD8+ T-Zell-Eigenschaften und stellen ein humanes Modell zur klinischen Untersuchung von Immunmechanismen dar.

Das Ziel der Studie war es, den Einfluss des humanen CXCR3-Chemokin-Systems auf die Migration und Effektorfunktion früh differenzierter CD8+ T$_{SCM}$ zu untersuchen. Die identifizierten Komponenten des Chemokinsystems wurden für die Immuncharakterisierung des Blasentumors angewandt und mit dem klinischen Verlauf der Patienten und weiterer solider Tumore verglichen.

Früh differenzierte CD8+CXCR3^{high} T$_{SCM}$ wurden im Blut gesunder Menschen und Blasenkrebspatienten nachgewiesen. CD8+CXCR3^{high} T$_{SCM}$ waren in Lymphknoten zusammen mit Tumor drainierenden CXCL9/10/11 angereichert. In Funktionsassays induzierten CXCL9/10/11 die Migration von CD8+ T$_{SCM}$, aber nur CXCL11 aktivierte antigen-spezifische CD8+ T$_{SCM}$. Die Analyse von CXCR3-Isoformen ergab eine hohe Expression von CXCL11-reaktivem CXCR3alt in CD8+ T$_{SCM}$. Die CXCR3alt-CXCL11 Immunachse wurde im Blasentumor analysiert. Intratumorale Spiegel von CXCR3alt und CXCL11 vor der Behandlung korrelierten positiv mit der Anzahl tumorinfiltrierender T-Zellen und dem Überleben der Patienten und sagten als Markerkombination das Ansprechen auf eine Chemotherapie bei 20/20 Patienten vorher. In einer zweiten Kohorte assoziierte ein CXCL11^{high} Tumorstatus vor Chemotherapie mit verbessertem Überleben (n=68). Letztlich wurde CXCR3-Expression in früh differenzierten CD8+ T-Zellen im

Blasentumor, Melanom und Nierenzellkarzinom bestätigt und erste Schritte für eine CD8$^+$CXCR3^{high} T$_{SCM}$-basierte adoptive T-Zell-Therapie entwickelt.

Zusammenfassend zeigen die experimentellen Daten, dass das CXCR3 Chemokinsystem die Migration und die Aktivierung früh differenzierter CD8$^+$ T-Zellen moduliert. Die klinischen Daten implizieren, dass das CXCR3 Chemokinsystem das Ansprechen auf eine Chemotherapie beim Blasenkrebs prädiziert und früh differenzierte CD8$^+$CXCR3^{high} T-Zellen in soliden Tumoren eine protektive Relevanz besitzen, was neue therapeutische Optionen eröffnet.

1.2 Abstract (English)

Migration of T cells into the tumor microenvironment and successful T cell survival at the tumor site are key for robust anti-tumor immune responses. The CXCR3 chemokine system grants CD4$^+$ and CD8$^+$ T cells entry to peripheral sites by interaction of the receptor CXCR3 with its interferon-inducible ligands CXCL9/10/11. Human CD8$^+$ stem cell memory T cells (T$_{SCM}$) form an early differentiated T cell subset characterized by a high CXCR3 *ex-vivo* expression state and the potency to execute robust antigen-specific responses yet to maintain proliferative potential *in-vivo*. To dissect the interlink how the CXCR3 chemokine system impacts the function of CD8$^+$ T$_{SCM}$ may reveal mechanistic components relevant for anti-tumor responses. T cell infiltration *per se* has been identified as a positive prognostic factor in multiple malignancies, *e.g.*, in bladder cancer. Bladder tumors harbor an immunogenic environment including cytotoxic CD4$^+$ and dysfunctional CD8$^+$ T cell states and represent a suitable human model to investigate immune mechanisms for clinical application.

The aim of this study was to investigate how the human CXCR3 chemokine system modulates early differentiated CD8$^+$ T$_{SCM}$ migration and effector function. The identified CXCR3 chemokine components were applied for the characterization of the tumor microenvironment in bladder cancer in relation to the clinical outcome and other solid tumors.

Early differentiated CD8$^+$CXCR3^{high} T$_{SCM}$ were found in the blood of healthy humans and bladder cancer patients at similar frequencies. CD8$^+$CXCR3^{high} T$_{SCM}$ were enriched in lymph nodes accompanied by tumor activity of CXCL9/10/11. In functional assays, CXCL9/10/11 induced migration of CD8$^+$ T$_{SCM}$, but only CXCL11 activated antigen-specific CD8$^+$ T$_{SCM}$. Dissection of CXCR3 into its isoforms revealed high expression of CXCL11-reactive CXCR3alt in CD8$^+$ T$_{SCM}$. The CXCR3alt-CXCL11 stimulatory immune axis was analyzed within the inflammatory tumor microenvironment of bladder cancer and tested for clinical relevance. Intra-tumoral, pre-treatment abundance of CXCR3alt and CXCL11 correlated with high levels of tumor-infiltrating T cells, associated with improved OS and *in combination*, predicted the response to chemotherapy in 20/20 patients. In a second patient cohort, a CXCL11^{high} tumor state before chemotherapy associated with improved OS (n=68). CXCR3 expression was confirmed in early differentiated CD8$^+$ T cell states in bladder cancer, melanoma and renal cell carcinoma. Ultimately, steps towards CD8$^+$CXCR3^{high} T$_{SCM}$-based adoptive T cell therapy were developed.

In conclusion, the results indicate a migratory but also stimulatory role of the human CXCR3 chemokine system for the function of early differentiated CD8^{+} T cells. The clinical data imply the CXCR3 chemokine system to be decisive for response to chemotherapy in bladder cancer and suggest protective pan-cancer relevance of intra-tumoral early differentiated CD8^{+}CXCR3^{high} T cells, opening novel therapeutic options.

2 Current State of Immune Research on CD8^{+} T cells in Cancer

2.1 The immunoediting concept describes the immune-cancer cell interplay

The immune system can fight solid tumors. One important strategy of the immune system is to control tumor growth by directly eliminating cancer cells. In order to do this, immune cells strive to reach the tumor site. The concept of *immunoediting* has been implemented that conceptualizes the dynamic immune-cancer cell interaction process (Fig. 1) *(1)*. First, the immune system can succeed to reduce the tumor burden, as immune cells can infiltrate into the tumor microenvironment to kill malignant cells (**1st stage: elimination**). Paul Ehrlich initially hypothesized that the immune system harbors a protective function against cancer *(2)*, which was ultimately confirmed in *in-vivo* models of immune-deficient inbred mice *(3, 4)*. In a second step, cancer cells can evade the immune response by de-novo mutations as well as suppress immune cells, *e.g.*, by the secretion of inhibitory stimuli *(5, 6)*. A steady state between both systems occurs which alternates between successful tumor clearing and cancer growth (**2nd stage: equilibrium**). Third, immune cells can fail to recognize cancer cells, as the malignancy continuously demasks antigens that are normally recognized by immune cells (*i.e.*, antigenic loss). This leads to a state of immune tolerance and eventual disease progression of the malignancy (**3rd stage: escape**) *(7, 8)*. The immunoediting concept illustrates that the tumor microenvironment is shaped by the immune system and robust immune responses are required to fight evasion strategies in cancer cells. *In clinical terms*, the immune system of patients with progressed solid tumor disease has failed to prevent the final immune escape stage. Nonetheless, a subgroup of patients maintains functional immune characteristics with implication for their prognosis and treatment choice. The overall approach of my MD/PhD thesis was to study such immune characteristics in cancer patients in order to find guidance for the clinical work-up. The precise objects of the immune system and the patients studied in this thesis are now described in the following chapters.
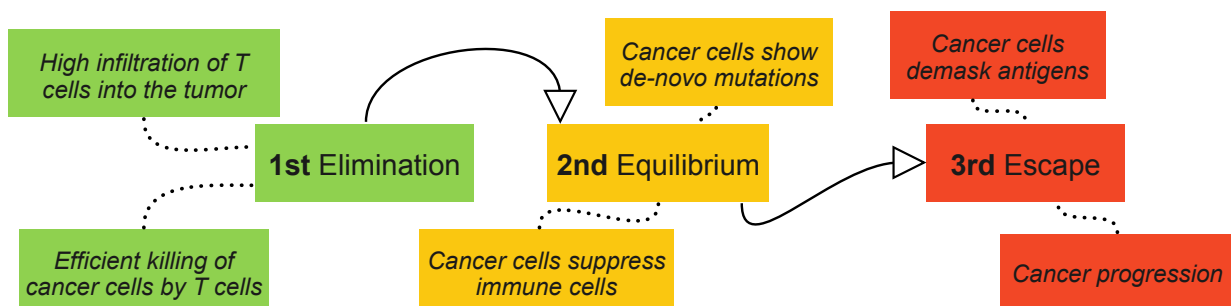

Figure 1: The cancer immunoediting concept.

2.2 T cell infiltration as prognostic and predictive variable for solid cancer patients

In my MD/PhD thesis, I focused on the human adaptive immune system as part of the tumor elimination machinery. CD3+ T lymphocytes (T cells) are central cellular players of the adaptive immune system and are directly involved in anti-tumor responses. Herein, CD8+ T cells are regarded as cytotoxic T cells, as they have the ability to kill pathogen infected and malignant cells. Their T cell receptor (TCR) can recognize tumor-related peptides presented on major histocompatibility complex I (MHC-I) molecules of the malignant cell *(9)*. When recognized, CD8+ T cells can induce apoptosis in the malignant cell by the expression of death receptors (*e.g.,* Fas ligand) and the secretion of granules that include cytotoxic granzymes and perforins *(10)*. In order to elicit killing and contribute to tumor elimination, CD8+ T cells have to overcome the immunosuppressive tumor microenvironment and infiltrate into the tumor.

The immune infiltrate in solid tumors has been investigated for its prognostic role in cancer patients by a myriad of studies. An early report in 2006 demonstrated that tumor infiltration of CD8+ T cells associated with diseases-free survival of patients with colorectal cancer. Interestingly, not only a high abundance of CD3 and CD8, but also their location at multiple sites within the tumor associated with a positive outcome in these patients *(11)*. In addition, the intra-tumoral presence of the T cell *memory* marker, and splice variant of the tyrosin kinase CD45, CD45RO correlated with a similar beneficial outcome in colorectal cancer *(12)*. Colorectal cancer has remained the pilot tumor entity for implementing immune criteria for the clinical evaluation of solid cancer patients. A recent multi-center validation study suggests an "Immunoscore" (derived from an automated intratumoral CD3/CD8 immunohistochemistry analysis) as a potent predictor for colorectal cancer recurrence *(13)*. The high accuracy for predicting colorectal cancer recurrence has initiated discussions to apply the "Immunoscore" in addition to standardized staging procedure (*e.g.*, TNM-score) *(14, 15)*. Beyond colorectal cancer, a cytotoxic, CD8-dominated immune infiltrate positively correlated with overall survival in 15 out of 17 investigated solid tumor entities, which included multiple types of adenocarcinoma and squamous cell cancers *(16)*. To translate prognostic immune markers for clinical application, *treatment-specific* immune signatures need to be defined that harbor *predictive* capacity for the therapeutic outcome.

Immunotherapies have been in the translational focus for testing T cell related signatures for treatment stratification approaches. Recently introduced immunotherapies,

e.g., immune checkpoint blockade (ICB) therapies (*e.g.,* anti-programmed cell death protein-1; anti-PD-1 and anti-PD-1 ligand; anti-PD-L1) underline that *in-vivo* T cell activation can lead to durable cancer remission. ICB therapies are now clinically approved in multiple tumor entities including melanoma and muscle-invasive bladder cancer *(17)*. Based on the T cell activating mechanism, a pre-existing T cell response was hypothesized to be relevant for response to immune treatment *(18)*. Multiple studies have now confirmed that the intra-tumoral abundance of CD8+ T cells (expressing PD-1) associates with response to immune checkpoint blockade *(19–22)*.

