IMmune MOdulating strategies for treatment of MErkel cell Carcinoma

Project Final Report

Grant Agreement Nº:	277775
Project Acronym:	IMMOMEC
Project Title:	IMmune MOdulating strategies for treatment of MErkel cell Carcinoma
Funding Scheme:	Collaborative Project (CP-STREP)
Date of latest version of Annex I against which the assessment will be made:	15th June 2015
Period Covered:	from M1 to M60 (1st January 2011 to 31st December 2016)

Project Coordinator Name:	Prof. Dr. Dr. Jürgen C. Becker
Project Coordinator's Organization:	Medical University of Graz (MUG)
Phone:	+43 316 385 71652 and +49 201 183 6727
Fax:	+43 316 385 72030 and +49 201 183 6945
E-mail:	juergen.becker@medunigraz.at j.becker@dkfz.de
Project Website:	http://www.immomec.eu
Date of Preparation:	27th February 2017

1. Final Publishable Summary Report

1.1. Executive Summary

Epidemiologic data suggest that there are approximately 2500 new Merkel cell carcinoma (MCC) cases per year within the EU; approximately 1000 of these patients will die from their disease. Although MCC is 40 times less common than melanoma, MCC has a dramatically higher mortality rate than melanoma rendering MCC as the most lethal skin cancer (37 versus 15 percent). This high mortality rate is largely due to the fact that until recently none of the available therapeutic interventions improved the overall survival of patients suffering from metastatic disease. Consequently, new therapeutic strategies were needed for metastatic MCC. Since several lines of evidence indicate the outstanding immunogenicity of MCC, immune modulating treatment strategies are of particular interest.

IMMOMEC was a project at the frontline of therapeutic, translational and medical research. The project tried to establish a novel immunotherapy taking advantage of the innovative concept of antibody-targeted cytokines to enrich the respective immune modulating agent at the tumor site. Until recently, treatment of MCC patients was solely based on anecdotal observations and case series, which implies a very low level of evidence. The prospectively randomized European multicenter clinical trial conducted within IMMOMEC aimed at establishing the clinical impact and immunological effects following therapeutically intended immune modulation by targeted interleukin-2 (IL2) (WP1).

However, the therapeutic landscape for advanced MCC changed dramatically and unexpectedly since the initiation of IMMOMEC in the beginning of 2011. At that time no competing therapeutic trials for advanced MCC patients existed. Early after initiation of the IMMOMEC trial, 3 competing trials had been activated which were designed for the same target population of metastatic MCC. Once preliminary data from these trials suggested a high efficacy of PD-1/PD-L1 blocking antibodies, recruitment into our trial comparing an immune intervention arm to a chemotherapy arm had become virtually impossible. Thus, while this was very fortunate for the patients suffering from advanced MCC, it was unfortunate for the IMMOMEC trial, which subsequently did not reach sufficient recruitment to provide any significant results with respect to the clinical efficacy of targeted cytokine therapy.

Nevertheless, IMMOMEC did not only aim at a new therapeutic option for MCC patients, but also at establishing new tools to monitor patients receiving immune modulating therapies in MCC (WP3, WP4, and WP5) as well as to compile prognostic and predictive biomarkers of MCC helping to individualize therapeutic indications (WP2 and WP5). To

this end the IMMOMEC project was very successful as the consortium reached the objectives strived for:

(i) to identify MCC-specific T-cell epitopes (WP3),
(ii) to establish the logistics to collect high quality peripheral blood lymphocytes (PBL) from patients across Europe recruited into the IMMOMEC trial, which are suitable for detailed characterization of specific T-cell responses (WP4)
(iii) to identify a blood-based surrogate biomarker for tumor burden of MCC (WP2)
(iv) to identify and characterize immune escape mechanisms of MCC and means to counteract them (WP2 and WP5)
(v) to establish methods to scrutinize tumor tissues with respect to the inflammatory infiltrate both at a morphological and molecular level including the T-cell receptor repertoire usage (WP2 and WP5)

However, due to the low recruitment into the trial and thus the limited number of patients receiving targeted IL2 as immunotherapy, no expedient correlative studies of the results obtained by the analyses addressing the systemic and localized immune responses could be performed. Instead we focused on comparative studies on these systemic and localized immune responses (WP5).

Since the IMMOMEC clinical trial was open for recruitment and ongoing therapy close until the end of the project, only some of the results have been published to date. However, the consortium has a specific list of publication plans for the next months.

Some of the translational data and results from IMMOMEC were presented at an open IMMOMEC International Symposium in Maastricht, The Netherlands, November 16th, 2016. On this occasion the stakeholders, IMMOMEC consortium members, invited guests as well as other participants were involved in fruitful discussions on how to use the obtained results from IMMOMEC, together with evidence from the literature and personal experiences to improve the clinical management of advanced MCC patients.

IMMOMEC has established a webpage at www.immomec.eu, which provides further information on the project and its results. The consortium aims at maintaining and updating this website to allow communication for future projects addressing immune modulating strategies for the treatment of Merkel cell carcinoma.

1.2. Project Context and Objectives

1.2.1. Project context

IMMOMEC represents a collaborative research effort of 9 academic and 2 industrial partners (SMEs) from 8 European countries.

MCC is a highly aggressive and often lethal neuroendocrine cancer of the skin, associated with the recently discovered common Merkel cell polyomavirus (MCPyV) or with chronic UV exposure. Epidemiologic data suggest that there are approximately 2500 new MCC cases per year within the EU; approximately 1000 of these patients will die from their disease. The incidence of MCC is considerably increasing: The reported incidence has more than tripled over the past 20 years. This increase can partially be explained by the demographic development, since MCC usually affects the elderly. The median age at diagnosis lies in the 8th decade of life, and there is a 5- to 10-fold increase in incidence after age 70 as compared with an age less than 60 years. Thus, it is likely that in an ageing European population the impact of this deadly cancer will further increase continuously. However, preliminary data from a MCC registry created within IMMOMEC suggest that besides an increasing incidence, the age distribution of patients is slowly shifting towards younger patients.

Notably, MCC has a dramatically higher mortality rate than melanoma, rendering MCC as the most lethal skin cancer. This high mortality rate is largely due to the fact that until recently none of the available therapeutics improved the overall survival of patients suffering from metastatic disease. Consequently, new therapeutic strategies were needed for metastatic MCC.

Since several lines of evidence indicate the outstanding immunogenicity of MCC, immune modulating treatment strategies are particularly attractive. To this end, the fundaments of the IMMOMEC project were based on a prospectively randomized phase II trial investigating the safety and efficacy of an innovative immunotherapy of advanced MCC (WP1). In detail, the trial was set up to compare the clinical efficacy of an - at that time - common chemotherapeutic intervention with a combination of this chemotherapeutic with an immune modulating therapy, i.e. the targeted delivery of IL2 to the tumor microenvironment by the tenascin C-reactive immunocytokine F16-IL2. The primary endpoint of this trial was overall survival; secondary endpoints included safety and the induction/boost of MCC-specific cellular immune responses. The translational research program, however, was not restricted to the identification and monitoring of MCC-specific T cell epitopes (WP3 and WP4), but also included the identification of blood and tissue based biomarkers of MCC as well as the detailed analysis of possible immune escape mechanisms (WP2 and WP5). Finally, two work packages covered coordination and management of the consortium (WP6), as well as dissemination and exploitation of the results (WP7).

All work packages with the exception of WP5 were run in parallel. Thus, the three main research areas of IMMOMEC comprised clinical testing, immune monitoring and biomarker identification.

1.2.2. Project Objectives

Specific objectives of IMMOMEC, which are also reflected in the respective work-package structure, include:

I. Establish an effective therapy for MCC evaluated in a multicentre randomized clinical phase II trial, thereby demonstrating the feasibility of immunotherapy for solid cancers (**WP1** Randomized phase II trial paclitaxel alone versus paclitaxel in combination with F16-IL2)
II. Identification of prognostic and predictive biomarkers (**WP2** Identification of biomarkers predicting patient prognosis, treatment outcome and immune response)
III. Identification and characterization of HLA-restricted immunodominant T cell epitopes specific for MCC to monitor the immune modulating effect and to develop specific therapeutics (**WP3** Identification and characterization of HLA-restricted immunodominant MCC-associated T cell epitopes; **WP4** Immune monitoring of MCC patients under therapy **& WP5** Correlation of systemic immunological responses and clinical benefit: impact of the microenvironment, immune escape mechanisms and regulatory circuits)
IV. Establish a European network for research and therapy of MCC (**WP6** Management of the project and the consortium & **WP7** Exploitation and dissemination)

The detailed objectives of the respective work packages were:

WP1	• Implementation and execution of a phase-II clinical trial investigating the efficacy of the immunocytokine F16-IL2 plus paclitaxel versus paclitaxel alone for the therapy of patients with metastatic Merkel cell carcinoma (MCC)
WP2	• Identification of surrogate, prognostic and/or predictive biomarkers of metastatic MCC
WP3	• Identification and characterization of immunodominant T cell epitopes restricted by HLA-A1, A2, A3, A24 derived from the MCPYV T antigens and novel MCC-associated novel antigens • Monitoring of immune responses against the identified peptides in normal donors and MCC patients
WP4	• Standardized collection of high quality PBMC patient samples in the multi-center trial by a proven European laboratory network.

	• Determination of immunological efficacy (proof of concept) of F16-IL2 by detecting and characterizing T cell activation • Validation of the relevance of novel MCPYV epitopes identified in WP3 will establish these as parameter for monitoring or even therapeutic applications in future MCC trials • Identification of new cellular markers and potential surrogates for immunological efficacy of F16-IL2 to discover patient populations with a better chance of clinical response
WP5	• Correlation of immunological and clinical responses to F16-IL2 immunotherapy • Correlation of systemic and intratumoral immune responses • Identification and characterization of immune escape mechanisms of MCC • Establish the impact of the microenvironment on the immune-mediated tumor eradication

Within **WP6** and **WP7** the Medical University of Graz (MUG) coordinated and managed IMMOMEC. The coordinator and his team managed the effective implementation of the ambitious work plan to ensure coordinated progress towards the projects aims and the optimal dissemination of its expected results to obtain optimal visibility. In addition, dissemination activities included the set-up and maintenance of the project website (www.immomec.eu), two public Workshops and a public International Symposium with invited external experts, in which IMMOMEC's results were discussed in the context of current evidence from the literature and personal experiences of experts in MCC to improve the clinical management of advanced MCC patients.

These seven WPs are closely interrelated (Figure 1). The consortium executing all tasks of the respective WPs comprised the following beneficiaries (Table 1), covering the different European countries to a large extent (Figure 2).

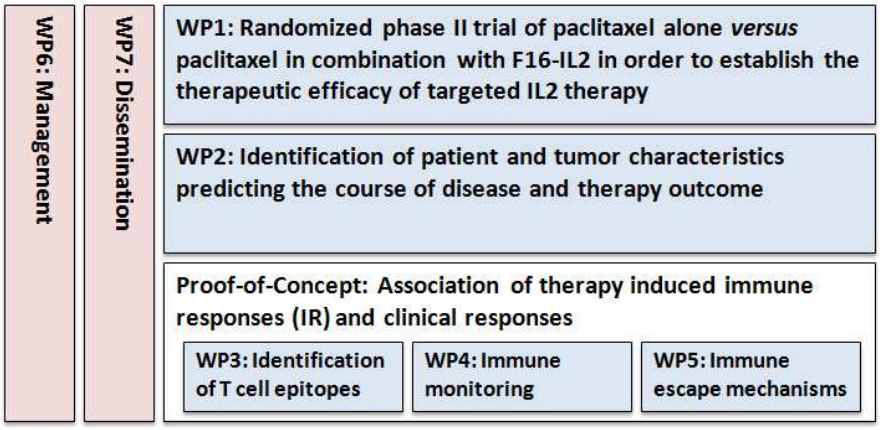


Figure 1. IMMOMEC work packages and their interdependencies

Table 1. List of Beneficiaries

Participant Number	Participant name	Participant short name	Country	Date enter project	Date exit project
1 (CO)	MEDICAL UNIVERSITY GRAZ	MUG	Austria	1	60
2	PHILOGEN SPA	PHG	Italy	1	60
3	IMMATICS BIOTECHNOLOGIES	IMM	Germany	1	60
4	REGION HOVEDSTADEN	CCIT	Denmark	1	60
5	ASSISTANCE PUBLIQUE - HOPITAUX DE PARIS	APHP	France	1	60
6	FUNDACIO PRIVADA CLINIC PER A LA RECERCA BIOMEDICA	FCRB	Spain	1	60
7	CHARITE - BERLIN	Charite	Germany	1	60
8	EBERHARD KARLS UNIVERSITAET TUEBINGEN	CDO	Germany	1	60
9	THE UNIVERSITY OF NOTTINGHAM	UNOTT	United Kingdom	1	36
10	UNIVERSITY CLINIC ESSEN	UKEssen	Germany	12	60
11	MARIA SKLODOWSKA-CURIE MEMORIAL CANCER CENTER	MCMCC	Poland	12	60

Figure 2: IMMOMEC Partners. Geographical distribution of the participating trial centers across Europe

1.3. The IMMOMEC Work Packages and Major Scientific Results

1.3.1. WP1 - Randomized phase II trial of paclitaxel alone versus paclitaxel in combination with F16-IL2 in order to establish the therapeutic efficacy of targeted IL2 therapy

The IMMOMEC trial was a phase II study of the tumour-targeting human F16-IL2 monoclonal antibody-cytokine fusion protein in combination with paclitaxel versus paclitaxel alone in patients with Merkel cell carcinoma.

This study was thought to explore an innovative immunochemotherapy for MCC which is based on the targeted delivery of interleukin-2 (IL2) to the tumour microenvironment via the armed antibody F16-IL2.

Study design and management of regulatory issues.

This phase II study was aimed at determining the anti-cancer activity of F16-IL2 in combination with paclitaxel in patients with advanced MCC, as measured by overall survival (OS) rate at 12 months after beginning of study treatment (primary objective). As secondary objectives, the study aimed at investigating safety and tolerability of the combination treatment of F16-IL2 and paclitaxel as well as efficacy in terms of response to treatment measured as overall response rate (ORR, rate of complete plus partial responses) or disease control rate (DCR, rate of complete plus partial responses plus disease stabilizations).

The preparation of the Clinical Study Protocol was an important commitment for the Sponsor, and for the Coordinator of IMMOMEC, together with all the Principal Investigators that were in charge of the trial. An unanimous agreement on the protocol was difficult, as the sponsor for safety reasons demanded an age limitation, which, however, was regarded by the clinical partners as a restrictive condition for a sufficient recruitment; a compromise was achieved in October 2012.

The Clinical Trial was planned to take place at nine different sites in seven countries:

1. Medizinische Universität Graz - MUG - Austria – Prof. J.C. Becker
2. Region Hovedstaden / CCIT Denmark – Dr. A. Krarup-Hansen
3. Assistance Publique - Hopitaux De Paris –APHP- France- Prof. C. Lebbè
4. Fundacio Privada Clinic Per A La Recerca Biomedica –FCRB- Spain – Prof. S. Puig
5. Charite - Universitätsmedizin Berlin - Germany – Dr. F. Kiecker
6. Eberhard-Karls-Universität Tübingen -CDO - Germany - Prof. C. Garbe
7. University of Nottingham – UNOTT - United Kingdom – Prof. P. Patel
8. Universitätsklinikum Essen –UKEssen - Germany – Prof. D. Schadendorf
9. Maria Sklodowska-Curie Memorial Cancer Center - MCMCC – Prof. P. Rutkowski

As a start, the approval of the different regulatory authorities of each country in which the trial was going to be managed was needed. An important number of documents were

prepared and submitted by the Sponsor to the Competent Authorities (CA) and Ethics Committees (EC) involved. When preparing the proposal, the time frame to obtain the approval was unpredictable, due to the number of CAs and ECs involved. Preparation and collection of documents from investigators and submissions of documents was done as scheduled, nevertheless ECs and CAs had different timelines in answering and requesting modifications or new documents. Approval timescales did not depend on the beneficiaries but on the authorities. The duration of the procedure varied from country to country and from EC to EC within the same country. In some countries, such as Spain and France, more details and documents were asked, which made regulatory processes more time consuming when compared to other countries such as e.g. Austria.

In the first 18 months, applications to Competent Authorities had been finalized in Germany (PEI), Austria (BASG), UK (MHRA), France (AFSSAPS), Spain and Denmark. MHRA and BASG had already approved the study in UK and in Austria, respectively. The submission to the Ethics Committees were completed in Graz (Austria), Berlin, Tübingen and Essen (Germany), Nottingham (UK), Barcelona (Spain) and Paris (France). Approval of the Clinical Trial was achieved from Competent Authorities in UK, Austria and Germany and from the Ethics Committees in Graz, Tübingen, Berlin, Barcelona.

