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Universitair Medisch Centrum
Utrecht

Novel immunotherapy strategies for multiple myeloma and other haematological malignancies

T. MUTIS

Since mid nineties, research at the Hemato-Oncology section of the dept. of Clinical Chemistry and Hematology in UMC Utrecht is focused on development and subsequent clinical translation of novel immunotherapy strategies for hematological malignancies, in particular for multiple myeloma (MM). These research activities are highly integrated with the clinical profile of the dept of Hematology. In this short overview, we will provide the specific goals and the current status of this research.

Multiple Myeloma

Multiple myeloma (MM), also known in the Netherlands as the 'disease of Kahler' after its Austrian discoverer Dr. Otto Kahler, is a hematological cancer originating from antibody producing plasma cell (1). MM is often mistakenly called a bone disease, as the cancer usually resides in the bone marrow and invades the bone tissue, resulting in multiple bone lesions, accidental and sudden break of bones. MM is a disease of middle aged or elderly people, with a median age of 59 years (range 26-84). Only 5 % of patients are under the age of 40 years, but MM has an important worldwide clinical impact: it is the second common hematological malignancy, accounts for 10% of all hematological disorders and 1% of all cancers, and yet it is incurable (2).

*Department of Clinical Chemistry and Hematology,
University Medical Center, Utrecht, the Netherlands*

E-mail: t.mutis@umcutrecht.nl

Multiple Myeloma therapy: a historical overview

Classical treatment of MM is chemotherapy. Before 1960's, the life expectancy of MM patients was less than one year (3). The introduction of melphalan, an alkylating agent, significantly improved survival up to two years. This trend was continued in the late nineties by the introduction of intensive chemotherapy, followed by autologous stem cell transplantation. Over the last decade, MM therapy has been further improved, first by the successful application of thalidomide, a teratogenic but an immunomodulatory agent (IMiD), and later by the introduction of a new series of novel anti-myeloma agents such as bortezomib and the thalidomide-analogue, lenalidomide. Currently, these novel agents are often used in combination with the classical agents to induce complete remission (4, 5). Nonetheless, virtually all patients eventually relapse from all forms of chemotherapy. The overall survival for younger patients is currently 5-6 years, and for elderly patients is only around 3-4 years. The prognosis for high risk patients, identified with specific chromosomal anomalies (17p deletion, t(4;14), t(14;16), t(14;20) translocations, deletion of Chr.13 or hypodiploidy) is still poor (6).

Immunotherapy of Multiple Myeloma

Achieving cure in MM by chemotherapy appears an extremely difficult task. This is mainly because myeloma cells are genetically highly instable and their growth is accelerated by their tight interactions with supportive cells and with the extracellular matrix in the bone marrow. Through these microenvironmental

interactions, myeloma cells not only gain survival advantage, but also develop chemotherapy resistance (7). Therefore, next to the classical chemotherapy, a completely different approach, 'immunotherapy' has also been developed and applied, especially for MM patients who do not benefit from chemotherapy (8).

Cellular immunotherapy of MM: possibilities and challenges.

To date, the most frequently applied form of immune therapy for MM in UMC Utrecht is the transplantation of the patient with the hematopoietic stem cells of an HLA-matched donor (allo-SCT). Allo-SCT is an illustrative form of cellular immunotherapy because the therapeutic 'graft vs tumor (GvT)' effect is actually not mediated by grafted stem cells but by the immune T cells present in the graft (9, 10). A large proportion of the grafted donor T cells are 'alloreactive' killer cells, i.e., they recognize recipient's cells as foreign organisms to destruct them. Hence, donor T cells, once activated properly, are highly efficient in tracing and eliminating the tumor cells of the patient. Therefore, in case of tumor relapses, treatment is usually followed by additional infusion of donor lymphocytes (DLI) (10). Allo-SCT/DLI can induce very long term remissions, but only in a proportion of MM patients. Improvement of the therapeutic effect of allo SCT/DLI a) by identifying the prerequisites of *in vivo* T cell activation after transplantation and b) by delineating tumor immune escape mechanisms are therefore our major goals. To this end, we have recently discovered that MM cells, through tight interactions the accessory cells of the tumor microenvironment can significantly downregulate T cell responses (van der Veer, M. et al. submitted). Through these interactions, MM cells also gain resistance against killer functions of T cells, probably due to the upregulation of anti-apoptotic proteins (de Haart, S. et al. ms in preparation). Towards improved therapies for MM, we are currently delineating the molecular basis of this cell adhesion mediated immune escape.

