Producing Colorectal Cancer Vaccine:

Combining Forces between Denmark and Estonia

PEER REVIEWED

DanDrit Biotech is a US biotechnology company developing a drug candidate that could become the world's first vaccine for the treatment of colorectal cancer. Springing from academic roots in Denmark, they built and patented immunotherapies, targeting initially advanced non-small cell lung cancer and colorectal cancer. The lead compound, MelCancerVac™ (MCV), is a dendritic cell immunotherapy vaccine that aims at preventing relapses of advanced colorectal cancer after resection and chemotherapy. Three single-arm Phase II clinical trials were conducted in Europe and Asia in cancer where MCV demonstrated efficacy. Based on the promising results from these clinical trials, the developer needed to confirm the efficacy of MCV in a larger, comparative randomised clinical trial. As a result, with the assistance of experienced practitioners in colorectal cancer treatment, a randomised Phase III trial (VIVA) with stage IV colorectal cancer patients with no evidence of disease after resection and chemotherapy was carried out. The vaccine GMP production for clinical trial is outsourced to a partnering company located in Tallinn, Republic of Estonia. Developers of the colorectal cancer vaccine believe that MCV has the potential to solve the problem of the high rate of relapses that dominates stage IV colorectal cancer patients with no evidence of disease.

MelCancerVac™

MelCancerVac™ (MCV) is a vaccine composed of the patient's dendritic cells (DC) generated from peripheral blood primary monocytes loaded with a lysate from an allogeneic melanoma cell clone with low expression of melanocyte-specific differentiation antigens[1]. For MCV to work as a therapeutic cancer vaccine, injected DCs need to be able to activate appropriate T cells in the draining lymph nodes and to generate effector T cells that are ultimately capable of destroying tumour cells. The dose of each vaccination is 3-5 x 10^6 DCs pulsed with allogeneic tumour cell lysate; the volume of vaccine is 0.2mL. The vaccine is administered intra-dermally in the upper front thigh (*trigonum femorale*). The developers believe that the next generation dendritic cell vaccine, MCV benefits from technological competitive advantages over other cancer vaccines, including:

- The vaccine is manufactured within eight days from a patient's peripheral blood. The producer will be able to generate the vaccine with only 250mL of blood. Leukapheresis, which is used in Dendreon's Provenge™ cancer vaccine preparation, is not required.
- The vaccine uses an allogenic (using cells, tissues, or organs, sourced from a genetically non-identical member of the same species as the recipient) tumour lysate as opposed to inconvenient autologous (from the patient) tumour lysate. The cancer-specific antigens are "off-the-shelf" and therefore patient's tumour cells are not needed to manufacture the vaccine.
- The vaccine is polytopic (targets several cancer-specific antigens). As a result, the risk of the tumour escaping is more limited and more T cells can be activated than if the vaccine was targetting one antigen only. However, MCV keeps a focus on melanoma-associated antigens (MAGE-A) that are only expressed by tumours and absent in normal tissues.

MCV Could Prevent Relapse of Advanced Colorectal Cancer

MCV demonstrated efficacy in three separate Phase IIa clinical trials in colorectal and non-small cell lung cancer[2]. Even if MCV can be used for various cancers, the developers decided to focus MCV's clinical development specifically on the prevention of relapses of advanced colorectal cancer (CRC). CRC is the second most frequent cause of cancer-related mortality in both Europe and the United States. The problem of the high rate of relapses dominates the stage IV patients with no evidence of disease (IV NED) condition. Despite the improved possibility of reaching the NED state in metastatic CRC, 90% of these patients eventually recur and die of their disease. Therefore, any systemic treatment aimed at reducing the chances of recurrence of stage IV patients who have reached the NED state should be considered.