In the second reporting period, i.e. month 19 to 36, the submission to Competent Authority in Poland and to Ethics Committees in Poland and Denmark was completed. We received approvals from the Competent Authorities in Germany, France, Spain, Denmark and Poland. The Ethics Committees of Paris, Essen, Copenhagen and Warsaw, besides, gave favorable opinion to the Study.

Basically, we received full approvals in seven sites which actually recruited into the Clinical Trial (Table 2). Two centers that were initially planned, Graz and Nottingham, did not take part in the multicenter study, due to internal difficulties. The fully authorized centers subsequently started recruiting patients.

Table 2. Summary of site initiation dates

Site	Date
Tübingen	24/9/2013
Paris	21/01/2014
Essen	24/3/2014
Barcelona	10/4/2014
Berlin	10/9/2014
Warsaw	25/11/2014
Copenhagen	9/12/2014

F16-IL2 is a novel bio-therapeutic agent, which had been tested so far up to a maximum dosage of 45 Mio IU. F16-IL2 is a recombinant fusion protein composed of a fully human recombinant monoclonal antibody (F16), and human recombinant interleukin-2 (IL2) (Figure 3). Clinical-grade F16-IL2 was manufactured in Philogen's GMP facility in sufficient amount for the execution of the clinical trial. Philogen owns a 2'000 m² GMP production facility, authorized to produce antibodies in mammalian cells. The production of biologic medicines is more complex and more variable than the production of chemical drugs. For such reason, manufacturing of biologics does not only require more steps, but also more stringent regulation and control of the processes. Critical quality attributes define the product properties of safety and efficacy, based on a thorough understanding of the product, including molecular properties and experience from pre-clinical and clinical evaluations. Quality controls were set in place to verify that manufacturing processes result in products of desired quality.

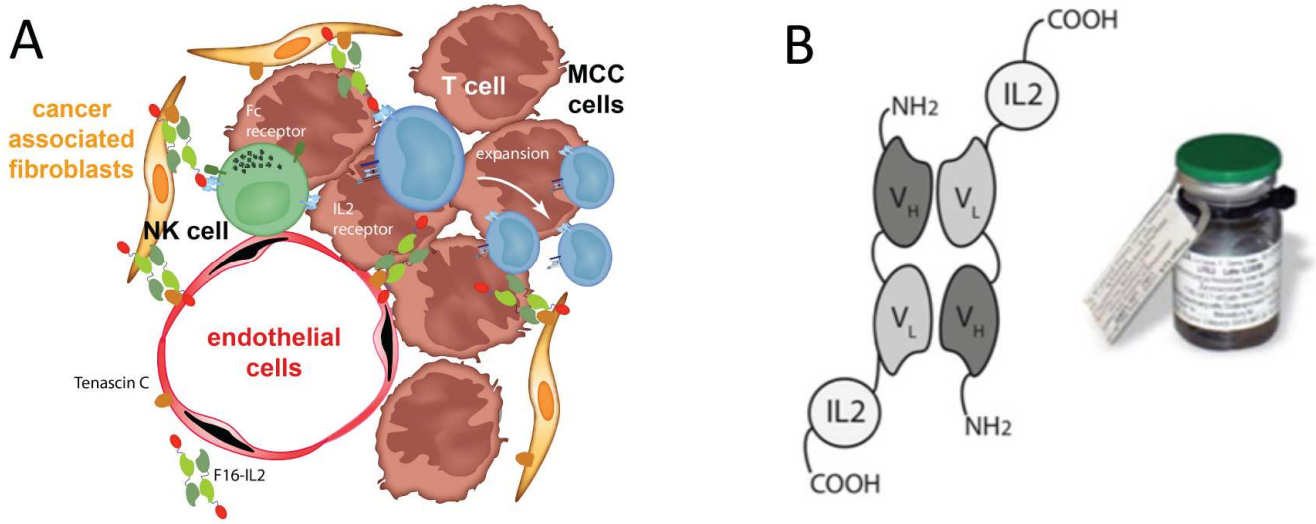


Figure 3. The immunocytokine F16-IL2. (A) F16-IL2 binds to tenascin C expressed by stromal cells of MCC tumors, and boost cellular immune responses (B) F16-IL2 consists of a stable non-covalent homodimer of the scFv F16 sequentially fused to human IL2, a strong pro-inflammatory cytokine. The N- and C-termini of the molecule as well as the VH and VL domains of the scFv moieties are indicated

Regulatory GMP guidelines ensure that personnel, infrastructure and logistics of manufacturing facilities provide optimal conditions for the implementation and validation of production processes. The study drug, F16-IL2, was manufactured, identified by a lot number, and shipped to the different clinical sites according to the requirements of Directive 2001/20/EC. F16-IL2 was supplied in pyrogen-free 10 ml glass containers as liquid, sterile, apyrogenous solution. All study drug supplies had to be stored at -80°C until use. Investigators were aware of and well instructed during the Site Initiation Visit about

the storage of the IMP, and knew that study drug doses that were stored differently from the recommendations were no to be used and had to be replaced with fresh doses.

Seventeen patients were screened for the Clinical Trial, of which 13 were enrolled and treated (summarized in Table 3). Seven patients were randomized in Arm A (F16-IL2 + paclitaxel), and six patients in Arm B (paclitaxel). All patients completed the first cycle of treatment. Five patients received 2 complete treatment cycles, the others discontinued previously, three of them due to progression of disease. The distribution of patients in the seven centers, the randomization in Arms A and B, and the number of treated patients per cycle are reported in Table 3.

Table 3. Patient enrollment and treatment summary

Patients enrolled			
	Tübingen	4	(30.77 %)
	Berlin	3	(23.08 %)
	Barcelona	1	(7.69 %)
	Paris	1	(7.69 %)
	Essen	2	(15.38 %)
	Copenhagen	1	(7.69 %)
	Warsaw	1	(7.69 %)
Patient randomization	Arm A (F16-IL2 + PACLITAXEL)	7	(53.85 %)
	Arm B (PACLITAXEL)	6	(46.15 %)
Treatment by cycle	Cycle 1	13	(100.00 %)
	Cycle 2	8	(61.54 %)
	Cycle 3	4	(30.77 %)
	Cycle 4	2	(15.38 %)
	Cycle 5	1	(7.69 %)
	Cycle 6	1	(7.69 %)
Patients who completed 2 cycles		5	(38.46 %)
Reason for NOT completing the treatment	Adverse Event	1	(12.50 %)
	Progressive Disease	3	(37.50 %)
	Serious Adverse Event	2	(25.00 %)
	Withdrawal of Informed Consent	2	(25.00 %)

Monitoring visits were programmed and scheduled in accordance to the needs of all clinical centers and depending on enrollment rate. The activities during the visit was done in compliance with the International Conference on Harmonization (ICH) guidelines for GCP and following Philogen Standard Operating Procedures. Aim of the Monitoring Visits

was to review the progress of the clinical study, hence to: (i) Ensure protocol adherence, (ii) assure accuracy of data, and (iii) assure regulatory compliance

Monitoring Visits were done by the Clinical Research Associate (CRA) of Philogen or by a local CRO (Warsaw and Copenhagen, as required by Ethics Committees to overcome language issues). All personnel involved in the trial had to be present (PIs, AIs, Research Nurses, Trial Coordinators, Data Managers, Pharmacists, Monitors).

The CRA or CRO ensured that (i) all participant original informed consents were filed in the medical record, signed and dated; (ii) medical records contained laboratory reports, X-ray, scan reports, physician notes, nursing notes, procedures documenting study parameters reported in Case Report Forms (CRFs), and (iii) all CRF's were complete, accurate, and up to date. At the end of the visit the monitor collected site queries/clarifications and prepared the monitoring visit report that was then placed in the Trial Master File.

Efficacy evaluation.

Of the 17 screened patients, 13 were enrolled and treated, with 8 of them evaluable, i.e. receiving at least two cycles of therapy, for primary efficacy outcome. Overall survival was calculated as the time between randomization and death due to any cause. Patients who were lost to follow-up were censored at the time when last known to be alive (Figure 4).

Overall survival (OS) was 100% at 2 months, 75% at 4 months, and 50% at 1 year for Arm A (F16-IL2 plus Paclitaxel) compared to 67% at 2 and 4 months, and 0% at 1 year for Arm B (Paclitaxel alone) (Figure 3). The analysis for differences in OS between the two arms was not significant due to low numbers (log rank test p=0.60). The median OS was equal to 303.5 days (95% C.I: 89-NE, upper limit not estimable) and 304 days (95% C.I: 44-304), respectively, in F16-IL2 plus Paclitaxel and Paclitaxel alone.

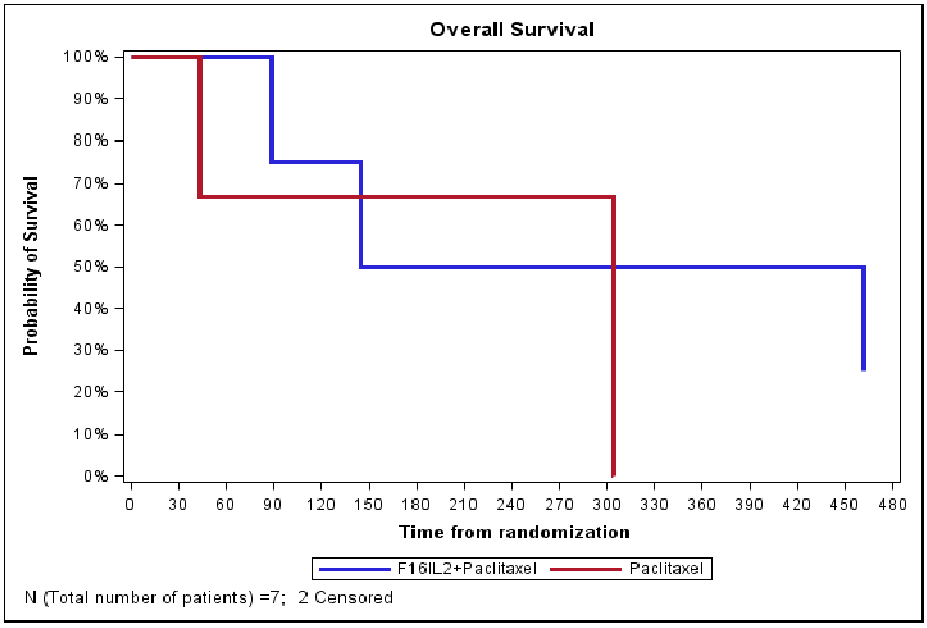
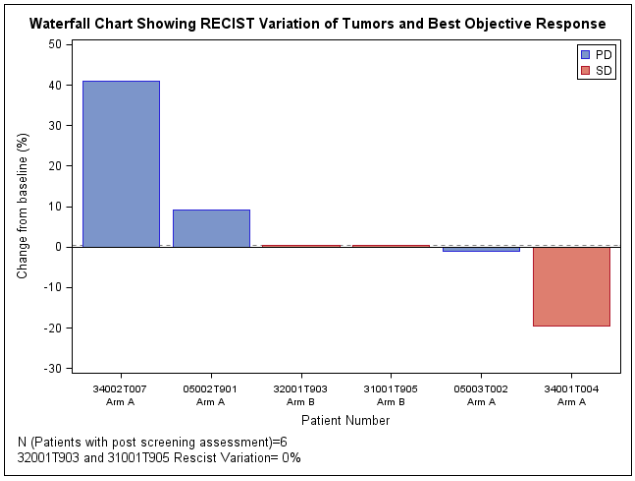


Figure 4. Overall survival in the per-protocol population

Response to treatment was evaluated in 8-week intervals (from week 8 up to week 24 or end of treatment) according to RECIST for measurable disease (target lesions), non-measurable disease (non-target lesions) and new lesions, and according to immune-related response criteria (irRC) for index and non-index lesions.

None of the patients treated in the study achieved a complete or partial response, neither in Arm A nor in Arm B, according to RECIST v. 1.1 or irRC at week 8, 16 or end of treatment. Figure 5 shows the maximum change of the sum of diameters of target lesions and the best objective response recorded for each study patient evaluable for efficacy during the study. The vertical bars indicate the most favorable RECIST score/irRC variation as a percent of the value recorded at baseline.



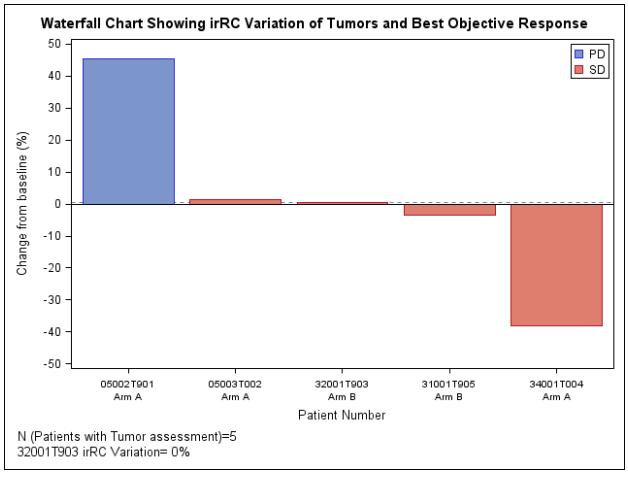


Figure 5. Objective responses. Change in diameters of target lesions and tumor response

Safety Evaluation.

Overall, all thirteen patients treated within the trial were evaluable for the secondary study endpoint safety (Figure 6). Seven patients were exposed to F16-IL2 plus Paclitaxel at least for one week of treatment, and six patients were exposed to Paclitaxel alone for at least one week of treatment. Overall, 12 out of 13 treated patients (92.3%) experienced at least one adverse event, among which in 3 (23.1%) patients these were classified as a severe adverse events were observed. Notably, all serious adverse events occurred in Arm A,

The most commonly involved system organ class for treatment-related AEs (any grade) were blood and lymphatic system disorders, general disorders, administration site conditions, and skin or subcutaneous tissue disorders.

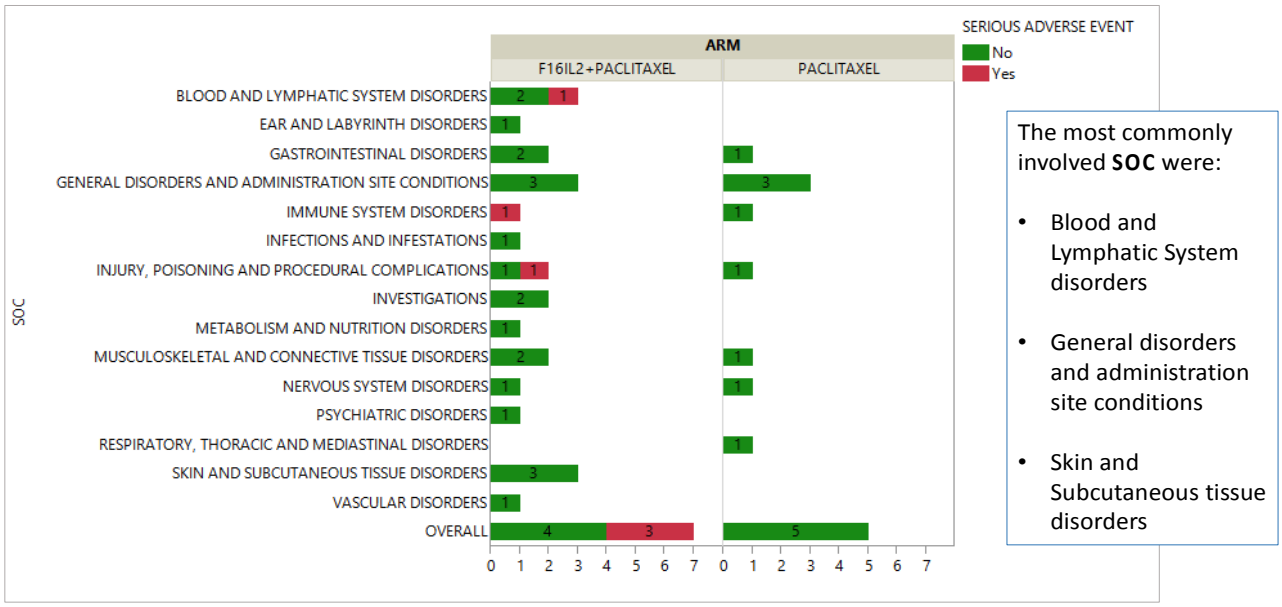


Figure 6. Safety. Number of patients showing adverse events, by system organ class (SOC) and total (overall).

In conclusion, the exiguous number of patients that could be recruited within the timeframe of the study implies that these results have to be considered preliminary have to be confirmed. Notably, patients treated with F16-IL2 plus Paclitaxel appeared to have a favorable outcome with 50% of patients alive after one year compared to 0% of patients treated with Paclitaxel alone.

