Cancer Vaccine development for Hepatocellular Carcinoma

Berichterstattung

Projektinformationen

HEPAVAC

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Final Report Summary - HEPAVAC (Cancer Vaccine development for Hepatocellular Carcinoma)

Executive Summary:
PROBLEM: Hepatocellular carcinoma - HCC is a disease with high unmet medical need. Indeed, it accounts for about 6% of all new cancer cases diagnosed worldwide (nearly 750,000 new cases/year), and is the third and the fifth leading cause of death from cancer globally in men and women, respectively. Given the current lack of available effective treatments, the overall prognosis for patients with HCC is poor with a dismal 5-year survival rate of approximately 5-6%. In such a framework, development of innovative and novel therapies for HCC is mandatory and immunotherapeutic interventions, including cancer

vaccines, may represent a valuable strategy.
AIM: The main objective of HepaVac is to develop a novel cancer vaccine approach for HCC based on epitopes naturally processed and presented by HLA class I and class II molecule (HLA-ligandome), to elicit both CD4+ T helper and CD8+ CTL tumor-specific effector and memory responses. Such an approach aims at improving clinical outcome in adjuvant HCC patients after standard treatment. Feasibility, safety and immunogenicity will be evaluated in a randomized, controlled European multi-centre phase I/II clinical trial.
EXPERIMENTAL APPROACH: The experimental approach undertaken by the HepaVac Consortium is based on development of an “off-the-shelf” vaccine comprising multiple newly identified tumor-associated peptides (TUMAPs) naturally presented on the surface of primary HCC cells. Upon immunological validation of HCC-specific TUMAPs, a peptide cocktail made of up to 40 HLA class I and II restricted epitopes will be designed for a multi-epitope and multi-HLA allele strategy, aiming at inducing both tumor-specific CD4+ T helper cell and cytotoxic CD8+ lymphocyte effector and memory immune responses. Furthermore, a sub-set of patients will be boosted with newly identified patient-specific HCC-associated mutated epitopes in an actively personalized vaccine (APVAC) approach. The “off-the-shelf” as well as personalized vaccine will be combined with a novel and potent RNA-based immunomodulator (RNAdjuvant®). Safety, feasibility and immunogenicity of the suggested approach will be tested in a randomised, controlled European phase I/II multi-centre clinical trial. A comprehensive T-cell immunomonitoring and biomarker program will be implemented to assess in detail the mechanism-of-action (MoA), identify immunological prediction markers of responsiveness and support further clinical development.
This will be one of the very few vaccine trials for HCC and the first multi-epitope, multi-target and multi-HLA allele therapeutic cancer vaccine for such a frequent and aggressive disease. Targeting the tumor with such a wide range of naturally occurring antigens will minimize the likelihood for tumor escape in vaccinated patients.

Project Context and Objectives:
1.1. Results of the whole project.
HEPAVAC is based on seven work packages (WPs) establishing a continuous workflow from tumor antigen identification to vaccine development and evaluation in a phase-I/II clinical trial.
The first task encountered in the implementation of a large project is the coordination of the activities of the different WPs in a given time frame. In the fourth reporting period of 18 months (total 72 months from the beginning of the project), Partners have met in many meetings and discussed in conference calls. The reserved area of the HEPAVAC website has been pivotal for sharing information among participants and archiving a spectrum of project-related documents. The website has also a public domain, which informs on the project features and provides information on issues of general interest.
The results so far obtained can be summarized as follows:
WP1 – Discovery of the hepatocellular carcinoma HLA class I-ligandome (M1 – M36)
Tumor associated antigens (TUMAPs) have been identified from primary HCC samples and tumor association was determined by mass spectrometry as well as gene expression profiling. These data sets supplemented by literature research lead to the selection of vaccine peptide candidates for non GMP synthesis and immunogenicity validation. After integration of all data sets, seven HLA-A*02 positive TUMAPs and five HLA-A*24 positive TUMAPs were chosen for the composition of the HepaVac cocktail. Additionally, the assessment of spontaneous immunogenicity of the selected TUMAPs was performed in

peripheral blood mononuclear cells (PBMC) from healthy subjects.

Details for each task:
Task 1. Tumor sample collection [TUE, IMM]
Sample collection started in September 2013. Tumor samples from 93 HCC patients were collected from the consortium members and additional 33 samples were acquired from 3 commercial biobanks. For detailed information please refer to deliverable report D1.1.
Task 2. Identification of HCC associated peptides from tumor samples by mass spectrometry [TUE, IMM]
Analysis by mass spectrometry was done for 17 A*02+ and 15 A*24+ HCC samples, identifying 7262 and 3297 unique TUMAPs respectively. Based on both the mass spectrometry data and the gene expression data that was acquired as part of Task 3 (see below) 34 HLA-A*02 peptides and 36 HLA-A*24 peptides were selected to be tested for immunogenicity in Task 5 of this WP. For detailed information please refer to deliverable report D1.1.
Task 3. Generation of gene expression profiling data from HCC samples by microarrays [TUE, IMM]
RNA was isolated from 12 HHC tissue samples and gene expression analysis was done using the Affymetrix Human Genome (HG) U133A or HG-U133 Plus 2.0 oligonucleotide microarrays. For several different healthy tissues and organs, a library of gene expression data was already available at IMM. For a more detailed description please also refer to deliverable report D1.1.
Task 4. Synthesis and purity testing of HCC class I TUMAPs [TUE, IMM]
Based on the results from task 2 and 3, 34 and 36 peptides, presented by HLA-A*02 and HLA-A*24 respectively, were selected for synthesis. All peptides were synthesized successfully in the needed purity. For all peptides solubility as well as stability at a higher temperature, in basic and in acidic media was investigated. For detailed information please refer to deliverable report D1.2.
Task 5. Immunologic validation of selected HCC class I TUMAPs with artificial antigen-presenting cells [TUE, IMM]
Candidate peptides for a multi-target vaccine were tested for in vitro immunogenicity, using standardized in vitro assays based on priming and expansion of isolated human CD8+ cells of healthy HLA-matched donors. 53 of 66 HCC-associated peptides (26 HLA-A*02- and 27 HLA-A*24-restricted peptides) were found to be immunogenic. For detailed information please refer to deliverable report D1.2.
Task 6. Assessment of the spontaneous immunogenicity of the selected TUMAPs in patients bearing antigen-expressing tumors [UN, former INSERM]
The presence of CD8+ T cell precursors specific for the HepaVac peptides was assessed in PBMC samples from healthy donors. We initially tested the HLA class-II restricted peptides including peptide 25-37 from the melanocyte derived antigen Melan-A for which a high frequency of CD8+ T cell precursors among CD45RA+ CCR7+ naive cells have been previously reported by us and others.
In these experiments circulating CD8+ T cell from healthy donors were isolated by magnetic cell sorting and re-stimulated (in average 10 million cell per peptide) with each of the peptides in the presence of cells from the CD8- fraction. These experiments were conducted with PBMC from 4 HLA-A*02 positive healthy donors. Cultures were assessed ten days later by intracellular IFNgamma secretion measurements following re-stimulation with each of the corresponding peptides. As anticipated responses to Melan-A (in average 1%) were detected in all donors. In contrast responses to HepaVac peptides were not generally detected except for a moderate response to QAR-5 peptide in 3 donors (0.06%), as represented in the following figure.
The analysis in HCC patients is still ongoing and will be finalized at the end of the clinical trial. To

overcome the limitation of assessing the responses by intracellular cytokine secretion, HLA-A*02 peptide multimers have been synthetized and used. This provides not only a methodology for higher sensitivity but will also make the assay independent from the production of specific cytokine. Finally, these data provide a baseline to assess the presence and frequency of HepaVac specific CD8+ T cells in HCC patients prior to and following vaccination. For more detailed information please refer to deliverable report D1.3.
WP2 – HLA class II-ligandome identification (M1 – M36)
Four HLA class II-bound peptides were selected for inclusion in the HepaVac cancer vaccine (IMA970A). IMM additionally analyzed the tumor cell lines provided by UNINS and additional tumor samples from patients infected with hepatitis B and C viruses (HBV and HCV) to confirm the suitability of the IMA970A TUMPAs for these patients. Thereafter the selection of class II-restricted peptides was repeated and revealed 4 class II-bound peptides. The synthesis and purity testing of HCC class II-restricted TUMAPS were achieved. Additionally, the assessment of spontaneous immunogenicity of the selected TUMAPs was performed in PBMC samples from healthy subjects.
As outlined in the second scientific report (months 19 - 36) four HLA class II-bound peptides were selected for inclusion in the HepaVac cancer vaccine (IMA970A). IMM additionally analyzed the tumor cell lines provided by UNINS and additional tumor samples from patients infected with HBV and HCV in order to confirm the suitability of the IMA970A TUMPAs for these patients. Thereafter the selection of class II-restricted peptides was repeated and revealed the 4 class II-bound peptides presented in Table 1. Assessment of spontaneous immunogenicity of the selected TUMAPs was performed in PBMC samples from healthy subjects.

Table 1: Names and class of HLA class II-restricted peptides contained in IMA970A
Item Immatics peptide name Structure TUMAP Type / HLA restriction Source gene name according to the “Human Genome Organization Gene Nomenclature Committee”
1 IMA-IGF2BP3-002 linear 14-AA peptide Class II / DR Insulin-like growth factor 2 mRNA binding protein 3
2 IMA-MET-005 linear 17-AA peptide Class II / DR MET proto-oncogene, receptor tyrosine kinase
3 IMA-MTT-001 linear 18-AA peptide Class II / DR Microsomal triglyceride transfer protein
4 IMA-SLC25A13-001 linear 21-AA peptide Class II / DR Solute carrier family 25 (aspartate/glutamate carrier), member 13

