Cellular immunotherapy : from stem cell to lymphocyte

Valorisation

Cancer is one of the major causes of death in the Western world and the second cause of death in developing countries, with a global growing incidence of 22.2 million estimated cases in 2030 [1-3]. In 2012, 14.1 million people were diagnosed with cancer and 8.2 million cancer deaths were counted worldwide [4]. Even though cancer mortality declines because of more effective, early detection in combination with early treatment, the burden on society remains enormous. The economic burden consists of costs associated with for example prevention, health-care and productivity losses due to morbidity and mortality. The estimated total cancer costs in the European Union were € 126 billion in 2009 [5]. Besides the economic burden, the social burden of cancer affecting the quality of life of both patients and their caregivers should also be acknowledged.

Because not all cancers can be cured with the current treatment protocols, an urgent need for novel therapies exists. More recently, therapies like immunotherapy became of great interest. Although great progress in the past decades has been made (e.g. the introduction of monoclonal antibodies), immunotherapy is often only effective in certain patients and only results in moderate prolonged survival instead of complete remission. Among cellular immunotherapies, adoptive T cell progenitor therapy and natural killer (NK) cell infusion are of great interest and are described in this thesis.

For adoptive cellular immunotherapy, the generation of high cell numbers of interest is often a requirement as well as a limitation. To treat for example a patient of 70 kg, somewhere between $70x10^6$ to $700x10^6$ cells are believed to be required for a single dose of T cell progenitors. In order to generate such great cell numbers, attempts to expand haematopoietic stem cells are of importance and of great interest.

In **chapter 3** we provide a method to expand haematopoietic stem cells. For human cells, this resulted in 160 fold more cells. Subsequent differentiation towards specific cell types could result in higher total cell numbers for therapy. However, since these cells produced by manipulation with a retroviral construct, first an alternative method should be generated to meet good manufacturing practices (GMP) and ensure safety. Nevertheless, this method provides a good basis for further research.

In **chapter 5**, we propose a clinical grade culture system for the production of T cell progenitors that can easily be translated to a clinical product and that could strengthen the patient's immune system after stem cell transplantation. Although stromal cell based systems that were previously developed by others and us could potentially be used for the generation of cellular therapy, getting approval for such product would be difficult, expensive and time consuming. Especially because cellular products are rather new and no extensive regulations are written. Moreover, the potential risks are not completely known. The stromal-based co-cultures use murine stromal cells lines that are retrovirally transduced to overexpress Delta

Like ligand. EMA and FDA advise against the use of products of animal origin, although it is not prohibited. In this case, the use of both the stromal cells and the FCS required to produce the cellular product will be debatable. Another concern is that the stromal cells are retrovirally transduced. Although these cells will not be in the actual product, it has to be assessed whether viral load is present in the cell products to assure safety. Besides safety regulations, there are other concerns regarding this product. Prior sorting to eliminate stromal cells and to select the proper T cell populations is required, which are as said time consuming and expensive. Moreover, reproducibility in a system with cell lines and FCS is more difficult than in a feeder- and serum-free culture. It is know that FCS and even cell lines have large batch to batch variation, resulting in lower reproducibility, increase in quality control and thereby increasing costs.

To circumvent the indicated undesired components, we generated a system using plate bound DLL4:Fc, fibronectin and a growth factor cocktail to differentiate haematopoietic stem cells to T cell progenitors, which can be clinical applicable. We discovered that ascorbic acid, also known as vitamin C, improves maturation of T cell progenitors and also improves the proliferation of these cells. Adoptive T cell progenitor therapy could provide faster recovery of patient's T cell levels and thus providing protection against infections. Currently, the low T cell levels of patients and subsequent infections account for high morbidity and mortality of these patients [6]. Moreover, the use of T cell progenitors is not limited to cancer patients, but could also be used to improve the immune system of other immunocompromised patient groups like AIDS patients. A first step towards the development of a clinical product would be the verification of the T cell progenitors *in vivo* in a humanized mouse model. Hereafter, production needs to be scaled up to obtain sufficient cell numbers for human cell infusions. Currently, the product is produced in 96 or 48 well plates resulting in limited yield and high maintenance. Culturing in special bags or bioreactors with controlled supplementation of required nutrients and cytokines could increase yield and reduce handling time resulting in a better product with less costs. Moreover, progenitor T cells need to be produced in a GMP facility before clinical trials can be initiated. Clinical trials should asses if injection of both stem cells and progenitor T cells will result in a faster recovery of the patient T cell pool and if this leads to reduced infection incidence.

