Intratumorally injected pro-inflammatory allogeneic dendritic cells as immune enhancers – A phase I/II study in patients with advanced hepatocellular carcinoma

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Introduction

Accumulating data indicate that the efficient induction of antigen-specific cytotoxic CD8+ T cells characterizing viral infections is caused by cross-priming where initially infected DCs produce a unique set of inflammatory factors that recruit and activate non-infected "bystander" DCs[1, 2]. Our DC-based vaccine concept (INTUVAX) is guided by such "bystander" view and accordingly, we have developed a cellular adjuvant consisting of pre-activated monocyte-derived DCs (activated by a novel activation cocktail) producing high levels of DC-recruiting and DC-activating factors in a sustained fashion (actively secreting these factors at the time of injection). This concept doesn´t require MHC-compatibility between injected cells and the patient and therefore introduces the possibility of using pre-produced and freeze-stored DCs from healthy blood donors as an "off-the- shelf" immune enhancer. The use of MHC-incompatible allogeneic DCs will further induce a local rejection process at the vaccination site that is expected to further enhance recruitment and maturation of endogenous "bystander" DCs[2].

The INTUVAX concept is aimed to target the patient-specific neoantigen repertoire and circumvent problems associated with ex-vivo production of neoantigen-based vaccines by using the patient's own tumor as a direct antigen source by in situ (intratumoral) administration of INTUVAX. The immune-enhancing role would be to recruit and activate the patient's endogenous DCs and DC precursors to the tumor site where they will encounter and engulf dying tumor cells and/or tumor cell debris, including the full array of "personal" neoantigens. These recruited endogenous DCs, loaded with relevant tumor antigens, will finally migrate to the tumor-draining lymph node where they will prime tumor-specific T cells, including cytotoxic CD8+ T cells.

Production of INTUVAX

INTUVAX cells were produced according to GMP guidelines, using leukapheresis-derived monocytes obtained from one healthy blood donor as starting material. The enriched monocyte fraction was differentiated into immature DC in culture bags using GM-CSF and IL-4 and matured for 18 hours using a novel activation cocktail. This vaccine production platform has been shown to be robust and reproducible resulting in a drug product (INTUVAX), releasing high levels of T-helper 1 associated chemokines and cytokines at the time of administration. Frozen vaccine cells have shown a high and reproducible quality after thawing and shelf life in frozen condition (-150°C) is > 1 year.

Study design

The study was a prospective single armed, open label phase I/II-study in patients with advanced hepatocelluar carcinoma (HCC) (ClinicalTrials.gov, NCT01525017). Study primary endpoint was safety and secondary endpoints included immunological response and clinical efficacy. Eleven HCC patients were included in the immunological and safety sets. After an amendment in the study protocol one patient with bile duct cancer was also incuded.

Three doses of vaccine cells (10 or 20 million cells/dose at day 0, 14 and 3 – 4 weeks after the second vaccination) were intratumorally injected into a liver lesion by ultrasonic guidance. The first seven HCC patients were treated with INTUVAX at the starting dose level of 10×10^6 cells, and the following four patients were treated with INTUVAX at the starting dose level of 20×10^6 cells. Nine out of 11 patients recieved all 3 vaccine doses. Six of these 9 fully treated patients had received prior first-line systemic treatment with sorafenib while 3 patients were treatment-naive as to systemic treatment.

Detection of tumor-specific CD8+ T cells

Frozen patient PBMC taken before the first dose of INTUVAX and one week after the third dose of INTUVAX were analysed. Mixes of overlapping peptides for alpha feto protein (AFP) and human telomerase reverse transcriptase (hTERT) were added at a final concentration for each peptide mix of 10 ug/mL. Using overlapping peptides spanning an entire protein sequence, CD8+ T-cell responses can thus be detected to multiple epitopes, regardless of HLA type[3]. The peptide mixes were produced by JPT Peptide Technologies GmbH, Berlin, Germany. A CD8 T Cell Detection cocktail consisting of a FITC-conjugated antibody against IFN gamma and APC-Cy7-conjugated anti-CD8 was used.

Patient characteristics

HCC patients: 9 males and 2 females. Age: Median 65 years (range 58 – 74). Child-Pugh (CP) status: 3 patients with CP status A5 and 9 patients with CP status A6. Four patients received Intuvax as first line systemic treatment while 7 patients received Intuvax as second-line systemic treatment after progression on sorafenib. Nine out of 11 patients where fully treated with 3 vaccine doses.