Classical cancer therapies - such as chemotherapy – are poorly investigated on immune markers for treatment success. Chemotherapeutic agents elicit unspecific toxic effects, *e.g.,* by inhibition of cell proliferation, but can also induce strong immune-stimulatory effects, which has only recently been recently appreciated *(23, 24)*. Exemplary, platinum-based chemotherapeutics (*e.g.,* cisplatin) bind to DNA and initiate crosslinking within one DNA strand that leads to insufficient DNA repair *(25)*. As a consequence, cancer cells die (causing tumor rejection), but also proliferating healthy cells die (causing treatment side effects: myelosuppression, nephrotoxicity, neurotoxicity) *(26)*. Mechanistic studies unveiled that platinum-based chemotherapy has distinct immunomodulatory effects. Platinum-based agents were shown to induce the up-regulation of PD-L1 in cancer cells of tumor patients *(27)* characterized as part of an immunogenic cell death program after platinum exposure to cancer cell lines *in-vitro (28–31),* synergize with anti-PD-L1 treatment *(32, 33)* and promote co-stimulation of CD8+ T cells by activating dendritic cells *(34)*. This indicates an activation of CD8+ T cells, which follows chemotherapy treatment and may support successful tumor rejection. In analogy to immunotherapies, it remains to be determined whether a *pre-existing* T cell infiltration state is pre-requisite for chemotherapy response and applicable for treatment stratification.

The identification of a T cell state in solid tumors before platinum-based chemotherapy was one primary clinical subject of my MD/PhD thesis. In the following chapter, I will outline CD8+ T cell migration and CD8+ T cell differentiation, two aspects of CD8+ T cell immunity investigated in this thesis.

2.3 Cell migration and differentiation state shape anti-tumor memory CD8^{+} T cell function

T cell infiltration is a positive prognostic factor in multiple tumor entities and can be interpreted as a sign of immune elimination (*see 2.1*). A significant role can be attributed to effector and memory CD8^{+} T cells that were shown to elicit (tumor related) antigen-specific responses in multiple solid tumor entities *(35)*. Beyond antigen recognition, two components are of importance for robust anti-tumor CD8^{+} effector and memory T cell function: **i)** productive T cell migration into the tumor and **ii)** successful T cell survival at the tumor site despite the immunosuppressive environment.

T cell migration is mediated by external signals, yet T cells harbor a receptor repertoire that can modulate their migratory response. Important external signals are chemotactic proteins (chemokines). Chemokines can be found in the tumor microenvironment and bind to chemokine receptors, which are expressed on immune cells inducing cell migration *(36, 37)*. Chemotaxis (*i.e.*, cell migration towards a chemokine gradient) is supposedly the primary mechanism how T cells migrate towards dynamic inflammatory environments such as the tumor site *(38)*. CXCR3 is a G-protein coupled chemokine receptor expressed on Th1 CD4^{+} and CD8^{+} T cells that mediates chemotaxis to inflamed tumors *(39, 40)*, inflamed peripheral sites *(41)* and the secondary lymphoid compartment *(42)*. The three interferon-inducible ligands CXCL9 (MIG), CXCL10 (IP-10) and CXCL11 (I-TAC) are the principal chemokines that bind to CXCR3 *(43)*. Notably, the CXCR3 chemokine system (receptor and ligands) has been primarily investigated in mice *(44)*, but significant differences to the human system exist (Table 1) *(45)*. Recent reports suggest that chemokines support non-migratory functions, which are relevant to orchestrate immune responses and add additional layers to the function of chemokine systems *(46–48)*. Exemplary, the CXCR3-CXCL9 axis was identified as a stimulatory (rather than migratory) axis in a murine tumor model *(49)*, however, CXCR3 receptor-ligand functionality needs to be re-evaluated in the human setting.

	Human	Murine differences	Study
CXCR3 chemokines	CXCL9/10/11	CXCL11 not expressed in C57BL/6 mice	*(43, 115)*
CXCR3 isoforms	CXCR3A/B/alt	One CXCR3 isoform	*(99, 100)*

Table 1: Main differences of murine models to the human CXCR3 chemokine system.

T cell survival is substantially guided by intrinsic cell programs that allow subtypes of T cells (*e.g.*, CD8⁺ memory T cell subsets) to endure in non-stimulatory or even immune-suppressive environments. Specifically, Rafi Ahmed`s group has established the concept that antigen-specific CD8⁺ T cell memory sustains longevity guided by *intrinsic* transcriptional and epigenetic differentiation programs *(50–52)*. In general, cell differentiation is an important concept in biology that describes the specialization of cells, *i.e.*, daughter cells are in a more professionalized state compared to their cellular origin, the adult stem cell *(53)*. In tumor-infiltrating T cells, single-cell analyses have likewise revealed distinct *T cell* differentiation states *(54–56)*. T cell differentiation states can be divided into **i)** early differentiated states that are endowed with a high self-renewing capacity and **ii)** late differentiated states that execute immediate T cell effector function. Interestingly, early differentiated CD8⁺ T cells were shown to be pivotal for maintaining T cell survival at the tumor site by providing a local "stem-like" T cell source *(57–59)*, actively responding to anti-PD-1 ICB therapies *(21, 60, 61)* and boosting the efficacy of adoptive T cell therapy approaches against cancer *(62, 63)*. However, there is limited research on how the early differentiated T cell state impacts the migratory capacity of human CD8⁺ T cells. To study this interlink may reveal functional insights into human signals that mediate homing of early differentiated CD8⁺ T cells to the secondary lymphoid compartment in cancer, but also directly to the tumor microenvironment.

CD8⁺ stem cell memory T cells (T$_{SCM}$) form a T cell subset with early differentiated cell properties and were the primary cellular object of this thesis. T$_{SCM}$ were identified in the (originally defined) naïve T cell compartment characterized as CCR7⁺CD45RA⁺CD45RO⁻CD62L⁺CD27⁺CD28⁺CD127⁺, which associated with naïve-like functional attributes such as longevity and high proliferative capacity. In addition, T$_{SCM}$ were characterized as CD95⁺CD122^{high}CXCR3^{high}, which associated with memory-like functional attributes such as effector function on antigenic rechallenge and tumor clearing in *in-vivo* models *(64)*. This initial report is now confirmed by multiple studies underlining that T$_{SCM}$ can exhibit potent antigen-specific responses *(65, 66)*, maintain longevity *(67, 68)* and execute high proliferative potential *in-vivo (69, 70)*. All of these characteristics are preferable for immunotherapeutic use and already exploited for T cell product generation approaches *(71, 72)*. This makes T$_{SCM}$ an excellent representative of the early differentiated T cell state and a therapeutically relevant T cell subset to study in solid cancer disease (*see 3.2* methods for overview on all CD8⁺ T cell subsets investigated including the flow cytometric gating strategy).

Our proof-of-concept study was performed in the disease of bladder cancer, which will be introduced in chapter (**2.4**) followed by a summary on the hypotheses and aims of my thesis (**2.5**).

2.4 Bladder cancer as a solid tumor disease modulated by T cell responses

The introduction of ICB treatment has successfully complemented the treatment arsenal against solid cancer disease (*see 2.2*). Mechanistic and clinical studies in the "frontrunner" disease of melanoma were pivotal for the implementation of ICB into clinical practice and have contributed to a thorough understanding of immune mechanisms in this skin cancer disease *(19, 73)*. Noteworthy, other immunotherapeutic strategies were already in place before ICB, but have gained less scientific attention. One of the first ideas to stimulate the immune system against cancer goes back to "Coley`s toxin", an approach to apply bacteria (*Streptococcus*) against sarcoma in 1891 *(74)*. A similar treatment approach was applied for 9 patients with bladder cancer in 1976 by the vesical installation of the attenuated bacteria *Mycobacterium bovis*, called Bacillus Calmette-Guérin (BCG), which showed clinical successes *(75)*. Nowadays, BCG installation is a standard treatment for patients with high-risk non-muscle invasive bladder cancer (NMIBC) inducing complete tumor rejection in more than half of the patients *(76)*. Despite widespread BCG application, mechanisms contributing to BCG response still need to be fully resolved. Primary studies report the significance of a pre-treatment BCG-specific T cell response for success and tumoral PD-L1 expression for failure of treatment *(77, 78)*. In addition, increased T cell infiltration and chemokine release were observed after BCG treatment *(79, 80)*. This links the induction of adaptive immune responses as a potent anti-bladder tumor treatment strategy and ultimately, bladder cancer has been acknowledged in literature as a human model disease to study anti-tumor T cell responses *(81, 82)*.

 The bladder tumor has been characterized as an active immune landscape by the identification of multiple immune cell types in the tumor microenvironment *(83)*. Bladder tumors were among the highest immune infiltrated tumors in a comparative transcriptomic signature analysis between multiple entities *(84)*. Immune infiltration was observed both in the NMIBC as well as the advanced muscle-invasive bladder cancer disease (MIBC) stage *(85–87)*. In MIBC, T cell analyses revealed clonal expansion of T cells in conjunction with a high neoantigen load as positive predictor of patient survival *(88)*. Neoantigen-reactive T cell responses were reported in MIBC and tumor-infiltrating T cells were characterized for the development of autologous T cell therapy against bladder cancer *(89, 90)*. Moreover, cytotoxic CD4+ T cell states were identified in human MIBC tumors that were activated following ICB and contributed to treatment response in murine models *(91, 92)*. A dysfunctional CD8+ T cell state was likewise reported to

associate with poor clinical outcome in MIBC *(93)*. However, the precise characteristics of a functional and robust CD8^{+} T cell response in bladder cancer remains unknown. Above all, immune state analyses need to be set in relation to the clinical situation of patients with MIBC to identify relevant prognostic and predictive biomarkers that may guide treatment decision and improve the outcome of patients with this disease.

Chemotherapy has remained a corner stone in the treatment for MIBC patients. Guidelines recommend the application of platinum-based chemotherapy to clinically fit MIBC patients prior to surgery, *i.e.*, neoadjuvant chemotherapy (NAC) *(94)*. These recommendations are based on prospective randomized trials that reported a NAC-related absolute increase of the 5-year overall survival (OS) by 5-8 % compared to surgery alone *(95, 96)*. However, only 25-40 % patients respond by histopathological downstaging after NAC treatment *(97)*. A pilot study on immune mechanisms identified an improved effector T cell response in MIBC after NAC treatment *(98)*, which confirms the observed immune-stimulatory effects of platinum-based agents in other entities (*see 2.3*). It remains unknown, however, whether responder patients harbor an immune state before treatment that associates with responsiveness to platinum-based NAC and allows treatment stratification.

To conclude, bladder cancer is an immunogenic tumor disease, and the clinical outcome can be modulated by effective adaptive immune responses. In this thesis, the distinct role of the CXCR3 chemokine system was studied as potential part of a beneficial intratumoral immune signature before NAC and tumor-infiltrating early differentiated CD8^{+} T cells were studied in MIBC compared to other solid tumors.

2.5 Hypotheses and aims of this thesis

The presented literature describes that migration of T cells into the tumor microenvironment (*e.g.*, by the CXCR3 chemokine system) and successful T cell survival at the tumor site (*e.g.*, of early differentiated CD8$^+$ T cells) are key for robust anti-tumor immune responses. However, mechanistical studies have been mostly conducted in murine models. Based on these premises, the hypothesis was laid out that the human CXCR3 chemokine system modulates early differentiated CD8$^+$ T cell function and its characterization may reveal novel and significant components for effective tumor eradication by the immune system. Moreover, the literature describes bladder cancer as an immunogenic solid tumor disease and a suitable model to examine immune mechanisms for clinical application. Based on these hypotheses, I pursued three principal aims during my MD/PhD studies:

i) to investigate how the CXCR3 chemokine system modulates early differentiated CD8$^+$ T$_{SCM}$ function relevant for anti-tumor immunity,

ii) to translate these findings for the characterization of the bladder tumor microenvironment in relation to the clinical outcome and

iii) to establish first T cell culture steps towards CD8$^+$ T$_{SCM}$ based adoptive T cell therapy.

3 In-depth Methods

This chapter gives a schematic overview on the methods that were established and applied in my MD/PhD thesis. Detailed descriptions of the methods are included the Material & Methods section in the study of this thesis (*see 10.* Publication "The intra-tumoral CXCR3 chemokine system is predictive of chemotherapy response in human bladder cancer").

3.1   Isolation of lymphocytes from human blood and lymph nodes

Cell isolation procedures were applied to access lymphocytes including T cells from the human veinous blood system and lymph nodes**. i)** From venous blood samples, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation in presence of a polymer substance (Biochrom). PBMC separated in a transferable cell layer (pellet) of macrophages/monocytes and lymphocytes including peripheral T cells. **ii)** Lymph node samples of patients were accessed after surgery, kept on ice, prepared and cut by a scalpel followed by filtering using a cell strainer. Lymph node derived cells including T cells were then applied to the subsequent analyses and experiments.