1.3.2. WP2 - Identification of biomarkers predicting patient prognosis, treatment outcome and immune responses

Over the past decade, biomarkers and targeted therapies have been essentially important to many of the major success stories in cancer treatment. Moreover, therapies are rarely

efficient for all patients of a certain cancer entity. Therefore, the inclusion of biomarker analyses into the context of clinical trials is desirable. The identification of biomarkers does, however, not only allow the selection of patients with a high probability to benefit from a specific therapy more accurately, but also improves the understanding of how the respective therapeutic works.

From large retrospective studies of MCC patients it is known that the survival curve of patients with advanced disease initially drops very fast, but stagnates at about 20% relative survival after two years. Thus, it seems that a subpopulation of patients is endowed with a constitutively better survival, or shows a better response to therapy.

One pre-requisite for studies on tissue- or blood-derived biomarkers is the existence of a set of biological specimens annotated with the respective clinical data. Thus, it was essential to establish a data base linked to biobanked patient materials. Long-lasting discussions within the IMMOMEC consortium, with the sponsor of the clinical trial, and the respective data protection officers revealed that the originally planned modus operandi of a Web-based open accessible data base would not sufficiently protect the privacy of the patients. An alternative, sufficiently secure version would have been possible, but beyond the planned budget as it would have to be adapted to the respective firewalls of the partner institutions. Consequently, we decided to establish a central data base with access rights for the partners. In addition, we established a web-based clinical registry for concurrent and retrospective MCC cases.

As a discovery set to identify a panel of candidate biomarkers we used a set of annotated biological samples from 77 advanced MCC patients. As the first step we determined the quality of the respective materials by H&E staining, demonstrating that 12 of these cases had insufficient quality for immunohistochemistry analyses. For 61 cases we could determine the mitotic rate, which was stratified into categories ranging for 0-1 to >10 mitoses/mm². In only 1 case we observed 0-1, the majority of cases, i.e. 37, had 1-5, 6 cases had 5-10, and 17 cases had >10 mitoses/mm². Next, the inflammatory infiltrate was analyzed. The quantity of infiltration was categorized from absent = 0 to strong = ++. In addition, the infiltrate was distinguished qualitatively between peritumoral and intratumoral distribution pattern. This discrimination was automated using the InForm™ software.

Since the status of the Merkel cell polyomavirus (MCPyV) has been suggested to be a prognostic marker, we evaluated the MCPyV status in our discovery sample set both by real time PCR and immunohistochemistry. MCPyV positivity by PCR was defined when at least two of three analyzed primer sets gave a positive result, i.e. a relative value presence of at least 0.01 compared to the MCPyV+ MCC cell line WaGa. Applying this definition, from the evaluable cases 29 were regarded as negative while 35 were MCPyV positive. Notably, among the 29 cases in which we could not clearly detect MCPyV DNA, 10 stained positive for MCPyV large T antigen in immunohistochemistry. Notably, for twelve cases we were able to only get either a PCR (six cases) or an immunohistochemistry result. Thus,

from the cases analysed for MCPyV status, 21 were negative (17 in both methods), 24 were positive in one assay (8 cases only analyzed by one method), and in 25 cases we obtained positive results by both methods. In total, 70% of the analyzed cases were positive for MCPyV.

Up to date, almost all studies on MCPyV prevalence are based on PCR techniques, which can not reveal the nature of the MCPyV DNA, i.e. episomal or integrated. Therefore, we performed MCPyV fluorescence *in situ* hybridization (FISH) on MCC tissue samples to determine whether this technique allows gathering information about the quality of the viral presence on the single cell level. To this end, MCPyV FISH was performed on tissue microarrays containing 62 tissue samples of 42 patients including all tumor grades.[1] The hybridization patterns were correlated to the qPCR data determined on corresponding whole tissue sections. Importantly, MCPyV FISH and qPCR data were highly correlated, i.e. 83% concordance for FISH-positive and 93% concordance for FISH-negative cores. Interestingly, two hybridization patterns were distinguishable in the MCPyV FISH specimen: a punctate pattern (85%) indicating viral integration correlating with a moderate viral abundance, and a combination of the punctate with a diffuse pattern (15%) indicating the coexistence of integrated and episomal virus DNA which was associated with very high qPCR values. Thus, MCPyV FISH seems to be useful to further elucidate MCPyV related carcinogenesis by adding important information about viral integration or episomal presence on the single cell level within the histomorphological context.

Nevertheless, since the presence of viral antigens in MCC cases should render this tumor specifically immunogenic, the tumor has to escape immune surveillance. This hypothesis is already sustained by the heterogeneous infiltration of MCC tissues with lymphocytes. To address this notion, we established a multiplexed immunofluorescence staining based on the Opal™ system, which allows for the detection of multiple antigens in a single tissue section. In order to use the full possibilities of this technique, a multispectral image acquisition platform is required. To this end, we used the Mantra System from PerkinElmer, which is an integrated workstation incorporating multispectral imaging technology, novel image acquisition and inForm analysis software. As exemplified in Figure 7, this method allowed us to scrutinize the lymphocytic and myeloid infiltrate in MCC in detail. The depicted example visualized the presence of tertiary lymphoid tissue, which has been described to be associated with an improved prognosis in other skin cancers.[2]

[1] Fluorescence *in situ* hybridization and qPCR to detect Merkel cell polyomavirus physical status and load in Merkel cell carcinomas. Haugg AM, Rennspiess D, zur Hausen A, Speel EJ, Cathomas G, Becker JC, Schrama D. Int J Cancer. 2014 Dec 15;135(12):2804-15

[2] Targeting of lymphotoxin-alpha to the tumor elicits an efficient immune response associated with induction of peripheral lymphoid-like tissue. Schrama D, thor Straten P, Fischer WH, McLellan AD, Bröcker EB, Reisfeld RA, Becker JC. Immunity. 2001 Feb;14(2):111-21

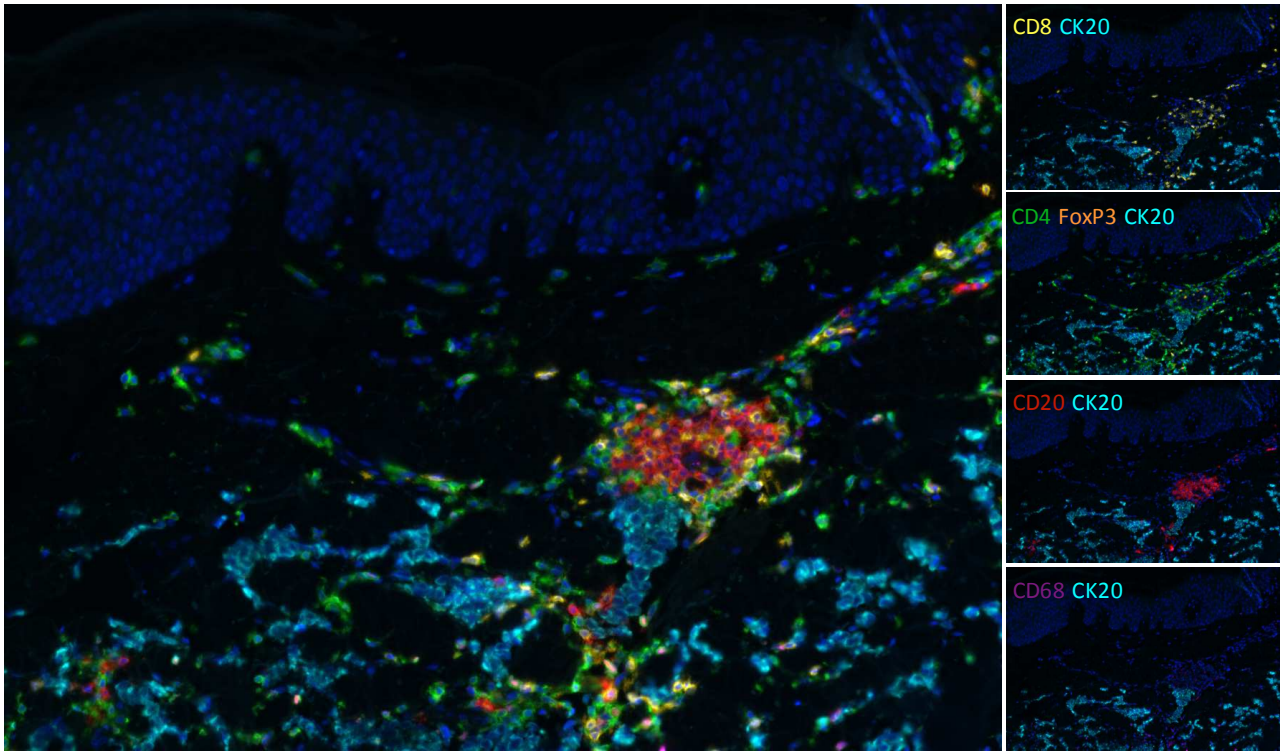


Figure 7. Multiplexed immunofluorescence. FFPE tissue sections of an MCC tumor were stained for T cell subtypes (CD4+ T helper cells, CD8+ cytotoxic T cells, FoxP3+ regulatory Tcells), B cells (CD20+), and macrophages (CD68+). Cytokeratin 20 was used to detect the MCC cells.

However, even by this means we could not detect a correlation between MCPyV status and TILs or clinical course.[3]. This is on one hand explained by the recently reported high mutational load in UV-associated MCC, but on the other hand, both MCPyV+ as well as MCPyV- MCC could either be characterized by a brisk, dim or absent T cell infiltrate. Consequently, we next tested for possible immune escape mechanisms. As we established that MCC tumors *in situ* do not express the NKG2D ligands MICA and MICB, which act activating on NK cells and costimulatory on T cells, we analyzed the expression of these ligands in MCC cell lines in vitro.[4]

In contrast to tissue analytics, blood-based biomarkers as a surrogate of tumor burden allow to be repeatedly checked to monitor the clinical course of cancer patients. Consequently, the American Joint Committee on Cancer (AJCC) incorporated several blood-based biomarkers into their staging systems, e.g., prostate-specific antigen (PSA)

[3] Merkel cell polyomavirus status is not associated with clinical course of Merkel cell carcinoma. Schrama D, Peitsch WK, Zapatka M, Kneitz H, Houben R, Eib S, Haferkamp S, Moore PS, Shuda M, Thompson JF, Trefzer U, Pföhler C, Scolyer RA, Becker JC. J Invest Dermatol. 2011 Aug;131(8):1631-8

[4] Reversal of epigenetic silencing of MHC class I chain-related protein A and B improves immune recognition of Merkel cell carcinoma. Ritter C, Fan K, Paulson KG, Nghiem P, Schrama D, Becker JC. Sci Rep. 2016 Feb 23;6:21678

and precursor PSA (proPSA) for prostate cancer, or α-fetoprotein and β-HCG for testicular cancer. Unfortunately, for the majority of solid cancers no reliable blood-based biomarkers have been established yet, including MCC. In order to establish a reliable blood-based biomarker for MCC, we first focused on the presence of neuron-specific enolase (NSE), which has been established as a surrogate marker for other neuroendocrine cancers. Unfortunately, however, we could not detect a significant correlation of NSE serum concentration with the tumor burden of MCC patients.

Advances in genomic technologies have boosted the number of candidate DNA- and RNA-based biomarkers. In this respect, circulating free (cf) DNA has been successfully demonstrated as a serum-derived biomarker for tumor burden in Langerhans cell histiocytosis. Although cancer in general is associated with increased serum DNA concentrations, most approaches using cf DNA as a blood based biomarker rely on tumor-specific DNA mutations, such as the BRAFV600E mutation in the given example. Unfortunately, due to the absence of specific hotspot mutations, this strategy is not applicable for MCC. An alternative approach takes advantage of cf miRNAs specifically overexpressed in certain cancer types. Since miRNAs are very resistant to degradation, sera from cancer patients contain large amounts of miRNAs derived from tumor cells. Indeed, miRNAs have been recognized as biomarkers for a variety of different cancer entities, such as miR-205 in breast cancer, or miR-19 in colorectal cancer. Since the analysis of MCC cells revealed a high expression of a set of specific microRNAs, we analyzed the respective microRNA expression in MCC cases. To this end, expression of this set of specific microRNAs normalized to the U6 snRNA was much higher in MCC cases compared to melanoma samples or the lung adenocarcinoma epithelial cell line A549. In a xenotransplantation model for MCC recently established by us,[5] we could clearly observe an increase of a set of MCC-specific microRNAs in mouse serum from a mean relative expression of 2.4 in 5 mice without tumor to 13.2 in 5 mice with tumor. Indeed, we not only demonstrated the abundant expression of this miRNA in MCC cell lines and tissues and its extracellular presence in MCC cell culture supernatants and sera of tumor-bearing preclinical animal models, but subsequently, we demonstrated the value of this cf miRNA as a surrogate marker of tumor burden in MCC patients. Notably, this cf miRNA not only discriminated patients with or without evidence of disease, but also correlated with the stage of disease. Additional studies including the comparison with other potential surrogate markers are needed to ascertain whether monitoring this cf miRNA levels in individual patients will be a useful tool for the early detection of disease recurrence or progression.

[5] Type I and II IFNs inhibit Merkel cell carcinoma via modulation of the Merkel cell polyomavirus T antigens. Willmes C, Adam C, Alb M, Völkert L, Houben R, Becker JC, Schrama D. Cancer Res. 2012 Apr 15;72(8):2120-8

We established a pseudonymized online registry for MCC patients meant for, but not restricted to the IMMOMEC consortium. In fact, the input boxes are open to any interested participant (http://ado-homepage.de/register/). Methods for data validation and safeguarding the uniqueness of each data set had been developed and implemented. Based on data from this registry, we were able to demonstrate that the MCPyV status of MCC is in general not associated with the clinical course of the disease.[6] However, in a subgroup of patients in which MCPyV appears to be initially involved in a hit-and-run carcinogenesis, but is subsequently lost, absence of MCPyV appears to be associated with an impaired prognosis.[7] Indeed, several reports in 2016 confirmed that in MCPyV-associated MCC there is a very low mutational burden; thus there are very few neo-epitopes apart from those derived from the virally encoded proteins. These observations emphasize the importance of re-inducing MHC class I expression without impairing the expression of the viral proteins. Retrospective analyses of data concerning the somatostatin receptor (SSTR) expression in MCC measured by PET revealed SSTR as a valuable target for molecular imaging of MCC.[8]

Data from the MCC registry which now comprises almost 1000 cases was also used in the development of the European guidelines for diagnosis and treatment of MCC.[9] Furthermore, we reported observations concerning the biology and clinical course of MCC.[10,11,12,13]

---

[6] Merkel cell polyomavirus status is not associated with clinical course of Merkel cell carcinoma. Schrama D, Peitsch WK, Zapatka M, Kneitz H, Houben R, Eib S, Haferkamp S, Moore PS, Shuda M, Thompson JF, Trefzer U, Pföhler C, Scolyer RA, Becker JC. J Invest Dermatol. 2011 Aug;131(8):1631-8. doi: 10.1038/jid

[7] Merkel cell carcinoma and Merkel cell polyomavirus: evidence for hit-and-run oncogenesis. Houben R, Grimm J, Willmes C, Weinkam R, Becker JC, Schrama D. J Invest Dermatol. 2012 Jan;132(1):254-6. doi: 10.1038/jid.2011.260

[8] Somatostatin receptor expression in Merkel cell carcinoma as target for molecular imaging. Buder K, Lapa C, Kreissl MC, Schirbel A, Herrmann K, Schnack A, Bröcker EB, Goebeler M, Buck AK, Becker JC. BMC Cancer. 2014 Apr 17;14:268

[9] Diagnosis and treatment of Merkel Cell Carcinoma. European consensus-based interdisciplinary guideline. Lebbe C, Becker JC, Grob JJ, Malvehy J, Del Marmol V, Pehamberger H, Peris K, Saiag P, Middleton MR, Bastholt L, Testori A, Stratigos A, Garbe C; European Dermatology Forum (EDF), the European Association of Dermato-Oncology (EADO) and the European Organization for Research and Treatment of Cancer (EORTC). Eur J Cancer. 2015 Nov;51(16):2396-403

[10] Left-sided laterality of Merkel cell carcinoma in a German population: more than just sun exposure. Gambichler T, Wieland U, Silling S, Dreißigacker M, Schaller J, Schulze HJ, Oellig F, Kreuter A, Stücker M, Bechara FG, Stockfleth E, Becker JC. J Cancer Res Clin Oncol. 2017 Feb;143(2):347-350)

[11] Treatment of MCC (Evaluation of real world treatment outcomes in patients with metastatic merkel cell carcinoma (MCC) following second line chemotherapy. Becker J, Lorenz E, Haas G, Helwig C, Oksen D, Mahnke L, Bharmal M. Ann Oncol (2016) 27 (suppl_6): 1154P.

