Praxisgemeinschaft für Zelltherapie
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Patient Identification:

Cozma, Alexandrina Liliana date of birth 27.10.1967 from Rumania

Diagnosis:

Advanced cholangiocarcinoma with liver and lymph node metastases originally diagnosed in March 2016 underwent incomplete surgery in May 2016

Current treatment at my office:

Dendritic cell based immunotherapy alongside with checkpoint inhibitor (Nivolumab) and infra-red laser therapy since August 2016; received therapy with Mifegyne until August 11th, received anti-inflammatory supplements (Curcuma; Frankincense)

Dear Colleague,

I saw Mrs. Cozma first in August 2016 when she presented a metastasized (liver) cholangiocarcinoma after incomplete surgery in May 2016. Because of the low efficacy and side effects she denied the standard chemotherapy. Several reports have demonstrated that specific cellular immunotherapy using dendritic cells is capable of inducing a clinical antitumor response in various kinds of tumors, including cholangiocarcinoma, with sometimes prolongation of the overall survival. Meanwhile, this kind of therapy is approved as *orphan drug* for the treatment of Glioblastoma multiforme WHO IV and got a FDA approval for the treatment of hormone refractory prostate cancer in the USA. Keeping in mind that this therapy strategy is based on a general immunological principle it is most likely that this therapy can be a successful therapy option for Mrs. Cozma.

We discussed the pros and cons of Dendritic Cell (DC) Vaccine Immunotherapy, including the blood specimen collection for harvesting the precursor cells, the monocytes. On August 2nd 2016 the leukapheresis was performed to harvest a high number of monocytes for preparing the autologous dendritic cells vaccines. The harvested monocytes were frozen for use in the DC vaccine preparation. The immature dendritic cells were generated ex vivo from the isolated patient's blood monocytes using a standard cytokine cocktail in a GMP certified lab. After priming of the DC with specific peptides the DC were further matured by certain cytokines. On day 6-7 the dendritic cells were harvested in 2 ml physiological salt

solution for intradermal application. For every vaccine preparation the quality of the dendritic cells were tested by measurement of characteristics surface markers using flow cytometry, microscopic control as well as by standard tests for sterility. The following markers were routinely tested: CD1a, CD83, CD80, CD86, CD11c, CD14, CD209, HLA-DR. Mrs. Cozma received the first injections of dendritic cells on August 9th 2016 and September 6th 2016 respectively by intradermal application route.

We discussed also a treatment option with the checkpoint inhibitor Nivolumab. The immunological control of a tumor growth is controlled via certain immune checkpoints, among them the PD-/PD-L1 pathway. PD-L1, expressed on dendritic cells and on tumor cells, delivers an inhibitory signal to T-cells upon binding to PD-1, expressed on activated T-cells. This could lead to inactivation of the activated T-cells, which are going into apoptosis. The blockade of this pathway may therefore lead to an improved clinical immune response against tumors. The systemic treatment with an antibody against PD-1 (Nivolumab, trade name Opdivo) has recently shown amazing results in various kinds of human tumors leading to approval of these new drugs in USA, Japan and Europe for treatment of melanoma, non-small-cell-lung cancer and renal cell cancer. Keeping in mind that this therapy strategy is based on an immunological principle it is likely that this therapy can be successful in the treatment of other cancer diseases including cholangiocarcinoma. In fact recent data are showing that parts of the cholangiacarcinoma are expressing the PD-L1.

A combination of checkpoint inhibitors with a vaccine strategy can enhance the therapy efficacy as it was recently shown for pancreatic cancer. Thus, we started to treat the patient also with the antibody against PD-1 (trade name: Opdivo) one day prior to the DC vaccine using a lower dose (0,5 mg/kg body weight).

Taken together the following treatments were done:

August 1st	First consultation and blood specimen collection for serology
August 2nd	Leukapheresis
August 4th	infra-red laser therapy
August 5th	infra-red laser therapy
August 8th	infra-red laser therapy Infusion of Nivolumab (0.5 mg/kg body weight)
August 9th	1st DC vaccine
September 5th	Infusion of Nivolumab (1 mg/kg body weight)
September 6th	2nd DC vaccine

Progesteron receptor overexpression is reported to be associated with immune evasion in different types of cancer and it could be shown that PR antagonists like Mifegyne can have anticancer therapeutic effect. Thus, Mrs. Cozma started to take Mifegyne in August 2016.

On August 11th the patient reported a heavy generalized allergic skin reaction. Thus, it was recommend to stop the therapy with Mifegyne. She was advised to take 20 mg Prednisolon and Ibuprofen for pain medication. On her recent appointment in September the skin reaction was improved. Furthermore, liver enzymes (GPT, GOT, AP, GGT) are also improved. However, the CRP as well as the neutrophils and Bilirubin are greatly increased supposing that the patient is suffering from a cholangitis. Thus, we restart a medication with Ciprofloxacin (2 x 500 mg/day).
She was advised to have a re-evaluation for clarifying whether the cholangitis is caused by a mechanical obstruction and could be addressed by a stent or a drain.

Any questions regarding this case please feel free to contact me.

Yours sincerely

Thomas Neßelhut