3.2   Flow cytometry

Flow cytometry was performed to investigate protein expression on CD8$^+$ T cells for analysis of CD8$^+$ T cell subsets, chemokine receptor and intracellular cytokine expression. Prior to analysis, fluorochrome-conjugated monoclonal antibodies were applied for T cell staining. Flow cytometry uses a fluidic system that aligns stained cells by a laminar flow. An optical system includes lasers that activate fluorochromes bound to antibodies and filters that direct emitted signals to the detectors. Light signals are converted into electronic signals for flow cytometric analysis. The stained cells were analyzed by the LSR II Fortessa flow (BD) or Cytoflex LX cytometer (Beckmann Coulter). Extracellular staining was performed to discriminate the CD3$^+$CD8$^+$ T cell subsets: CCR7$^+$CD45RA$^+$CD95$^-$ naïve T cells (T$_{naïve}$), CCR7$^+$CD45RA$^+$CD95$^+$ stem cell memory T cells (T$_{SCM}$), CCR7$^+$CD45RA$^-$ central memory T cells (TCM), CCR7$^-$CD45RA$^-$ effector memory T cells (TEM), and CCR7$^-$CD45RA$^+$ terminally differentiated effector-memory T cells (TEMRA). This gating strategy was likewise used to study chemokine receptor expression (CXCR1/3/4 and CCR3/5/6/7) on CD8$^+$ T cell subsets. After antigenic

stimulation, fixation/permeabilization procedure was performed to identify intracellular expression of cytokines (IFN-γ, TNF-α, IL-2) and activation markers (*e.g.*, CD137). This allowed the identification and characterization of cytomegalovirus (CMV)-specific and tumor-reactive T cells after an overnight stimulation set-up.

3.3 Fluorescently activated cell sorting (FACS)

FACS was performed to isolate human CD8+ T cell subsets prior to the experimental or analytical setting (*e.g.*, T$_{SCM}$ expansion assay or analysis of CXCR3 isoforms, *see below*). FACS is based on flow cytometric analysis of cells stained by fluorochrome-conjugated antibodies (*see above*). In addition, FACS includes electric charging to single cell containing droplets for polarization. Polarized droplets can be subsequently electromagnetically separated based on the single-cell marker expression selected by the operator. The Aria II Calliope (BD) was used for cell sorting of the distinct human CD8+ T cell subsets.

3.4 Functional T cell assays

T cell assays were applied to test for effects of chemokines on CD8+ T cell migration, T cell effector function and chemokine receptor expression.

The T cell chemotaxis assay was performed to investigate CD8+ T cell chemotaxis directed to chemokines. Short term culture was performed using transwell assays (Corning) with a porous membrane between the lower chamber (including chemokines) and the upper chamber (including PBMC). Flow cytometry was applied to analyze transmigrated CD8+ T cells.

The T$_{SCM}$ expansion assay was performed to investigate stimulatory effects of the chemokines on early differentiated CD8+ T cell function. Long term culture was performed using a T$_{SCM}$-based expansion set-up, as previously published by our group *(65)*. PBMC were enriched for a CD3+CCR7+CD45RA+ T cell population by FACS followed by stimulation with CD3-depleted and CMV peptide pool (JPT) pulsed PBMC and IL-7 and IL-15 (CellGenix) supplemented complete medium. At day 7, restimulation was performed by peptide-pool pulsed, CD3-depleted PBMC. At day 14 and 21, antigen-specificity was assessed by restimulation with peptide-pool pulsed antigen presenting cells.

The T cell proliferation assay was performed to investigate the proliferative effect of the chemokine CXCL11 on early differentiated CD8$^+$ T cell function. T$_{SCM}$ were labeled with Carboxyfluorescein succinimidyl ester (CFSE) and spiked into the T$_{naïve}$ population at the initial *ex-vivo* frequency. CMV stimulation was conducted analogous to the T$_{SCM}$ expansion assay set-up (*see above*). CFSE dilution in CD8$^+$ T cells after 96 hours indicated T cell proliferation and was analyzed by flow cytometry.

The chemokine receptor ligation assay was performed to investigate chemokine mediated downregulation of CXCR3 on CD8$^+$ T$_{SCM}$. Chemokines were incubated with PBMC in different concentrations. Acidic washing was applied to exclude receptor occupancy prior to flow cytometric analysis of CXCR3 expression.

3.5   Real time-quantitative polymerase chain reaction (RT-qPCR)

RT-qPCR was performed to analyze mRNA abundance of the alternatively spliced isoforms of CXCR3 in CD8$^+$ T cell subtypes and in bladder tumors. Isolated mRNA was transcribed into complementary DNA (cDNA) by reverse transcriptase (Quiagen). Taqman RT-qPCR uses probes with attached fluorochrome (5' end) and quencher (3' end) that hybridize with the target sequence during PCR amplification. The polymerase replicates the amplicon and thereby, the polymerase cleaves the fluorescent probe leading to decoupling of the quencher from the fluorochrome. A fluorescence signal is emitted by the fluorochrome and detected by the thermal cycler at real time, which is proportional to the cDNA amount of the target. For analysis of the CXCR3 isoforms, an RT-qPCR panel was established that discriminates the CXCR3B and CXCR3alt isoform based on their differentially spliced gene sequences (Fig. 2A).

CXCR3A is the main and initially described isoform *(45)* and was measured by a commercially available TaqMan gene expression assay (Thermo Fisher Scientific).

CXCR3B is the second isoform described and contains an additional, unique sequencing "tail" (*i.e.,* alternative splicing by a new acceptor site) *(99)*. A TaqMan assay was designed with a probe that recognizes the extended CXCR3B-specific sequence (Fig. 2B).

CXCR3alt is the third isoform described and contains a skipped sequence (*i.e.,* alternative splicing by exon skipping) *(100)*. This results into a reduced protein structure and limits CXCR3alt affinity mainly to the chemokine CXCL11, and less to CXCL9. *(45,*

100, 101). A TaqMan assay was designed with a probe that spans the CXCR3alt-specific sequencing gap (Fig. 2C).
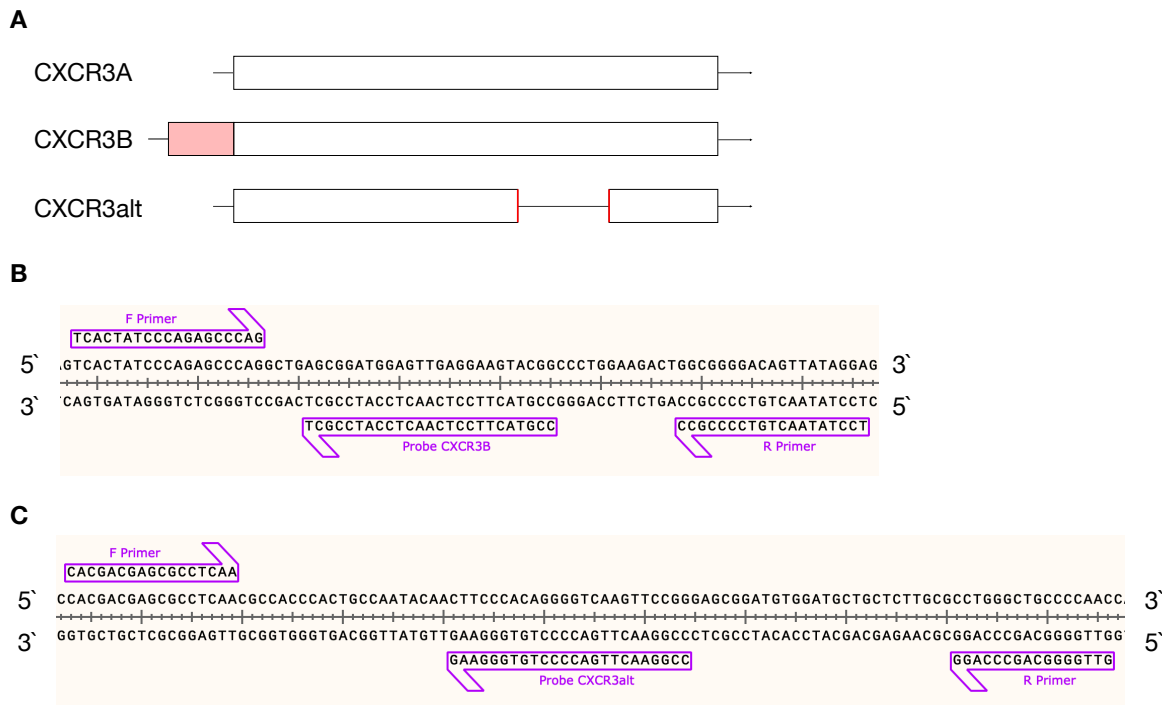

Figure 2: Established RT-qPCR panel to analyze the isoforms CXCR3B and CXCR3alt.
The figure is derived from the supplementary material of the study by Vollmer et al., 2021.
(**A**) Scheme of all CXCR3 isoforms. Red area indicates location for the designed CXCR3B and CXCR3alt probes. (**B**) Sequence of primers and probe to analyze the CXCR3B isoform.
(**C**) Sequence of primers and probe to analyze the CXCR3alt isoform.

3.6 Patients included in this study

All patients (n=46) underwent the diagnostic procedure of TURBT (transurethral resection of the bladder tumor) to establish the diagnosis of bladder cancer (17 NMIBC and 29 MIBC patients) (Fig. 3). From TURBT, the treatment-naïve tumor samples were gained for analysis in this study. After TURBT, 9/29 MIBC patients were clinically unfit to receive neoadjuvant chemotherapy (no-NAC), whereas 20/29 MIBC patients received NAC. In the NAC receiving cohort, 9/20 patients responded to NAC and 11/20 patients did not respond to NAC. Response was defined as downstaging in the histopathology of the cystectomy specimen and subdivided into complete response (pT0N0M0; CR=5), partial response (pTa, pT1, or pTis and all N0M0; PR=4), stable disease (≥ pT2N0M0; SD=4), and progressive disease (any pT with pN1-2 and/or pM1; PD=7).

The sentinel node detection method was applied to analyze tumor-specific activity of the CXCR3 chemokines. A radioactive substance (technetium) was injected into the

tumor base before surgery by a transurethral access and sentinel node detection was performed after surgery by means of a Geiger meter.
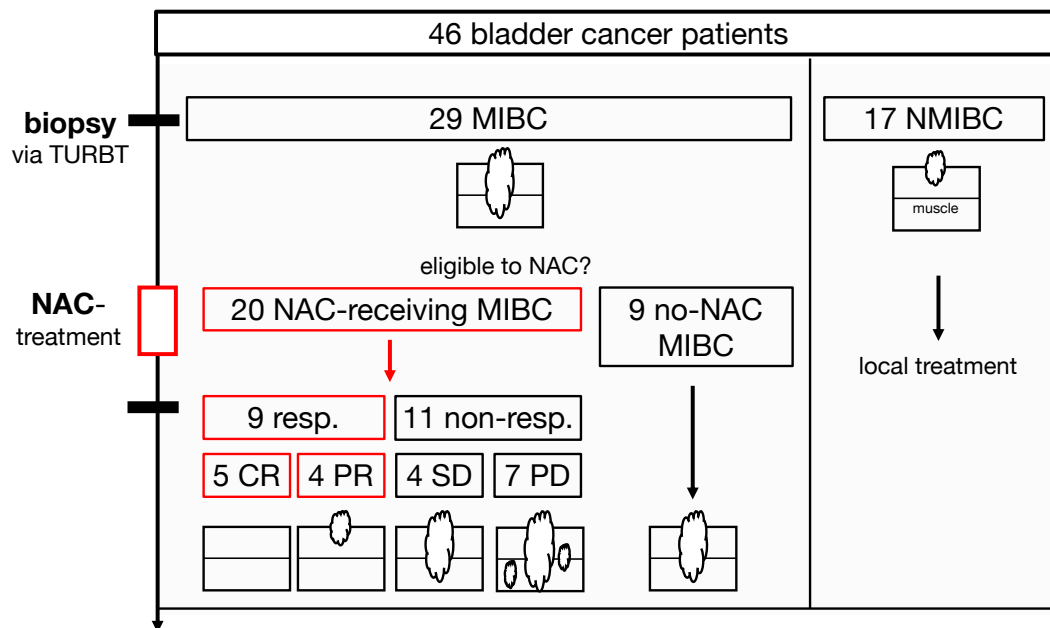
Figure 3: **Diagnostics and treatment of patients with bladder cancer investigated in this study.** The figure is derived from the supplementary material of the study by Vollmer et al., 2021.