[12] Merkel cell carcinoma: Epidemiology, prognosis, therapy and unmet medical needs. Schadendorf D, Lebbé C, Zur Hausen A, Avril MF, Hariharan S, Bharmal M, Becker JC. Eur J Cancer. 2017 Jan; 71:53-69

[13] New developments in the biology and the treatment of metastatic Merkel cell carcinoma. Terheyden P and Becker JC. Curr Opin Oncol (2017), in press

1.3.3. WP3 - Identification and characterization of HLA-restricted immunodominant T cell epitopes derived from the MCPYV T antigens

Discovery of CD8+ T cell epitopes in MCC is important for understanding the immune recognition of cancer cells. This cellular component plays a major role in mediating tumor cell eradication and should help us to understand the induction of T cell reactivity following F16-IL2 therapy. Indeed, MCPyV-encoded proteins are likely targets for cytotoxic immune responses to MCC as they are both foreign to the host and necessary to maintain the oncogenic phenotype. However, prior to the IMMOMEC project only a single MCPyV-derived CD8 T-cell epitope has been described, thus impeding specific monitoring of T-cell responses to MCC. Consequently, the purpose of this study was to identify and validate a sufficient number of MCPyV-derived T-cell epitopes for future characterization of spontaneous, modulated, or induced immune responses to MCC.

Virus antigens are degraded intracellulary, and thereafter processed and presented on the cell surface by MHC class I molecules - the classical pathway for the immune system to survey changes in intracellular compartments. T cells of the host will recognize fragments (peptides) from the virus proteins on the surface on tumor cells embedded in the MHC class I molecules, dependent on the patients' HLA type. T cell epitopes from MCPyV-encoded proteins are ideal targets to kill cancer cells, since these are recognized as "foreign" by the host immune system and not subjected to tolerance mechanism as observed with most self-antigens. Thus, the goal of this work package was to identify MHC class I restricted T-cell epitopes derived from MCPyV-encoded proteins (Figure 8).

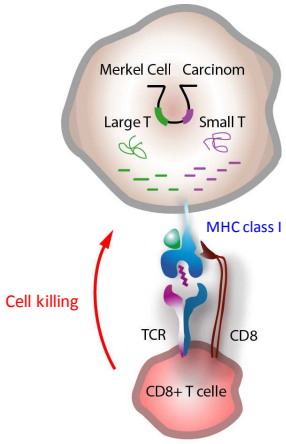


Figure 8. T-cell mediated tumor cell killing. Cytotoxic T cells require T-cell receptor mediated recognition of specific epitopes presented by MHC class I on the targeted cell for cognate killing.

This goal was achieved by scanning the MCPyV oncoprotein large T (LTA) and small T antigens (STA) and the virus capsid protein (VP1) for potential T-cell epitopes, and testing for MHC class I affinity. We confirmed the relevance of these epitopes using a high-throughput platform for T-cell epitope identification to characterize MCPyV-specific CD8 T-

cell epitopes in LTA, STA, and VP1. This platform is based on a parallel enrichment of peptide–MHC-reactive T cells and the detection of specific responses by combinatorial encoding with peptide–MHC multimers, enabling multi-epitope identification using limited patient material.[14],[15] Using this approach, we detected T-cell responses among 398 predicted T-cell epitopes restricted to HLA-A1,-A2, -A3, -A11, and -B7. In total, 56 MCPyV-specific T-cell responses were detected in 38 individuals, representing 35 different specificities (Figure 9).

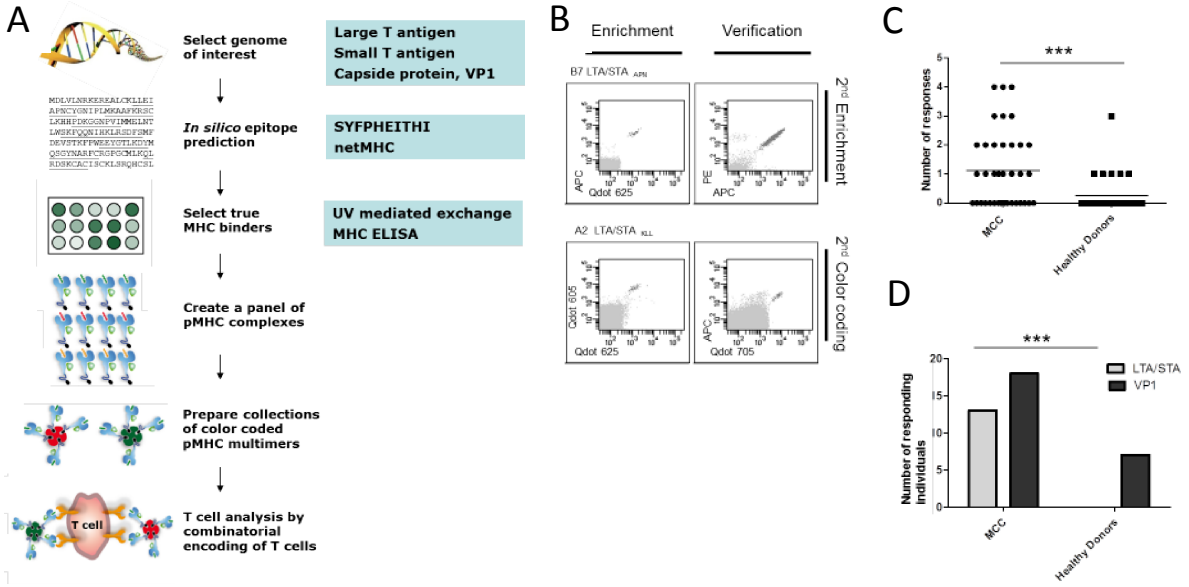


Figure 9. T-cell responses against MCPyV in MCC patients and healthy donors (adapted from [14] and [16]). (*A*) Schematic overview of the applied methodology (B) Specific responses were detected using flow cytometry and combinatorial encoded MHC- multimers followed by verification with either a 2nd enrichment (top) or a 2nd MHC-multimer detection (bottom). (C) Number of MCPyV specific T-cell responses per individual in MCC patients and healthy donors. (D) MCC patients or healthy donors with LTA/STA or VP1 specific T-cell responses. Asterisk indicate significant differences: *: p<0.05, **: p < 0.01, *** p < 0,001.

In parallel, we attempted to identify T cell epitopes by mass spectrometry through the elution of peptides presented in MHC class I on the surface of three MCC tumor cell lines. However, this analysis did not reveal any MCPyV-derived epitopes.

[14] Parallel detection of antigen-specific T-cell responses by multidimensional encoding of MHC multimers. Hadrup SR, Bakker AH, Shu CJ, Andersen RS, van Veluw J, Hombrink P, Castermans E, Thor Straten P, Blank C, Haanen JB, Heemskerk MH, Schumacher TN. Nat Methods. 2009 Jul;6(7):520-6[15] Parallel detection of antigen-specific T cell responses by combinatorial encoding of MHC multimers. Andersen RS, Kvistborg P, Frøsig TM, Pedersen NW, Lyngaa R, Bakker AH, Shu CJ, Straten Pt, Schumacher TN, Hadrup SR. Nat Protoc. 2012 Apr 12;7(5):891-902

[15] Parallel detection of antigen-specific T cell responses by combinatorial encoding of MHC multimers. Andersen RS, Kvistborg P, Frøsig TM, Pedersen NW, Lyngaa R, Bakker AH, Shu CJ, Straten Pt, Schumacher TN, Hadrup SR. Nat Protoc. 2012 Apr 12;7(5):891-902

Strikingly, although T cells specific for VP1-derived epitopes were detected both in patients with MCC and healthy donors, T-cell responses against oncoproteins were exclusively present in patients with MCC, but not in healthy donors. We further demonstrated both the processing and presentation of the oncoprotein-derived epitopes, as well as the lytic activity of oncoprotein-specific T cells toward MHC-matched MCC cells. Demonstrating the presence of oncoprotein-specific T cells among tumor-infiltrating lymphocytes further substantiated the relevance of the identified epitopes.[16]

We have observed T-cell based recognition in MCC patients of both the MCPyV-derived oncoproteins and the capside protein VP1. Importantly T cells directed against the oncoproteins are exclusively found in MCC patients and not healthy virus-infected individuals, indicating that these epitopes are exclusively found on cancer cells. Furthermore, we have shown for a number of these epitopes that T cell recognition can lead to killing of MCC cells. Thus, the host T cells do recognize the virus-derived T cell epitopes, but they are obviously not sufficiently effective in inducing cancer cell killing *in vivo*, but can be boosted with therapeutic measures. MCC tumor infiltrating lymphocytes do express a series of markers indicating T cell exhaustion and an immunosuppressive environment within the tumor. However, very relevant for the current application, the observation that MCC develops more frequently in immuno-compromised individuals suggests that the boosting of immune responses in these individuals will have a clinical benefit.

1.3.4. WP4 - Immune monitoring of MCC patients under therapy to correlate therapeutic effects with therapy-induced immune responses

In the IMMOMEC clinical trial standardized and centralized analysis of specific and functional T lymphocytes was performed in order to monitor each patient's immune competence during successful F16-IL2 delivery. To this end, a network of 7 specialized cell culture laboratories had been successfully established and trained for the standardized and GCP-compliant collection and isolation of patients' peripheral blood mononuclear cells (PBMC).

As the available PBMC cell numbers per patient were expected to be limited and as the number of immune-dominant T-cell epitopes identified from MCPyV and MCC tumor antigens identified in WP3 were supposed to vary, *Immatics*' established immune monitoring assays were adapted to include important cell-saving technologies in preparation for the IMMOMEC clinical trial: Successful implementation, validation and improvement of the Multimer Multiplexing technology followed by benchmarking the

[16] T-cell responses to oncogenic merkel cell polyomavirus proteins distinguish patients with merkel cell carcinoma from healthy donors. Lyngaa R, Pedersen NW, Schrama D, Thrue CA, Ibrani D, Met O, Thor Straten P, Nghiem P, Becker JC, Hadrup SR. Clin Cancer Res. 2014 Apr 1;20(7):1768-78

sensitivity of this new technology to Immatics' previously used standard Multimer technology.[17] Additionally, *Immatics* has implemented further cell-saving technologies (e.g. *ex vivo* analysis of rare cells as compared to methods using an *in vitro* amplification step), with the objective that a maximal data set can be generated out of the inherently limited biological material within the IMMOMEC clinical trial. For the characterization of changes in additional immunological parameters during F16-IL2 therapy, such as other relevant cell populations like immunosuppressive Foxp3$^+$ regulatory T cells (Tregs) or myeloid derived suppressor cells (MDSCs), *Immatics* developed and established a highly standardized proprietary flow cytometry detection panel for detailed characterization and quantification of numerous cell populations contained within patient PBMC samples.

Within the IMMOMEC clinical trial a total of 26 PBMC samples from 16 patients at 7 participating clinical centers have been successfully collected. Of these 16 patients, 7 patients were enrolled in the experimental arm (arm A: paclitaxel plus F16-IL2) and 6 patients were enrolled in the control arm (arm B: paclitaxel only). Moreover, 4 baseline samples were taken from MCC patients who were finally not enrolled into the trial and PBMC samples of 5 healthy donors were included in the baseline Treg and MDSC analyses.

F16-IL2 administration is expected to lead to an activation of MCPyV- and MCC-specific cytotoxic T cells (CTLs) directed towards naturally occurring epitopes, and to the proliferation and acquisition of effector functions in these CTLs. Thus, T-cell responses to published MCC peptide antigens and peptide antigens identified in WP3 were monitored in detail. MCC-specific CD8+ T cells were assessed *ex vivo* to monitor specific T cell response profiles before and after application of F16-IL2. These analyses enabled the validation of the relevance of novel MCPyV epitopes and may establish these novel targets as MCC antigens for monitoring or even therapeutic applications in other MCC trials. 11 of the enrolled patients exhibited a suitable HLA-type (HLA-A*02, -B*07 or -A*24) and were evaluable for determination of MCC-specific peptide reactivities. Of these patients 55% (6/11) showed CD8$^+$ T-cell responses at baseline to at least one of the MCC-specific peptides (exemplarily shown in Figure 10, for one MCC-specific antigen and HIV as control antigen).

[17] Parallel detection of antigen-specific T-cell responses by multidimensional encoding of MHC multimers. Hadrup SR, Bakker AH, Shu CJ, Andersen RS, van VJ, Hombrink P, Castermans E, thor SP, Blank C, Haanen JB, Heemskerk MH, Schumacher TN (2009). Nat. Methods 6, 520-526

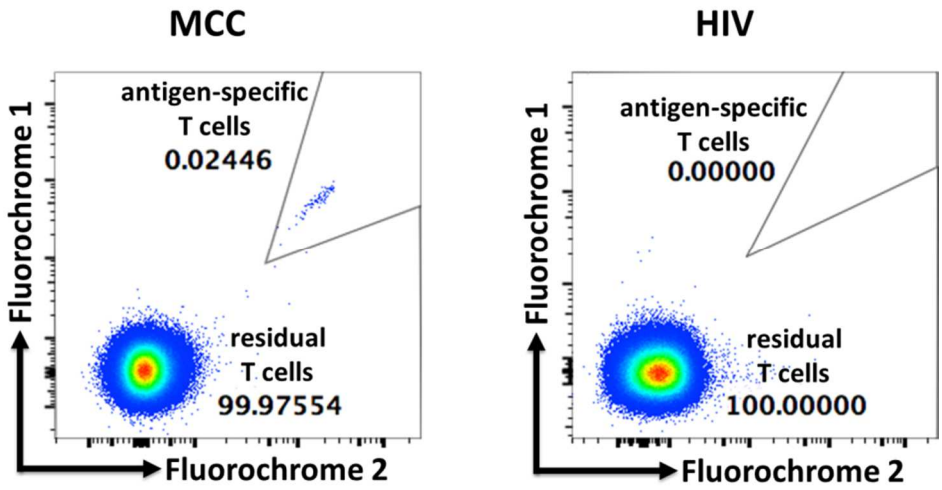


Figure 10. *Ex vivo* measured, MCC-peptide specific T-cell response and negative control.

MCC-specific responses are in the range of <0.001% - 0.024% of $CD8^+$ T cells. However, neither for the 3 patients of the experimental arm nor for the 2 patients of the control arm, who were evaluable for pre- and post-treatment $CD8^+$ T cell reactivities, a treatment-induced increase in MCC-specific $CD8^+$ T cells was detected.

Moreover, immune cell populations influencing specific T cell responses were assessed in order to compare pre-treatment Treg and MDSC levels of healthy donors and cancer patients, to assess Treg and MDSC levels before and after application of F16-IL2, as well as to identify Treg or MDSC populations that are potentially predictive biomarkers for the clinical outcome or for the immune responses.

All 11 Treg populations analyzed had comparable baseline levels in MCC patients and healthy donors (data not shown). However, during treatment with F16-IL2 plus paclitaxel (experimental arm) Treg levels strongly increased in most of the Treg populations analyzed, while treatment with paclitaxel only (control arm) had no influence on Treg levels. This is exemplarily shown in Figure 11 for one Treg population at baseline and at week 6 (and week 24) for patients evaluable in Arm A (A) and Arm B (B). Even though the effect of IL-2 on Tregs is well known, the strength of the induction is impressive.

In addition, 6 different MDSC populations were analyzed in detail (for details about the different populations, please refer Table 4). While baseline levels of MDSC populations 1, 2 and 4 seemed to be comparable between MCC patients and healthy donors, levels of the MDSC populations 2, 5 and 6 tended to be numerically higher in MCC patients (Figure 12). However, due to the limited sample numbers these differences did not reach statistical significance. Because of the small patient number and the patient variability no clear trend over time could be observed for the different MDSC populations at baseline, week 6 (W6) and week 24 (W24).

In summary, cellular biomarker analyses showed 100 % evaluability rate for Treg and MDSC analyses. 65% of patients (11/17) were evaluable for CD8 MCC-specific T cell responses on the basis of their HLA restriction.

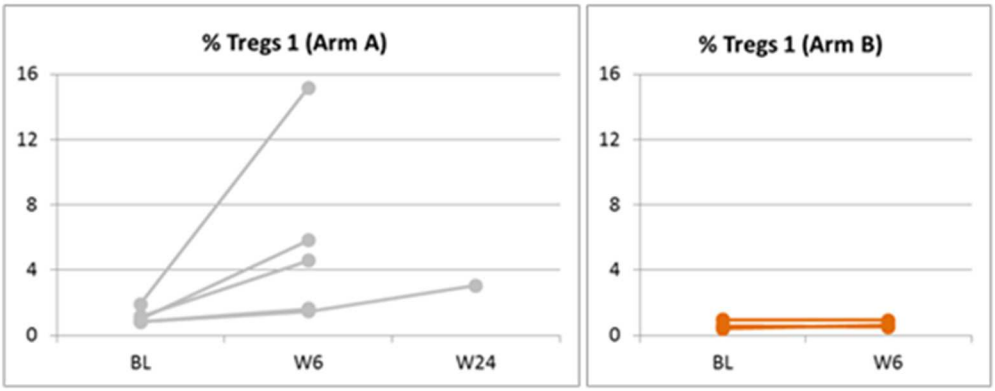


Figure 11: Quantification of regulatory T cells (Tregs). Percentage of Tregs 1 ($CD3^+$ $CD4^+$ $CD8^-$ $CD25^{high}$ $CD127^{low}$ $Foxp3^+$) before and after treatment in patients of Arm A (F16-IL2 plus paclitaxel) and Arm B (paclitaxel alone).