Details for each task

Task-1: Tumor sample collection and HCC cell lines culture [UNINS, INTNA]
Both partners have implemented the collection of a substantial number of tumor sample tissues, beyond the collection of HCC tumor cell lines already described in the first and in the second reporting periods (months 1 - 18 and months 19 - 36, respectively). Fresh tumor tissues were frozen following the protocol established by the consortium. In parallel, HLA genomic typing for major HLA class I (A, B, C loci) and HLA class II (DR, DQ, DP) was performed. In order to assess the HLA class I and class II expression in hepatic carcinoma, UNINS unit completed the immunohistochemical analysis (IHC) of 43 HCC tumor tissues from 43 different patients. Paraffin-embedded tumor tissues were characterized for both their tumor cells and the tumor-infiltrating components. Classical hematoxylin-eosin (HE) staining was first performed to assess the cellular and stroma composition of the tumor tissue. Markers such as Cytokeratin 8, Cytokeratin 18 and HepPar-1, were used to distinguish tumor cells from other cellular components. The

tumor infiltrating cells were characterized to assess leukocyte subpopulations such as myeloid (monocyte-macrophages, granulocytes) and lymphoid (T, B, NK) cells. Considering the T lymphocyte subpopulation, beside the classical markers identifying the CD4 and CD8 cell subsets, additional markers, designated checkpoint inhibitors, such as PD1 and CTLA-4, whose expression has been recently shown to be of prognostic value, were assessed. Within this frame, the possibility that PDL1, the PD1 ligand, was expressed on tumor cells and associated with worst prognosis, was also assessed. While normal hepatocytes displayed no HLA class I and HLA class II expression, HCC cells strongly upregulated HLA class I while remaining mostly negative for HLA class II expression. Interestingly, absence of HLA class II expression in HCC cells correlated with lack of expression of the major HLA class II transactivator, CIITA, which could not be rescued even after treatment with its major inducer, IFN-γ. HCC tumor tissues displayed distinct degree of lymphocyte infiltration which did not correlate with pre-existing diagnosis status of HBV or HCV infection. Expression of PD-1 was detected in infiltrating cells, mainly represented by lymphocytes, interspersed within the HCC tumor tissue. PD-L1 expression was seen in cells with monocyte-macrophage morphology at the margin of tumor cell areas and usually not infiltrating the tumor mass. We did not find HCC PD-L1-positive tumor cells. Our results strongly suggest that HCCs, in contrast to other tumor types, by upregulating their HLA class I may favor the display and presentation of possible tumor antigens to the immune system, although they cannot act as surrogate APCs for HLA class II-restricted tumor antigens. The variability of immune checkpoint expression, particularly PD-L1 expression in infiltrating cells but not in tumor cells, should also be taken into account when envisaging strategies of anti-checkpoint inhibitors treatment in order to select the appropriate HCC that express high levels of PD-1/PD-L1 markers.

Task-2 Establishment of HLA class II positive HCC cells [UNINS, INTNA]
This task was accomplished during the first reporting period (months 1-18). For detailed information please refer to deliverable report D2.1.
Further implementation was performed during the second reporting period (months 19 - 36) particularly as far as the immunohistochemical characterization of HCC which will be relevant also for the implementation and achievements of the identification of potential cellular biomarkers to predict responding patients (see WP6).

Task-3: Identification of HLA class II associated peptides from HCC tumor cells [IMM, UNINS, INTNA]
A protocol applying the anti-DP monoclonal antibody provided by UNINS was established at IMM. The cell line HEP3B-fCIITA (1st reporting period - months 1 - 18) and additionally the cell line PLC/PRF5-fCIITA (2nd reporting period - months 19 - 36), and 10 primary HCC samples (1st reporting period - months 1 - 18) and additionally a number of viral infected tumor samples (2nd reporting period - months 19 - 36) were analyzed by mass spectrometry (LC-MS/MS using e.g. an LTQ-Orbitrap mass spectrometer, Thermo Scientific, connected to a nanoAcquity uPLC, Waters). As outlined in the first reporting period, from the primary HCC samples 11,001 unique HLA-DR-restricted TUMAP sequences could be identified. From the cell line HEP3B-fCIITA 6368 unique TUMAPs were identified. As outlined above, although the minimal requirements for the accomplishment of Task 3 was reached on time for the first reporting period (months 1 - 18), at the Paul-Ehrlich Institute (PEI) preparation meeting in Mainz (May 2015) it was decided to make additional efforts to identify additional HLA class II-restricted TUMAPs to potentiate the CD4+ T helper cell stimulation arm of the anti-HCC vaccine. Moreover, additional HCC tumor samples were analyzed in order to confirm the suitability of the HepaVac vaccine (IMA970A) for HBV and HCV infected patients. This

approach was considered by the HepaVac consortium as suitable, since the chance for superior vaccine efficacy was increased, adding substantial value to the main goal of the HepaVac-101 clinical trial. In this direction we assessed the TUMAPs of an additional, highly informative HCC cell line PLC/PRF5-fCIITA, expressing an HLA class II allelic genotype present in 25% of the Caucasoid population and of additional viral infected tumor samples. From the cell line, 3775 peptide sequences were isolated, of which 2252 were HLA-DR-specific. After in-depth analysis, 4 HLA-DR TUMAPs were identified by comparative expression in HCC fresh tumor samples and CIITA-transfected HCC tumor cell lines and selected for purification and inclusion in the final vaccine cocktail (see Table 1). For detailed information please refer to deliverable report D2.2.
In addition, results were confirmed at INTNA with a novel two-step bioinformatics strategy, used in HCC samples to identify signatures with pathogenetic, diagnostic and, most importantly, therapeutic implications in hepatitis-associated HCC. HCC-specific signatures were identified by a meta-analysis of publicly available microarray databases. Results were fully validated by 1) deep sequencing analysis (e.g. RNASeq) on totally unrelated HCC samples; 2) immunohistochemistry analysis on HCC as well as 20 normal tissues, available at the Human Protein Atlas (Petrizzo et al., Identification and Validation of HCC-specific Gene Transcriptional Signature for Tumor Antigen Discovery. 2016. Sci Rep 6: 29258 .

Task-4: Synthesis and purity testing of HCC class II TUMAPs [TUE, IMM]
After the cumulative re-evaluation of the HLA class II-bound peptides, the 4 peptides described in task 3 and listed in Table 1 were finally selected for synthesis. These peptides were chosen based on them being coded by genes that especially showed a high expression on fresh hepatocellular carcinoma samples and in CIITA-transfected cell lines. All selected peptides have been successfully synthesized and tested by RP-HPLC. Purity was between 25.7% and 77.2%. Although the work related to this task was initiated during the first reporting period (28-Feb-2015, months 1-18), the selection of the HLA class II TUMAPS was completed in August 2015. Please also refer to deliverable report D2.2.

Task 5. Assessment of the spontaneous immunogenicity of the selected TUMAPs in patients bearing antigen- expressing tumors [UN, former INSERM].
The assessment of spontaneous immunogenicity of the selected TUMAPs has been performed so far only in samples from healthy subjects, since the PBMC sample collection from HCC patients started with a delay. This assessment in HCC patients, as well as the evaluation of the role of CD4+ Treg in HCC has already begun, and it is currently in progress. These two evaluations are going to be completed at the end of the HepaVac-101 clinical trial.

Task 6. Analysis of the Treg infiltrate in HCC [UN, former INSERM].
The work related to this task is currently in progress.

Significant results achieved

D2.1 and D2.2 were completed and MS6 to MS8 were successfully achieved. Considering the aforementioned delay in the beginning of the HepaVac-101 clinical trial, and consequently in the PBMC sample collection, D2.4 was completed and MS9 and MS10 were achieved although in delay.
Task 1.
Immunohistochemical characterization of 43 HCC with several important findings:

• 41/43 samples strongly express HLA class I compared to a relatively low expression in normal hepatocytes. This finding establishes that the tumor HLA class I ligandome can be representative of the class I TUMAPs and is significant in view of the possible immunogenicity of HLA class I-bound TUMAPs selected for the vaccine;
• only 3/43 HCC cells expressed in a very low percentage of cells low levels of HLA class II. These results further justify the approach of CIITA transfection of HCC cells to assess the HLA-II ligandome of HCC cells potentially useful for characterizing TUMAPs.
• For the first time an extensive analysis on the HCC tumor tissues has been initiated and strongly indicates that most HCC have an infiltrate with an heterogenous pattern of expression of PD-1 checkpoint marker. More importantly, our results indicate, at variance with previous reports, that the HCC tumor cells are virtually negative for the ligand of PD-1, the PD-L1 molecule, whereas positivity for this marker is essentially confined to myeloid cells (monocytes and DC) infiltrating the tumor. This finding may have a relevant impact on the outcome of the vaccination, since the tumor microenvironment seems not to be particularly immunosuppressive. Please refer also to Deliverable report D2.1.
Task 2.
• Construction, isolation and phenotypic characterization of CIITA-transfected HCC lines newly expressing HLA class II genes and corresponding molecules.
• HLA class I and class II expression of HCC cell lines before and after the inflammatory stimulus of IFN-γ. Here we obtained the unprecedented and very significant result that while HCC cell lines can upregulate their HLA class I expression, they could not be induced to express HLA class II genes and corresponding molecules, due to the blocking of expression of CIITA. This result is very important because suggests that in vivo HCC could not present HLA class II-restricted tumor peptides to CD4 TH cells, further emphasizing the importance of our protocol of genetic transfer of an expressable CIITA to these cells in order to make them HLA class II-positive and forcing them to display potential HLA class II-restricted tumor peptides for scrutiny by TH cells. This has been the rationale to select the potential tumor peptides that are now used in our vaccine peptide cocktail.
Task 3.
• Purification of HLA class II molecules and HLA-II-bound peptides from CIITA-transfected HCC and from fresh tumor tissues has been finally achieved with the selection of 4 representative TUMAPs to include in the HepaVac vaccine cocktail. The decision of peptide selection has taken advantage from the more extensive characterization of the HLA class II-bound peptide repertoire expressed in CIITA-transfected HCC cell lines which have elucidated the presence of additional, more suitable peptide candidates based on their tumor-specific expression and capacity to be loaded on the tumor HLA class II molecules.
Task 4.
• All selected peptides have been successfully synthesized and tested by RP-HPLC. Purity was between 25.7% and 77.2%. Please also refer to deliverable report D2.2.
Task 5.
• It will be reached by the end of the clinical trial in 2020.
Task 6.
• It will be reached by the end of the clinical trial in 2020.
WP3 – Development and GMP manufacturing of an off-the-shelf multi-epitope vaccine (M1 – M47)
WP3 related objectives were so far completely achieved. The formulation/development and the GMP manufacturing of an off-the-shelf multi-epitope vaccine (IMA970A) was successfully completed (GMP vendors were selected and contracted). Likewise, the GMP manufacturing of RNAdjuvant® was reached.