The classical paradigm of cancer treatment is that whatever works in the advanced setting, like useful clinical responses, prolonged progression-free survival (PFS) or overall survival (OS), should work with curative intent when used as adjuvant therapy. This paradigm has held up in CRC: Fluoropyrimidines (FU) affording a six-month gain in the advanced setting produce an additional cure rate of about 10% when used as adjuvant therapy in stage III. Based upon this paradigm, the benefit of adjuvant chemotherapy (CT) in the setting of stage IV NED should be even higher, considering the 90% chances of relapsing of these patients. But, unfortunately, adjuvant therapies of stage IV do not work too well. The efficacy of pure adjuvant CT is debatable: FU affords a borderline significant benefit in OS compared to surgery alone (absolute delta 7% at five years) and FOLFIRI produced a numerically better OS that was not statistically significant compared to FU alone[3]. FOLFOX has not been studied (the NSABP trial C09 was discontinued for lack of accrual), although FOLFOX remains the most commonly employed regimen in clinical practice around the world. In the light of these marginal results obtained by the adjuvant strategies, "neo-adjuvant CT" of resectable metastases has been investigated within the frame of a "perioperative strategy" i.e., preoperative CT, then surgery, then CT after surgery. The EORTC produced the only randomised Phase III study available, that failed to demonstrate an OS advantage of the interventional strategy as compared to surgery alone[4,5]. The developers of the vaccine believe that an immunotherapy with a good safety profile such as MCV may be the perfect adjuvant therapy for CRC.

Immunotherapy has gained substantial interest in cancer therapy lately. Since the early steps taken in this field in the 1980s, and after a lag phase of decades during which much effort has been put into basic research, in the last few years several new immune modulatory agents showed activity in a

number of solid tumours, both as single agents and in combination with other anti-cancer therapies (chemotherapy, radiotherapy, vaccines). Strategies designed for "removing brakes" from the antigen-mediated immune response, for example anti-CTL4 and anti-PD-1/PDL-1 antibodies, have proven effective against cancer [6]. An alternative approach involves stimulation of innate immunity, based on the rationale that activation of this system may have both direct anti-tumour effects and a role in enhancing tumour antigen presentation. In particular, agonists of Toll receptor 9 have shown promising anti-tumour activity by enhancing the clinical outcomes from cancer vaccination, conventional chemotherapy and other therapeutic modalities[7]. The knowledge of a growing number of tumour-associated antigens has provided a major stimulus for the development of cancer vaccines, i.e. active immunisations designed to treat growing tumours. In this context, recent studies have shown that high infiltration of primary CRC by CD3(+) and CD8(+) lymphocytes are associated with a better prognosis [8] independently from the CRC TNM classification. This finding suggests that the immune response plays an important role in CRC natural history and that boosting this response by vaccination may lead to clinical benefit.

Currently, the use of dendritic cells pulsed with tumour lysate, such as MCV, for anti-cancer immunotherapy vaccines is of great interest, and the vaccines have been investigated in more than 150 clinical trials to date. The results demonstrate that these vaccinations are safe and in some cases can increase anti-tumour immunity in cancer patients and eventually induce clinical response in some patients. Although surgery is the primary effective treatment for CRC, combination regimens with conventional oncological therapy and new immunotherapeutic treatments can improve CRC treatment efficacy, resulting in reduced relapses and improved overall survival. The early experience in patients with progressive stage IV CRC treated with MCV showed 4/17 SD with 2 of these 4 lasting more than 27 and 37 months. Because the refractory, advanced setting is worse for vaccine therapy, these results, although very limited and preliminary, constitute a hint of activity for running this randomised trial. Therefore, the evidence available from pre-clinical and early-phase clinical trials justifies the study of the effect of dendritic cell-based vaccines in formal, controlled, randomised studies in stage IV CRC patients.

VIVA: Phase III Clinical Trial

The development programme is centred on VIVA, a randomised, open label, multicentre Phase III clinical trial in stage IV colorectal cancer patients with no evidence of disease after standard of the care (SoC). VIVA will be conducted in Italy in collaboration with GISCAD (Group for the Study of Digestive Tract Cancer). VIVA's principal investigator is Prof Alberto Sobrero at IRCCS AOU San Martino-IST-Istituto Nazionale per la Ricerca sul Cancro, Genova, Italy. The primary objective of this study is to investigate the efficacy of MCV in stage IV CRC patients rendered disease-free after completion of standard treatment according to local practices. The primary endpoint is Relapse Free Survival (RFS). Prior to randomisation, patients will have completed all treatment for colorectal cancer (CRC), including complete resection of the primary CRC tumour and any liver metastases and all SoC including chemotherapy (neo-adjuvant/adjuvant).