3.7   Analysis of the inflammatory tumor microenvironment

The tumor microenvironment was analyzed for the components of the CXCR3 chemokine system (chemokines and CXCR3 isoforms) and compared to inflammatory mediators (cytokines) and tumor-infiltrating T cells (CD3). A tissue method was established that homogenizes the tumor material by chemical and mechanical lysis procedure. DNA/RNA extraction was performed by the AllPrep DNA/RNA Micro kit (Qiagen) and protein isolation by use of protein extraction buffer (Thermo Fisher Scientific).

Protein was used for multiplex analysis to measure intra-tumoral cytokines including the CXCR3 chemokines (CXCL9/10/11). The ELISA-based Luminex platform was applied that uses cytokine-specific antibodies conjugated to magnetic beads. The beads or microspheres harbor differential fluorescent signatures and can be detectable individually by the system. This enabled the simultaneous measurement of multiple analytes (cytokines) in one well of tumor lysate.

mRNA was used for RT-qPCR to measure intra-tumoral abundance of CXCR3 isoform expression (*see 3.5*) and T cell infiltration (CD3 mRNA). 34 house keeping genes (HKG) were tested in bladder tumors and a statistical algorithm applied to select the most

stable HKG candidates. CDKN1B and IPO8 were identified as the most stable HKG and the geometric mean of CDKN1B and IPO8 was used for normalization of the target genes.

DNA was used for T cell receptor (TCR) sequencing to analyze intratumoral TCR diversity. The complementary determining region 3 (CDR3) within the TCR-β chain was amplified. The CDR3 contains regions of all three TCR gene segments (V/J/D) that are recombined (*i.e.*, during somatic recombination). Thus, CDR3 represents an extremely variable part of the TCR representing suitable to discriminate TCRs. Amplified CDR3 loci were processed by the Illumina NGS platform. TCR diversity was estimated by the Shannon and Berger-Parker index.

3.8 Second bladder cancer cohort and single-cell analysis of the CXCR3 chemokine components

Sequencing data of the intra-tumoral CXCR3 chemokine components were accessed for two purposes: **i)** testing the prognostic relevance of the CXCR3 chemokine system in a second patient cohort and **ii)** elucidating the single-cell origin of the CXCR3 chemokine components in different tumor entities.

Bulk RNA-sequencing data was accessed from The Cancer Genome Atlas (TCG) and gene expression values were processed for normalization. Gene expression was analyzed in treatment-naïve tumor samples of 68 MIBC patients who received chemotherapy in the follow-up and compared to 292 MIBC patients who did not receive chemotherapy.

Single-cell RNA-sequencing (scRNAseq) data were accessed under the respective Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information (NCBI). scRNAseq data was derived from tissue samples of healthy bladder and tumor samples of MIBC, melanoma and renal cell carcinoma. Cells with low quality and genes with low abundance were removed. For analysis of CD8+ T cells, pre-sorting was applied by filtering on CD4- and CD8+ T cells.

4 Essential Results

This chapter describes the experimental and clinical insights gained during my MD/PhD thesis (Fig. 4). The experimental results describe how the CXCR3 chemokine system contributes to human early differentiated CD8+ T cell migration and T cell effector function relevant for anti-tumor responses (**4.1** and **4.2**). The clinical results describe the characteristics of the intratumoral CXCR3 chemokine system in bladder cancer. The identified CXCR3 chemokine components were evaluated in the context of precision medicine for chemotherapy treatment and a precise CXCR3-based biomarker candidate was established (**4.3**). CXCR3 chemokine components were tested for prognostic relevance of chemotherapy treatment in a second patient cohort, their single-cell origin was elucidated in the tumor microenvironment and tumor-infiltrating early differentiated CD8+ T cell states were investigated in MIBC, melanoma and renal cell cancer (**4.4**). These results were published as research article "The intra-tumoral CXCR3 chemokine system is predictive of chemotherapy response in human bladder cancer" in *Science Translational Medicine* (2021). Ultimately, first establishment steps towards CD8+CXCR3^{high} T$_{SCM}$-based adoptive T cell therapy were developed (**4.5**).
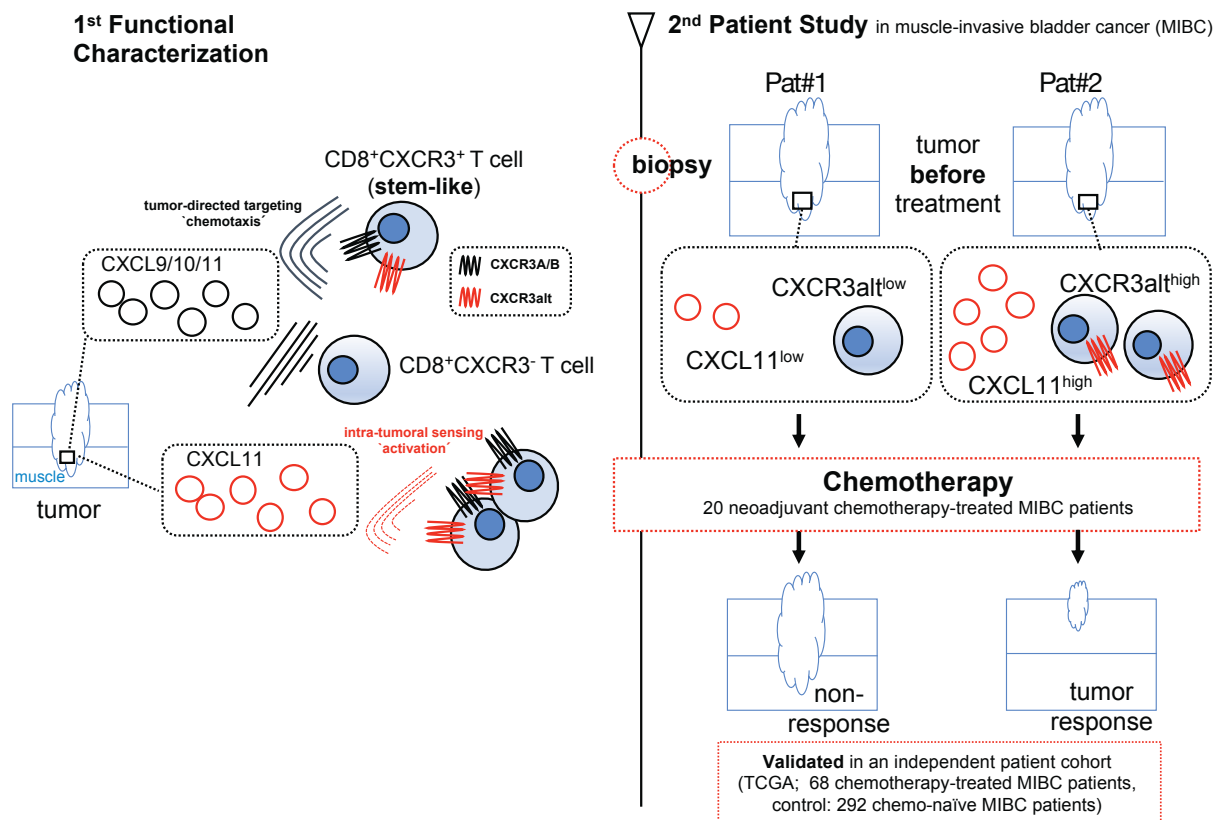

Figure 4: Summary of essential results. The figure is derived from the supplementary material of the study by Vollmer et al., 2021.

4.1 Identification and characterization of early differentiated CD8$^+$ T cells in healthy humans and bladder cancer patients

Experiments of the first data set objected to identify and characterize early differentiated CD8$^+$ T$_{SCM}$ in healthy humans and bladder cancer patients (Fig. 5). T$_{SCM}$ were identified by flow cytometry as CD3$^+$CD8$^+$CCR7$^+$CD45RA$^+$CD95$^+$CXCR3^{high} in the blood of healthy humans in confirmation with previous reports *(64, 65)*. High expression of the chemokine receptor CXCR3 in the early differentiated T cell states (T$_{SCM}$ and T$_{CM}$) contrasted low CXCR3 expression in the late differentiated T cell states (T$_{EM}$ and T$_{EMRA}$) and T$_{naïve}$. In addition, CD8$^+$ T$_{SCM}$ expressed higher CXCR3 levels compared to the chemokine receptors CXCR1/4 and CCR3/5/6. The characterization of CD8$^+$ T$_{SCM}$ in healthy humans was complemented by the characterization of CD8$^+$ T$_{SCM}$ in bladder cancer patients. CD8$^+$CXCR3^{high} T$_{SCM}$ were observed in the peripheral blood of bladder cancer patients at similar frequencies compared to healthy humans. High CXCR3 expression indicated that CD8$^+$ T$_{SCM}$ may home to CXCR3 chemokine^{high} environments, *e.g.*, the secondary lymphoid compartment *(42, 102)*. Access of lymph nodes derived from MIBC patients allowed to apply flow cytometric analysis of nodal CD8$^+$ T cell subsets. In this analysis, CD8$^+$CXCR3^{high} T$_{SCM}$ were enriched in lymph nodes compared to blood and strikingly, node derived T$_{SCM}$ showed tumor reactivity after *ex-vivo* stimulation with bladder tumor lysate. This implicates an active role of CD8$^+$CXCR3^{high} T$_{SCM}$ in anti-tumor responses in bladder cancer and characterizes the human secondary lymphoid compartment as a potential homing target for CD8$^+$CXCR3^{high} T$_{SCM}$. To identify tumor activity of the CXCR3 chemokines (CXCL9/10/11), the nodal chemokine abundance was analyzed and set in relation to tumor drainage estimated by the sentinel node detection method. Here, an overall positive association between CXCR3 chemokine abundance and tumor drainage was observed. This indicates that CXCR3^{high} T$_{SCM}$ are enriched in human lymph nodes, which is accompanied by an active CXCR3 chemokine system. CXCR3 chemokines may direct and regulate CD8$^+$ T$_{SCM}$ function relevant for anti-tumor responses.
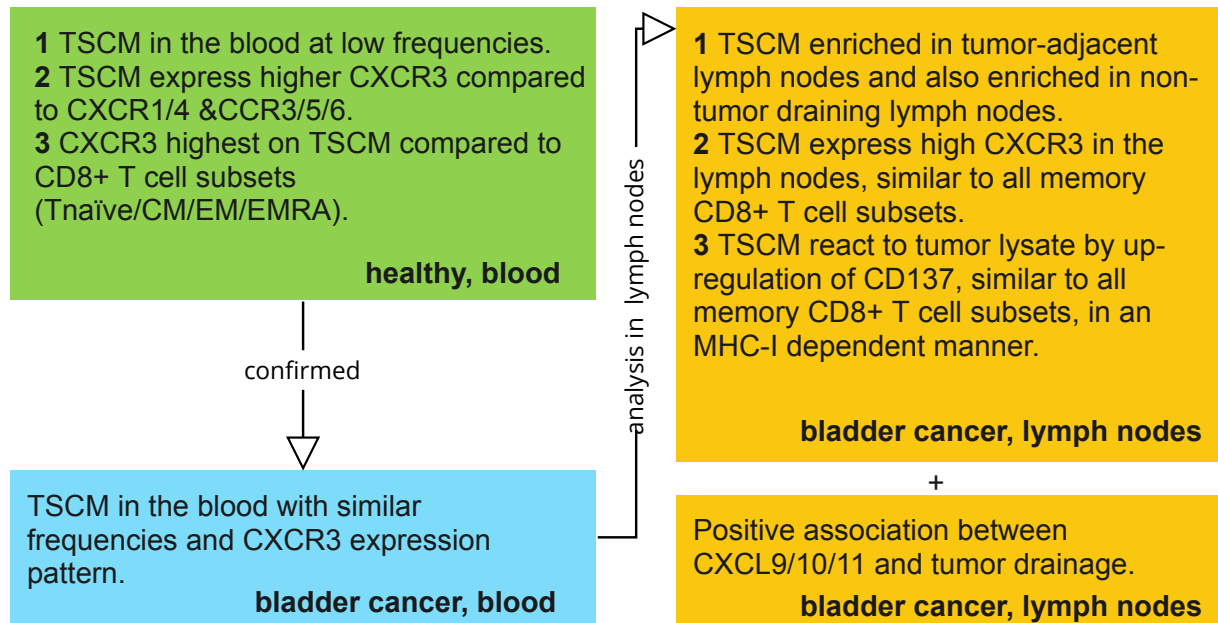
1 TSCM in the blood at low frequencies. **2** TSCM express higher CXCR3 compared to CXCR1/4 &CCR3/5/6. **3** CXCR3 highest on TSCM compared to CD8+ T cell subsets (Tnaïve/CM/EM/EMRA). **healthy, blood**	**1** TSCM enriched in tumor-adjacent lymph nodes and also enriched in non-tumor draining lymph nodes. **2** TSCM express high CXCR3 in the lymph nodes, similar to all memory CD8+ T cell subsets. **3** TSCM react to tumor lysate by up-regulation of CD137, similar to all memory CD8+ T cell subsets, in an MHC-I dependent manner. **bladder cancer, lymph nodes**

confirmed

analysis in lymph nodes

TSCM in the blood with similar frequencies and CXCR3 expression pattern. **bladder cancer, blood**	Positive association between CXCL9/10/11 and tumor drainage. **bladder cancer, lymph nodes**