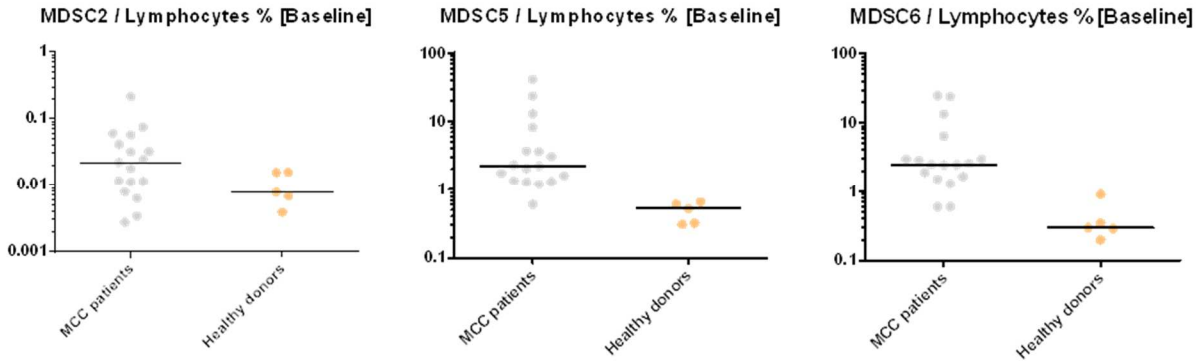


Figure 12. Quantification of Myeloid-derived Suppressor Cells (MDSCs). Percentage of MDSCs 2, 5 and 6 (normalized to lymphocytes) in PBMCs of MCC patients and healthy donors.

Table 4: Prospectively defined MDSC populations within multicolour panel

Population	Phenotype	Type	Reference
MDSC 1	CD14+ CD124+	monocytic	J Immunol. *182*, 6562-6568
MDSC 2	CD15+ CD124+	granulocytic	J Immunol. *182*, 6562-6568
MDSC 3	Lin- HLA-DR- CD33+	other	Cancer Res *66*, 9299-9307
MDSC 4	CD14+ HLA-DR- SSCim	monocytic	J Clin Oncol *25*, 2546-2553
MDSC 5	CD11b+ CD14- CD15+	granulocytic	Cancer Res. *65*, 3044-3048
MDSC 6	CD15+ FSClo SSChi	granulocytic	Sci. Rep. *5*, 15179

1.3.5. WP5 - Correlation of systemic immunological responses and clinical benefit: impact of the microenvironment, immune escape mechanisms and regulatory circuits

Given the difficulty in obtaining biological specimens from MCC patients, it is obligatory that specimens are not squandered on unfocused exploratory studies, or evaluated using assays that are seriously lacking in robustness and reproducibility. Consequently, innovative methods and the respective standard operating procedures (SOPs) had been established within the IMMOMEC project.

While the sample acquisition for FFPE blocks occurred within the anticipated extent, the acquisition of cryopreserved tissue samples was frustratingly slow and low. Thus, we decided to focus on the FFPE tissue samples and to establish the respective analyses planned for the cryopreserved tissue samples on FFPE samples, which was possible by the advent of new technologies such as gene expression profiling by the nCounter Analysis System (nanoString Techologies), TCR clonotype mapping by immunoSEQ (Adaptive Biotechnologies) or immune phenotyping by the Opal System (PerkinElmer). For example, next generation sequencing (NGS)-based T-cell repertoire analyses by ImmunoSeq allows the comprehensive characterization of FFPE tumor tissues, which is the prerequisite for T cell clonality analyses and clonotype tracking, which was formerly only possible if fresh frozen (*aka* cryoconserved) tumor tissue was available.[18] By comparing 2 cases for which both FFPE and cryopreserved tissue samples were available, we confirmed that these analyses indeed yield comparable results. Unfortunately, however, *in situ* peptide/MHC-multimer staining to detect specific T cells in tissue, a technique we had previously established for cryopreserved tissue sections, did not work at all in FFPE sections.[19]

With respect to the characterization of MCC- and MCPyV-specific T-cell responses, peripheral blood lymphocytes obtained at screening were available for all 17 patients screened for the IMMOMEC clinical trial. However, only 11 of these samples could be evaluated for specific T-cell responses. This restriction is based on the fact, that only these 11 patients had an HLA-phenotype for which specific MCC- and MCPyV-epitopes are known. For 5 patients MCC- and MCPyV-specific T-cell responses were also evaluated at post-screening.

Over the past year it became increasingly obvious that cancers contain a heterogeneous and dynamic microenvironment communicating with the immune system. The immune contexture of the tumor microenvironment showed to be able to influence the course of the

[18] T cell receptor repertoire usage in cancer as a surrogate marker for immune responses. Schrama D, Ritter C, Becker JC. Semin Immunopathol. 2017 Jan 10. doi: 10.1007/s00281-016-0614-9. [Epub ahead of print]

[19] Spontaneous cytotoxic T-cell responses against survivin-derived MHC class I-restricted T-cell epitopes *in situ* as well as ex vivo in cancer patients. Andersen MH, Pedersen LO, Capeller B, Bröcker EB, Becker JC, thor Straten P. Cancer Res. 2001 Aug 15;61(16):5964-8

disease. Hence, pre-existing immunity is determining the fate and survival of the patient and the likelihood of response to immunotherapy. Indeed, several reports suggest in different tumor entities ranging from colorectal cancer to melanoma, that the course of cancer disease is controlled by the host's immune system underlying the importance of including immunological biomarkers for the prediction of prognosis and response to therapy. Quantification of immune cell densities revealed the major positive role of infiltrating T cells for patient's survival. These reports, however, also revealed that the localisation of the immune infiltrate is equally important than the mere quantification. These findings became the foundation of a new concept: immune contexture, which also takes functional orientation, density, and location within distinct tumor regions of a natural *in situ* immune reaction into account. The evaluation of the immune contexture, however, is a complex challenge. In our studies we took advantage of the InForm software, which can be trained to quantify not only cells based on their nuclei, their specific form, but also their immunohistological or immunofluorescent staining pattern (e.g. expression of cytokeratin 20 for MCC tumor cells). Moreover, the counted cells can be categorized according to their localisation, e.g. in the stroma surrounding or within the tumor or the tumor itself. Such an analysis is exemplified in Figure 13 for CD8+ T cells infiltrating MCC tumors.

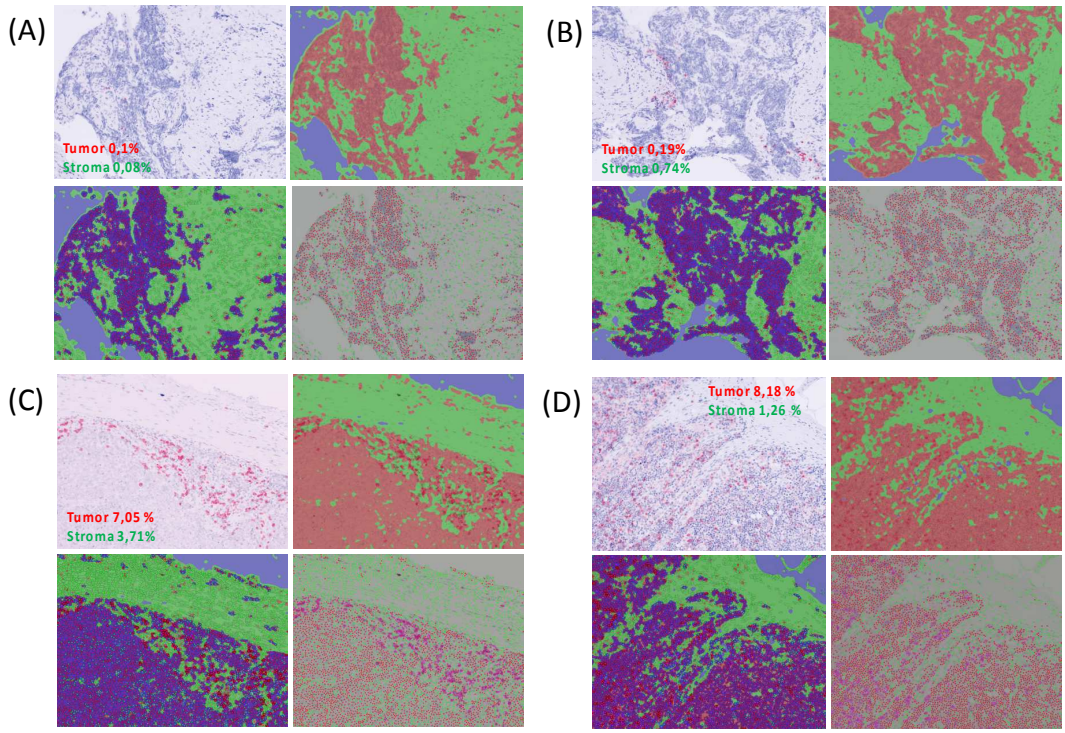


Figure 13. Quantification and categorization of CD8+ T cells in MCC. (A) Low stroma and tumor infiltration, (B) low tumor, dim stroma infiltration, (C) mostly tumor border infiltration, and (D) brisk tumor infiltration.

Using this approach, we scrutinized the lymphocytic (i.e. CD4 – T helper and regulatory T cells; CD8 – cytotoxic T cells; PD-1 – exhausted T cells; FoxP3 – regulatory T cells; CD20 – B cells) and the myeloid infiltrate (CD68 – macrophages; CD163 – tumor associated macrophages/M2 macrophages; arginase – tumor associated macrophages; PD-L1 – presumably tumor associated macrophages), as well as the expression of MICA, MHC class I and PD-L1 on CK20+ tumor cells (MICA – NKG2D ligand; HLA-A – MHC class I; PD-L1 – PD-1 ligand; CK20 – MCC tumor cells).

These analyses demonstrated a very heterogeneous presence and composition of infiltrating lymphoid or myeloid cells. Interestingly, there were a number of trends with respect how these infiltrates were composed: (i) a strong infiltration of M2 polarized PD-L1 expressing macrophages is inversely correlated to infiltration of CD4 and CD8 T cells; (ii) strong infiltration with CD8+ T cells was correlated with the presence of FoxP3 positive regulatory T cells; and (iii) MICA and HLA-A expression on tumor cells was correlated with a stronger CD8+ T-cell infiltration, which was associated with the PD-L1 expression on tumor cells (but not PD-L1 expression on stroma cells). However, probably due to the high number of tested variables with regard to the limited number of analysed samples, none of these trends reached statistical significance.

These morphological characterizations were expanded by a NanoString nCounter platform-based molecular analyses for an immune-related gene expression profile previously described to be a predictive marker for the clinical outcome of an immune checkpoint blocking antibody treatment such as pembrolizumab or nivolumab. The original codeSet was supplemented by 4 genes specifically expressed in MCC cells, i.e. CK20, neuron-specific enolase and the two MCPyV-encoded early genes. After normalization for the tumor content and expression of classical housekeeping genes as well as CK20 and neuron-specific enolase, a hierarchical clustering using either Euclidean distance or Spearman correlation was performed. While neither clustering method clearly separated MCC lesions characterized by brisk or dim CD8+ infiltrate, individual genes did. As exemplified for mRNA expression encoding the non-classical MHC molecule HLA-E, granzyme B and interferon gamma, these were either inversely (HLA-E) or directly (granzyme B and interferon gamma) correlated with the degree of the CD8+ T-cell infiltrate.

The next series of experiments addressed the T-cell receptor repertoire usage of the T-cell infiltrate in MCC lesions by ImmunoSeq technique. Both the number of TCR transcripts as well as the number of overexpressed TCR clonotypes varied substantially in the analysed samples. Notably, the TCR high clonality group was associated with a brisk CD8+ infiltrate, higher numbers of FoxP3+ cells as well as a low numbers of M2-polarized macrophages. Again, these correlations did not reach statistically significance, probably due to the high number of tested variables in regards to the limited number of analysed samples. When we correlated the clonality of the TCR repertoire usage in the MCC lesions with the

immune-related gene expression profile described above, none of the applied hierarchical clustering methods clearly separated MCC lesions characterized by high or low TCR BV clonality. However, similar to the brisk and dim CD8+ T cell infiltrate the same individual genes did.

When we set obtained results of the characterization of systemic and localized immune responses into an associative context, a number of conclusions became obvious: (i) systemic and localized T cell responses are comparable if the tumor cells express MHC class I molecules and there is a low abundance of M2-polarized macrophages in the tumor, (ii) a high PD-L1 expression on tumor cells is associated with both systemic and *in situ* T cell responses, and (iii) a TCR repertoire usage of TIL characterized by a high clonality seems to be associated with systemic T cell responses.

Based on the disease-specific mortality rate, MCC is more lethal than melanoma. Still, spontaneous remissions of both primary MCC as well as metastatic lesions are frequently reported and explained by adaptive immune responses; thus, MCC appears to be a prime candidate for immunotherapy. Indeed, recent clinical trials demonstrated the efficacy of immune checkpoint blocking antibodies. However, at least half of the patients in these trials were characterized by a primary resistance to checkpoint blockade, and a relevant proportion of the responding patients developed secondary resistance. Characterization of the mechanisms underlying immune-resistant cancer progression may contribute to the rational design of strategies to improve the efficacy of immunotherapy in patients suffering from advanced MCC.

Natural Killer group 2D (NKG2D) is a lectin-like type 2 transmembrane receptor encoded by the gene *Klrk1* (killer cell lectin-like receptor subfamily member 1), and is part of a critical pathway signaling cellular stresses to the innate and adaptive immune system. Charged residues in the transmembrane region enable NKG2D to pair with the signaling adaptor protein DAP10, which is essential for NKG2D surface expression and downstream signaling to PI3K and GRB2. These signaling molecules then stimulate proliferation, cytokine production, immune cell activation, and cytotoxic potential of NK and T cells. A recent study suggested a link between the Natural Killer group 2D (NKG2D) receptor system and up-regulation of immune responses to MCC. Specifically, transcriptional analyses of MCC tumors revealed that NKG2D was among the highest expressed mRNAs in tumors obtained from patients with a good prognosis. However, these tumors represented a minority of patients, suggesting most MCCs evade NKG2D signaling as a means of immune escape.

The NKG2D ligands include UL16-binding proteins (ULBPs) as well as the MHC class I chain-related protein (MIC) A and B family. MICA and MICB are present at low to undetectable levels in normal cells, but are induced by cellular stresses including infectious agents and neoplastic transformation. Indeed, MICA and MICB are highly expressed in a number of solid tumors like carcinomas of the breast, colon, kidney, ovary,

or prostate, as well as in melanoma. However, NKG2D expression renders tumor cells more susceptible to elimination by the immune system. The importance of MICA and MICB induced NKG2D-signaling for immune surveillance of virally infected and transformed cells is highlighted by the fact that viruses and cancer cells have developed mechanisms to interfere with this interaction. These mechanisms include shedding of surface-expressed molecules, binding and retaining of MICA and MICB proteins in the cytoplasm, over-expression of MICA and MICB mRNA-targeting microRNAs, as well as other epigenetic mechanisms such as chromatin remodeling.

MICA and MICB are not or only slightly expressed by MCC tumors *in situ* and completely absent on MCC cell lines in vitro. We demonstrated that the lack of MICA and MICB mRNA and protein expression in MCC is largely due to epigenetic silencing via histone hypo-acetylation in their promoter region. This epigenetic silencing is very robust even in the presence of several well-established stress factors known to induce their expression. However, this silencing can be abrogated by treatment with HDAC inhibitors both in vitro and *in vivo*. Since the ultimate goal of our studies was to establish a therapeutic approach for MCC, we used a clinically relevant concentration, i.e. concentrations attained in patients treated with the FDA approved HDAC inhibitor vorinostat (Zolinza™). Although this concentration of vorinostat increased histone acetylation at the MICA and MICB promoter as well as subsequent mRNA and protein surface expression in MCC cell lines, the effects were not very strong. Classical MCC cell lines grow as 3D-cultures in large spheroids and therefore represent the *in vivo* situation of a solid tumor much closer than other cancer cell lines. To increase the susceptibility of cancers to HDAC inhibitors, they are frequently combined with other drugs. Mithramycin A is a gene-selective Sp1 inhibitor, which has been reported to potentiate HDAC inhibitor-induced transcriptional activation. To this end, promoter acetylation of MICA and MICB genes and subsequent mRNA and protein expression were markedly enhanced in MCC cells upon this combined treatment, which resulted in an increased susceptibility to cellular cytotoxicity (Figure 14).

