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MEETING REPORT

Immunotherapy: from basic research to clinical applications

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Introduction

Activation or modulation of host immune responses is emerging as a promising strategy to combat many diseases, including autoimmune disorders and cancer. However, although initial successes have sometimes been encouraging, larger trials have unfortunately often shown only limited efficacy. There is clearly much room for improvement. The development of new therapies and their translation into clinical practice is the aim of the Collaborative Research Centre (Sonderforschungsbereich, SFB) 685, Tübingen, coordinated by Professor Hans-Georg Rammensee. To provide a forum for discussion of new developments, the SFB 685 hosted a symposium on immunotherapy, between 6 and 7 March 2008. The scope of this symposium encompassed basic research topics including NK cell, T cell and antigen-presenting cell (APC) biology as well as clinical applications of therapies developed from such research. Especially, the potentiation or attenuation of specific T cell responses is the common goal of many immunotherapeutic strategies. Since these responses are to a great extent determined by the delicate interplay between T cells and APC, manipulation of either cell type offers the opportunity for selective, yet at the same time powerful intervention. Dendritic cells (DC) are particularly interesting in this context as they bridge innate and adaptive immunity. Being able to harness their full potential would allow recruitment of both arms of the immune system for the desired therapeutic response. This, however, requires a good understanding of the basic processes governing the fate and actions of the cells involved. Although first successes are clearly being achieved with immunotherapeutic interventions, especially with soluble molecules such as antibodies or cytokines, the breakthrough in cellular immunotherapies crucially depends on knowledge gained by further research, both basic and clinical.

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T cells and NK cells

The outcome of the T cell—APC interaction is determined by many different factors, including type and activation state of the APC, signal duration and strength, antigen concentration and cytokines present [32]. Federica Sallusto (Bellinzona, Switzerland) presented data on the newest addition to the T helper cell subsets, Th17 cells, which are considered to be important for the protection against extracellular pathogens, but can also be highly pathogenic when reacting to self-antigens, such as in several animal models of autoimmune disease [6]. She showed that these cells can be distinguished from other T helper cell subsets by their co-expression of chemokine receptors (CCR) 4 and 6 [2]. While in mice, Th17 differentiation depends on TGFβ and IL-6 [43], in humans, it is driven by IL-1β and IL-6 and inhibited by high doses of TGFβ [1]. Interestingly, some of these IL-17-producing T cells also produce IL-22 and/or IFNγ, suggesting further heterogeneity within the subset.

CCR6 expression might be important for migration of Th17 cells into inflamed tissues and the CCR6 knockout mouse is resistant to experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis. Selective blockade of CCR6 might, therefore, be an interesting therapeutic perspective for Th17-mediated autoimmune diseases. Correlations between chemokine receptor expression and function have been found in many cases. For example, naïve T cells and central memory T cells (T_{CM}) can home to lymph nodes by virtue of CCR7 expression, while effector and effector memory (T_{EM}) cells lacking CCR7 are considered unable to do so [40]. However, Sallusto's group was able to demonstrate that the latter two $CD8^+$ T cell subsets, through expression of CXCR3, are able to enter reactive lymph nodes and kill DC presenting the cognate antigen, thereby probably limiting immune responses [22]. $CD4^+$ T_{EM} can also enter reactive lymph nodes and, through constitutive expression of CD40L, can act like an "endogenous adjuvant", maintaining DC in a mature, immunostimulatory state even in the absence of microbial stimuli (F. Sallusto and A. Martin-Fontecha, unpublished results). By triggering DC maturation within lymph nodes, $CD4^+$ T_{EM} might also contribute to autoimmunity.

