1.4 Aims and outline of this thesis

This thesis aims to substantiate the therapeutic benefit of loco-regional immune modulation in early stage melanoma. It does so by adding to the insight in the loco-regional biology and pathophysiology of the immune system in early stage melanoma by describing the physiological and melanoma-induced pathophysiological effects in the SLN. Through clinical studies in which immune modulatory agents GM-CSF and CpG-B are locally administered, we aim to reverse these melanoma-induced immunosuppressive effects, raise an effective anti-tumor immune response, and ultimately improve the prognosis of early stage melanoma patients.

Chapter 1 serves as a general introduction in which the historical background of melanoma is highlighted, followed by the epidemiology, staging principles and some general comments on the etiology and genetics of melanoma. Current and past treatment modalities, approved for melanoma, are delineated with an emphasis on immunotherapy with its recent breakthrough events. The history and current position of the SLN in clinical medicine and research is described as well as accumulated knowledge on the immune response in relation to melanoma and the immunotherapeutic windows these accomplishments have opened.

In Chapter 2, four cDC subsets from the melanoma SLN are presented and phenotypically and functionally characterized through multiparameter flow cytometric and functional analysis. Two CD1a⁺ skin derived cDC subsets (i.e. LCs and DDCs) are distinguished and two CD1a⁻CD11c⁺BDCA3⁺ cDC subsets, presumably derived from the blood. Despite their phenotypically higher maturation state, the CD1a⁺ skin derived cDC subsets proved inferior T-cell activators compared with the CD1a⁻ subsets residing in the lymph nodes. These data represent the first functional characterization of human lymph node cDC subsets and supply new leads for the development of human DC-targeted immunotherapies.
As an addendum to Chapter 2, Chapter 2.1 presents a response to a letter to the editor of Blood by Gerlini et al. claiming that LCs in melanoma SLNs are immature, at variance with our data represented in Chapter 2 [148]. In response, we show that this difference in LC maturation in melanoma SLNs may be explained by the difference in patient groups between both studies: both Breslow thickness (i.e. invasion depth of the primary melanoma) and the time interval between primary melanoma excision and SLN harvest play a significant role in the maturation status of LCs in the melanoma SLN.

Chapter 3 builds on these observations and further explores the immunosuppressive effects of melanoma on the regional immune system by determining the maturation and activation state of SLN DC subsets in relation to melanoma stage. In addition, melanoma induced suppressive effects on the T cell compartment are described. Whereas in stage I and II melanoma, increased Breslow thickness is associated with progressive suppression of skin-migrated CD1a+ DC-subsets, LN-resident DC-subsets and T cells are only affected once metastasis to the SLN occurs (i.e. stage III disease). Follow up of these patients showed that local melanoma recurrence is correlated to lower frequencies of skin-migrated CD1a+ DC-subsets, whereas distant recurrence and reduced melanoma-specific survival are associated with reduced maturation of LN-resident DC-subsets. These findings offer a rationale to target migratory as well as LN-resident DC subsets for early immunotherapeutic interventions to prevent local and distant melanoma recurrence and spread.

In Chapter 4, a clinical phase II trial is presented which aims to target the migratory as well as LN-resident DC subsets by locally administering GM-CSF and/or the TLR9-activating agent CpG-B in early stage melanoma patients. 28 Clinically stage I-II melanoma patients were randomized to receive intradermal injections around the primary tumor excision site of saline or low-dose CpG-B, alone or combined with GM-CSF, before excision of the SLN. Combined CpG/GM-CSF administration resulted in enhanced maturation of the conventional as well as
plasmacytoid SLN DC subsets and selectively induces increased frequencies of LN-resident BDCA3+ cDC subsets. Correlative in vivo analyses and in vitro studies provide evidence that these subsets are derived from BDCA3+ cDC precursors in the blood that are recruited to the SLN in a type I IFN-dependent manner and subsequently mature under the combined influence of CpG and GM-CSF. In line with their reported functional abilities, frequencies of these BDCA3+ DCs correlated with increased cross-presenting capacity of SLN suspensions. Combined local CpG/GM-CSF delivery thus supports protective anti-melanoma immunity through concerted activation of pDC and cDC subsets and recruitment of BDCA3+ cDC subsets with T cell stimulatory and cross-priming abilities.