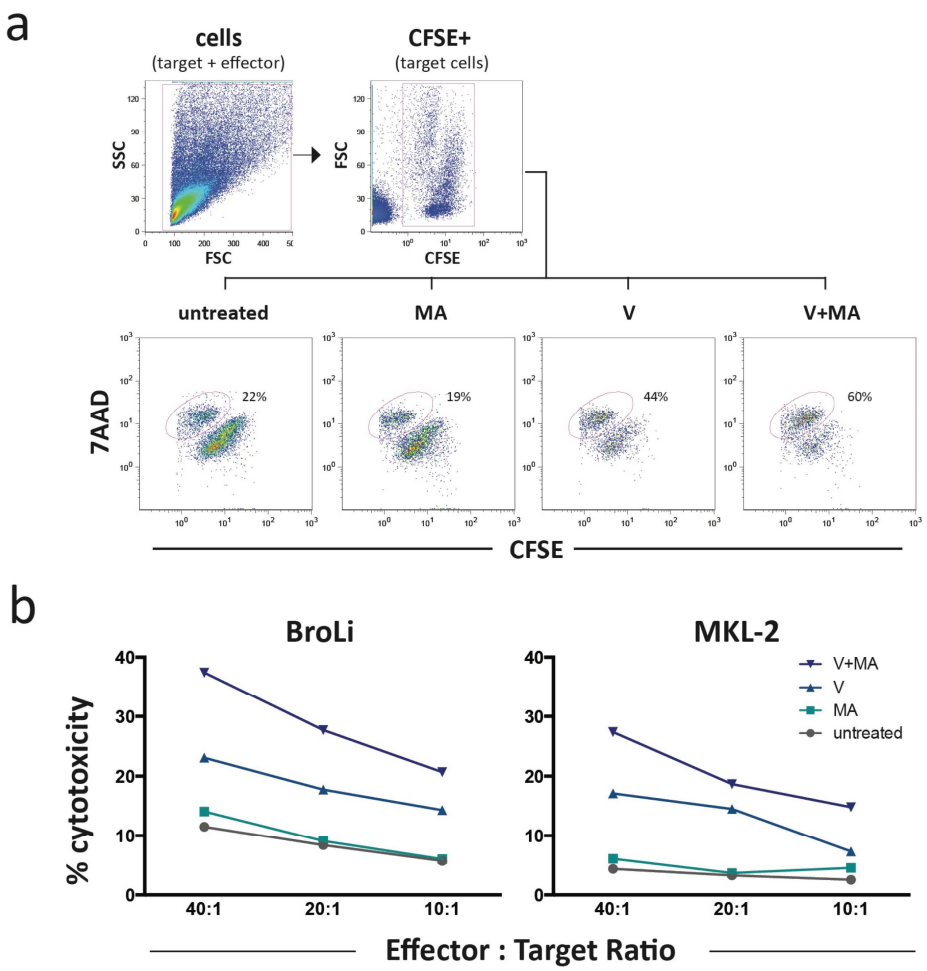


Figure 14. Inhibition of HDACs in MCC cell lines increased their susceptibility to LAK cell mediated lysis, which is is subdued by MICA/B blockade. (A) The gating strategy of the flow cytometry based cytotoxicity assay is illustrated for untreated and treated BroLi cells; target cells were gated as CFSE positive cells in an FSC/CFSE plot, lysed target cells were defined as 7AAD/CFSE double positive cells. (B) Untreated (grey), vorinostat (V, light blue), mithramycin A (MA, turquoise), or the combination thereof (V+MA, dark blue) treated BroLi and MKL-2 cells served as target cells for LAK cells in a 4h cytotoxicity assay.

1.4. Potential Impact

The overall aim of IMMOMEC was an increase in public health with respect to a rare, but highly aggressive skin cancer, i.e. MCC, affecting mostly the elderly, not only in the member states, but worldwide. Moreover, we aimed at strengthening the competitiveness of biomedical research in Europe with respect to both clinical as well as translational research. The ambition was to provide a proof-of-concept study of an innovative form of immunotherapy using antibody-targeted interleukin-2 in a well characterized, highly immunogenic cancer entity. The clinical trial was accompanied by a comprehensive

translational research program comprising newly established immune monitoring techniques allowing the analyses of localized immune responses in the tumor microenvironment and systemic responses in the circulating blood. We further assumed that the obtained results would open new avenues for a generic adaptation of this concept of immunotherapy to other cancer entities.

1.4.1. Potential impact of the results of WP1

Unfortunately, due to the reasons described above, which were at least in part beyond the potential of the consortium, the clinical trial implemented within IMMOMEC did not reach sufficient recruitment to provide significant results. A cornerstone of the Lisbon Strategy is to improve the competitiveness and increased productivity of the European research community and industry. Even though the IMMOMEC consortium despite all efforts was not able to recruit a sufficient number of patients into the clinical trial to deliver informative evidence of the clinical efficacy of an antibody-targeted interleukin-2 treatment, it still had a substantial impact on the standing of the consortium and thus the European Community in the field of clinical and translational research and development in rare cancers and tumor immunology. Most European centers caring for patients with MCC are organized within the EORTC. However, no multicenter trials for MCC had been organized by this European organization in the past. The direct interactions of European sites with respect to clinical and translational research in MCC have now been established as IMMOMEC has intensified the cooperation of centers from all over Europe with regard to both MCC patient care and translational research. The implementation of such a tightly organized network had strengthened the importance of European researchers in the field of MCC. Until recently, most clinical trials initiated by large pharmaceuticals companies (*'big pharma'*), even those with a European background, are coordinated by the US. The demonstration of a well functioning European network conducting a randomized clinical trial in a very rare cancer improved the standing of European groups to such a degree, that it was subsequently possible to launch an European investigator initiated trial (IIT) for the adjuvant treatment of MCC using the CTLA-4 blocking antibody Ipilimumab (ADMEC, EudraCT 2013-000043-78). This trial is conducted largely with participation of the IMMOMEC consortium funded by an educational grant provided by Bristol-Myers Squibb. Notably, this trial would not have been possible without the network established in IMMOMEC and the experience the consortium accumulated during the term of the project. To date more than 40 patients had been screened and almost 35 enrolled into the ADMEC clinical trial. Similar to IMMOMEC, ADMEC is accompanied by an innovative translational research program based on the established techniques, results and experience obtained within IMMOMEC – however, lacking the same generous financial support by the EC, less comprehensive.

1.4.2. Potential impact of the results of WP2

Major advances in cancer therapy over the past years were due to a better understanding of the tumor biology and immunology, thereby allowing a more personalized therapy improving the lifespan and quality of life for the concerned patients and their relatives. Within IMMOMEC we analysed an archived set of clinically annotated tumor tissues and serum samples, thereby we identified a number of biomarkers serving as surrogate for tumor burden and in part predicting patient prognosis. Moreover, we established the relevance of the biologic pathways underlying these biomarkers. However, retrospective analyses of archived bio-samples, even collected in a prospective observational registry, have to be confirmed in a prospective study. While this validation could not be sufficiently done using the prospectively collected bio-samples within the IMMOMEC trial, this validation is planed. Indeed, within the ADMEC trial, which is active in several of the IMMOMEC centers, tumor samples are prospectively collected.

We established a pseudonymized online registry for MCC patients meant for, but not restricted to the IMMOMEC consortium. In fact, the input boxes are open to any interested participant. Methods for data validation and safeguarding the uniqueness of the data set had been developed and implemented. Data from the MCC registry which now comprise almost 1000 cases was used in the development of the European guidelines for diagnosis and treatment of MCC. In the future this database is likely to be used as a benchmark to compare new approaches in the clinical management of MCC patients. These approaches are not necessarily restricted to immunotherapy of advanced disease, but also comprise the initial therapy of primary or locally advanced MCC such as safety margins, sentinel lymph node dissection or adjuvant radiation.

We established several techniques for the comprehensive immune profiling using FFPE samples. These techniques include complex gene expression analyses, T-cell receptor repertoire analyses, and multiplexed immunofluorescence for immune infiltrate characterization. These techniques are currently used to scrutinize MCC lesions to dissect possible predictive biomarkers as well as escape mechanisms to immune checkpoint blocking antibodies. Furthermore, the detailed SOPs will be published and made available via www.immunomec.eu as soon as the respective publications have been accepted for publication.

1.4.3. Potential impact of the results of WP3

MCC is one among several cancers that are known to have a viral origin. Worldwide, the WHO International Agency for Research on Cancer estimated that in 2002 17.8% of human cancers were caused by infection, with 11.9% being caused by one of seven different viruses. The oncogenic process in most cases originates from the integration and expression of oncogenes in the host genome – taking the first step towards the development of a cancer cell. This is of essential importance as far as these cancers hold

viral components that are ideal targets for immune recognition, since these are both foreign to the immune system and essential for cancer cell survival. Thus, immunological recognition of the integrated oncogenes was shown to be a very relevant strategy for cancer therapy of e.g. cervical cancer induced by human papilloma virus, and with novel knowledge about immune recognition of MCPyV from our laboratory, we hypothesize that also this virus-induced cancer can be efficiently targeted by immunotherapeutic strategies. Especially in the light of a number of new immune modulatory drugs (antibodies blocking CTLA4, PD1, PD-L1, and other immune checkpoints) that have recently been approved for other types of solid cancer. These immunomodulating agents may induce a shift towards an immune-stimulatory environment in the patient – and together with specific targeting of T cells towards MCPyV-encoded antigens enable the generation of efficient anti-tumor immune responses against MCC.

Understanding the precise peptide-MHC targets on tumor cell that are relevant for immune-cells recognition and killing will allow the monitoring of immunotherapeutic strategies to understand and foresee the efficacy. Further, such therapies, like F16-IL2 may be combined with specific immune cell targeting toward MCC-derived epitopes, through vaccination strategies or adoptive cell therapy.

1.4.4. Potential impact of the results of WP4

IMMOMEC has a substantial impact on the standing of the European Community in the field of immune monitoring. For the IMMOMEC clinical trial, we showed that it is feasible to establish a network of specialized cell culture laboratories for the standardized and GCP-compliant collection and associated logistics of patients' PBMC with a success rate of 100%. Such validated and highly standardized PBMC collection is a prerequisite for every clinical trial including elaborated immune response analyses, and the associated laboratories are now trained to collect PBMCs of the same quality in future trials.

Because the available PBMC numbers per patient were limited (due to limited availability of blood for each blood drawing time point during immunotherapeutic intervention), the establishment of cell saving techniques was an important step to generate a maximal data set out of the inherently limited biological material in the IMMOMEC trial. These new tools will be used in future clinical trials to monitor patients.

Moreover, the standardized and centralized T-cell immune monitoring in this multi-center, randomized Phase II clinical trial using cutting edge methods define a new state-of-the-art and enabled the collection of proof-of-concept data of the immune competence in MCC patients before and after treatment with paclitaxel alone or in combination with F16-IL2. One of the findings of the immune monitoring was the strong and consistent increase of Tregs after treatment with F16-IL2. Interestingly, even if the prognostic value of regulatory T cells in cancer remains controversial, high Treg infiltration seems to be significantly

associated with shorter overall survival in the majority of solid tumors.[20]. However, Sihto et al. published that in a cohort of more than 100 Finnish MCC patients with MCPyV DNA-positive cancer FoxP3+ tumor-associated lymphocytes were correlated with better prognosis.[21] Considering the F16-IL2 induced increase in Tregs observed in the IMMOMEC trial, this publication gains in importance even if it must be considered that for IMMOMEC only peripheral Tregs were measured.

Unfortunately, the number of samples collected for IMMOMEC was small, and thus the possibilities for comparison of immune cell populations between both treatment arms were limited. However, the pre-treatment analyses of the blood cell population were helpful to get more insights into the basic principles of MCC and may help to develop new therapy options. For example, one option could be a peptide vaccination using peptides for which pre-existing T cell reactivities were found in the MCC patients analyzed. As more than 50% of the evaluable patients showed at baseline MCC-specific T-cell responses to at least one of the MCC-specific peptides, this may lead to a strategy to identify patients which are more likely to respond to vaccination with MCC-specific peptides. The feasibility of such a concept of pre-immunogenicity testing has recently been shown within GAPVAC, another EU FP7-funded project. In addition, the immunogenic MCC epitopes identified could be used for immune monitoring in other MCC trials.

In summary, work performed in WP4 of the IMMOMEC project has a potential impact on technical advances in cutting edge immune monitoring that may be beneficial for many future trials in the growing field of cancer immunology as well as on our understanding of the immune biology of MCC and potential future immunotherapeutic approaches for this disease.

1.4.5. Potential impact of the results of WP5

Recently, many exciting developments have led to new, effective cancer immunotherapies. Immune checkpoint blockade, cytokines with and without tumor targeting, as well as adoptive T-cell transfer with and without chimeric antigen receptors results in objective, long lasting clinical responses with response rates, speed and depth even in advanced tumor stages. However, a majority of patients still do not benefit from therapy. Predictive biomarkers for response to immunotherapy are immune response gene signatures or the presence of clonally expanded CD8+ T cells within the tumor. Unfortunately, only 20% of the patients' MCC lesions are characterized by such a favorable immune signature.

[20] Prognostic value of tumor-infiltrating FoxP3(+) regulatory T cells in cancers: a systematic review and meta-analysis. Shang B, Liu Y, Jiang SJ, Liu Y (2015). Sci. Rep. 5, 15179

[21] Tumor infiltrating immune cells and outcome of Merkel cell carcinoma: a population-based study. Sihto H, Bohling T, Kavola H, Koljonen V, Salmi M, Jalkanen S, Joensuu H (2012). Clin Cancer Res 18, 2872-2881

As reported from WP4, MCPyV-specific CD8+ T cells are present in the peripheral blood of more than half of the MCC patients tested, and the intra-tumoral infiltration of CD8+ lymphocytes is a positive prognostic marker for these patients. Unfortunately, MCPyV-reactive CD8+ T cells are not fully functional in most MCC patients. This exhausted phenotype was associated with expression of PD-1 on the MCPyV-reactive T cells; notably, PD-L1 expression has been reported for both MCC cells and myeloid cells infiltrating the tumor microenvironment. Signaling via NKG2D may prevent the exhaustion of MCPyV-reactive CD8+ T cells. In addition to restoring pre-existing T-cell responses, induction of NKG2D ligand expression on MCC cells is likely to trigger new T-cell responses. Activation of NK and γδ T cells via NKG2D increases tumor cell killing and thus cross-presentation of antigens, as well as production of chemokines and cytokines attracting and activating CD8+ T cells. Furthermore, naïve CD8+ T cells express NKG2D as a co-activating receptor and binding to NKG2D ligands boosts their activation. In pre-clinical models it is well established that NKG2D ligand over-expression on tumor cells results in an increased priming and activation of tumor-specific CD8+ T cells and long lasting T-cell memory responses even against NKG2D-negative tumor cells: (i) Induction of NKG2D ligands on carcinoma cells boosts anti-tumor effects of CTLA-4 blockade, and (ii) treatment with immune stimulating cytokines such as IL-2 and IL-12 is more effective against NKG2D ligand expressing tumors.

The lack of MICA and MICB expression on MCC cells is likely to contribute to this immunological state as the re-induction of these NKG2D ligands by HDAC inhibition restores the susceptibility of MCC cells to cytotoxic lymphocytes. Thus, HDAC inhibitor-mediated MICA and MICB induction in MCC is likely to enhance the effects of immune therapeutic approaches currently tested in the clinic: (i) autologous MCPyV specific CD8+ T cell transfer (NCT01758458), (ii) the CTLA-4 blocking antibody ipilimumab (NCT02196961), (iii) the PD-L1 blocking antibody MSB0010718C (NCT02155647), or cytokine based therapies using (iv) tumor-stroma targeting antibody-IL2 fusion proteins (NCT02054884) or (v) IL12-encoding plasmids delivered by electroporation (NCT01440816). Consequently, “epigenetic priming” of cancer cells for immune recognition appears to be a valuable addition to current immune therapeutic interventions in MCC.

1.5. Address of Project Public Website and Contact Details

The IMMOMEC logo (**Figure 15**) was designed in order to identify the project. It is used together with the Grant Agreement number HEALTH-F2-2012-277775, the FP7 logo and the European flag, on any printed and electronic issues for the communication with the European Commission and for any other official contact.

Figure 15: IMMOMEC Logo

Public awareness of IMMOMEC has been addressed by the website www.immomec.eu. This web page is hosted by beneficiary 1 (MUG) and contains general information about the IMMOMEC-project, its beneficiaries and contact persons.