Additionally, the way of applying the vaccine was evaluated, as well as its in vivo tolerance (in vivo safety). Both IMPs (IMA970A and RNAdjuvant®) were successfully study-specific labeled, packaged and received final release by a Qualified Person.
The formulation of the HepaVac off-the-shelf vaccine (IMA970A) was completed - sixteen TUMAPs were selected within WP1/WP2 and characterized regarding their physico-chemical characteristics. Respective analytical method- and formulation development was completed. GMP manufacturing of IMA970A was successfully completed, as well as the GMP manufacturing of RNAdjuvant® (GMP vendors were successfully selected and contracted). The way of applying the vaccine was evaluated (IMA970A mixed with RNAdjuvant® or two separate injections in close proximity) as well as its in vivo tolerance (in vivo safety). The in-use-stability for both drug candidates was determined. Additionally, both IMPs (IMA970A and RNAdjuvant®) were successfully study-specific labeled, packaged and received final release by a Qualified Person.
Task 1. HepaVAC off-the-shelf vaccine formulation [IMM, TUE]
Up to 40 TUMAPs, synthesized in research grade quality by solid phase peptide chemistry for WP1 and WP2 was evaluated regarding their physico-chemical characteristics (e.g. solubility, stability at different temperatures and pH-values) as a prerequisite for a subsequent formulation development process. Test formulations containing the final selected peptide set was prepared optional by using different excipients and investigated regarding stability and reconstitution characteristics in order to get a vaccine candidate applicable on clinical sites. Final release specification for APIs (individual peptides) and Drug Product has been defined. An analytical PR-HPLC release method has been in-house developed as part of the release specification methods and for stability assessment including a real-time and accelerated stability program allowing defining a sufficient shelf life for the vaccine based on extrapolation of the accelerated data as accepted by European authorities. The analytical methods for release have been implemented at qualified analytical GMP contract laboratories. A batch production instruction has been developed and transferred to a qualified contract GMP manufacturer. Task 1 was performed by IMM and TUE in close collaboration. Please refer also to Deliverable report D3.2.
Task 2 GMP manufacturing of active pharmaceutical ingredient (API; single peptides) [INTNA, IMM]
API manufacturing has been subcontracted to BCN Peptides S.A. (Barcelona, SPAIN) a GMP compliant, experienced peptide manufacturer based in Europe. Identification was coordinated by partner INTNA assisted by partner IMM who provided its experience in selecting and contracting such manufacturers. Peptides were manufactured by solid phase peptide synthesis, purified by high-performance liquid chromatography and lyophilized. Release of the individual peptides was performed according to pre-defined specifications.
Task 3: GMP manufacturing of RNAdjuvant® [CureVac]
Production of RNAdjuvant® was transferred into a GMP process, including the establishment of analytic processes as well as the performance of stability tests, already established at partner CUREVAC. This provided GMP-quality RNAdjuvant® in a sufficient amount to conduct the planned HepaVAC clinical trial.
Task 4. GMP manufacturing of HepaVAC vaccine. Packaging, labelling, final QP-release and storage of the vaccine [INTNA (subcontracted), IMM, CureVac].
GMP compliant manufacturing of Drug Product (HepaVAC “off-the-shelf” vaccine) has been performed by a subcontracted, experienced GMP manufacturer GP-Pharm (Barcelona, SPAIN) . The “off-the-shelf” vaccine has been manufactured by combining the GMP grade APIs (Task 2) according to a developed formulation process (Task 1). Release of the final bulk material will be performed according to pre-defined specifications. The bulk material was labelled, packaged and final released by a subcontracted,

experienced manufacturer Nuvisan GmbH (Neu-Ulm, GERMANY).
Task 5. Stability assay of the adjuvanted vaccine (peptides mixed with RNAdjuvant®) [CureVac]
Analysis of the in-use-stability has been performed to define the maximal tolerable time period between assembly of the adjuvanted vaccine and its injection. At several time points after mixing the peptides and the RNAdjuvant®, the complete vaccine has been analysed. Main parameters to be evaluated included quality of RNA, solubility of the peptides in RNAdjuvant® and the immunostimulatory capacity in vitro by investigating activation and cytokine secretion of human peripheral blood monocytes (PBMCs) loaded with vaccine. Please refer also to Deliverable report D3.1.

Task-6: In vivo tolerance of the adjuvanted vaccine (peptides mixed with RNAdjuvant®) [CURE]
To evaluate the in vivo tolerance of the complete vaccine systemic cytokine release has been analysed at several time points after single intradermal injection of various vaccine doses in C57Bl/6 mice. In addition, C57Bl/6 mice were analysed after repeated intradermal injection of the vaccine product for local reactions at the injection site, changes in health status or behavior, and major systemic side effects.
Significant results achieved
D3.1 to D3.7 were completed and MS11 to 19 were successfully achieved
Task 1.
• Physico-chemical characterization: All 17 selected peptides (7 HLA*A02 peptides; 5 HLA*A24 peptides; 1 viral control peptide; 4 HLA class II peptides) were characterized regarding their physico-chemical characteristics (solubility properties, stability at different pH and at higher temperatures). All tested peptides showed acceptable stability and solubility results. No stability and solubility issues were identified which could be critical for the stability of the later clinical trial material.
• Analytical PR-HPLC release method: An RP-UHPLC procedure has been developed which is able to separate all 17 peptides in one method. The method has been used for research stability and research in-use stability experiments. Results of stability tests have been included into the Investigational Medicinal Product Dossier (IMPD) for submission to regulatory agencies. The research stability data are a fundamental part of the planned shelf-life definition/extension plan for the GMP batch which shall be used in the proposed clinical trial. A test instruction has been prepared for transfer and implementation of this method at the GMP manufacturer.
• Formulation Development: During formulation development several different excipients (solubilizing agents) have been tested to verify a stable lyophilizate which can be easily reconstituted for intradermal application during the clinical trial. Several suitable solubilizing agents in combination with a bulking agent have been tested during a deeper testing phase. Final formulation has been studied regarding stability and detailed information has been included into the IMPD for submission.
Task 2.
• European public tender activities regarding API and Drug Product manufacturing has been completed. API manufacturing has been started on July 2015 at the well experienced and selected manufacturer BCN Peptides S.A. (Barcelona, Spain). Manufacturing has been completed in March 2016 and 17 peptides (APIs) have been produced and released in accordance with predefined specifications.
• One experienced drug product manufacturer (GP Pharm S.A. Barcelona, Spain) has been selected as the final step of the public procurement procedure. Several GMP relevant and product specific documents have been prepared together with the manufacturer and the production process as well the analytical UHPLC procedure has been discussed and successfully transferred. Production of the unlabeled HepaVac off-the-shelf vaccine (formulation, filling and lyophilization) took place in July 2017. Release and

stability testing as well as validation activities were finalized by the end of 2016. Stability testing will be continued until approx. end of July 2019 in accordance with a predefined stability schedule.
Task 3.
• Manufacturing SOPs for the RNA component R2025 and RNAdjuvant® are available.
• Manufacturing process for the RNA component R2025 and RNAdjuvant® are established at CureVac, validation batches were prepared.
• Set of QC methods is established for RNAdjuvant® (SOPs are available).
• A stability study for the RNA R2025 was performed to show long term stability at -20°C. A long term stability for storage of R2025 at -20°C for 24 months was confirmed with real time data. Stability studies for RNAdjuvant® are ongoing, sufficient data for a stability claim for RNAdjuvant® of 36 months for the clinical study are available.
• The RNA component R2025 and RNAdjuvant® is covered by the manufacturing license of CureVac.
• The first RNAdjuvant® batch was already fully released and it is stored at Nuvisan. A second batch was already successfully manufactured and its packaging and labeling are planned for March 2018.
Task 4.
• A public tender has been launched by INTNA in order to subcontract the GMP manufacturing regarding labelling, packaging, QP release and shipment of final product. In August 2016 the well experienced manufacturer Nuvisan GmbH (Neu-Ulm, Germany) has been selected and the final contract was signed. Investigational Medicinal Products (IMPs) are stored at Nuvisan and are ready for shipment to clinical sites (labeled, packaged and release by a Qualified Person).
Task 5.
• As already mentioned in the last scientific report (months 19 - 36), the mix of RNAdjuvant® with the selected peptides contained in the HepaVac vaccine was not stable throughout the experiments performed for the in-use stability study. Therefore, no data confirming the stability of this mix and in particular the contained RNA component could be obtained, and consequently they will be i.d. administered separately in the HepaVac-101 clinical trial. Data for the in-use stability of RNAdjuvant® at 25°C are available confirming stability over a period of 48 h.
Task 6.
• Ethics and regulatory approvals were obtained and forwarded also to the EC. The in vivo safety of the complete vaccine was confirmed - for detailed information please refer to delivery report D3.5./D3.6. As mentioned in the previous scientific report (months 19 - 36), no significant increase in serum levels of proinflammatory cytokines or chemokines was observed after intradermal injection of the complete vaccine at both analyzed time points (14h and 48h post injection) demonstrating the in vivo tolerability of the complete vaccine.

WP4 – Establishment, preparation and GMP manufacture of an actively personalised boost vaccine complementary to the HepaVAC vaccine (M1 – M72)
In spite of considerable efforts and using all techniques available to us in a multi-omics approach, in contrast to the initial objective to reliably discover naturally presented HLA-ligands from distinct tumor mutations (mutated neo-epitopes), this aim proved unattainable in HCC. Respective findings and limitations of the approach have been published on behalf of the HepaVAC consortium in a high-ranked peer-reviewed scientific journal 1. To deal with this setback a revised strategy was devised including alternative tumour-specific T cell targets, comprising also non-mutated HLA ligands and a flexible approach, to select the best optional peptides for each patient, based on the well-established OptiTope

framework2. The revised strategy was instructed to account for comparability and to conform to high regulatory requirements enabling the approval of a respective clinical trial, based on a discovery platform producing stable and reproducible results, which could be accomplished with relevant efforts, since at various stages during the project modified requirements had to be accommodated.
In spite of considerable efforts and using all techniques available to us in a multi-omics approach, in contrast to the initial objective to reliably discover naturally presented HLA-ligands from distinct tumor mutations (mutated neo-epitopes), this aim proved unattainable in HCC. Respective findings and limitations of the approach have been published on behalf of the HepaVAC consortium in a high-ranked peer-reviewed scientific journal 1.
To deal with this setback a revised strategy was devised including alternative tumour-specific T cell targets, comprising also non-mutated HLA ligands and a flexible approach, to select the best optional peptides for each patient, based on the well-established OptiTope framework2. The revised strategy was instructed to account for comparability and to conform to high regulatory requirements enabling the approval of a respective clinical trial, based on a discovery platform producing stable and reproducible results, which could be accomplished with relevant efforts, since at various stages during the project modified requirements had to be accommodated.
The implementation and translation of the actively personalised vaccine (APVAC) concept into a clinical trial design approvable by regulatory authorities proved a major challenge. Not only was the APVAC topic a constant matter of debate during annual meetings of the HepaVac consortium but it also became clear that this endeavour would require close exchange and guidance by regulatory authorities, since this area remained new ground to all parties involved. It should be noted that the APVAC concept was first discussed with EMA officials in January 2012 and deemed generally feasible but without any role model at the time of submission of the HepaVac project 3. In this regard, important input was received from competent authorities (Paul Ehrlich Institut, Langen/ Germany) on two occasions, first in an informal consultation on the APVAC concept in July 2015 and subsequently on the proper trial design and exact details relevant for regulatory submission during a formal scientific advice, conducted in March 2018. Respective guidance involved changes to the initial trial design, inter alia the decision that the HepaVac-101 clinical trial and the APVAC trial would not fit together and therefore had to be included in different trials for a variety of reasons, a decision which was approved by the HepaVAC consortium in November 2016 and implemented in an EC approved project amendment. Additionally the modified trial design now includes the administration of a checkpoint inhibiting antibody, directed against PD-1 in a platform/ basket trial design, encompassing various hard-to-treat malignancies. Ensuing revisions of documentation needed for regulatory submission, now required with a stand-alone trial design did not provide any synergy effects by jointly submitting documentation with the HepaVac-101 clinical trial, as initially planned. This step was challenging and demanded multiple revisions during trial planning and re-/ submissions. Overall standalone documentation included documentation for manufacturing of new molecular entities (investigational medicinal product dossier) and respective authorizations, clinical trials documentation encompassing clinical study protocol, investigator´s brochure as well as dossiers and standard operating procedures for discovery and selection of vaccine targets, which had to be drafted and multiply revised. Changes in the adjuvantation were also necessary, since adjuvantation with RNAdjuvant for the platform/ basket trial was declined as unfeasible and as an alternative, Montanide® ISA 51 VG was included and a manufacturing authorization applied for and granted in 2018, enabling the manufacturing and release of a mixing kit containing a vaccine together with a respective adjuvant for clinical trial use. The platform/ basket trial (entitled PepIVAC) was submitted for regulatory review in December 2018, received a