Another cellular immunotherapy that is currently under investigation is NK cell therapy as treatment for types of cancer that are not curable by the conventional therapies as surgery, chemotherapy or radiation. Cell numbers required for adoptive therapy are estimated to run in the billions especially because only a minor population of the total NK cell fraction is capable of eliminating tumour cells [7-8]. In **chapter 6**, we show that ascorbic acid has a positive influence on the expansion of peripheral NK cells, a finding that can be of great value for the improvement of the production of adoptive NK cell therapy. The generation of more cells in a shorter time could lead to a better cost-effective product. Since ascorbic acid is already a

FDA-approved compound, this can easily be implemented in clinical protocols and clinical trials. First, the current proposed culture method needs to be upgraded to a GMP compliant method. Currently, this is performed in our lab in collaboration with the German company Zellwerk. The culture system is a bioreactor in a GMP qualified safety cabinet with a constant supply of fresh media. Also glucose, pH, temperature are constantly monitored and adjusted if needed. Currently, NK cells generated in this bioreactor are evaluated on their proliferative capacity, phenotype and function. After subsequent verification of our results *in vivo* mouse models, phase I clinical trials will be started.

As mentioned shortly, for both T cell progenitor and NK cell therapy, clinical product translation is still required. For GMP, products need to be prepared in closed systems in special facilities with high quality control and standardized protocols. Before starting clinical research, approval by the Medical Research Ethical Committee needs to be granted. Financial support for the trial can be obtained from private funding or funding from organizations like the Dutch Cancer Society (KWF) and the Cancer research Fund of the Limburg Health Foundation. To ultimately bring these products to the patients, charities will not be able to provide sufficient money to pay for the involved costs. It is foreseen that spin-off companies that attract venture capital can bring these needed therapies several steps further. Phase III clinical trials will need so much money that the big pharmaceutical companies will be needed to further co-develop the products.

Besides the developed and improved culture methods to generate cells applicable for adoptive cellular therapy, we investigated the vitamin C status of patients with a haematological malignancy. In **chapter 7** we show that these patients, either treated with stem cell transplantation and/or chemotherapy have significantly reduced vitamin C levels. This information has a high new value and further research could be performed to investigate whether these low vitamin C levels correlate to patients' lymphocyte counts. This information, especially in combination with the *in vitro* effects of vitamin C shown in **chapter 5 and 6**, could therefore be used to start clinical trials. For example, the effect of vitamin C supplementation on lymphocyte recovery and infection incidence could be studied. Since vitamin C is inexpensive to produce, readily available, known for many years and already proven to be safe when supplemented in high doses, clinical trials could be initiated fast. Furthermore, it would be of great interest to investigate the effect of vitamin C supplementation on thymus regeneration, potentially resulting in increased thymic output and higher T cell levels.

These findings contribute to the current promising progress in the field of cellular immunotherapy. Our results promise a good feasibility; however, these data need to be strengthened with *in vivo* experiments with our proposed clinical products. Subsequently clinical trials need to demonstrate the scientific and clinical value of these therapies. Cellular immunotherapies require patient specific products that are labour-intensive and therefore

high costs will be involved in the production [9-10]. Since several cellular immunotherapies are already in use, for example stem cells for transplantation and a dendritic cell based vaccine for prostate cancer (Provenge®), cellular immunotherapies already prove to be effective. This opened the road for novel therapies as proposed in this thesis. These therapies could improve the life expectancy of patients with cancer in the near future and should therefore be of main interest for current research and clinical translation.

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