Contact details - Project Coordinator

Prof. Dr. Dr. Jürgen C. Becker

Medical University of Graz

General Dermatology

Auenbruggerplatz 8
8036 Graz, Austria

Phone: +43 316 385 71652
Fax: +43 316 385 72030
E-mail: juergen.becker@medunigraz.at

German Cancer Consortium (DKTK)
Partner Site Essen/Düsseldorf
University Hospital Essen

Translational Skin Cancer Research – tscr

Hufelandstrasse 55
45147 Essen, Germany

Phone: +49 201 183 6727
Fax: +49 201 183 6945
E-mail: j.becker@dkfz.de

Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES

NO	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers (if available)	Is/Will open accessprovided to this publication?
1	Reversal of epigenetic silencing of MHC class I chain-related protein A and B improves immune recognition of Merkel cell carcinoma	C Ritter , K Fan , KG Paulson , P Nghiem , D Schrama , JC. Becker	Scientific Reports	Vol. 6	Nature Publishing Group		2016	21678		Yes
2	T-cell responses to oncogenic merkel cell polyomavirus proteins distinguish patients with merkel cell carcinoma from healthy donors	Lyngaa R, Pedersen NW, Schrama D, Thrue CA, Ibrani D, Met O, thor Straten P, Nghiem P, Becker JC, Hadrup SR	Clinical Cancer Research		AACR		2014	1768-78	doi: 10.1158/1078-0432	No
3	Immunocytokines: a novel class of products for the treatment of chronic inflammation and autoimmune conditions	Franziska Bootz, Dario Neri	Drug Discovery Today	Vol. 21/ Issue 1	Elsevier Limited		2016	180-9	doi: 10.1016/j.drudis.2015.10.012 ISSN: 1359-6446	No

4	More tricks with tetramers: a practical guide to staining T cells with peptide-MHC multimers	Garry Dolton, Katie Tungatt, Angharad Lloyd, Valentina Bianchi, Sarah M. Theaker, Andrew Trimby, Christopher J. Holland, Marco Donia, Andrew J. Godkin, David K. Cole, Per Thor Straten, Mark Peakman, Inge Marie Svane, Andrew K. Sewell	Immunology	Vol. 146/ Issue 1	Wiley-Blackwell		2015	11-22	doi: 10.1111/imm.12499 ISSN: 0019-2805	Yes
5	Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing	Mahesh Yadav, Suchit Jhunjhunwala, Qui T. Phung, Patrick Lupardus, Joshua	Nature Publishing Group	Vol. 515/ Issue 7528	Nature Publishing Group		2014	572-576	doi: 10.1038/nature14001 ISSN: 0028-0836	No

		Tanguay, Stephanie Bumbaca, Christian Franci, Tommy K. Cheung, Jens Fritsche, Toni Weinschenk, Zora Modrusan, Ira Mellman, Jennie R. Lill, Lélia Delamarre								
6	Fluorescence in situ hybridization and qPCR to detect Merkel cell polyomavirus physical status and load in Merkel cell carcinomas	Haugg AM, Rennspiess D, zur Hausen A, Speel EJ, Cathomas G, Becker JC, Schrama D	Int J Cancer.	Vol. 135/ Issue 12			2015	2804-15	doi: 10.1002/ijc.28931. PubMed PMID: 24771111.	Yes
7	Response to Shuda et al.	Angermeyer S, Hesbacher S, Becker JC, Schrama D, Houben R	J Invest Dermatol.	Vol. 134/ Issue 5			2014	1481-2	doi: 10.1038/jid.2013.486. PubMed PMID: 24217012.	Yes
8	Immunocytokines: a novel class of potent armed antibodies	Pasche N, Neri D.	Drug Discov Today		Elsevier		2012	583-90	doi: 10.1016/j.drudis.2012.01.007	No

9	Downregulation of MHC-I expression is prevalent but reversible in Merkel cell carcinoma	Paulson KG, Tegeder A, Willmes C, Iyer JG, Afanasiev OK, Schrama D, Koba S, Thibodeau R, Nagase K, Simonson WT, Seo A, Koelle DM, Madeleine M, Bhatia S, Nakajima H, Sano S, Hardwick JS, Disis ML, Cleary MA, Becker JC, Nghiem P.	Cancer Immunol Res.	Vol. 2/ Issue 11			2014	1071-9	doi: 10.1158/2326-6066.CIR-14-0005. PubMed PMID: 25116754; PubMed Central PMCID: PMC4221542.	Yes
10	Prognostic relevance of high atonal homolog-1 expression in Merkel cell carcinoma.	Gambichler T, Mohtezebsade S, Wieland U, Silling S, Höh AK, Dreißigacker M, Schaller J, Schulze HJ, Oellig F, Kreuter A,	J Cancer Res Clin Oncol.	Vol.143/ Issue 1			2017	43-49	doi: 10.1007/s00432-016-2257-6. PubMed PMID: 27624714	No

		Stockfleth E, Stücker M, Bechara FG, Becker JC.								
11	T cell receptor repertoire usage in cancer as a surrogate marker for immune responses	Schrama D, Ritter C, Becker JC.	Semin Immunpathol				2017		doi: 10.1007/s00281-016-0614-9 PubMed PMID: 28074285	No
12	Merkel cell carcinoma: Epidemiology, prognosis, therapy and unmet medical needs.	Schadendorf D, Lebbé C, Zur Hausen A, Avril MF, Hariharan S, Bharmal M, Becker JC	Eur J Cancer	Vol.71			2017	53-69	doi: 10.1016/j.ejca.2016.10.022 PubMed PMID: 27984768	Yes
13	Left-sided laterality of Merkel cell carcinoma in a German population: more than just sun exposure.	Gambichler T, Wieland U, Silling S, Dreißigacker M, Schaller J, Schulze HJ, Oellig F, Kreuter A, Stücker M, Bechara FG, Stockfleth E, Becker JC	J Cancer Res Clin Oncol.	Vol.143/ Issue 2			2017	347-350	doi: 10.1007/s00432-016-2293-2. PubMed PMID: 27778198.	No

14	Voluntary Running Suppresses Tumor Growth through Epinephrine- and IL-6-Dependent NK Cell Mobilization and Redistribution.	Pedersen L, Idorn M, Olofsson GH, Lauenborg B, Nookaew I, Hansen RH, Johannesen HH, Becker JC, Pedersen KS, Dethlefsen C, Nielsen J, Gehl J, Pedersen BK, Thor Straten P, Hojman P.	Cell Metab.	Vol. 23/ Issue 3			2016	554-62.	doi: 10.1016/j.cmet.2016.01.011. PubMed PMID: 26895752.	No
15	Immunocytokines and bispecific antibodies: two complementary strategies for the selective activation of immune cells at the tumor site	Jonathan D. Kiefer, Dario Neri	Immunological Reviews	Vol. 270/ Issue 1	Blackwell Publishing		2016	178-92	doi: 10.1111/imr.12391	No
16	Diagnosis and treatment of Merkel Cell Carcinoma. European consensus-based interdisciplinary guideline	Celeste Lebbe, Jürgen C. Becker, Jean-Jacques Grob, Josep Malvehy, Veronique del Marmol, Hubert	European Journal of Cancer	Vol. 51/ Issue 16	Elsevier Limited		2015	2396-2403	doi: 10.1016/j.ejca.2015.06.131 ISSN: 0959-8049	No

		Pehamberger, Ketty Peris, Philippe Saiag, Mark R. Middleton, Lars Bastholt, Alessandro Testori, Alexander Stratigos, Claus Garbe								
17	Pre-Vaccination Frequencies of Th17 Cells Correlate with Vaccine-Induced T-Cell Responses to Survivin-Derived Peptide Epitopes.	Køllgaard T, Ugurel-Becker S, Idorn M, Andersen MH, Becker JC, Straten PT	PLoS One	Vol. 10/ Issue 7			2015	e0131934	doi: 10.1371/journal.pone.0131934 PubMed PMID: 26176858; PubMed Central PMCID: PMC4503613.	Yes
18	Antibody–Drug Conjugates and Small Molecule–Drug Conjugates: Opportunities and Challenges for the Development of Selective Anticancer Cytotoxic Agents	Giulio Casi, Dario Neri	Journal of Medicinal Chemistry	Vol. 58/ Issue 22	American Chemical Society		2015	8751-8761	doi: 10.1021/acs.jmedchem.5b00457 ISSN: 0022-2623	No

19	Redefining cancer immunotherapy-optimization, personalization, and new predictive biomarkers: 4th Cancer Immuno-therapy and Immunomonitoring (CITIM) meeting, April 27-30, 2015, Ljubljana, Slovenia	Aptsiauri.N.	Cancer Immunol Immunother	Vol.65/ Issue 7			2016	875 - 83	https://www.researchgate.net/publication/291823048_Redefining_cancer_immunotherapy-optimization_personalization_and_new_predictive_biomarkers_4th_Cancer_Immunotherapy_and_Immunomonitoring_CITIM_meeting_April_27-30_2015_Ljubljana_Slovenia	Yes
20	Antibody Format and Drug Release Rate Determine the Therapeutic Activity of Noninternalizing Antibody-Drug Conjugates.	Gébleux R, Wulhfard S, Casi G, Neri D.	Molecular Cancer Therapeutics	Vol. 14/ Issue 11	American Association for Cancer Research Inc.		2015	2606-2612	doi: 10.1158/1535-7163.MCT-15-0480	Yes
21	Tryptophan 2,3-dioxygenase (TDO)-reactive T cells differ in their functional characteristics in health and cancer.	MD Hjortsø, SK Larsen, P Kongsted, Ö Met, TM Frøsig, G Holmen, A Shamaila, M Ahmad, IM Svane, J C Becker, P thor Straten, MH Andersen	Onco-immunology	Vol. 4/ Issue 1	landes bioscience		2015	e968480	doi: 10.4161/21624011.2014.968480	Yes
22	Spontaneous presence of FOXO3-specific T cells in cancer patients.	Stine Kiaer Larsen, Shamaila Munir Ahmad, Manja	Onco-immunology	Vol. 3/ Issue 8	landes bioscience		2014	e953411	doi: 10.4161/21624011.2014.953411	Yes

		Idorn, Özcan Met, Evelina Martinaite, Inge Marie Svane, Per thor Straten, Mads Hald Andersen								
23	Cells of Origin in Skin Cancer	Jürgen C. Becker, Axel zur Hausen	J Inve Dermatol	Vol.134/ Issue 10	Nature Publishing Group		2014	2491-2493	doi: 10.1038/jid.2014.233	Yes
24	Intralesional treatment of stage III metastatic melanoma patients with L19-IL2 results in sustained clinical and systemic immunologic responses	Weide B, Eigentler TK, Pflugfelder A, Zelba H, Martens A, Pawelec G, Giovannoni L, Ruffini PA, Elia G, Neri D, Gutzmer R, Becker JC, Garbe C.	Cancer Immunol Res	Vol. 2/ Issue 7			2014	668-78	doi: 10.1158/2326-6066.CIR-13-0206. PubMed PMID: 24906352.	Yes
25	Somatostatin receptor expression in Merkel cell carcinoma as target for molecular imaging	Buder K, Lapa C, Kreissl MC, Schirbel A, Herrmann K, Schnack A, Bröcker EB, Goebeler M, Buck AK, Becker JC	BMC Cancer	Issue 14			2014	268	doi: 10.1186/1471-2407-14-268. PubMed PMID: 24742330; PubMed Central PMCID: PMC4021101.	Yes

26	The HSP70 modulator MAL3-101 inhibits Merkel cell carcinoma	Adam C, Baeurle A, Brodsky JL, Wipf P, Schrama D, Becker JC, Houben R	PLoS One	Vol. 9/ Issue 4			2014	e92041		Yes
27	Self-reactive T cells: suppressing the suppressors	Becker JC, thor Straten P, Andersen MH.	Cancer Immunol Immunother		Springer		2014	313-9	doi: 10.1007/s00262-013-1512-9	No
28	Merkel Cell Carcinoma: Epidemiology, Target, and Therapy	Hughes MP, Hardee ME, Cornelius LA, Hutchins LF, Becker JC, Gao L.	Curr Dermatol Rep.		Springer		2014	46-53	PMID: 24587977	Yes
29	Expression of stress ligands of the immunoreceptor NKG2D: regulation and clinical significance	Paschen A, Baingo J, Schaden-dorf D.	Eur J Cell Biol.		Elsevier		2014	49-54	doi: 10.1016/j.ejcb.2014.01.0	No
30	Tumor-educated myeloid cells: impact the micro- and macroenvironment	Becker JC	Exp Dermatol.	Vol. 23/ Issue 3			2014	157-8	doi: 10.1111/exd.12241. PubMed PMID: 24102950.	Yes
31	Clinical remission of Merkel cell carcinoma after treatment with imatinib.	Loader DE, Feldmann R, Baumgartner M, Breier F, Schrama D, Becker JC, Steiner A.	J Am Acad Dermatol.	Vol. 69/ Issue 4			2013	e181-3	doi: 10.1016/j.jaad.2013.03.042. PubMed PMID: 24034390.	
32	Functional characterization of	Larsen SK, Munir S,	Leukemia	Vol. 27/ Issue 12			2013	2332-40	doi: 10.1038/leu.2013.196.	No

	Foxp3-specific spontaneous immune responses	Woetmann A, Frøsig T, Odum N, Svane IM, Becker JC, Andersen MH							PubMed PMID: 23812418.	
33	Immune oncology in focus	Wölfel T, Becker JC, Schmitt M	Onkologie	Vol. 36/ Suppl 3			2013	7-11	doi: 10.1159/000350921. German. PubMed PMID: 23797364	Yes
34	Brief S2k guidelines--Merkel cell carcinoma	Becker JC, Assaf C, Vordermark D, Reske SN, Hense J, Dettenborn T, Seitz O, Grabbe S	J Dtsch Dermatol Ges.	Vol. 11/ Suppl 3			2013		doi: 10.1111/ddg.12015_6. PubMed PMID: 23734895	No
35	The dark side of cyclophosphamide: cyclophosphamide-mediated ablation of regulatory T cells	Becker JC, Schrama D	J Invest Dermatol	Vol. 133/ Issue 6			2013	1462-5	doi: 10.1038/jid.2013.67. PubMed PMID: 23673502	Yes
36	Immune-suppressive properties of the tumor microenvironment	Becker JC, Andersen MH, Schrama D, Thor Straten P.	Cancer Immunol Immunother		Springer		2013	1137-48	doi: 10.1007/s00262-013-1434-6	No
37	Merkel cell polyomavirus-positive Merkel cell carcinoma cells do not require expression of the viral small T antigen	Angermeyer S, Hesbacher S, Becker JC, Schrama D, Houben R	J Invest Dermatol	Vol. 133/ Issue 8			2013	2059-64	doi: 10.1038/jid.2013.82. PubMed PMID: 23439392.	Yes

38	Survivin downregulation is not required for T antigen knockdown mediated cell growth inhibition in MCV infected merkel cell carcinoma cells	Schrama D, Hesbacher S, Becker JC, Houben R	Int J Cancer	Vol. 132/ Issue 12			2013	2980-2	doi: 10.1002/ijc.27962. PubMed PMID: 23180604.	
39	Antibody-drug conjugates: basic concepts, examples and future perspectives	Casi G, Neri D. J	J Control Release		Elsevier		2012	422-8	doi: 10.1016/j.jconrel.2012	No
40	Type I and II IFNs inhibit Merkel cell carcinoma via modulation of the Merkel cell polyomavirus T antigens	Willmes C, Adam C, Alb M, Völkert L, Houben R, Becker JC, Schrama D	Cancer Res	Vol. 72/ Issue 8			2012	2120-8	doi: 10.1158/0008-5472.CAN-11-2651. PubMed PMID: 22389452.	Yes
41	Activation of the PI3K/AKT pathway in Merkel cell carcinoma	Hafner C, Houben R, Baeurle A, Ritter C, Schrama D, Landthaler M, Becker JC	PLoS One	Vol. 7/ Issue 2			2012	e31255	PubMed PMID: 22363598; PubMed Central PMCID: PMC3281946.	Yes
42	P-cadherin expression in Merkel cell carcinomas is associated with prolonged recurrence-free survival	Vlahova L, Dorfling Y, Houben R, Becker JC, Schrama D, Weiss C, Gobeler M, Helmbold P, Goerdt S, Peitsch WK	Br J Dermatol	Vol. 166/ Issue 5			2012	1043-52	doi: 10.1111/j.1365-2133.2012.10853.x. PubMed PMID: 22283194.	No

43	Merkel cell carcinoma: recent insights and new treatment options	Schrama D, Ugurel S, Becker JC	Curr Opin Oncol	Vol. 24/ Issue 2			2012	141-9	doi: 10.1097/CCO.0b013e32834fc9fe. Review. PubMed PMID: 22234254.	No