deficiency letter in April 2019 and regulatory approval in August 2019 after revision and resubmission, making it the leadoff trial with a respective design in Europe, which is indeed a crucial achievement. This unprecedented trial will be conducted according to all applicable legislation and guidelines including ICH/GMP and GCP.

References

1. Löffler M.W. et al. (2019) Multi-omics discovery of exome-derived neoantigens in hepatocellular carcinoma. Genome Med.; 11(1):28.
2. Touissaint., ., et al. (2009) OptiTope--a web server for the selection of an optimal set of peptides for epitope-based vaccines. Nucleic Acids Res. 2009;37 (Web Server issue):W617-22.
3. Britten C.M. et al. (2019). The regulatory landscape for actively personalized cancer immunotherapies. Nat Biotechnol.; 31;(10):880-2.

Task 1. Identification of patient specific tumour mutations in HCC [TUE]

The described task was accomplished during the first reporting period (month 1-18).

Required patients were recruited for analysis of patient materials for WP4 (for details please refer to Deliverable Report D4.1). Sequencing of HCC and corresponding liver tissue was performed for evaluation of reproducibility and suitability of different sample materials. WES and WTS quality controls were performed to optimize the used pipeline at TUE for WES that would subsequently be used to determine mutations that can be searched for in mass spectrometry data (HLA-ligandome), including benchmarking experiments and assessment of tumor heterogeneity. During the third reporting period (months 37-54) the data basis for HCC samples could be expanded as well as the sequencing pipeline was reorganized in cooperation with Quantitative Biology Center (QBiC) at the University of Tübingen, implementing latest software and setting up optimized pipelines and easier to use user interfaces for NGS data analysis. (see also Task 3). Further tumor heterogeneity in HCC was assessed in more detail (by NGS as well as on HLA-ligandome level; Task 2). We were able to define respective mutations in time for all samples analyzed by NGS and used the respective information for analysis of the HLA-ligandome for discovery of respective naturally presented neo-epitopes. Please also refer to Deliverable Report D4.1. Respective results were published on behalf of the HepaVac Consortium consortium in a high-ranked peer-reviewed scientific journal during the fourth reporting period 1.

Task 2. Identification of tumor specific peptide ligands [TUE]

This task could not be accomplished concerning the identification of mutation derived HLA-ligands by mass spectrometry (MS) (mutated neo-epitopes) during all reporting periods (months 1-72). For respective scientific reasons please refer to 1. During the second reporting period alternative strategies to identify wild-type sequence tumor-specific peptide ligands as vaccine candidates have been successfully developed and effectuated (Please also refer to Task 4 below), which were further improved and adapted, according to clinical trial requirements within the following reporting periods.

Alternative strategies were implemented for the discovery and selection of vaccine targets including i) wildtype sequence tumor-specific HLA ligands, ii) work regarding protocols for TiL isolation from tumor tissue and expansion as well as characterization, started during the third reporting period.

During the fourth reporting period (months 55-72) a flexible approach, to select the best optional peptides for each patient, based on the well-established OptiTope framework2 in an iterative process was revised and implemented in the meanwhile approved APVAC clinical study protocol (see also Task 3).

Task 3. Data processing and discovery platform for APVAC [TUE]

The required task was accomplished during the first reporting period but due to changes in the state of the art and compliance with regulatory demands, required frequent implementation of updates and revision of

strategies.
Handling of high throughput Omics data (WES, WTS and HLA-ligandomics) for personalized therapies (APVAC) inevitably requires a suitable data processing and discovery platform, including the establishment of complex bioinformatics pipelines and their optimization. In cooperation with the Quantitative Biology Center (QBiC) at Tübingen University strategies to identify tumor-specific wild-type sequence HLA-ligands that are suitable vaccine candidates on an individual basis were implemented, including alternative targets such as tumor-specific wild type sequence antigens in a multi-tier approach, that accommodates an optimized automated iterative peptide selection algorithm conforming with regulatory requirements and allows for individual selection of optimal vaccine targets in a reproducible and comparable manner. This platform was implemented into the OptiTope framework (see task 4) with container-based solutions allowing for reproducible software usage and reanalysis independent from the computing environment as well as storage of an analysis pipeline essentially unchanged and conform with FAIR principles. It further provides required sample tracking and accountability.
During the first reporting period an integrated platform including sample tracking and data integration was established for use in this project in cooperation with QBiC at TUE (D4.1/ D4.2). In this context subsequently container based solutions have been implemented, with readjustments and optimizations, where necessary, and adaptations according to the revised final trial design, ensuring compliance with regulatory requirements throughout the third and fourth (months 55-72) reporting period.
Task 4. Composition of actively personalised boost vaccines [TUE]
Based on the results of task 1 and 2, described in detail elsewhere, and also with support of improved and alternate technical approaches MS/MS validated mutated HLA ligands remained elusive in HCC 1. In this regard the approach for selection of suitable vaccine targets was revised and now includes various alternative targets that may be selected in a multi-tier approach of optimal and reproducible choice of peptides enabled through the OptiType framework. The approach (D4.3) had to be revised according to regulatory guidance and due to respective requirements throughout the project on various occasions, and now endorses a selection algorithm for patient-specific target discovery based on pre-described constraints producing a vaccine selection that when approved by a target selection committee can be manufactured as an APVAC for clinical trial use. The respective pipeline has been last modified during the fourth reporting period and was ultimately approved by regulatory authorities (PEI) and can therefore be used for individual vaccine selection in a clinical trials environment for the upcoming PepIVAC trial, including patients with HCC as well as various other malignancies.
Task 5. Manufacturing of ready-to-use GMP-grade actively personalised boost vaccine [TUE]
Due to factual constraints inter alia the regulatory approval of the APVAC clinical trial in August 2019, manufacturing of peptides according to individual patient-specific specifications for clinical trial use was impossible, since these sequences naturally remained unknown before trial initiation (D4.4; rejected due to this fact). Nevertheless, in lieu of such peptides, manufacturing of peptides generically included as active pharmaceutical ingredients (API) in the designated multi-peptide vaccine as viral marker peptides, including (TFYLNHTFKKVAITF derived from human adenovirus) as well as a promiscuous HLA class II peptide (TLGEFLKLDRERAKN derived from BIRC5), were initiated according to full GMP during the fourth reporting period as well as the required stability testing performed. Additionally, during the project duration various essential adaptations in the GMP environment had to be accommodated to comply with the updated requirements of the platform/ basket trial design that was implemented in an EC approved project amendment granted in 2018. As such, changes in adjuvantation were included (now comprising Montanide instead of RNAdjuvant, which was unavailable for this setting). These modifications also

required the preparation of standalone documentation according to the modified trial design, including full paperwork for regulatory submission and SOPs, handling instructions and dossiers drafted and revised within the fourth reporting period (months 55-72) and applications for a new manufacturing authorization for a mixing kit and various consultations and scientific advices on several occasions before. Nevertheless, inter alia due to the fact that the GMP facility at TUE holds a valid manufacturing authorization limited to patient specific preparations, containing one to ten peptides of synthetic origin as active pharmaceutical ingredients for use in clinical trials, the APVAC trial (PepIVAC) received regulatory approval in the last month of the funding period and will enter clinical stage development soon.
Due to delays experienced and unanticipated obstacles that needed to be overcome, the final goal of APVAC administration within a clinical trial has not been reached yet. Nevertheless, since all necessary provisions have been implemented and received ethics and regulatory approval their remains a future task currently en route, which is why the remaining milestones (M23/ M24) were unattainable so far and Deliverable D4.4 was rejected as unattainable when amending the DOW.
Task 6. Stability assay of the adjuvanted vaccine (peptides mixed with RNAdjuvant®) [CureVac]
The respective task has been performed within WP3 for off-the-shelf vaccine peptides. However, due to a separate application mode of RNAdjuvant and vaccine peptides, the respective task is inapplicable and has been omitted.
As an adjuvant for the approved APVAC clinical trial Montanide® ISA 51 VG has been chosen and a manufacturing authorization has been obtained for release of a mixing kit, containing vaccines and adjuvants for clinical trial use.
Task 7. In vivo tolerance of the adjuvanted vaccine (peptides mixed with RNAdjuvant®) [CureVac]
The respective task has been performed within WP3 for off-the-shelf vaccine peptides. However, due to a separate application mode of RNAdjuvant and vaccine peptides, the respective task is inapplicable and has been omitted.
Significant results achieved
Task 1.
• Sufficient high quality samples for analyses obtained in time.
• Identification of tumor mutations performed and benchmarked such as checked for feasibility, internal such as external validity. (MS20; D4.1)
• Identification of tumor-specific peptide ligands performed on time.
(MS21; D4.1)
• Respective results were published in a peer reviewed scientific journal 1.
Task 2.
• Mass spectrometry measurements showed good quality/ high peptides yields.
• Targeted mass spectrometry approaches established for target discovery.
• Shotgun proteomics yielded mutated proteins.
• Characterization of the HLA ligandome of HCC and liver tissue intensified and extended. (D4.1)
• Respective results were published in a peer reviewed scientific journal 1. Analyses for mass spectrometry validation to establish the reproducible use in the framework of a clinical trial performed.
Task 3.
• WES and WTS feasible according to accredited routines and SOPs. (D4.1)
• Discovery platform and data integration available and implemented. (D4.1)
• OptiType available for high-resolution HLA-typing from WES data.
• Sample tracking system available (in cooperation with QBiC at TUE). (D4.2)