VIVA will enrol 174 patients with stage IV CRC and no evidence of disease within two years. 87 patients will be randomised to active treatment with MCV; 87 patients will be randomised to no further treatment (control group). Eligible patients randomised to active treatment will be vaccinated with MCV and receive a total of 15 vaccinations. Patients randomised to no further treatment (control) will receive SoC. Vaccine group patients will have three blood draws for the production of the vaccine. The first blood draw for vaccine production will be after randomisation. Patients will be followed for RFS and OS for five years after randomisation. For active treatment patients, plasma samples obtained from blood collected for vaccine production will be frozen and stored for possible analysis of immunological response at a later date, given that a specific informed consent form has been signed by patients. Response will be assessed according to Response Evaluation Criteria in Solid Tumours (RECIST). At the discretion of the investigator, patients with confirmed relapse during the treatment early phase and before receiving all the 15 planned vaccinations may continue to receive treatment and undergo all scheduled assessments, unless clinically indicated. Adverse events (AEs) will be assessed according to the National Cancer Institute's Common Terminology Criteria for adverse events.

MCV is Produced by Estonian Company

MCV manufacturing is outsourced from a contract manufacturing organisation – Cellin Technologies (CT) based in Tallinn, Estonia – under a pharmaceutical agreement. The colorectal cancer vaccine will be produced at CT's GMP facility and returned to the study site for administration. CT uses the state-of-the-art GMP cleanroom facilities dedicated and equipped for the production of cell therapy medicinal products. They hold a manufacturer's authorisation for preparing of sterile products and an activity licence for handling cells, tissues and organs, issued by the Estonian State Agency of Medicines (SAM). They have extensive know-how and a competent quality management system, as well as a highly experienced and quality-driven team consisting of qualified medical and scientific specialists. Ultimately, they are a reliable and loyal partner with Scandinavian work ethics. CT has a contract to manufacture the MCV for the whole clinical trial programme for up to 174 patients. Moreover, CT is also responsible for manufacturing the melanoma cell lysate (MCL), a critical raw material used for MCV production, and preparing and storage of melanoma working cell bank cells needed for MCL production.

In addition to CT's practical approaches to provide GMP contract manufacturing services, they are actively involved in an EU-funded research and development project, which studies the use of regenerative cells from human adipose tissue in the creation of new blood vessels, directed by Dr Andrus Loog. Furthermore, CT's own research focus is to investigate the molecular mechanism of stem cells proliferation and differentiation, with a main emphasis on development of techniques to manipulate homing, differentiation and integration of cells following transplantation[9–10].

The collaboration of the two organisations began in January 2014, with the MCV technology transfer to CT's GMP facility. The process to get the manufacturer's authorisation for MCV production started in March 2014 and was successfully evaluated by the Estonian competent authority (SAM) in

June 2014. Due to Estonian innovative e-services, the processes with government organizations are fast and efficient compared to many other countries nearby, without losing the compliance to high quality demands.

Benefits of e-Estonia

The term "e-Estonia" is often used to describe Estonia's emergence as one of the most advanced e-countries in the world. For the citizens of Estonia, e-services have become routine: e-voting, e-taxes, e-police, e-banking, and e-school[11]. In addition, there are some e-services in healthcare that are widely used in Estonia, e.g. the digital prescription system and e-Health record – a medical information system with which people can view their own digital medical history[11]. Recently this year, Estonia announced the e-residency that has gained popularity worldwide, giving the opportunity to others than Estonians to use secure e-services that have been accessible to Estonians for years already[12].

Based on Estonian outstanding e-capability and the high quality of the state-of-the-art GMP laboratory and competent personnel, the Estonian company has become a partner enterprise, in which to develop an innovative personal medicine. Furthermore, never should anybody underestimate two very important assumptions, when starting to solve great tasks: the common understanding of the work ethics, and the sense of mission.

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