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO	Type of activities	Main leader	Title	Date/Period	Place	Type of audience	Size of audience	Countries addressed
1	Press release	MUG (Beneficiary 1)	Hautkrebs-Therapie: Med Uni Graz koordiniert EU-Projekt "IMMOMEC"	July 19, 2012	Press release	Civil Society		Austria
2	Press release	MUG (Beneficiary 1)	Skin Cancer Therapy: Medical University of Graz coordinates EU-project „IMMOMEC“ searching for new ways to treat Merkel cell carcinoma	July 19, 2012	Press release	Civil Society		Austria
3	articles published in the popular press	MUG (Beneficiary 1)	Grazer Forscher entwickeln im Verbund Immuntherapie gegen Hautkrebs	July 19, 2012	Wiener Zeitung Online	Civil Society		Austria
4	articles published in the popular press	MUG (Beneficiary 1)	Grazer Forscher entwickeln Immuntherapie gegen Hautkrebs	July 19, 2012	Der Standard Online	Civil Society		Austria
5	articles published in the popular press	MUG (Beneficiary 1)	Immuntherapie gegen Hautkrebs entwickelt	July 19, 2012	APA online	Civil Society		Austria
6	articles published in the popular press	MUG (Beneficiary 1)	Grazer Forscher entwickeln im Verbund Immuntherapie gegen Hautkrebs	July 19, 2012	Kleine Zeitung Online	Civil Society		Austria
7	articles published in the popular press	MUG (Beneficiary 1)	Immuntherapie gegen Hautkrebs aus Graz	July 20, 2012	Salzburger Nachrichten	Civil Society		Austria
8	articles published in the popular	MUG (Beneficiary 1)	Med Uni Graz zieht Fäden bei EU-Projekt	July 25, 2012	Grazer Woche	Civil Society		Austria

	press							
9	articles published in the popular press	MUG (Beneficiary 1)	Med Uni koordiniert EU-Projekt „IMMOMEC“ – Neue Behandlungsmethoden für aggressive Hautkrebsart	July 26, 2012	Kronen Zeitung	Civil Society		Austria
10	articles published in the popular press	MUG (Beneficiary 1)	Gold aus Dänemark	December, 2012	Das Magazin der Ärztekammer Steiermark	Civil Society		Austria
11	articles published in the popular press	MUG (Beneficiary 1)	Großprojekt gegen tödlichen Hautkrebs	January 17, 2013	Kleine Zeitung	Civil Society		Austria
12	Other	MUG (Beneficiary 1)	ForscherInnen hoffen, eine Therapie für seltenen Hautkrebs zu finden	September, 2012	MEDITIO 02/2012	Civil Society		Austria
13	interview	MUG (Beneficiary 1)	Kampf dem Hauttumor	December 10, 2012	uni.webradio – Webradio der Grazer Universitäten	Civil Society		Austria
14	Web	IMM (Beneficiary 3)	EU-funded IMMOMEC consortium to develop novel methods for treatment of skin cancer patients	July 17, 2013	Immatics website: http://www.immatics.com/	Civil Society		Germany
15	Flyers	MUG (Beneficiary 1)	IMMOMEC - IMmune MOdulating strategies for treatment of MErkel cell Carcinoma	June 01, 2013	http://www.immomec.eu/project/flyer/	Civil Society		Austria
16	Presentation	MUG (Beneficiary 1)	Melanoma, Basalioma and Merkel Cell Carcinoma	September 26, 2014	ESMO 2014, Madrid, http://www.i-med.institute/_mulitmedia/2014/esmo/wcp_becker/html5_player.html	Civil Society		Austria
17	Web	APHP (Beneficiary5)	Protocole carcinoma á cellules de Merkel IMMOMEC	August 29, 2013	Assistane Publique-Hôpiteux de Paris- website: Http://cancer-ghparis10.aphp.fr/protocole-carcinome-a-cellules-de-	Civil Society		France

					merkel-immomec/			
18	Web	MUG (Beneficiary 1)	MCC Database	July 7, 2014	http://www.immomec.eu/mcc-database/	Civil Society		Austria
19	Presentation	MUG (Beneficiary 1)	'Immune escape mechanims of Merkel cell carcinoma' (22-24 January 2015: 1st International Symposium on Tumor-Host Interaction in Head and Neck Cancer, in conjunction with the 5th Annual Meeting on Experimental and Translational Head and Neck Oncology. www.headandneck2015.org, Essen)	January 22, 2015	Essen	Scientific Community		Germany
20	Presentation	MUG (Beneficiary 1)	'Merkel Cell Carcinoma' (•?11-14 March 2015: ENETS – European Neuroendocrine Tumor Society, 12th Annual ENETS Conference, http://www.enets.org/barcelona 2015.html, Barcelona)	November 11, 2015	Barcelona	Scientific Community		Spain
21	Presentation	MUG (Beneficiary 1)	'Biology and Immunology of MCC' (13-14 April 2015: Third scientific retreat - German Cancer Research Center (DKTK ? Deutsches Konsortium für Translationale Krebsforschung)	April 13, 2015	Heidelberg	Scientific Community		Germany
22	Presentation	MUG (Beneficiary 1)	'Skin Cancer - an update' (24-25 April 2015: Wilsede School of Oncology, Wilsede)	April 24, 2015	Wilsede	Scientific Community		Germany

23	Presentation	MUG (Beneficiary 1)	Merkel cell carcinoma' (29 April - 2 May 2015: 48. DDG-Tagung (Deutsche Dermatologische Gesellschaft ? German Dermatological Society), http://www.derma.de/de/fuer-aerzte/48-ddg-tagung/, Berlin)	April 29, 2015	Berlin	Scientific Community		Germany
24	Presentation	MUG (Beneficiary 1)	'Tumor escape mechanisms: Immune checkpoint inhibition in MCC' (29 May - 2 June 2015: ASCO (American Society of Clinical Oncology), Annual Meeting 2015, http://am.asco.org/, Chicago)	May 29, 2015	Chicago	Scientific Community		USA
25	Presentation	MUG (Beneficiary 1)	'Clinical trials in MCC: ADMEC and IMMOMEC' (24 June - 27 June 2015: 5th European Post-Chicago Meeting on Melanoma / Skin Cancer Meeting, http://www.melanomaglobal2015.org/, Munich)	June 24, 2015	Munich	Scientific Community		Germany
26	Presentation	MUG (Beneficiary 1)	'Clinical trials in MCC: ADMEC and IMMOMEC' (9-12 September 2015: The 25th Skin Cancer Congress ADO, http://www.ado-kongress.de/, Munich)	September 9, 2015	Munich	Scientific Community		Germany
27	Presentation	MUG (Beneficiary 1)	Immune therapeutic approaches in skin cancer' (26-28 September 2015: 18th	September 26, 2015	Vienna	Scientific Community		Austria

			ECCO – 40th ESMO European Cancer Congress, Reinforcing multidisciplinarity, https://www.europeancancercongress.org/, Vienna)					
28	Interview	UKEssen (Beneficiary 10)	Malignes Melanom: Immuntherapie gewinnt an Bedeutung (26-28 September 2015: 18th ECCO ? 40th ESMO European Cancer Congress, Reinforcing multidisciplinarity, https://www.europeancancercongress.org/, Vienna)	September 26, 2015	Vienna	Scientific Community		Austria
29	Presentation	MUG (Beneficiary 1)	'Tumor immunology' (28-31 October 2015: 11th EADO Congress & 8th World meeting of Interdisciplinary Melanoma/Skin Cancer Centres, http://www.eado.org/event/11th-eado-congress-and-8th-world-meeting-of-interdisciplinary-melanoma-skin-centers_9, Marseille)	October 28, 2015	Marseille	Scientific Community		France
30	Press release	UKEssen (Beneficiary 10)	DKTK Essen: Gefährliches Merkelzellkarzinom der Haut enttarnt	March 2, 2016	http://www.derma.de/de/news/uebersicht/detail/article/4085/1049/	Civil Society		Germany
31	articles published in the popular press	UKEssen (Beneficiary 10)	Merkelzellkarzinom: Reaktivierte Zielscheibe	March 7, 2016	http://news.doccheck.com/de	Civil Society		Germany
32	Presentation	IMM (Beneficiary 3)	Presentation at the 4th Cancer Immunotherapy and Immuno-monitoring (CITIM) meeting	April, 2015	Immunomonitoring for personalized cancer immune-therapies	Scientific Community		Slovenia
33	Presentation	MCMCC	Presentation at the Warsaw	October, 2015	Merkel Cell Carcinoma –	Scientific		Poland

		(Beneficiary 11)	Skin Cancer Conference		treatment perspectives	Community		
34	Web	UKEssen (Beneficiary 10)	Immuntherapie beim metastasierten Merkelzellkarzinom	2016	https://www.krebsgesellschaft.de/onko-internetportal/kongresse/asco-annual-meeting/asco-annual-meeting-2016.html#haut	Scientific Community		Germany
35	Web	UKEssen (Beneficiary 10)	Immuntherapie beim metastasierten Merkelzellkarzinom	2016	https://www.krebsgesellschaft.de/onko-internetportal/kongresse/asco-annual-meeting/asco-annual-meeting-2016/immuntherapie-beim-metastasierten-merkelzellkarzinom.html	Scientific Community		Germany
36	Web	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	International Symposium on the Immunology and Immunotherapy of Merkel cell Carcinoma	2016	http://www.hpvconference2016.nl/programme/immomec_meeting	Scientific Community		Austria/ Germany
37	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	International Immunooncology Meeting, 20. -22.1 in Philadelphia, PA, USA	January, 2016	Immune escape mechanisms of MCC, Jürgen C. Becker	Scientific Community		USA
38	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	Deutscher Krebskongress, 24. bis 26.2. in Berlin	February, 2016	Loss of non classical MHC molecules as immune escape mechanism of MCC, Cathrin Ritter und Jürgen C. Becker	Scientific Community		Germany
39	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	Deutscher Krebskongress, 24. bis 26.2. in Berlin	February, 2016	Immuntherapie des MCC, Jürgen C. Becker	Scientific Community		Germany
40	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	Pathologie, UK Essen, 10.3. in Essen	March, 2016	Immunologie des MCC, Jürgen C. Becker	Scientific Community		Germany
41	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	Prime Oncology, 21.5. in Amsterdam	May, 2016	Immunotherapy of Skin Cancer, Jürgen C. Becker	Scientific Community		Netherlands
42	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	ASCO 2016, 2.6. bis 7.6 in Chicago	June, 2016	Immune escape mechanisms of MCC, Jürgen C. Becker	Scientific Community		USA

43	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	Viruses and Cancer, 10 -12.6 in Bologna	June, 2016	MCPyV and MCC, Jürgen C. Becker	Scientific Community		Italy
44	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	Microbiome and Cancer, 23. - 25.8. in Bochum	August, 2016	Immunology and biology of MCC, Jürgen C. Becker	Scientific Community		Germany
45	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	Dermatologische Fortbildung Universität Bochum, 27.8. in Bochum	August, 2016	Diagnostik und Therapie des MCC, Jürgen C. Becker	Scientific Community		Germany
46	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	EADO, 31.8 - 3.9. in Wien	September, 2016	Immunologic Therapy of MCC, Jürgen C. Becker	Scientific Community		Austria
47	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	Wilseder Schule - Onkologie Kompakt, 15.9. - 17.9. in Wilsede	September, 2016	Neue Entwicklungen in der Behnadlung von Hauttumoren, Jürgen C. Becker	Scientific Community		Germany
48	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	DKTK-PEI Workshop on Therapeutic Vaccines, 20.9. in Langen	September, 2016	Vaccines and immune modulation in MCC	Scientific Community		Germany
49	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	ADO-Tagung, 21.9. - 24.9. in Dresden	September, 2016	PD-L1 expression and MHC class I loss in MCC, Jürgen C. Becker	Scientific Community		Germany
50	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	ESMO Perceptorship, 30.9. - 1.10. in Amsterdam	October, 2016	Innovative Therapy pf MCC, Jürgen C. Becker	Scientific Community		Netherlands
51	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	ESMO 2016, 7.10 - 10.10 in Kopenhagen	October, 2016	Second line chemotherapy in MCC, Jürgen C. Becker	Scientific Community		Denmark
52	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	IMMOMEC final Meeting and International Symposium	November, 2016	International Symposium on the Immunology and Immunotherapy of Merkel cell Carcinoma, Jürgen C. Becker	Scientific Community		Netherlands
53	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	8th HPV and Polyomvirus Concerence 16.11 - 18.11 in Maastricht	November, 2016	Immuneescape mechanisms of MCC, Jürgen C. Becker	Scientific Community		Netherlands
54	Presentation	MUG (Beneficiary 1)/	AEK Autumn School on	November,	Characterization of the	Scientific		Germany

		UKEssen (Beneficiary 10)	Immuno-oncology, 21.11 - 22.11. in Berlin	2016	immune infiltrate in MCC, Linda Kubat	Community		
55	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	AEK Autumn School on Immuno-oncology, 21.11 - 22.11. in Berlin	November, 2016	miR375 as a surrogate biomarker of MCC tumor burden, Kaiji Fan	Scientific Community		Germany
56	Presentation	MUG (Beneficiary 1)/ UKEssen (Beneficiary 10)	AEK Autumn School on Immuno-oncology, 21.11 - 22.11. in Berlin	November, 2016	TCR Repertoire analyses in MCC, Ivelina Spassova	Scientific Community		Germany

Section B (confidential)

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.			
Type of IP Rights: Patents, Trademarks, Registered designs, Utility models, etc.	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)

TEMPLATE B2: OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND					
Exploitable Foreground (description)	**Exploitable product(s) or measure(s)**	**Sector(s) of application**	**Timetable, commercial use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**

1.6 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	277775
Title of Project:	IMMOMEC
Name and Title of Coordinator:	Prof. Dr. Dr. Jürgen C. Becker

B Ethics

1. Did you have ethicists or others with specific experience of ethical issues involved in the project?	○	**Yes**

2. Please indicate whether your project involved any of the following issues (tick box) :	**YES**
INFORMED CONSENT	
• Did the project involve children?	
• Did the project involve patients or persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human Genetic Material?	
• Did the project involve Human biological samples?	X
• Did the project involve Human data collection?	X
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)	
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	
• Were those animals transgenic small laboratory animals?	
• Were those animals transgenic farm animals?	
• Were those animals cloning farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Use of local resources (genetic, animal, plant etc)	
• Benefit to local community (capacity building ie access to healthcare, education etc)	
DUAL USE	
• Research having potential military / terrorist application	

C Workforce Statistics

3 Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	0	1
Work package leader	3	4
Experienced researcher (i.e. PhD holders)	25	16
PhD Students	3	1
Other	35	11

4 How many additional researchers (in companies and universities) were recruited specifically for this project?	**10**
Of which, indicate the number of men:	4
Of which, indicate the number of women:	6

D Gender Aspects

5	**Did you carry out specific Gender Equality Actions under the project ?**	**X**	Yes

6 Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	X
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	X
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	X
○	Other:					

7 Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No

E Synergies with Science Education

8 Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

X No

9 Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

X Yes- please specify

- http://www.immomec.eu
- www.merkelzell.de in cooperation with Merck Serono

○ No

F Interdisciplinarity

10 Which disciplines (see list below) are involved in your project?

x	Main discipline: 3.2 Clinical Medicine		
x	Associated discipline: 3.1 Basic Medicine	x	Associated discipline: 1.5 Biological Sciences

G Engaging with Civil society and policy makers

11a Did your project engage with societal actors beyond the research community? *(if 'No', go to Question 14)*	**X** ○	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

- ○ No
- ○ Yes- in determining what research should be performed
- ○ Yes - in implementing the research
- **X** Yes, in communicating /disseminating / using the results of the project

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ **X**	Yes No

12 Did you engage with government / public bodies or policy makers (including international organisations)

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- **X** Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- **X** Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	x
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	x
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- **X** Local / regional levels
- **X** National level
- **X** European level
- ○ International level

H Use and dissemination

14 How many Articles were published/accepted for publication in peer-reviewed journals?	**43**
To how many of these is open access provided?	**22**
How many of these are published in open access journals?	**22**
How many of these are published in open repositories?	
To how many of these is open access not provided?	**19**
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository **X** no suitable repository available **X** no suitable open access journal available **X** no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other: ……	

15 How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**None**
16 Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**None**
	Registered design	**None**
	Other	**None**
17 How many spin-off companies were created / are planned as a direct result of the project?		**None**
Indicate the approximate number of additional jobs in these companies:		

18 Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	**X**	None of the above / not relevant to the project
❑	Difficult to estimate / not possible to quantify	❑	

19 For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs	*Indicate figure:*
Difficult to estimate / not possible to quantify	**X**

I Media and Communication to the general public

20 As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes **X** No

21 As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes **X** No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

X	Press Release	**X**	Coverage in specialist press
X	Media briefing	**X**	Coverage in general (non-specialist) press
❑	TV coverage / report	❑	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
X	Brochures /posters / flyers	**X**	Website for the general public / internet
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

X	Language of the coordinator	**X**	English
X	Other language(s)		