In some instances, such as autoimmune disease or graft-versus-host-disease (GVHD) after bone marrow transplantation (BMT), T cell responses can be detrimental. Regulatory T cells (Treg) constitute one of the most important physiological mechanisms for controlling such responses. Due to their low numbers, efficient isolation and in vitro expansion protocols are needed in order to obtain sufficient amounts of functional Treg for therapeutic interventions. Matthias Edinger (Regensburg, Germany) highlighted pitfalls and solutions of such procedures. Having successfully established a protocol for GMP isolation and in vitro expansion of Treg [24, 26], his group went on to further characterise the expanded cell population in detail [25]. They found that only $CD45RA^+CD4^+CD25^{hi}$ Treg maintain expression of FoxP3, a transcription factor required for Treg function. In vitro expanded $CD127^{lo/-}CD4^+CD25^{hi}$ Treg on the other hand show a variable phenotype, with some cells (mainly $CD45RA^-$) losing FoxP3 expression, possibly due to methylation at the TSDR (Treg-specific demethylated region) of the FoxP3 locus [4, 16]. Expanded $CD45RA^+$ Treg, therefore, appear to be the most suitable Treg population for use in the clinic. Such a clinical trial with co-transfusion of donor Treg and conventional T cells after stem cell transplantation is currently underway and one more without additional immunosuppressive treatment is in preparation.

While adoptive transfer of Treg can be used to dampen immune responses, adoptive transfer of tumour-specific $CD8^+$ T cells can enhance anti-tumour activity. Andreas Mackensen (Erlangen-Nürnberg, Germany) addressed challenges and limitations of such approaches. The ability to deliver large numbers of these cells offers the possibility to overcome immunological unresponsiveness, for example, due to expression of down-modulatory cytokines in an immunocompromised host. In addition, co-administration of T helper cells or appropriate cytokines can further enhance anti-tumour activity. The potential of this therapeutic approach was demonstrated in a phase I clinical trial with melanoma patients [35]. Melan-A-specific cytotoxic lymphocytes (CTL) can be expanded up to 500-fold in vitro when repeatedly stimulated with peptide-pulsed autologous DC. In vitro expanded and adoptively transferred cells can be detected in patients up to 2 weeks after transfer. They accumulate at the tumour site and induce tumour regression in some patients. While these results clearly demonstrate the potential of adoptive T cell transfer for cancer treatment, one of the limitations of any antigen-specific approach became also apparent. Selective loss of Melan-A expression in metastases following T cell transfer was observed in two patients, preventing recognition by the transferred CTL. Other mechanisms which can prevent or impair T cell-mediated tumour eradication include metabolic regulators, such as IDO (indoleamine 2,3-dioxygenase) and lactic acid, cellular regulators, such as Treg, and expression of inhibitory molecules such as PD-L1 (programmed death-ligand 1) by the tumour [7, 9, 15, 17, 18]. PD-L1 is constitutively expressed on many tumours [8] and its expression on human renal cell carcinoma correlates with tumour-specific survival [48]. Blocking of PD-L1 enhances antigen-specific T cell responses, and inhibiting the function of PD-L1 or other co-inhibitory molecules might represent a strategy to potentiate these responses also in patients [8, 9]. Alternatively, identification of essential growth factors, such as those present in T cell conditioned medium, which are superior to IL-2 for expansion of antigen-specific T cells, would allow the design of optimal culture media and conditions for generating large numbers of highly active CTL, further improving adoptive T cell therapy.

While T cells and DC are currently the focus of most immune interventions, the potential of NK cells for immunotherapy is slowly re-emerging as new aspects of their regulation and function are being uncovered [41, 42]. Originally, NK cells were considered to be mainly innate immune effector cells mediating a cytolytic response against tumour cells and virus-infected cells in the absence of specific immunization. However, the term "natural killer" does not reflect the maturation requirements for NK cells in order to be able to kill, nor the other biological functions NK cells can exert. Based on new functional assays, genetic models and genomic analysis, Thierry Walzer (Marseille, France) proposed a more accurate definition of NK cells, including the phenotypic identification of NK cells as $CD3^-$ $NKp46^+$ cells across mammalian species

[52]. Through the identification of an NKp46 promoter region and the subsequent use of this NKp46 promoter, they generated conditional transgenic mice expressing EGFP and the diphtheria toxin receptor in NK cells [50]. Injection of diphtheria toxin into these mice leads to a selective and complete NK cell ablation, introducing a new and attractive model for the in vivo characterization of NK cell function.