• Integrated platform for data storage, processing, tracking and integration available in cooperation with QBiC at TUE. (D4.1/ D4.2)
• Update of software environment to the current state of the art and implementation of containerized solutions for pipelines storage used required in a clinical trial environment and conforming with regulatory requirements
• Strategy and platform implemented (D4.3) revised and approved within a clinical study protocol for APVAC.
Task 4.
• Selection procedure encompassing different sets of peptides of interest devised. (D4.3)
• Adapted flexible bioinformatics platform for optimal (iterative) peptide selection implemented (based on OptiTope). (D4.1)
• Revised and optimized stepwise selection approach for individualized multi-peptide vaccines on several occasions and security measures. (D4.3)
• Clinical platform/ basket trial approved by the competent authority (Paul Ehrlich Institute, Langen).
Task 5.
• GMP certificate for clinical grade peptide manufacture available.
• Manufacturing Authorization according to national German Pharmaceuticals Law available.
• Dry run of on-demand peptide manufacture performed and respective problems solved on time as well as continuous optimizations. (MS22)
• Stability testing of produced peptides/ multi-peptide cocktails performed and data on shelf-life of different peptides available.
• Creation of required documentation for the APVAC trial including manufacturing and quality finalized.
• Clinical trial design revised according to regulatory advice and amendment.
• Manufacturing Authorization for Montanide mixing kit in combination with peptide vaccines granted.
• Clinical platform/ basket trial approved by the competent authority (Paul Ehrlich Institute, Langen).
Task 6.
• none/ task is inapplicable and has been omitted. Adjuvantation for the APVAC clinical trial will include Montanide® ISA 51 VG (and imiquimod).
Task 7.
• none/ task is inapplicable and has been omitted.

WP5 – European multi-centre phase-I/II clinical trial (M24 – M72)
The clinical trial has been extensively discussed by all partners through many meetings [Kick off Meeting in Naples (November 2013), first HepaVac annual meeting in Tübingen (October 2014), second HepaVac annual meeting in Antwerp (October 2015), third HepaVac annual meeting in Benevento (November 2016), fourth HepaVac annual meeting in Pamplona (November 2017), fifth HepaVac annual meeting in Birmingham (November 2018) and sixth HepaVac annual meeting in Naples (June 2019)]. Additionally, a scientific advice meeting with the German regulatory authorities (Paul Ehrlich Institute - PEI) was conducted in order to discuss the clinical study design and the pre-clinical package required for submission of a clinical trial application (CTA). The CTA was already submitted in all five countries involved in the trial (Belgium, Germany, Italy, Spain and UK). Additionally, regulatory and ethical committee approvals were reached in all five countries. Afterwards, clinical sites were initiated/trained, received (non) Investigational Medicinal Product for the conduction of the trial, and started screening, enrollment and treatment of patients.

The HepaVac clinical study protocol specified that about 20 patients treated with IMA970A and CV8102 are considered sufficient to evaluate trial's endpoints safety and immunogenicity comprising adverse events profile and immune responses, respectively. Overall 22 patients started study treatment (i.e. received at least the single pre-vaccination infusion of cyclophosphamide) and for that reason the screening of further patients was officially closed on 02-Jul-2019 and patient enrollment for study treatment was closed right after (17-Jul-2019). All six clinical sites have enrolled and treated at least one patient in the study, which was an extraordinary achievement not often achieved in clinical trials.
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As illustrated by the figure below, a total of 82 patients were screened for the HepaVac clinical trial: 41 of them did not meet the inclusion/exclusion criteria at the first screening (Visit A / Screening 1) or did not present a suitable allotype for the study; 41 patients received HCC's standard treatment, but 8 out of them did not continue within the trial due to different reasons (additional HCC's standard treatment was needed, etc.); from the patients that continued in the trial (33 patients) and had their second screening visit (Visit B / Screening 2), 11 did not meet the inclusion/exclusion criteria at that time. The remaining 22 patients were put on study treatment (i.e. received at least the single pre-vaccination infusion of cyclophosphamide / Visit C) and one out of them dropped out of the study (due to personal reasons the patient decided to not continue the trial). Twenty-one patients were vaccinated with the HepaVac approach (IMA970A plus CV8102) and at the moment 13 patients completed the study treatment and are being followed-up (FU phase). Eight patients are still under study treatment and the last regular study visit within the mains phase (i.e. last patient last visit [LPLV] defining the end of the trial) anticipated for January 2020.

Task 1: Preparation of the clinical trial [INTNA + FGK - subcontracted CRO].
By contrast to the description in the DOW, the preparation of the clinical trial already started in month 1 (plan-start at month 24 as specified in the DOW) and involved all partners (only INTNA and CRO were planned to be involved as specified in the DOW). INTNA, supported by a sub-contracted CRO (FGK Clinical Research GbmH), led this task. A clinical study protocol (CSP) has been prepared describing

objectives, design, methodology, statistical considerations and organization of the trial. An Investigational Medicinal Product Dossier (IMPD) corresponding to the required CTD format and an Investigator Brochure (IB) have been prepared and updated annually or as required, respectively, describing the quality and preclinical development of the investigational medicinal product to be used in the clinical trial along with an overall risk-benefit assessment. Before the clinical trial started, all relevant documents were submitted and approved by the Competent Authorities and Ethics Committees of the five European clinical sites. For detailed information please refer to deliverables D5.1 to D5.4.
Task 2. HCC Patient enrolment [INTNA, TUE, UNINS, UoB, UNAV, UZA].
Patient enrolment has started in September 2017 and all six clinical sites (Naples and Varese/Negrar in Italy, Tübingen in Germany, Birmingham in UK, Pamplona in Spain and Antwerp in Belgium) have enrolled at least one patient into the study treatment group (at least one patient at each clinical site has received the treatment with IMA970A plus CV8102 following a single pre-vaccination infusion of cyclophosphamide). As previously mentioned, eighty-two patients were screened and twenty-two of them entered study treatment. Detailed information regarding the patient screening/enrollment per site are shown in the table below.

All available options to us for validation of mutation-derived HLA ligands as naturally presented in HCC patients´ autologous tumor tissue were unsuccessfully exhausted. Respective results have been published on behalf of the HepaVac consortium in a peer-reviewed scientific journal (Löffler et al. Multi-omics discovery of exome-derived neoantigens in hepatocellular carcinoma, Genome Med. 2019; 11(1):28). For underlying reasons please consult the mentioned publication as well as reasons outlined in WP4. As a contingency, the strategy for individualized target discovery was extensively revised and reassessed to also include alternate tumour-specific T cell targets, comprising also non-mutated HLA ligands and a flexible approach, to select the best optional peptides for each patient. Since mutations as T cell targets for the APVAC part proved unfeasible and regulators advised against submission as part of the HepaVac trial this was accounted for also in an amendment approved by EC in 2018. A basket/ platform trial (PepiVAC) comprising several hard-to-treat malignancies and with full standalone documentation was discussed, submitted and approved by regulatory (August 8th 2019). The innovative trial design makes the PepIVAC trial a leadoff trial without respective example, which has overcome unprecedented hurdles, being designed as a basket/ platform trial and would have resulted insurmountable otherwise. This PepiVac clinical trial including several (stat-of-the-art) modifications of the trial design will be conducted and completed involving additional financial sources, including HCC patients as initially planned (for further detail please see also WP4).
As previously mentioned, the last patient is anticipated to have her/his last visit by January 2020. This patient, enrolled in Spain, is currently receiving the study treatment (received her/his 5th vaccination on August 28th, 2019). To achieve the required number of treated patients described in the protocol (20) as suitable to evaluate trial’s endpoints, patients were screened and enrolled into the HepaVac trial until July 2019. Consequently, some patients are still under study treatment and this milestone will be reached later than originally expected. The graphic bellow illustrates the cumulative screening and enrollment curves, demonstrating that most of the patients could be enrolled into the HepaVac clinical trial during the current year (2019).

Since some patients are still under study treatment, a complete safety analysis of all vaccinated patients could not yet be performed. Nevertheless, first preliminary safety data can be presented, based on the data recorded so far and reported by the clinical sites:
- In general, treatment-emergent adverse events (TEAEs) reported were usually mild (CTC grade 1) to sometimes moderate (CTC grade 2); only two TEAEs with CTC grade 3 (severe) were observed, one 'influenza like illness' (recovered; related to study drugs) and one 'upper respiratory tract infection' (recovered; not related to any study drugs). - Only four Adverse Events of Special Interest (AESIs) were reported; three of them (reaction to contrast medium, twice; and contact allergic dermatitis) were recorded and assessed as not related to any investigational medicinal product (events related to patients' clinical conditions and/or to contrast medium used for the conduction of the Computed Tomography); and only one AESI (flu-like symptoms) was reported as probably related to IMA970A and CV8102 and unlikely related to cyclophosphamide. The aforementioned AESIs were not classified as Serious Adverse Events (SAEs) and all patients recovered;
- Four SAEs were reported so far; three of them (hepatic encephalopathy, bronchopulmonary infection, and decompensated liver cirrhosis) were documented as not related to any investigational medicinal product (events related to patients' clinical conditions); and only one of them (fever) was recorded as possibly related to cyclophosphamide, IMA970A and CV8102. The aforementioned events were classified as SAEs, since they required patient's hospitalization and all SAEs resolved;
- Overall, 154 AEs assessed as at least possibly related to the study treatment were reported, most of them included clinical signs or symptoms of injection site reactions as expected for this trial (intradermal IMPs administration during trial-specific vaccinations). Thus, general disorders and administration site reactions were the most frequently reported TEAEs so far. Among the safety data collected, 'injection site erythema' and 'injection site oedema' were most prevalent;
- With regards to other significant adverse events, until now, no TEAE was reported that lead to delay of or permanent discontinuation of vaccinations except for one 'influenza like illness' (CTC grade 1; recovered; not related to any study drugs) causing delayed administration of CV8102/IMA970A;
- No patient deceased during treatment phase and non-interventional follow-up;
- Six DSMB meetings (Data and Safety Monitoring Board Meetings [DSMB meetings]) were performed and after each meeting, DSMB members decided, based on safety results, that the trial may continue without modifications. The next scheduled meeting (7th DSMB meeting) will take place by the end of the year.
Since study treatment of 8 patients is ongoing, final safety analysis is pending and will be conducted as soon as the last patient had his/her last visit and end of trial is reached. In preparation of final data analysis including safety as a primary endpoint, preparatory activities were or are being conducted (please see also Milestone MS34). In particular, this includes reconciliation of SAEs reported by clinical sites to the Pharmacovigilance Department at the CRO and relevant AE data entered into and collected within the clinical data base were performed twice already.
With eight patients still being under trial-specific vaccination treatment and last patient last visit not reached yet, since the end of the trial is anticipated for January 2020, a complete efficacy analysis of all vaccinated patients could not be performed yet. To ensure reliability of final trial results, the data management plan (DMP) describing all study specific data management activities and a data validation plan (DVP) specifying the checks of the data for plausibility and completeness were implemented and updated in the meantime (December 2018). In preparation of final data analysis after data base closure planned for Q1/2020, the statistical analysis plan (SAP) describing the statistical methods and procedures