Chapter 5 describes the subsequent effects of local administration of CpG and GM-CSF on effector and regulatory T and NK cell subsets in the melanoma SLN. On the one hand, effects favoring anti-tumor immunity like lower CD4/CD8 ratios, Th1 skewing, possible recruitment of effector NK cells and increased frequencies of melanoma-specific CD8+ T cells are presented. But, on the other hand, these immune-potentiating effects are shown to be counterbalanced by increased IL-10 production by T cells and significantly higher levels of FoxP3 and CTLA4 in Tregs with correspondingly higher suppressive activity in the SLN. The significantly lower numbers of SLN metastases observed in the CpG/GM-CSF administered patients compared to the saline control patients however, suggest that the increase in local suppressive markers after CpG/GM-CSF administration does not outweigh the immune stimulatory properties of these agents, but that additional measures to minimize loco-regional Treg activity might optimize clinical efficacy of CpG and GM-CSF as immune-potentiating agents for early stage melanoma patients.

To facilitate functional testing of T cells from small SLN samples, high-efficiency polyclonal T cell expansion is required. In the comparative study described in Chapter 6, melanoma SLN cells were expanded via classic methodologies with anti-CD3/CD28 antibodies and with the K32/4-1BBL artificial
APC system, and analyzed for responsiveness to common recall or TAA-derived peptides. K32/4-1BBL-expanded T cell populations contained significantly more effector/memory CD8+ T cells. Moreover, recall and melanoma antigen-specific CD8+ T cells were more frequently detected in K32/4-1BBL-expanded samples as compared with anti-CD3/CD28-expanded samples. Thus, K32/4-1BBL aAPC appear to be superior to anti-CD3/CD28 antibodies for the expansion of in vivo-primed specific CD8+ T cells and their use may facilitate the sensitive monitoring of functional anti-tumor T cell immunity in SLN.

Chapter 7 presents the long term follow-up results of the randomized phase II trial described in chapter 4 and 5, combined with the follow-up results of a previous trial on the local administration of CpG-B as a single agent in early stage melanoma patients. Identical enrollment criteria and consistent immune modulating effects of CpG-B in both trials support this pooled analysis. After a median clinical follow-up of 88.8 months, a clearly improved recurrence-free survival (RFS) for the CpG/GM treated patients as compared with the placebo (saline) controls was shown (p=0.008). Also for pathologically confirmed stage I–II patients separately, a significant difference in RFS in favor of the treated group was demonstrated (p=0.02). These clinically favorable results were further supported by a pooled analysis of immune monitoring data from both trials which confirmed the selective recruitment and activation of both CD14- and CD14+ LN-resident cDC subsets with cross-presentation ability in the melanoma SLN of patients receiving local CpG-B injections as described in Chapter 4. Combined T cell monitoring data confirmed that higher melanoma-specific T-cell response rates were found in SLN of CpG-B–treated patients as put forward in Chapter 5. In addition, complementary peripheral blood T-cell monitoring showed that systemic melanoma-specific T-cell response rates were also significantly increased, indicative of systemic immune protection following local CpG-B administration, consistent with an observed near-significant improvement in distant RFS upon local CpG-B and/or GM-CSF administration. In conclusion, local low dose CpG-B administration in early stage melanoma patients
provides an adjuvant treatment option offering durable protection for a large group of patients currently going untreated despite being at considerable risk for disease recurrence.

The studies described in this thesis are further discussed and integrated in Chapter 8 which serves as a general discussion and offers a view on future prospects. It positions our findings in the context of recent trials on (neo-)adjuvant treatment with immune checkpoint inhibitors in patients with stage II and III melanoma, and proposes a possible clinical decision tree for therapy options in early and advanced stage melanoma.