Next to the important issue of tumor escape, our work focus on the improvement of the specificity and hereby the safety of allo-SCT, because in its current form, allo-SCT is a double-edged sword, which is also associated with severe complications in 30-40% of the patients (11). Such complications mainly arise because the infused donor T cells, as they are not specifically directed to tumor cells, also attack normal tissues, resulting in Graft vs Host Disease (GvHD). To separate the therapeutic and detrimental effects of allo-SCT, we focus on the identification and clinical application of target antigens and relevant T cell subsets that are specifically associated with the therapeutic GvT effect.

Hematopoietic system specific minor Histocompatibility antigens: tissue specific alloantigens to separate GvT from GvHD

Human Leukocyte (HLA) antigens are the 'major transplantation antigens' recognized by alloreactive donor T cells. Allo-SCT is therefore usually applied between fully HLA-matched donor-patient pairs to reduce the risk of harmful alloreactivity. However,

potent alloreactivity leading to beneficial GvT and detrimental GvHD also occur after HLA-matched transplantations, due to the mismatching of the patient and the donor for the so called 'minor transplantation antigens' (12). Fortunately, this type of mismatching provides opportunities to separate GvT from GvHD, because minor antigens, -unlike the major HLA antigens, can be expressed in a tissue specific manner. In fact, minor antigens are HLA-bound polymorphic peptides derived from intracellular proteins encoded by allelic genes (13). Several of these allelic genes are lineage or tissue specific. In particular, a specific group of minor antigens are solely expressed in hematopoietic cells, including the tumor cells derived from these cells. Using *in vitro* and *in vivo* experimental models, we and others have shown that T cells specific for such 'hematopoietic minors' can establish strong anti-tumor effects without harming the cells from any other tissue (13-17). Hence, 'hematopoietic minors' are the ideal target antigens to induce GVT without the risk of GvHD. A significant part of our research is therefore devoted to the identification of hematopoietic minors to enable a broad application of minor-based immunotherapy. Towards this end, we have recently developed a powerful, convenient and rapid genetic method, the genome-wide zygosity-genotype correlation analysis (18, 19). With this method, we now identified two new hematopoietic minor antigens; one on the B cell specific molecule CD19 (18) and the other on the hematopoietic specific gene C12orf35 (Oostvogels R et al, ms submitted), which gives us the possibility to develop immunotherapy for MM and other B cell malignancies. Indeed, we have shown that killer T cells recognizing these antigens are highly effective in eliminating malignant B cells and/or MM cells in an antigen specific fashion (18, 20, 21). Using these two new antigens and a number of previously identified hematopoietic minors we have now initiated a phase I/II clinical 'minor vaccination' trial for allo transplanted patients with MM and other relevant malignancies. In this trial, allotransplanted patients who are not responding to a first course of DLI, receive a second course of DLI and are at the same time vaccinated with dendritic cells loaded with the peptides of hematopoietic minors. Dendritic cells are superior antigen presenting cells required for initial priming of T cells against their antigens. Thus in this trial we aim at improving the GvT effect by specifically skewing the donor T cells towards hematopoietic minor antigens. The first results are expected in 2012.

Regulatory T cells: nature's cellular tools for separating GvT from GvHD.

Regulatory T-cells (Tregs) are a specific subset of CD4⁺ T cells with immune suppressive capacities. In our research program, we investigate the relevancy of Tregs to effectively prevent GvHD after allo-SCT. In a xenogeneic GvHD model, we have recently shown the potent capacity of human Tregs to control GvHD (22). Toward clinical application of huTregs we also explored their impact on the GvT effect. Again, in a humanized murine model, we demonstrated that human Tregs, while effectively controlling GvHD, can

permit GvT effects, but only against tumors residing in the bone marrow (Guichelaar T. et al ms submitted). Exploring this remarkable phenomenon, we discovered that the suppressor functions of Tregs are neutralized by bone marrow stromal cells, indicating the possibility that Tregs can be used as therapeutical tools for the prevention of GvHD but only in patients such as MM, as in this disease the tumor resides usually in the bone marrow.

Immunotherapy of MM with Antibodies.