was generated and finalized in July 2019. Data management activities (including a setup of a final transfer of immunomonitoring data into clinical data) and programming of final statistical analysis are currently ongoing.
Task 3: Performance of Phase I/II clinical trial [INTNA, TUE, UNINS, UoB, UNAV, UZA].
The HepaVac-101 clinical trial started patient recruitment in September 2017 and the screening and enrollment of new patients was officially closed in July 2019.Isolation of PBMC samples from patients who have suitable HLA-allotype(s) for the study were performed and subsequent immune-monitoring is being carried out (for detailed information please refer to WP 6).
Significant results achieved
D5.1 to D5.5 were successfully completed and MS25 to MS30 were achieved.
The HepaVac clinical study protocol specified that about 20 patients treated with IMA970A and CV8102 are considered sufficient to evaluate the trial endpoints. To achieve this number, some patients were enrolled later on and for that reason they will have their last visit by January 2020. Consequently, the remaining deliverables (D5.6 to D5.8) and milestones (MS31 to MS34), which depend on the finalization of the clinical trial were not yet completely achieved.
Task 1 - 3.
- A PEI scientific advice meeting was conducted in July 2015 and questions with respect to RNAdjuvant® produced by CureVac (safe dose) and the HepaVac vaccine (no toxicological data before first-in-man) were discussed. Furthermore, the combination of the vaccine plus RNAdjuvant®, required safety measures, mixing at the bed side and the study design was discussed. The outcome of the meeting was very positive and impacted the clinical trial design;
- Final design of the Hepa-Vac-101 clinical trial has been approved by the whole consortium on the second annual meeting (in October 2015, in Antwerp). This task was the basis for the establishment of a clinical study protocol and all other clinical core documents. At present all clinical core documents were finalized and approved. After the acceptance/approval of the CSP one amendment was written (15-May-2017) and was approved (CSP version 1.3 24-Sep-2018);
- All six clinical sites successfully had their site initiation visit (SIV) and enrolled at least one patient into the clinical trial (i.e. at least one patient was treated with the study approach at each clinical site). Additionally, all sites have being regularly monitored (Monitoring visit);
- The clinical trial is registered in a WHO – or ICMJE-approved registry;
- HepaVac-101 clinical trial was approved by Ethics Committee (EC) and Regulatory Agency. The EC’s approval was obtained for all clinical sites.
- At least one PBMC laboratory close to/at every clinical site was identified, trained and successfully released. Isolation of PBMC samples from patients that presented suitable HLA-restricted peptides for the study was already performed;
- All required vendors (drug substance manufacturer, drug product manufacturer, secondary packaging and release company, clinical research organization) were identified according to the strict rules for vendor selection (e.g. public procurement).

WP6 - Immune monitoring and biomarker identification to determine immunological efficacy of HepaVAC (M12 – M72)
The design and the setup of immune-monitoring assays, as well as the design of the PBMC isolation/collection and of the logistic network involved in all six participants clinical sites were successfully achieved. At least one PBMC laboratory close/at every clinical site was identified, trained and released,

allowing them to perform PBMC isolation. The isolation of 182 PBMC samples from patients screened in the HepaVac-101 clinical trial has been performed so far. The setup of study-specific immune-monitoring and immunogenicity biomarker evaluation, as well as technical setup and implementation of cell-saving methods in order to allow a highly sensitive potential ex vivo measurement of vaccine-induced immune responses have been achieved. As of 31.08.2019 112 samples from 8 of the 21 treated patients were analysed for vaccine-induced immune responses to the IMA970 vaccine using an ex vivo immunomonitoring workflow which combines MHC Class I 2D multimer staining and MHC Class II intracellular cytokine staining.

To achieve the aim of extensive immune monitoring of patients during the Hepavac clinical trial, task 1, task 2 and task 4 of WP6 have been started. This included:

- Planning of design and setup of immune-monitoring assays as well as design of the PBMC collection and logistic network involved in all 06 participating clinical sites;
- Setup of study-specific immune-monitoring and immunogenicity biomarker evaluation, as well as technical setup and implementation of cell-saving methods in order to allow a highly sensitive potential ex vivo measurement of vaccine-induced immune responses;
- Establishment of PBMC aliquoting table to ensure optimal use of PBMC samples;
- Establishment of PBMC collection laboratory network, as well as identification, training and release of at least one PBMC laboratory close/at every clinical site;
- Study-specific adaptations to the Immunomonitoring Database have been completed
- Isolation of 182 PBMC samples from patients successfully screened in the HepaVac-101 trial.
- Ex vivo analyses of vaccine-induced CD8 T cell responses by 2DM multimer analysis for 8/21 patients
- Ex vivo analyses of vaccine-induced CD4 T cell responses by intracellular cytokine staining for 8/21 patients

Task 1. Immune monitoring assay setup and standardization for specific use in HepaVAC [IMM].

To analyze vaccine-induced T cell responses an ex vivo workflow was established which combines HLA class I multimer staining and HLA class II intracellular cytokine staining (ICS) in one assay. This novel cell saving technology allows the simultaneous assessment of ex vivo CD8 and CD4 immune responses. Besides the quantification of vaccine-induced T cells, also their characterization in terms of phenotype (CD8) and functionality (CD4) is possible. This ex vivo assay has already been used as primary assay using SAP-defined analyses in the GAPVAC-101 clinical trial and will be used for the immune-monitoring of the HepaVac-101 trial. The HLA class I multimer staining uses a two-dimensional combinatorial approach (2D multimer staining, 2DMM) adapted from Andersen et al. 2012, which allows the simultaneous analysis of up to 21 CD8+ HLA-peptide specificities within a single sample. Therefore, this is a novel cell saving technology. The HLA class I multimer staining in the routinely used ex vivo workflow has so far been performed using UV exchange multimers. Setup assays, e.g. to test the optimal concentration of classically refolded monomers/multimers in the flow cytometry staining have been performed in Q3/2016.

Task 2. Standardised PBMC collection during the Phase I/II clinical trial [IMM, all clinical centers].

Multi-centric PBMC collection will be coordinated by partner IMM and carried out in a highly standardized manner. At least one suitable PBMC laboratory has been identified for all six clinical sites participating in the HepaVac-101 trial. All these laboratories have been supplied with the required documents and materials, and suitable technicians have been identified and trained according to IMM’s SOPs (= theory training completed). The quality of tests isolations in every PBMC laboratory was evaluated at IMM and it was in accordance with all IMM’s requirements. It should be noted that a respective laboratory was just

fully released and thus allowed to conduct PBMC sample isolations for the HepaVac-101clinical trial once at least two technicians have successfully performed test isolations. As previously mentioned, at least one suitable PBMC laboratory was fully release for each clinical site involved in the HepaVac-101 trial. The current status at the respective laboratories is as follows:
- L182, Birmingham (UK): Theory training completed, all test isolations completed, quality of test isolations adequate, lab fully released.
- L183, Naples (Italy): Theory training completed, all test isolations completed, quality of test isolations adequate, lab fully released.
- L184, Pamplona (Spain): Theory training completed, all test isolations completed, quality of test isolations adequate, lab fully released.
- L185, Antwerp (Belgium): Theory training completed, two test isolations done, quality of test isolations adequate, lab fully released.
- L188, Negrar (Italy): Theory training completed, all test isolations completed, quality of test isolations adequate, lab fully released.
- L101, Tübingen (Germany): IMM internal laboratory, fully released.
A premium class courier specialized on clinical trial logistics has been identified and contracted. Scientific evaluation of timing of blood drawing / PBMC isolation time points is completed and included into the clinical study protocol. Please also refer to deliverable report D6.1.
Task 3. Immune monitoring of vaccine-induced T-cell responses [IMM, INSERM].
Considering the delay in the start of the HepaVac-101 clinical trial, i.e. start of patient enrollment and consequently in PMBC sampling collection, the task 3 was also delayed. The analysis of the immune response of vaccinated patients (immune-monitoring) started in Q2 2019, considering that PBMC samples collected from the same patient, but in different times, are pooled in order to perform laboratory assays. Immune monitoring of vaccine-induced T cell responses is ongoing and has been completed for 8 patients. Please refer to deliverable report D6.3 for immunomonitoring results of these 8 patients.
Task 4. Cellular biomarker analyses [IMM, INSERM].
As described in Task 3, PBMC samples from patients were collected just at the end of 2017/beginning of 2018 and for that reason this task is delayed. Suppressive immune cell populations as Foxp3+ regulatory T cells (Tregs) and myeloid-derived suppressor cell (MDSC) populations might be analyzed. As stated in the DOW, the flow cytometric panels have previously been established at IMM and UN.
Task 5: Evaluation of biomarker and immune monitoring analyses and association to clinical and biological efficacy [IMM, INTNA].
As described in Task 3, PBMCs collection from patients and immune response analyses are still ongoing. The present task has not yet started but will be completed in February 2020.
Significant results achieved
D6.1 and D6.2 are completed and MS35 and MS36 were achieved. The remaining deliverables (D6.3 and D6.4) and milestones (MS37 to 40) are related to the conduct of the HepaVac-101 clinical trial and mainly to the patient enrollment/PBMC sample collection and, for that reason, they were not completed yet. According to the new Gant chart proposed in the prolongation letter these deliverables and millstones will be reached by 31st August 2019.
Task 1.
- Establishment of ex vivo workflow combining HLA class I multimer staining and HLA class II intracellular cytokine staining (ICS);
- Establishment and final setup of 2-dimensional multiplexed tetramer staining for detection of TUMAP-

specific T cells ex vivo;
- Classical refoldings of HLA monomers finished
- Reagent testing and validation completed
Task 2.
Setup of all SOPs;
- At least one PBMC laboratory close/at every clinical site has been trained and fully released in order to isolate and cryopreserve PBMC samples;
- Design of immunomonitoring (and PBMC time points) for clinical trial.
Task 3.
- Ex vivo analyses of vaccine-induced CD8 T cell responses by 2DM multimer analysis for 8/21 patients and interim analysis of vaccine-induced Class I immune responses of 8/21 patients
- Ex vivo analyses of vaccine-induced CD4 T cell responses by intracellular cytokine staining for 8/21 patients and interim analysis of vaccine-induced Class I immune responses of 8/21 patients
Task 4.
- Establishment of biomarker (Treg) staining.
- Establishment of biomarker (MDSC) staining.
General.
- Preparation of PBMC aliquoting table.