Figure 5: **Identification and characterization of early differentiated CD8⁺ T cells.**

4.2 Functional investigation of the CXCR3 chemokine system in human early differentiated CD8⁺ T cells

Experiments of the second data set aimed to investigate the functional response of the distinct human CD8⁺ T cell subsets – *with focus on the CD8⁺CXCR3^{high} T$_{SCM}$* – to the CXCR3 chemokines (Fig. 6). T cell assays were applied to investigate the effects of the CXCR3 chemokines on CD8⁺ T cell migration and early differentiated CD8⁺ T cell effector function.

First, T cell chemotaxis assays were applied to test for chemotactic effects of the chemokines. All CXCR3 chemokines (CXCL9/10/11) induced migration of the CD8⁺ T cell subsets and CD8⁺ T$_{SCM}$ showed the highest chemotactic response of all CD8⁺ T cell subsets towards the CXCR3 chemokines. This implies that the CXCR3^{high} state endows CD8⁺ T$_{SCM}$ with a functional responsiveness to the CXCR3 chemokine family.

Second, CD8⁺ T$_{SCM}$ culture assays of two antigenic models were applied to test for stimulatory effects of the chemokines. Only CXCL11 (and not CXCL9/10) amplified the enrichment of antigen-specific CD8⁺ T$_{SCM}$ in both antigenic models. In addition, CXCL11 induced *in-vitro* proliferation of antigen-specific T$_{SCM}$ and CXCL11 induced significant down-regulation of CXCR3 on T$_{SCM}$ in short term culture assays. The combined experimental data suggest a stimulatory role of CXCL11 for CD8⁺ T$_{SCM}$ function.

Third, screening for a CXCL11-reactive receptor in CD8$^+$ T$_{SCM}$ was performed. A CXCL11-reactive receptor expressed in CD8$^+$ T$_{SCM}$ may mediate the CXCL11-specific functional response. The expression of the (non-CXCR3) CXCL11-receptors CXCR7 and DARC on CD8$^+$ T$_{SCM}$ was excluded by flow cytometry. Next, CXCL11 reactivity was screened within the CXCR3 isoform repertoire. Due to unavailable CXCR3 isoform-specific antibodies, an RT-qPCR panel was established that detects all human CXCR3 isoforms (CXCR3A/B/alt) (Fig. 2). FACS was applied to sort on the CD8$^+$ T cell subsets, which were analyzed for CXCR3 isoform expression. Strikingly, CD8$^+$ T$_{SCM}$ were confirmed to express the highest transcript amounts of the CXCL11-reactive CXCR3alt of all CD8$^+$ T cell subsets.

In conclusion, CXCL11 attracted and stimulated CD8$^+$CXCR3^{high} T$_{SCM}$, and associated with high expression of the CXCL11-reactive isoform CXCR3alt in T$_{SCM}$. Ultimately, CXCR3alt-CXCL11 was identified as a potential stimulatory immune axis relevant for CD8$^+$ T cell function.
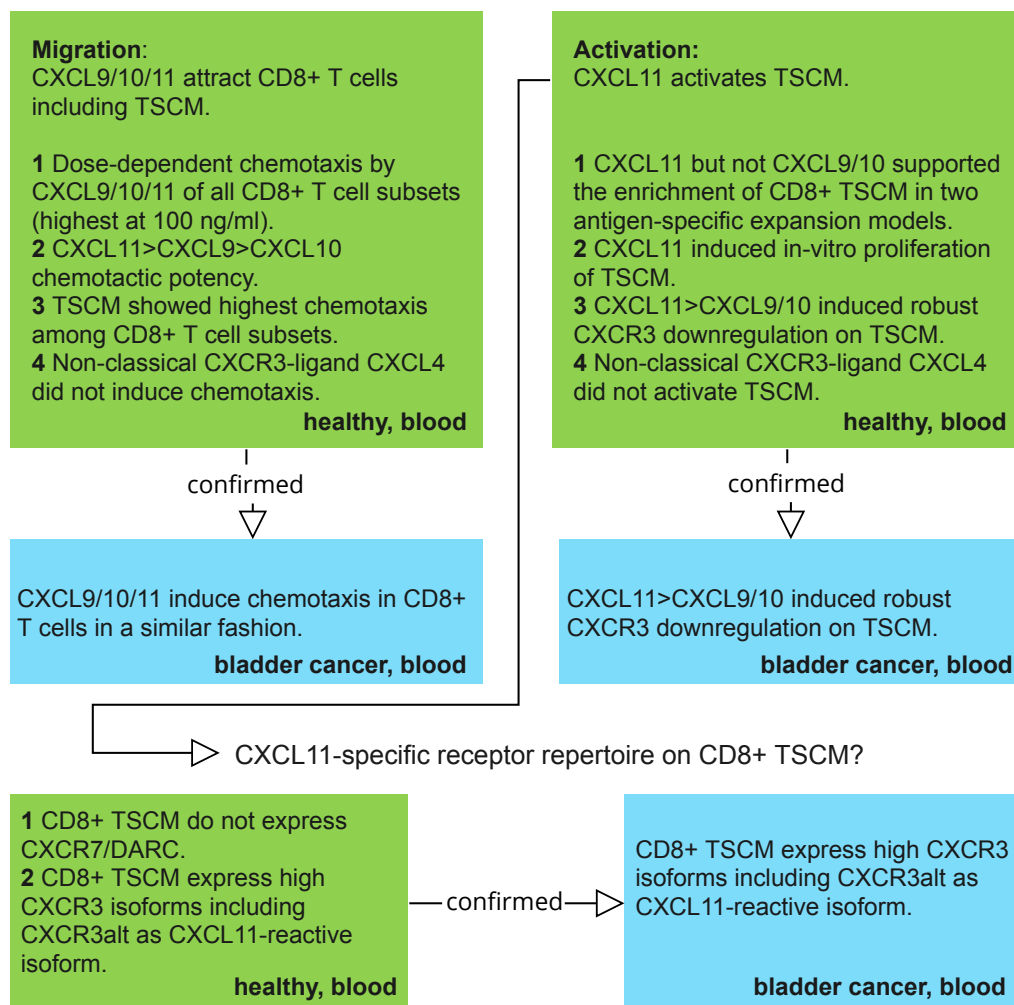

Figure 6: Functional investigation of the human CXCR3 chemokine system.

4.3 Characterization of intratumoral T cell infiltration and the CXCR3 chemokine system in bladder cancer

Experiments of the third data set investigated the role of the CXCR3 chemokine system in the tumor microenvironment within inflammatory networks and for clinical relevance. 46 patients with bladder cancer were recruited in this thesis and treatment-naïve tumor samples were studied (*see 3.6* for detailed patient description). For analysis, a tissue method was established to mechanically disrupt and chemically lyse the tumor sample, which was followed by **i)** protein extraction to analyze intratumoral cytokines including the CXCR3 chemokines by multiplex-based ELISA technique, **ii)** RNA extraction to analyze intratumoral mRNA transcripts of the CXCR3 isoforms and CD3 by RT-qPCR and **iii)** DNA extraction to analyze TCR diversity by TCR sequencing. As control, tumor biopsy matched patient sera were analyzed for protein cytokine levels.

First, analysis on the abundance of tumor infiltrating T cells (CD3) revealed no differences between the three main patient groups: NMIBC, no-NAC MIBC and NAC-receiving MIBC. However, within the NAC-receiving MIBC group responder patients had significantly higher intratumoral T cell levels compared to non-responder. The increased infiltration of T cells in the responder tumors did not associate with changes in the diversity of TCR repertoire compared to non-responder.

Second, a comprehensive screen on intratumoral cytokines revealed that CXCL11 was most significantly associated with NAC response and harbored the highest sensitivity for response prediction. In detail, CXCL11 clustered with CXCL9/10, IFN-y, CCL2/3/4/19, CXCL12/13, IL-16 and correlated significantly with the abundance of tumor-infiltrating T cells. Ultimately, intratumoral CXCL11 concentrations were significantly higher in responder compared to non-responder NAC-receiving MIBC, no-NAC and NMIBC patients, and a CXCL11high tumor state associated with improved OS in MIBC.

Third, the assessment of intratumoral CXCR3 isoforms revealed that CXCR3A/alt but not CXCR3B significantly correlated with the abundance of tumor-infiltrating T cells, CXCR3alt most rigorously predicted NAC response and interestingly, non-responder showed a significant decrease in intratumoral CXCR3A/alt concentrations compared to responder NAC-receiving MIBC, no-NAC and NMIBC patients. Last, a CXCR3althigh tumor state associated with improved OS in MIBC.

Fourth, the interactions and dependencies between the ligands (CXCL9/10/11) and the CXCR3 isoforms (A/B/alt) were analyzed within an intra-tumoral inflammatory network analysis that included the response to NAC. Here, a correlation between

CXCL11 and the response and CXCR3alt and the response was observed, independently. Application of combined CXCL11 and CXCR3alt as pre-treatment biomarkers within a logistic regression model resulted into complete discrimination between the 9 responder and the 11 non-responder MIBC patients.

4.4 Second biomarker testing, identification of the cellular origin of intratumoral CXCR3/CXCL11 and analysis of early differentiated CD8^{+} T cell states in MIBC, melanoma and renal cell carcinoma

Analysis of openly accessible data sets were conducted for testing purpose of the CXCR3-based biomarker and to gain improved mechanistic insights of the intra-tumoral CXCR3 chemokine system in solid tumors.

First, components of the CXCR3-biomarker were investigated in a second MIBC cohort of the TCGA data bank, which comprises bulk RNA sequencing data of human tumors. Treatment-naïve tumor samples of 68 MIBC patients who in the follow-up received adjuvant chemotherapy were compared to 292 MIBC patients who did not receive chemotherapy. Interestingly, a CXCL9/10/11[high] and CXCR3^{high} state associated with improved OS only in the chemotherapy receiving cohort, but not in the cohort that did not receive chemotherapy.

Second, single-cell RNA sequencing data of healthy bladder samples and MIBC tumors samples were analyzed. Here, CXCR3 expression associated to tissue-resident and tumor-infiltrating CD8^{+} T cells in the bladder, but not to non-immune healthy bladder cells or cancer cells. In addition, CXCR3 expression was confirmed in tumor-infiltrating CD8^{+} T cells in the disease melanoma and on the ligand site, CXCL9/10/11 expression was detected in tumor-infiltrating CD14^{+} macrophages.

Third, single-cell analysis was exploited to decipher CXCR3 expression in differentiation states of tumor infiltrating CD8^{+}T cells in human MIBC, melanoma and renal cell carcinoma. Strikingly, CXCR3 expression could be confirmed in early and in late CD8^{+} T cell differentiation states in all three tumor entities investigated.