Cholangiosarcoma: One 65 year old male patient with CP A6 liver status who received all three Intuvax doses in conjuction with trans-arterial chemoembolization (TACE).

Safety

The safety profile was excellent except for the two SAEs that were assessed as related to INTUVAX and which consisted of fever (up to 39°C) that led to hospitalization for 3 – 5 days following vaccination. Urine and blood cultures were negative in both patients.

Immune response in peripheral blood

Among the 9 fully treated patients, 6 patients exhibited an increase of AFP and/or hTERT specific and IFN-gamma producing CD8+ T cells 1 week after the third vaccine dose as compared to base-line levels (figure 1 – 2). Data on tumor-specific CD8+ T cells before and one week after 3 INTUVAX doses in a patient who was treated with Intuvax as first-line systemic treatment and who lived for 18 months is shown in figure 3.

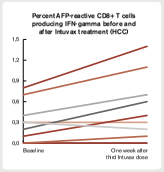

Figure 1

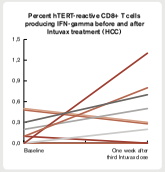

Figure 2

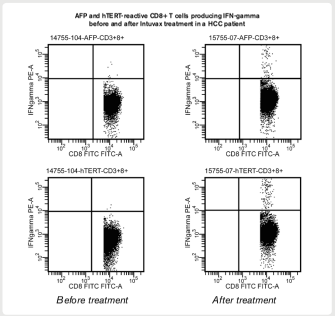

Figure 3

Overall survival

Median overall survival (mOS) for the subgroup of HCC patients who received all three INTUVAX-doses as second line systemic treatment (n=6; CP status A5 in 3 patients and A6 in 3 patients) (figure 4) was 9.4 months while mOS for the subgroup of patients who recieved INTUVAX as first line systemic treatment (n=3; Child-Pugh status A6 in all three patients) (figure 5) was 11.9 months. These mOS data compares favourably with historical data[4, 5]. The bile duct cancer patient who was included in the study and who received INTUVAX as first line systemic treatment progressed after 6 months and was then given additional systemic treatment with gemcitabine (known to inhibit myeloid-derived suppressor cells) + cisplatin which induced a durable objective tumor response. This patient is still (24/10/2016) alive 33 months after start of INTUVAX treatment (figure 6) which compares highly favourably with historical data[6].

Overall survival vs. tumor-specific immune response

4 out of 5 HCC patients who passed their expected mOS (2 patients receiving INTUVAX as second line and 2 patients receiving INTUVAX as first line systemic treatment) as well as the bile duct cancer patient exhibited an increased frequency of tumor-specific CD8+ T cells in peripheral blood after vaccination. Notably, 2 out of 3 patiens who did not respond with an increase in tumor-specific T cells deceased within 4 months after the first INTUVAX dose.

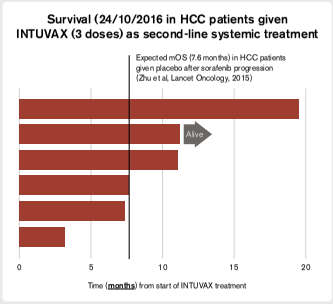

Figure 4

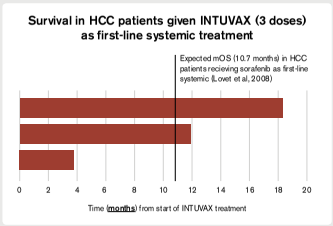

Figure 5

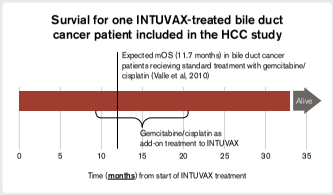

Figure 6

Conclusions

Our findings indicate that intratumoral administration of proinflammatory allogeneic DCs is safe and induces a systemic CTL-mediated anti-tumor response in a majority of HCC patients. Data on a patient with bile duct cancer who has received additional treatment with gemcitabine (known to deplete myeloid-derived suppressor cells) indicate a synergistic anti-tumor effect. Approval has recently been received from the Swedish Medical Products Agency to expand the study by including up to six additional HCC patients. These patients are approved to receive INTUVAX as first-line systemic treatment in combination with locoregional chemoembolization or in combination with the tyrosine kinase inhibitor sorafenib.

This study was conducted by:

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