Next to cellular immune therapy, a rapidly upcoming form of immunotherapy is the antibody-based targeted immunotherapy. Although the idea is not new, the field is in a rapid movement, thanks to the development of technologies to generate fully humanized antibodies against relevant tumor associated surface molecules (23). Furthermore, this form of immunotherapy can be combined with chemotherapy. For instance in several B cell malignancies, antibodies against CD20 (rituximab) are now successfully combined with chemotherapeutics such as fludarabine, cyclophosphamide or lenalidomide. To achieve a similar goal in the MM setting, we recently collaborated with the biotech company Genmab, which developed a humanized CD38 specific antibody, daratumumab (DARA). In our *in vitro* and *in vivo* models, we demonstrated that this antibody mediates strong lysis of MM cells via direct apoptosis, complement dependent cytotoxicity, and antibody dependent cellular cytotoxicity (24). In further *in vitro* studies we combined DARA with novel chemotherapeutics and demonstrated that DARA significantly improves the MM cell lysis induced by novel anti-MM drugs lenalidomide and bortezomib in a synergistic manner, even in samples of patients who are poor responders for these drugs (25). DARA also significantly improves the MM lysis achieved by triple combinations of lenalidomide, bortezomib, dexamethasone and Melphalan (van der Veer et al 2011, Blood Cancer Research, in press), illustrating its strong therapeutic potential and warranting the clinical evaluation of DARA alone or in combination with other anti MM drugs for MM treatment.

In summary, towards successful cellular immunotherapy of MM, we have now novel technologies, which are successfully applied for the identification of relevant antigens. We have also been able to translate the concept of minor-antigen therapy to the clinics and in the near future we will be able to evaluate the safety and efficacy of minor antigen based immunotherapy. Towards this end, delineating the immune escape mechanisms of MM cells and developing adequate strategies to tackle this is still an important challenge. In addition, exploiting specific GvHD-downregulatory capacities of Tregs, first in animal models, subsequently in clinical trials will also be a future focus, especially for patients who are not eligible for minor antigen based therapies. Finally, contingent on the premise that combination therapies are required for the successful induction of initial complete responses, our future investigation will also focus on strategies by which cellular immunotherapy and antibody therapy

will be combined with each other and with other chemotherapeutic agents that can improve immune effector functions against MM cells.

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Placental DNA as a source for clinical diagnostics and a cause of maternal diseases

C.B.M. OUDEJANS and M. van DIJK

The placenta is essential for embryonic growth and development, informative for genetic disorders of the fetus and a cause of maternal diseases. Here we describe the latest accomplishments in the use of placental DNA in maternal plasma as a source for non-invasive prenatal diagnostics of fetal genetic diseases, i.e. trisomy 21 and placental DNA as the cause of prevalent maternal diseases, i.e. pre-eclampsia.

The placenta as source for clinical diagnostics: from invasive to non-invasive, from indirect to direct, from focal to genome-wide and from protein to DNA

The genetic material (DNA sequence) retained in placental cells is identical to the genetic blueprint of the fetus and as such used routinely for the diagnosis of aneuploidy and other chromosomal anomalies of the fetus. However, to obtain placental cells for this clinical purpose invasive procedures like chorionic villus sampling are needed (1). These procedures carry a risk of miscarriage due to the procedure. Non-invasive methods with the same sensitivity and specificity were considered urgently wanted, both by patients and doctors. The landmark discovery in 1997 that placental DNA is present in maternal plasma from early pregnancy onwards in quantities that permit reliable and amenable molecular analysis reactivated the search for the holy grail: non-invasive prenatal diagnosis (NIPD) of common fetal genetic diseases (trisomy 21,

13 and 18) using placental i.e. fetal DNA in maternal plasma (2, 3). Pregnant plasma contains packages of released cellular particles (exosomes) of the placenta, nicely packed and thereby protected that contain all the genetic material (DNA and RNA) of the child, are present in large quantities in the plasma and can be analyzed by modern DNA methods for their contents. The clinical breakthrough came two years ago with the introduction of the second generation DNA sequencers and the vision, intelligence and fund raising power of Dennis Lo. It had been proven by Dennis Lo and Rossa Chiu that this test permitted diagnostics of Down's syndrome (trisomy 21). We, our colleague Kypros Nicolaides in London and Dennis Lo in Hong Kong had sufficient sample numbers to perform the ultimate test in 2010 performed in Hong Kong. In a study of about 800 pregnant women containing 90 proven cases of trisomy 21 the presence or absence of Down's syndrome was diagnosed with near perfect accuracy (2).

The principle is as follows. During early pregnancy (week 9-13) blood is withdrawn from the mother by a standard procedure. The blood is processed with removal of the cells by means of centrifugation. In the remaining fraction (plasma) the total amount of DNA is subsequently isolated. This DNA originates from the particles (exosomes) and contains maternal (90%) and fetal (10%) DNA. The ends of the DNA are polished, glued to pieces of synthetic DNA that subsequently, by a combination of enzymes and DNA fragments complementary to the glued DNA, are recognized and transcribed leading to the simultaneous million fold amplification of the original DNA. All these DNA fragments are then immobilized on a car-

Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands

E-mail: cbm.oudejans@vumc.nl