4.5 Establishment steps towards CD8^{+}CXCR3^{high} T$_{SCM}$ based T cell therapy

Adoptive T cell therapy, *e.g.*, the transfer of CAR T cells, has shown limited clinical successes against solid tumors, which associated with reduced infiltration and persistence of transferred T cells in the tumor microenvironment *(103–109)*. Early differentiated CD8^{+}CXCR3^{high} T$_{SCM}$ are a promising T cell source for use in adoptive T

cell therapy due to their high survival and migratory capacity, as demonstrated in this thesis. Until now, characterization of tumor-specific T_{SCM} has been mostly performed in murine models *(110, 111)*. Human CMV-specific T_{SCM} can be found in the peripheral blood at low frequencies (Fig. 7A). Notably, human CMV-specific CD8+ T cells share exhaustion signatures with tumor-reactive CD8+ T cells *(112)* and exploit the CXCR3 chemokine system for infiltration into the inflamed tissue *(113)*. Hence, CMV-specific T_{SCM} are a reasonable and accessible human model to establish a good manufacturing practice (GMP)-compatible T cell culture process that yields high amounts of antigen-specific T_{SCM}. To increase T_{SCM} frequencies, T_{SCM} were initially pre-enriched on a CCR7+CD45RA+ phenotype followed by CMV-specific T cell expansion in accordance with a protocol previously published by our group (Fig. 7B) *(65)*. To increase the robustness of the protocol, **i)** the influence of GMP-grade cytokine regimes (IL-7/IL-15, IL-2, IL-4/IL-7) *(114)*, **ii)** the role of CD4+ T cells (*i.e.*, the depletion of CD4+ regulatory T cells (T_{REG}) and addition of bulk CD4+ T cells) and **iii)** post-stimulation selection strategies were tested on antigen-specific CD8+ T_{SCM} expansion.

First, both IL-7/IL-15 and IL-2 supplementation enriched antigen-specific T_{SCM} during cell culture, whereas IL-4/IL-7 did not. Moreover, solely IL-7/IL-15 maintained proportions of the initial CCR7+CD45RA+ naïve-like T cell phenotype, whereas IL-2-cultured antigen-specific CD8+ T_{SCM} vastly lost CCR7 and CD45RA expression during the expansion process (Fig. 7C, D).

Second, initial depletion of T_{REG} from pre-enriched CCR7+CD45RA+ T cells did not alter CD8+ T_{SCM} expansion (Fig. 7E). Surprisingly, the depletion of T_{REG} strongly reduced CD8+ T_{SCM} expansion, when the starting cells were initially sorted on the T_{SCM} phenotype (Fig. 7F). The addition of irradiated CD4+ T cells to the starting T cell pool increased, whereas CD4-depletion decreased the frequency of antigen-specific CD8+ T_{SCM} from pre-enriched CCR7+CD45RA+ during cell culture (Fig. 7G).
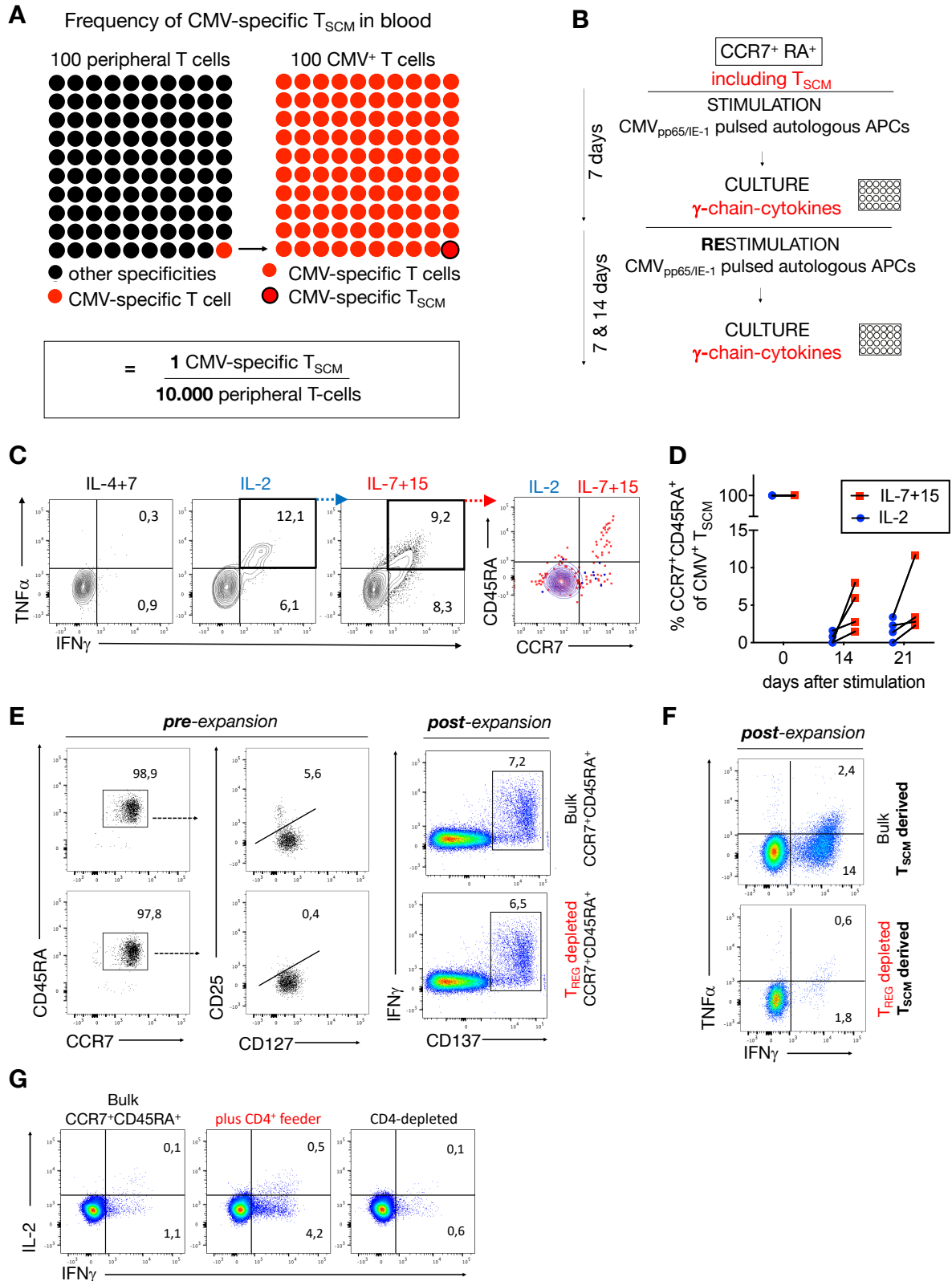
Figure 7: Impact of cytokine regimes and CD4+ T cells on antigen-specific CD8+ T_SCM expansion. (**A**) Scheme of *ex-vivo* frequency of CMV-specific T_SCM within peripheral T cells as reported by Schmueck-Henneresse et al. *(65)*. (**B**) Cell culture workflow to expand CMV-specific T_SCM from CCR7+CD45RA+ T cell pool. (**C**) Intracellular cytokine staining after antigenic restimulation to identify post-expansion frequencies of IFNγ+TNFα+ T_SCM under different cytokine regimes (left) and the CCR7+CD45RA+ phenotype of IL-2

and IL-7/IL-15-expanded antigen-specific T$_{SCM}$ as overlay plot (right) at day 14. (**D**) Quantified data of CCR7$^+$CD45RA$^+$ T$_{SCM}$-phenotype based on identification strategy presented in (C) at day 14, n=4 healthy donors. (**E**) *Ex-vivo* CD127$^-$CD25$^+$ T$_{REG}$ depletion of CCR7$^+$CD45RA$^+$ T cell pool by FACS before expansion (left) and intracellular cytokine staining after antigenic restimulation to identify frequencies of CD137$^+$IFNγ$^+$ CD8$^+$ T$_{SCM}$ derived from CCR7$^+$CD45RA$^+$ T cell pool in the presence or absence of T$_{REG}$ at day 21 (right). (**F**) Intracellular cytokine staining after antigenic restimulation to identify frequencies of IFNγ$^+$TNFα$^+$ CD8$^+$ T$_{SCM}$ derived from T$_{SCM}$ pool in the presence or absence of T$_{REG}$ at day 14. (**G**) Intracellular cytokine staining after antigenic restimulation to identify frequencies of IFNγ$^+$IL-2$^+$ CD8$^+$ T$_{SCM}$ derived from a CCR7$^+$CD45RA$^+$ T cell pool in the presence or absence of CD4$^+$ irradiated feeder T cells compared to a CD4-depleted CCR7$^+$CD45RA$^+$ T cell pool at day 14.

Third, post-expansion selection on activated CD45RO$^+$ T$_{SCM}$ (after activation-induced CD45RA→CD45RO switch) significantly increased the frequency of antigen-specific CD8$^+$ T$_{SCM}$ from pre-enriched CCR7$^+$CD45RA$^+$ during cell culture (Fig. 8A-C).

In summary, IL-7/IL-15 cytokine culture supplementation, bulk untouched CD4$^+$ T cell support and CD45RO-based selection were established as robust and GMP-compatible culture strategies to purify an antigen-specific CD8$^+$ T$_{SCM}$ product (Fig. 8D).
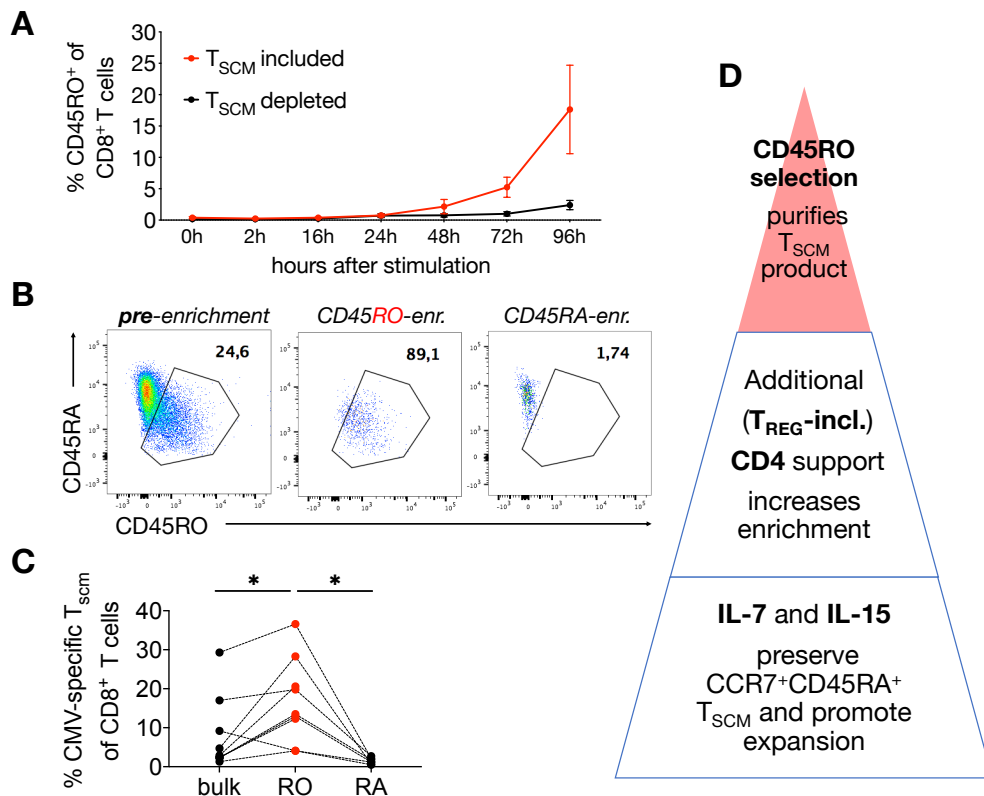

Figure 8: **CD45RO selection for improved CD8$^+$ T$_{SCM}$ expansion.** Cell culture protocol based on workflow presented in Fig. 7B. (**A**) Kinetics of CD45RO expression on CD8$^+$ T cells derived from a T$_{SCM}$ included CCR7$^+$CD45RA$^+$ T cell pool (bulk) versus a T$_{SCM}$ depleted

CCR7$^+$CD45RA$^+$ T cell pool after antigenic stimulation, n=9 healthy donors. (**B**) CD45RO selection (and CD45RA selection as control) by FACS of a CCR7$^+$CD45RA$^+$ T cell derived pool after antigenic stimulation at day 4. (**C**) Frequency of antigen-specific CD8$^+$ T$_{SCM}$ derived from a CCR7$^+$CD45RA$^+$ T cell pool, bulk versus selected on CD45RO/RA according to (B) and identified by antigenic restimulation at day 14. Wilcoxon rank-sum test applied, *p < 0.05, n=9 healthy donors. (**D**) Cell culture steps towards application of antigen-specific T$_{SCM}$ therapy.