WP7 – Coordination and dissemination (M1 – M72)
The overall coordination of the project has been pursued with continuity and a balanced attention to both scientific and administrative aspects at INTNA by the Scientific Coordinator Dr. Luigi Buonaguro supported by the Project Manager Dr. Serena Salerno and the Research Services Office (RSO), and with the contribution at IMM provided by the co-coordinator Dr. Harpreet Singh. At the end of 2017, Dr. Salerno left the project for another job and the role of the Project Manager has been taken over by Dr. Maria Luigia Mazzone.
Summary of progress towards objectives
The overall coordination of the project has been pursued with continuity and a balanced attention to both scientific and administrative aspects at INTNA by the Scientific Coordinator Dr. Luigi Buonaguro supported by the Project Manager Dr. Serena Salerno and the Research Services Office (RSO), and with the contribution at IMM provided by the co-coordinator Dr. Harpreet Singh. At the end of 2017, Dr. Salerno left the project for another job and the role of the Project Manager has been taken over by Dr. Maria Luigia Mazzone.
Task 1. Results delivery, to achieve the objectives of each WP in a timely and cost-effective fashion; INTNA have actively coordinated scientific activities; in particular the Coordinator with the Project Manager have made sure that all the deliverables deadlines, foreseen for this reporting period, were fulfilled and uploaded on EC website. No formal Steering Committee consultations have been organized (D7.11 and D7.12). The following planning activities (in which Consortium was involved) have been conducted:
1. Conference calls between the Coordinator and the Vice-Coordinator (Dec.19 2013- June 12, 2014)
2. Interim meeting in Mainz at CIMT 2014(May 7, 2014). Participating partners were: Intna, University of Insubria, University of Tuebingen, Immatics, Curevac. Minutes are available on Website.
3. Conference call between the coordinator, the Vice- Coordinator and Curevac (Aug 27, 2014-Sep 12, 2014)

4. Interim meeting in Mainz at CIMT 2015 (May 13, 2015). Participating partners were: Intna, University of Insubria, University of Tuebingen, Immatics, Curevac. Minutes are available on Website.
5. Interim meeting in Mainz at “Third CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference” (September 7, 2017). Participating partners were: INTNA, University of Insubria, University of Tuebingen, Immatics, Curevac.
6. Interim meeting in Mainz at “16th CIMT Annual Meeting” (May 15-17, 2018). Participating partners were: INTNA, University of Insubria, University of Tuebingen, Immatics, Curevac.
7. Interim meeting in Mainz at “17th CIMT Annual Meeting” (May 21-23, 2019). Participating partners were: INTNA, University of Insubria, University of Tuebingen, Immatics, Curevac.
8. Several conference call between the coordinator, the Vice- Coordinator and Curevac.
Pivotal part is the discussion of results obtained and/or upcoming problems occurring during implementation of the project. These conference calls typically last one hour each and are initiated by INTNA. They are preceded by a preparation phase where an agenda is set by the coordinator/project manager and sent, together with any related document, to the relevant HEPAVAC members (see also Deliverable D7.7; D7.8; D7.9; D7.10; D7.11; D7.12). After the CC a report is written by the project manager, distributed to the SC for comments/corrections and finally uploaded in the website (reserved area).
Task 2. Integration activities, aimed to promote integration among complementary tasks of the different WPs;
An initial task has been the organization of a full two-day “Kick-off” meeting which took place in Naples, Italy, on Nov. 14-15, 2013. Our Legal Officer (SO) Anita Kucharska and Financial Officer Christoforos Oikonomidis as well as all Principal Investigators (PI) of the 9 participating Institutions attended the meeting. The meeting had lively discussions and helped the scientist to know each other, get together and focus on early planning. (see also Deliverables DL 7.1).
The first annual meeting has been organized in Tuebingen, thanks to the hospitality of Curevac and Immatics partners, on October 16,17 2014. The meeting had a full two-day format and was attended by all HEPAVAC participants as well as by one of the three members (Tim Greten) of the scientific advisory board (SAB). Minutes of the Meeting as well as a Report from the SAB are uploaded in the website. It has been a very fruitful meeting with frank but constructive discussions (see also Deliverables DL 7.13). The SAB reported to be impressed with the quality of work carried out so far, in particular the scientific achievements the consortium was able to make in such short time; moreover, it appreciated the spirit of collaboration along with the constant comparison existing in the consortium, without neglecting the organization and the direction of the meeting have been judged very positively.

The second annual meeting has been organized in Antwerp last year, on October 15 and 16, thanks to the hospitality of Dr. Sven Francque who welcomed us at the his University in Antwerp Hospital. The meeting had a full two-day format and was attended by all HEPAVAC participants as well as by two of the three members (Tim Greten and Vincenzo Bronte) of the scientific advisory board (SAB). Minutes of the Meeting as well as a Report from the SAB are uploaded in the website. It has been a very fruitful meeting with frank but constructive discussions The SAB reported to be impressed with the quality of work carried out so far, in particular the scientific achievements the consortium was able to make in such short time; moreover, it appreciated the spirit of collaboration along with the constant comparison existing in the consortium, without neglecting the organization and the direction of the meeting have been judged very positively.

The third and fourth annual meetings have been organized in Benevento, on October 24 and 25 2016, and in Pamplona, on November 16 2017. Both meetings were attended by all HEPAVAC participants. Unfortunately, none of the three members of the scientific advisory board (SAB) were able to participate. Minutes of the Meeting were uploaded in the website. They were very fruitful meetings with frank but constructive discussions. Please refer to deliverable D7.15 and D7.16.

The fifth and sixth annual meetings have been organized in Birmingham, on November 21 and 23 2018, and in Naples, on June 27-28 2019. Both meetings were attended by all HEPAVAC participants. Unfortunately, none of the three members of the scientific advisory board (SAB) were able to participate. Prof. Bolondi, Coordinator of the SDMB, attended the meeting in Naples. Minutes of the Meeting were uploaded in the website. They were very fruitful meetings with frank but constructive discussions. Please refer to deliverable D7.17 and D7.18.
Task 3. Dissemination activities, aimed to spread significant scientific results developed by each Participant group within the different WPs;
Establishment of a HEPAVAC website has been accomplished. First the reserved area was implemented and is organized in three main folders: Administration, Forms, Meetings, and Science, each of which contains several subfolders with relevant documents. Registered users can freely upload and download documents. An automatic notification e-mail reaches all users once a new document is uploaded showing the name of who is uploading it and the location of the document. This tool is extremely useful as an archive of all HEPAVAC related documents and for their dissemination among all HEPAVAC participants (see also Deliverables DL 7.3).
The public part of the HEPAVAC website was launched online at http//:www.hepavac.eu on Feb.1 2014. This has required a lot of background work together with an ePress professional (sub contractor) in charge of this task. An original HEPAVAC logo is displayed in the website and was chosen among several candidates created by the HEPAVAC participants. The pages of the website include a home, project summary, clinical trial, news, the consortium (with active links to all participating Institutions' websites), publications (organized in background and foreground), contact us, and the access to the reserved area. The HEPAVAC represents the main source for the dissemination of all activities concerning the project. The project manager continually updates it; first of all she takes care to make public all the relevant events involving the project. Furthermore, the website has been enriched with two additional pages: publications and links. In particular, in the first one all the article abstracts, in chronological order, together with the indications about the Journals where they have been published, are listed; in the second one, the most important liver cancer foundations and meetings are listed. In addition, the most prestigious journals focusing on immunology, immunotherapy and liver cancer are listed. The reserved area has been adjourned with the WP reports from the last HEPAVAC annual meeting. Results obtained by the HepaVac Consortium have been presented and discussed in International Meetings focussing on cancer immunotherapy.
A presentation and description of the HepaVac project is present in the HorizonHealth.eu (www.horizonhealth.eu) an initiative of CommHERE (Communicating European Health Research) (www.commhere.eu). CommHERE is a FP7 EU-funded program aiming at improving communication on the outcome of EU funded health research projects to the general public and the media. An article about the Project has been recently published on Platinum magazine, distributed all over the world and available on-line (http://www.platinum-online.com/marzo-2016-speciale-ricerca-innovazione/).
Results obtained by the HepaVac Consortium have been presented and discussed in International

Meetings focussing on cancer immunotherapy.
An informative brochure has been produced and posted on line on the Project Website as well as distributed at International Meetings focussing on cancer immunotherapy.
Task 4. Administrative services, with economic and financial management;
A Consortium Agreement (CA) was written by Dr. Iolanda Attanasio (INTNA, LEAR) and signed by the legal representatives of all participating Institutions. This is an important document which regulates the several complex aspects of the project and the relationships among consortium partners. A HEPAVAC material transfer agreement (MTA) template has to be used to send reagents from one laboratory to another.
HEPAVAC first instalment funding was received by INTNA at the end of October 2013 and funds were transferred to all Institutions within forty days from receipt (pre-financing). Dr. Farinari (INTNA, RSO) followed this initial step and carefully supervised all the financial aspects of HEPAVAC at each reporting period.
An External Advisory Board was established. Ten candidates were suggested by the HEPAVAC members and the following three persons were elected according to the votes obtained from the whole consortium: Enzo Bronte, Massimo Colombo, Tim Greten. All three accepted the invitation to act as members of the external advisory committee.
An external Data Safety Monitoring Board (DSMB) was established (see also Deliverable D7.2). Ten candidates were suggested by the HEPAVAC members and the following three persons were elected according to the votes obtained from the whole consortium: Giorgio Parmiani (Coordinator), Stefan Endres, Pedro Romero. All three accepted the invitation to act as members of the Data Safety Monitoring Board. They have been acting also as Ethics Advisory Board (EAB) providing the ethical periodic reports (see also Deliverable D7.4; D7.5; D7.6; D7.19).
Subsequently, the external Data Safety Monitoring Board (DSMB) was enlarged. The original Members Giorgio Parmiani (Coordinator), Stefan Endres and Pedro Romero were joined by Dr. Tim Greten, Prof. Massimo Colombo The latter, therefore, are both Members of the AB and the DSMB. Lastly Prof. Luigi Bolondi joined the DSMB and was appointed as Coordinator of the DSMB, replacing Prof. Parmiani who had to step down for personal reasons.
In Dec. 2016 it was finalized the exit from the Consortium of the Partner INSERM and the entrance of the Partner UN (Univ. of Nantes). The people involved in the project remained the same with the same expertises but the affiliation changed.
In June 2018 it was requested to European Commission an extension of the project for a total of 12 months, bringing the ending date to August 31st 2019. The need for such request of extension was to ensure the completion of the clinical trial which was already in place in June 2018 with patients on vaccination protocol, when the request was submitted. The extension was granted and notified on July 12 2018.
Task 5. Project reporting to EC;
The present Final Report is an integrated effort by the HepaVAC Management Team at INTNA.
Task 6. Project administration.
The daily project administration is an integrated effort by the HepaVAC Management Team at INTNA.
Significant results achieved
Task 1.
• Conference calls between the Coordinator and the Vice-Coordinator (Dec.19,2013- June 12, 2014)
• Interim meeting in Mainz at CIMT 2014(May 7, 2014). Participating partners were: Intna, University of

Isubria, University of Tuebingen, Immatics, Curevac. Minutes are available on Website.
• Conference call between the coordinator, the Vice- Coordinator and Curevac (Aug 27, 2014-Sep 12, 2014)
• Interim meeting in Mainz at CIMT 2015 (May 13, 2015). Participating partners were: Intna, University of Insubria, University of Tuebingen, Immatics, Curevac. Minutes are available on Website.
• Conference call between the coordinator, the Vice- Coordinator and Curevac (Aug 27, 2014-Sep 12, 2014)
• Interim meeting in Mainz at "Third CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference" (September 7, 2017). Participating partners were: Intna, University of Insubria, University of Tuebingen, Immatics, Curevac.
• Interim meeting in Mainz at "16th CIMT Annual Meeting" (May 15-17, 2018). Participating partners were: Intna, University of Insubria, University of Tuebingen, Immatics, Curevac.
• Interim meeting in Mainz at "17th CIMT Annual Meeting" (May 21-23, 2019). Participating partners were: INTNA, University of Insubria, University of Tuebingen, Immatics, Curevac
• Bimonthly conference call between the coordinator, the Vice- Coordinator and Curevac (March 2018-Aug 2019)
Task 2.
• Kick-off Meeting, Naples, Italy, Nov. 14-15, 2013
• First Annual Meeting, Tuebingen, October 16,17 2014
• Second Annual Meeting, Antwerpen, October 15,16 2015
• Third Annual Meeting, Benevento - IT, October 24-25, 2016
• Fourth Annual Meeting, Pamplona - ES, November 16 2017
• Fifth Annual Meeting, Birmingham - UK, November 21 - 23, 2018
• Sixth Annual Meeting, Naples - IT, June 27 - 28 2019
Task 3.
• Website implemented at www.hepavac.eu
• Website manteinance at www.hepavac.eu
• Publication about Hepavac http://www.horizonhealth.eu/project/cancer-vaccine-development-hepatocellular-carcinoma/240#w3s-allow-cookies=false. and http://www.horizonhealth.eu/article/vaccines-third-kind/255#w3s-allow-cookies=true.
• Presentation at CIMT Annual Meeting 2014 (May 6 – May 8) in Mainz, Germany
• Presentation at "14th International Conference on Progress in Vaccination against Cancer – PIVAC-14 (Sept 24 – 26) in Rome, Italy
• Presentation at "Cancer Bio-Immunotherapy in Siena – XIIth NIBIT Meeting" (Oct 9 – 11) in Siena, Italy
• Publication of brochure http://www.hepavac.eu/wp-content/uploads/BROCHURE_eng.pdf
• Presentation at CIMT Annual Meeting 2015 (May 11-May 13) in Mainz, Germany
• Presentation at 9th Annual Conference of International Liver cancer association Paris 2015 (Sep 4- Sep 6)
• Presentation at "15th International Conference on Progress in Vaccination against Cancer – PIVAC 2015 (Oct,6-8) in Tuebingen, Germany
• Cancer Bio-Immunotherapy in Siena – 13TH NIBIT Meeting" 2015 (Oct 8 -10) in Siena,
• Presentation at CIMT Annual Meeting 2016 (May 10 – May12) in Mainz, Germany
• Presentation at AACR-CRI-EATI-CIMT Annual Meeting 2016 (Sept 25-28) in New York, USA.
• Presentation at "Third CRI-CIMT-EATI-AACR International Cancer Immunotherapy Conference", Mainz,

Germany (September 7, 2017)
• Presentation at “EASL - THE INTERNATIONAL LIVER CONGRESS™ 2017” Amsterdam (April 19-23, 2017)
• Presentation at “EASL – HCC Summit” Geneva (Feb, 2-3, 2017)
• Presentation at “IHV Meeting” Baltimore (Oct, 22-26, 2017)
• Presentation at “Immunotherapy Bridge” Naples (Nov, 29-30, 2017)
• Presentation at “EASL – HCC Summit” Geneva (March, 1-3, 2018).
• Presentation at “16th CIMT Annual Meeting” Mainz (May 15-17, 2018)
• Presentation at “BioBeirut 8” Beirut (Oct, 24-26, 2018)
• Presentation at “NIBIT Annual Meeting” Milan (Oct, 11-13, 2018)
• Presentation at “16th Naples Workshop on Bioactive Peptides” Naples (June, 7-9, 2018)
• Presentation at “60th Annual Meeting of the Italian Cancer Society” Milan (Sept, 19 - 22, 2018).
• Presentation at “2018 ASCO Annual Meeting”, Chicago (June 1 – 5, 2018)
• Presentation at “12th International Liver Cancer Meeting” London (Sept, 14 – 16, 2018).
• Presentation at “17th CIMT Annual Meeting” Mainz (May 21-23, 2019)
• Presentation at “CRI – CIMT –AACR – EATI Meeting” Paris (Sept 25 – 28, 2019)
• Presentation at “NIBIT Annual Meeting” Verona (Oct, 11-13, 2019)
Task 4.
• Drafting of the Consortium Agreement duly signed by all Partners
• Receiving and distributing pre-financing
• Appointment of External Advisory Board as well as of Data Safety Monitoring Board (DSMB)/ Ethics Advisory Board (EAB).
• Enlargement of External Advisory Board as well as of Data Safety Monitoring Board (DSMB)/ Ethics Advisory Board (EAB).
• Coordination of External Advisory Board as well as of Data Safety Monitoring Board (DSMB)/ Ethics Advisory Board (EAB).
Task 5.
• Generation of 18-month Interim Scientific and Financial Report
• Generation of 36-month Interim Scientific and Financial Report
• Generation of 54-month Interim Scientific and Financial Report
• Generation of 72-month Interim Scientific and Financial Report
• Generation of the final Scientific and Financial Report
Task 6.
• Daily project administration

Project Results:
Hepatocellular carcinoma (HCC) is the most common primary liver malignancy, with both viral and non-viral pathogenesis, and accounts for about 6% of all new cancer cases diagnosed worldwide (nearly 750,000 new cases/year). It is the third and the fifth leading cause of death from cancer globally in men and women, respectively. The age-standardized incidence rate (ASR) per 100,000 men per year for HCC greatly varies in different regions. It is about 9.5 in Southern Europe and Northern America but increases to

31.9 and 22.2 in Eastern and South-Eastern Asia, respectively (http://globocan.iarc.fr/).
There is a growing incidence of HCC worldwide mostly due to long-lasting chronic HBV and HCV infections acquired in the last century, although incidence and mortality rates are greatly heterogeneous. The most frequent risk factors for HCC include chronic viral hepatitis (types B and C), alcohol intake and aflatoxin exposure. However, even though their geographical distribution is uneven, more than 50% of HCC cases can be attributed to HBV infection, more than 30% can be attributed to HCV infection and approximately 15% can be associated with other causes.
The overall prognosis for HCC patients is poor, with a dismal 5-year survival rate of approximately 5-6%. Indeed, the number of medical interventions tested in HCC are significantly lower compared to other cancers with a high prevalence/incidence worldwide (e.g. lung, breast, colorectal cancers). Therefore, a limited range of therapies are available to be used in the management of HCC according to the extent and severity of liver disease.
Surgery (i.e. liver resection and transplantation) represents the first choice of treatment for HCC in patients with early tumors on an intention-to-treat perspective, achieving a survival of 60–80% at 5 years. However, 70% of patients undergoing liver resection show tumor recurrence within 5 years characterized by either intrahepatic metastases or appearance of de novo tumor lesions and several adjuvating treatments to prevent recurrence have been evaluated, but none of these has provided a clear body of evidence for efficacy.
However, the majority of patients are diagnosed when disease is not treatable by surgical strategies anymore and can be approached only with loco-regional therapies which include a large panel of choice. Among such panel, local ablation is the first option for HCC patients at early stages and radiofrequency ablation (RFA) provides up to a 40-70% survival rate at 5 years. Indeed, RFA has been considered a possible alternative to surgical resection in HCC patients with single small lesions but results on clinical outcome have been contrasting. Transcatheter chemoembolization (TACE) is the first option for treatment of intermediate stage and unresectable HCC. Partial response is observed in almost 50% of patients treated with TACE showing a delayed tumor progression, although survival benefits have not been fully established.
Finally, systemic therapeutic options in advanced unresectable HCC are limited to Sorafenib which is the only approved therapy confirmed to provide a limited increase in survival of 2.3–2.8 months. Different studies have addressed the HCC pathogenesis in order to identify possible additional targets for systemic therapies, suggesting that multiple concurrent molecular mechanisms or pathways (e.g. vascular growth factor [VEGF] signaling; epidermal growth factor [EGF] signaling; Ras MAPK signaling; insulin-like growth factor receptor [IGFR] signaling) are involved, requiring a combination or targeted therapies to possibly achieve a clinical improvement.
All of the above highlights an urgent unmet medical need for new therapeutic strategies to confer durable clinical benefit for HCC patients. To this aim, the main goal of HEPAVAC is to develop a novel therapeutic cancer vaccine strategy for HCC based on a cocktail of newly identified HLA class I and II restricted tumor-associated peptides – TUMAPs (“off-the-shelf” vaccine) boosted by an actively personalized vaccine (APVAC) approach, including patient-specific naturally presented mutated peptides (www.hepavac.eu).

Potential Impact:
www.hepavac.eu
l.buonaguro@istitutotumori.na.it

List of Websites:
in Attachments:
1) Publishable Summary
2) Brochure of the HEPAVAC project
3) Final picture of the HEPAVAC -101 clinical trial

Verwandte Dokumente

final1-publishable-summary.pdf

Letzte Aktualisierung: 16 Juni 2020

Permalink: https://cordis.europa.eu/project/id/602893/reporting/de