5 Clinical Applications and Outlook

The mechanistic data reveal that the CXCR3 chemokine system modulates the function of human early differentiated CD8^{+} T cells in a migratory but also stimulatory manner. The clinical data reveal that the CXCR3 chemokine system is decisive for the response to chemotherapy in bladder cancer. Ultimately, early differentiated CD8^{+}CXCR3^{high} T cells were identified in the tumor microenvironment of MIBC, melanoma and renal cell carcinoma patients. The data suggest pan-cancer relevance of the intra-tumoral CXCR3 chemokine system for early differentiated CD8^{+} T cells and offer an experimental strategy to exploit CD8^{+}CXCR3^{high} T_{SCM} for adoptive T cell therapy approaches.

5.1 Human analysis unveils a stimulatory role of the CXCR3 chemokine system in early differentiated CD8^{+} T cell function and solid tumors

The approach of this thesis included a thorough functional investigation of the CXCR3 chemokine system in the human setting. Most murine models are markedly reduced in the complexity of the CXCR3 chemokine system *(45, 115)*. Notably, CXCR3 isoform expression and CXCL11 abundance showed high relevance within the experimental and clinical analyses of this thesis: **i)** the CXCR3 isoforms CXCR3A/alt positively and CXCR3B negatively associated with chemotherapy outcome and **ii)** CXCL11 was defined as a stimulatory regulator of T cell function. Hence, these data offer strong experimental and clinical arguments in favor of *human* studies that facilitate the dissection of the non-redundant function of the CXCR3 isoforms and CXCL9/10/11 chemokines within the versatile CXCR3 chemokine system.

Novel insights were gained in this thesis for the functional requirements of early differentiated CD8^{+} T cells in cancer disease. Their identified intratumoral niche in proximity to antigen presenting cells *(58)* and their pivotal role for response to anti-PD-1 ICB *(21)* is now complemented by their functional dependency on the CXCR3 chemokine system. First, the presented data indicate that early differentiated CD8^{+}CXCR3^{high} T cells preferentially locate in tumor-adjacent lymph nodes and are attracted by all three chemokines CXCL9/10/11. Second, early differentiated CD8^{+}CXCR3^{high} T cells are present in the tumor microenvironment and harbor a repertoire of CXCR3 isoforms, which defines their functional outcome to the chemokines. Third, the selective (tissue) abundance of CXCR3 chemokines may stimulate early differentiated CD8^{+} T cell function (*e.g.*, by CXCL11 expressed and presented by tumor infiltrating CD14^{+} macrophages). In summary, these data imply that early differentiated CD8^{+} T cell migration and T cell

survival can be regulated within one chemokine system based on two modalities, *i.e.*, chemokine receptor isoform expression and ligand abundance.

The functional dependency between receptor (CXCR3 isoforms) and ligands (CXCL9/10/11) was clinically relevant for MIBC patients, *i.e.*, utilizing intratumoral *co-abundance* of CXCR3alt and CXCL11 resulted into perfect separation of responder and non-responder patients. Hence, clinical benefits can be observed, when the CXCR3 chemokine system is complete, which facilitates an active immune axis between receptor and ligand. The pivotal cellular players within this immune axis are likely tumor infiltrating CD8+CXCR3alt+ T cells stimulated by CD14+CXCL11+ macrophages.

To broaden the mechanistic understanding, one important next step will be to assess single-cell expression of the CXCR3 isoforms in tumor infiltrating CD8+ T cells. In this thesis, the CXCR3 isoform repertoire of early differentiated CD8+ T cells was defined as CXCR3A/B/alt^{high} in peripheral blood of healthy humans and bladder cancer patients. Yet, the precise CXCR3 isoform state in tumor infiltrating early differentiated CD8+ T cells remains to be determined. This analysis could not be performed due to the snap freezing procedure of the tumor material that was necessary for biomarker evaluation.

This thesis lays the groundwork for clinical application of the CXCR3 chemokine system for cancer diagnostics, *i.e.*, predictive and prognostic strategies in solid tumors (**5.2**) and for therapeutic application to modulate the tumor microenvironment (**5.3**).

5.2 Biomarker validation in bladder cancer and transferability to other solid tumor diseases

A plethora of studies has pursued to identify biomarkers that predict NAC response in MIBC, however, robust stratification systems have not been translated for clinical use *(116–131)*. The primary clinical benefit of NAC stratification will be to exclude non-responder MIBC patients from ineffective chemotherapy. Non-responder patients have a high tendency to progress and metastasize which cannot be prevented by chemotherapy. The identification of non-response at the diagnostic stage could initiate immediate radical surgery that halts disease progression *(132)*. In this study, intratumoral CXCR3alt/CXCL11^{high} segregated all 9 responder patients from the 11 non-responder MIBC patients before NAC. In the TCGA cohort, an intratumoral CXCL11^{high} state before adjuvant chemotherapy associated with improved OS compared to a CXCL11^{low} state in 68 MIBC patients. These clinical data strongly support the utility of a CXCR3-based biomarker for NAC stratification and identification of non-responder patients. However, a

thorough validation study is required that applies the same study conditions to a second MIBC patient cohort including tumor sample preparation, ELISA-based analysis of CXCL11 protein and RT-qPCR analysis of CXCR3 isoform mRNA abundance. A validation study is already ongoing in joined work with Umea University, Sweden.

The transferability of a CXCR3-based biomarker to other tumor diseases is supported by the data within this thesis: melanoma tumors were confirmed to be strongly infiltrated by CD8+CXCR3high T cells and most importantly, protective early differentiated CD8+ T cells were identified to express CXCR3 within the melanoma immune infiltrate. Previous studies confirm a positive prognostic role of CD8+CXCR3high T cells for the outcome of melanoma patients and describe melanoma-directed recruitment of CD8+ T cells by the CXCR3 chemokine system *(39, 133, 134)*. Hence, combined analysis of intra-tumoral CXCR3 isoform and ligand abundance may be a promising biomarker candidate, *e.g.*, for patient stratification of routinely applied ICB treatment in progressed melanoma.

Early differentiated CD8+CXCR3high T cells were also identified in the tumor microenvironment of renal cell carcinoma patients within this study. Previous clinical studies unveiled an ambiguous prognostic role of the CXCR3 chemokine system in this disease, which may be explained by the additional involvement of CXCR3 in renal cancer cell function *(135, 136)*. In detail, renal cancer cell derived CXCR3A associates to metastatic function, whereas CXCR3B mediates apoptotic renal cancer cell signaling *(137, 138)*. Hence, cancer cell derived CXCR3 isoforms may impede the clinical utility of a biomarker that is based on the absolute abundance of the CXCR3 isoforms. Single-cell strategies are warranted to untangle renal cancer and immune cell related origin of the CXCR3 isoforms, which needs to set in relation to the clinical outcome, respectively. In addition, the role and cellular source of CXCR3alt remains to be elucidated in renal cell carcinoma.

Ultimately, gained mechanistic insights behind the CXCR3-based biomarker allow a hypothesis driven, yet adjustable approach for testing in other solid tumor diseases. A "copy and paste" approach (*i.e.*, analysis of intra-tumoral CXCR3alt-CXCL11) may be applicable in the disease of melanoma, whereas cell source characterization of the CXCR3 isoforms may result into a redefined CXCR3-based biomarker for patients with renal cell carcinoma. Moreover, patients with solid tumor diseases that receive analogous platinum-based NAC (*e.g.*, cohorts of breast cancer and rectal carcinoma patients) are of concrete interest to study for transferability of the CXCR3-based biomarker.

5.3 Therapeutic modulation of the tumor microenvironment by CXCR3-based approaches

The study describes the beneficial role of a T cell-inflamed tumor microenvironment equipped with an active state of the IFN-γ-associated CXCR3 chemokine system in treatment responding patients. In general, this supports an accepted immunotherapeutic concept that aims to induce a favorable "immune hot" tumor microenvironment *(139)*. In detail, the presented data confirm the role of stimulatory and regulatory CXCR3 components and strikingly, add new functional layers to the CXCR3 chemokine system. The dissection of the distinct CXCR3 components allows targeted strategies to modulate the expression and abundance of single CXCR3 isoforms and ligands. On the receptor side, CXCR3A-mediated stimulatory signaling (inducing cell chemotaxis) should be favored over CXCR3B-mediated inhibitory signaling (inducing apoptosis) in CD8$^+$ T cells *(45)*. This antagonistic function was supported in this study by the observed CXCR3A-related good prognostic versus a CXCR3B-related poor predictive outcome in MIBC patients. In addition, CXCR3alt-related immune stimulatory effects were identified as anti-cancer objectives. On the ligand side, this study describes CXCL9/10/11 as potential T cell migratory targets in confirmation with previous results *(140)*, and identified CXCL11 as a stimulatory therapeutic target for human CD8$^+$ T cell function. Hence, therapeutic interventions of the CXCR3 chemokine system can be categorized into **i)** CXCR3 isoform modulation and **ii)** CXCR3 ligand modulation.

CXCR3A and CXCR3alt are highly expressed in peripheral blood derived CD8$^+$ T$_{SCM}$ of healthy humans and cancer patients. Thus, CXCR3A/alt^{high} T$_{SCM}$ display a direct native source for the stimulatory CXCR3 isoforms accessible by the veinous blood system. This makes CD8$^+$ T$_{SCM}$ an attractive candidate for adoptive T cell therapy approaches in the solid tumor setting, *i.e.*, the transfer of antigen-specific T cells to the patient. In this thesis, first establishment steps were developed that seek to exploit the CXCR3A/alt^{high} *ex-vivo* state in CD8$^+$ T$_{SCM}$ for an autologous cell transfer set-up of antigen-specific T cell products. The presented data confirm the previously described stimulatory role of the common gamma-chain cytokines IL-7 and IL-15 for antigen-specific T$_{SCM}$ function *(141)*. Further, the data in this thesis highlight the relevance of a complete CD4$^+$ helper T cell pool for sufficient CD8$^+$ T$_{SCM}$ expansion and define a selection method based on activation induced CD45RO expression to accelerate T$_{SCM}$ enrichment. The parallel application of the identified culture requirements allows a GMP-compatible and robust enrichment process of antigen-specific CD8$^+$ T$_{SCM}$ from peripheral blood. The

established protocol is suitable for transfer to a neoantigen or tumor-associated antigen T_{SCM} expansion set-up. In a next step, T_{SCM} enrichment may be further enhanced by supplementation of the stimulatory chemokine CXCL11, as characterized in this thesis. However, a GMP-compatible production process of recombinant CXCL11 protein will be required for ultimate clinical translation. Moreover, T_{SCM} enriched populations may be used for genetic incorporation of, *e.g.*, chimeric antigen receptors (CAR) that ensure anticancer recognition. Further, genetical engineering by (viral or non-viral) knock-in strategies of CXCR3A/alt may be applied to enhance the migratory and survival function of established T cell products. Last, CXCR3A/alt may be directly targeted by agonizing antibodies that recognize these CXCR3 isoforms.

CXCR3B represents the opposing immune inhibitory CXCR3 molecule. In analogy to ICB, CXCR3B may be a reasonable blocking target by CXCR3B-specific antibodies in order to enhance CD8+ T cell function. In this study, the additionally investigated non-classical CXCR3B-ligand CXCL4 *(142–145)* did not interfere with CD8+ T_{SCM} function; neither in a stimulatory nor inhibitory manner. Hence, thorough mechanistical analysis of CXCR3B-ligand interactions will be pre-requisite before the initiation of blocking approaches to fully clarify the role of CXCR3B for early differentiated CD8+ T cell function.

CXCL9/10/11 were identified as potential targeting candidates to enhance tumor infiltration of early differentiated CD8+ T cells in humans. Future mechanistic studies that dissect non-redundant relevance for migratory function of CXCL9/10 or 11 in distinct tumor entities and tissue compartments (tumor versus lymph node) are pivotal to prioritize modulatory strategies on *one* CXCR3 chemokine. This study suggests that increased CXCL11 expression in the tumor microenvironment may favor both CD8+ T cell migration and CD8+ T cell survival. In addition, increased CXCL11 gene expression was observed in tumor-infiltrating CD14+ macrophages in this study. Notably, CXCL11 gene expression was previously reported to be STAT3-dependent *(146)*, to be induced by IFNγ and IFNβ (but not by IFNα) *(43, 147)* and enhanced by TNFα *(148)*. Cell-type specific modulation of CXCL11 expression (*e.g.*, by *ex-vivo* cell culture or genetical manipulation) may benefit ongoing antigen presenting cell based immunotherapeutic approaches *(149)*.

In summary, this thesis contributes to the mechanistic understanding how the human CXCR3 chemokine system modulates early differentiated CD8+ T cell function. Beyond diagnostic application, this opens new therapeutical paths for targeted modulatory strategies of the distinct human CXCR3 components in solid tumors.

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7 Statutory Declaration

"I, Tino Vollmer, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic "The human CXCR3 chemokine system in early differentiated CD8+ T cell function and solid tumors"/"Das humane CXCR3 Chemokinsystem in der früh differenzierten CD8+ T-Zellfunktion und in soliden Tumoren", independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

[In the case of having conducted your doctoral research project completely or in part within a working group:] Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

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Date   Signature

8 Detailed Description of Own Achievements

The publication: **Vollmer T**, Schlickeiser S, Amini L, Schulenberg S, Wendering DJ, Banday V, Jurisch A, Noster R, Kunkel D, Brindle N, Savidis I, Akyüz L, Hecht J, Stervbo U, Roch T, Babel N, Reinke P, Winqvist O, Sherif A, Volk HD, Schmueck-Henneresse M. **The intra-tumoral CXCR3 chemokine system is predictive of chemotherapy response in human bladder cancer.** *Sci Trans Med, 2021.*

Overall, I contributed with conceptualization of the study, planning and execution of experiments, selection and inclusion of the patients, analysis of data, composition of the figures and writing the manuscript.

Initially, I executed thorough literature search to identify new hypotheses and distinct methods for the investigation of the human CXCR3 chemokine system in CD8+ T cells and solid tumors. In accordance and discussions with Dr. Michael Schmueck-Henneresse, Prof. Dr. Hans-Dieter Volk and Prof. Dr. Petra Reinke, I set up the principal hypotheses, aims, experimental design and methods for this study.

Establishments: To complement existing T cell-based techniques of our group for this study, I established the setups for *flow cytometry panels of chemokine receptors*, *T cell chemotaxis assay* and the *chemokine receptor ligation assay*. In addition, and with support of Katrin Vogt, I designed the *RT-qPCR panel* for analysis of the CXCR3 isoforms including the blasting of the isoform-specific probes/primers, selection of HKG in bladder tumors, and the experimental establishment of the qPCR assays. To analyze protein and DNA/RNA from the inflammatory tumor environment, I established a *tumor lysis* method (including mechanical and chemical lysis steps) followed by a *protein and DNA/RNA extraction method*. For the tissue methods, I benefitted from the knowledge of Dr. Janosch Schoon in handling of murine tumors. In accordance with Prof. Winqvist, I established a set-up for *ex-vivo stimulation of node-derived CD8+ T cells* with tumor lysate to estimate tumor reactivity. All of these establishments were the basis for the majority of the data in the publication *(Fig. 1-7 and Supp. Fig. 1-11, 15)*.

Experimental data (Fig. 1,2 and Supp. Fig. 1, 3, 4, 5, 6): I conducted all T cell-based experiments, *e.g.*, flow cytometry and functional T cell assays including long-term cell culture in this study. Occasionally, Dr. Leila Amini, Sarah Schulenberg, Dr. Desiree J. Wendering, Anke Jurisch and Dr. Schmueck-Henneresse supported with, *e.g.*, medium change during cell culture. I prepared T cell samples for FACS, which was conducted by the Core Facility for Cell Sorting of the Berlin-Brandenburg Center for Regenerative

Therapies. I performed all analyses from T cell-based readouts (*e.g.*, flow cytometric gating/compensation/analysis and qPCR normalization/analysis) in accordance with Dr. Schmueck-Henneresse.

Clinical data bladder cancer, 1st cohort (Fig. 3-7 and Supp. Fig. 2, 3, 8, 9): In the primary clinical study, I conducted most of the experiments and was supported by my colleagues in handling multiple patient specimens. Initially and in accordance with Dr. Amir Sherif, I selected the patients and set-up the chemotherapy response criteria for this study. I lysed and processed the tumor samples with support of Anke Jurisch, extracted DNA/RNA with support of Sarina Richter and performed qPCR with support of Rebecca Noster. The Luminex analysis was performed at the Immunological Study Lab of the BCRT and Dr. Sherif and Kerstin Almroth helped with the initial clinical sampling of bladder tumors after surgery. I contributed to the application of TCR sequencing for this study together with Dr. Ulrik Stervbo who analyzed the data. Alltogether, I analyzed the clinical data in accordance with by Dr. Schlickeiser and Dr. Schmueck-Henneresse. Further, I supported Dr. Schlickeiser who applied *in-silico* methods to analyze the protein data (clustering in heatmap) and established the logistic regression model including the biomarkers (CXCR3alt and CXCL11) challenged by cross validation techniques.

Clinical analysis 2nd bladder cancer cohort and melanoma/renal cell carcinoma (Supp. Fig. 10-14): I selected the appropriate patient cohort in the TCGA data bank and the scRNA sequencing data from recent studies on intra-tumoral T cell states and tumor-infiltrating immune cells based on our hypotheses. I supported Dr. Schlickeiser who accessed the data and I supported him throughout the analysis (spectral seriation and dimensionality reduction techniques).

Eventually, I interpreted and reviewed all data from the initial manuscript submission, data generated during the revision process and data from the CD8+ T_{SCM}-based adoptive T cell therapy approaches and discussed it together with all co-authors, mainly with Dr. Schmueck-Henneresse, Prof. Dr. Volk and Prof. Dr. Reinke. I designed the graphs for the publication and wrote the initial version of the manuscript, which was optimized mainly by Dr. Michael Schmueck-Henneresse, and by all co-authors. During the revision process, I designed new data graphs and improved the manuscript together with Dr. Michael Schmueck-Henneresse based on the reviewers` comments.

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Datum   Unterschrift

9 Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2018** Selected Editions: SCIE,SSCI
Selected Categories: **"CELL BIOLOGY"** Selected Category Scheme: WoS
Gesamtanzahl: 193 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	NATURE REVIEWS MOLECULAR CELL BIOLOGY	45,869	43.351	0.091360
2	CELL	242,829	36.216	0.571850
3	NATURE MEDICINE	79,243	30.641	0.162840
4	CANCER CELL	36,056	23.916	0.091050
5	Cell Metabolism	34,829	22.415	0.099550
6	Cell Stem Cell	24,628	21.464	0.087030
7	CELL RESEARCH	15,131	17.848	0.038680
8	NATURE CELL BIOLOGY	40,615	17.728	0.082430
9	Science Translational Medicine	30,485	17.161	0.121980
10	TRENDS IN CELL BIOLOGY	14,380	16.588	0.034120
11	MOLECULAR CELL	62,812	14.548	0.170680
12	NATURE STRUCTURAL & MOLECULAR BIOLOGY	27,166	12.109	0.069440
13	EMBO JOURNAL	65,212	11.227	0.067930
14	Autophagy	16,161	11.059	0.032630
15	TRENDS IN MOLECULAR MEDICINE	9,946	11.028	0.018900
16	Journal of Extracellular Vesicles	3,675	11.000	0.012110
17	Annual Review of Cell and Developmental Biology	9,734	10.833	0.016750
18	AGEING RESEARCH REVIEWS	6,539	10.390	0.015890
19	CURRENT BIOLOGY	60,772	9.193	0.135820
20	DEVELOPMENTAL CELL	28,572	9.190	0.068550

Selected JCR Year: 2018; Selected Categories: "CELL BIOLOGY"

10 Publication

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

CANCER

The intratumoral CXCR3 chemokine system is predictive of chemotherapy response in human bladder cancer

Tino Vollmer[1,2,3], Stephan Schlickeiser[1,2], Leila Amini[1,2,3], Sarah Schulenberg[2], Desiree J. Wendering[1,2,3], Viqar Banday[4,5], Anke Jurisch[1,2,3], Rebecca Noster[2], Desiree Kunkel[2], Nicola R. Brindle[2], Ioannis Savidis[2], Levent Akyüz[1,2], Jochen Hecht[6,7], Ulrik Stervbo[8], Toralf Roch[2,8], Nina Babel[2,8], Petra Reinke[2,3], Ola Winqvist[9], Amir Sherif[4], Hans-Dieter Volk[1,2], Michael Schmueck-Henneresse[1,2,3]*

Chemotherapy has direct toxic effects on cancer cells; however, long-term cancer control and complete remission are likely to involve CD8+ T cell immune responses. To study the role of CD8+ T cell infiltration in the success of chemotherapy, we examined patients with muscle invasive bladder cancer (MIBC) who were categorized on the basis of the response to neoadjuvant chemotherapy (NAC). We identified the intratumoral CXCR3 chemokine system (ligands and receptor splice variants) as a critical component for tumor eradication upon NAC in MIBC. Through characterization of CD8+ T cells, we found that stem-like T cell subpopulations with abundant CXCR3alt, a variant form of the CXCL11 receptor, responded to CXCL11 in culture as demonstrated by migration and enhanced effector function. In tumor biopsies of patients with MIBC accessed before treatment, CXCL11 abundance correlated with high numbers of tumor-infiltrating T cells and response to NAC. The presence of CXCR3alt and CXCL11 was associated with improved overall survival in MIBC. Evaluation of both CXCR3alt and CXCL11 enabled discrimination between responder and nonresponder patients with MIBC before treatment. We validated the prognostic role of the CXCR3-CXCL11 chemokine system in an independent cohort of chemotherapy-treated and chemotherapy-naïve patients with MIBC from data in TCGA. In summary, our data revealed stimulatory activity of the CXCR3alt-CXCL11 chemokine system on CD8+ T cells that is predictive of chemotherapy responsiveness in MIBC. This may offer immunotherapeutic options for targeted activation of intratumoral stem-like T cells in solid tumors.

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11 Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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12 Complete List of Publications

T cell-based system for stratified application of chemotherapy (thesis)

Vollmer T, Schlickeiser S, Amini L, Schulenberg S, Wendering DJ, Banday V, Jurisch A, Noster R, Kunkel D, Brindle N, Savidis I, Akyüz L, Hecht J, Stervbo U, Roch T, Babel N, Reinke P, Winqvist O, Sherif A, Volk HD, Schmueck-Henneresse M
The intra-tumoral CXCR3 chemokine system is predictive of chemotherapy response in human bladder cancer
Science Translational Medicine, 2021

Cell model for toxicity testing in uremic patients

Vollmer T, Ljungberg B, Jankowski V, Jankowski J, Glorieux G, Stegmayr BG
An in-vitro assay using human spermatozoa to detect toxicity of biologically active substances
Scientific Reports, 2019

T cell product for adoptive immunotherapy, characterization

Amini L, **Vollmer T**, Wendering DJ, Jurisch A, Landwehr-Kenzel S, Jürchott K, Volk HD, Reinke P, Schmueck-Henneresse M
Comprehensive characterization of a next-generation antiviral T-cell product and feasibility for application in immunosuppressed transplant patients
Frontiers in Immunology, 2019

T cell product for adoptive immunotherapy, knockout for specific application

Amini L, Wagner DL, Uta Rössler, Zarrinrad G, Wagner LF, **Vollmer T**, Kornak U, Volk HD, Reinke P, Schmueck-Henneresse M
CRISPR-Cas9-Edited Tacrolimus-Resistant Antiviral T Cells for Advanced Adoptive Immunotherapy.
Molecular Therapy, 2020

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