NK cell trafficking in steady-state and inflammatory situations is another research topic of Walzer's group. They could show that lysophospholipid sphingosine 1-phosphate regulates recirculation of NK cells by binding to its receptor $S1P_5$ [51]. In the context of the nervous system, $S1P_5$ regulates migration and survival of oligodendrocytes in vitro. $S1P_5$ is also expressed on NK cells in humans and mice and $S1P_5$ deficiency in mice results in a modified homing of NK cells during steady-state conditions. $S1P_5$ is also required for the mobilization of NK cells to inflamed organs. Manipulating NK cell trafficking by targeting $S1P_5$ might represent an attractive immunotherapeutic approach in the future.

In humans, many activating NK cell receptors have been characterised. Frequently, however, the corresponding cellular ligands remain to be identified, rendering further advances in understanding the involvement of these receptors in NK cell-mediated immunoregulation and immunosurveillance difficult. The group of Alexander Steinle (Tübingen, Germany) identified an interaction partner of NKp80, a stimulating Natural Killer Gene Complex (NKC)-encoded human NK receptor. They characterised the orphan C-type lectin-like receptor (CTLR) activation-induced C-type lectin (AICL) as a novel ligand of NKp80 and defined AICL as a myeloid-specific, activating receptor, which is upregulated by Toll-like receptor (TLR) stimulation [53]. Both NKp80 and AICL are absent in rodents and both are encoded in the human NKC in close vicinity. AICL engagement by NKp80 promotes NK cell-mediated cytolysis of malignant myeloid cells. Since NKp80 stimulates the release of proinflammatory cytokines by both monocytes and NK cells, the NKp80-AICL interactions may contribute to the initiation and maintenance of immune responses at sites of inflammation by specifically bridging those two cell types.

Antigens and antigen-presenting cells

Many immunotherapies, especially vaccination regimes, rely on the manipulation of APC, master regulators of the immune response. Antigen-loaded monocyte-derived DC have been used as vehicle in many clinical trials, albeit with varying success. However, presentation by other APC also critically influences the immune response. To address their contribution, the group of Thomas Brocker (Munich, Germany) analysed vaccination protocols in which antigen presentation is restricted to DC [21]. Such immunization resulted in profoundly stronger primary as well as memory responses. While no differences in T cell surface markers could be detected, their number was clearly increased, indicating that antigen presentation by non-DC might be responsible for the down-modulation of CTL responses. Microarray analysis of T cells from these animals revealed strong downregulation of pro-apoptotic factors, suggesting that antigen presentation by non-DC might negatively influence the T cell response via regulation of apoptosis.

Insight into another pathway by which APC attenuate T cell responses was provided by Waldemar Kolanus (Bonn, Germany). Although the importance of the interaction of B7-H1 on APC and co-inhibitory molecules such as PD-1 on T cells for anergy induction is well known, the downstream signalling events are incompletely understood. He showed that the inhibition of PI3-kinase activation leads to strong induction of the guanine-nucleotide exchange factor (GEF) cytohesin-3 (Cyh3) [31] via FoxO1. Enhancing activity of Cyh3 blocks IL-2 production, resulting in T cell anergy, and its knock-down in effector cells abrogates Treg-mediated inhibition of proliferation. Cytohesin-1, a close homologue, has opposing effects. It is phosphorylated upon T cell activation and activates MAP kinase signalling and transcription factors like NFκB. Cyh3 appears to be an essential component of a novel type of inhibitory pathway involved in B7-H1-induced attenuation of immune responses.

Signalling influencing DC fate is mainly mediated by TLR and other pattern recognition receptors (PRR) [49]. While the molecular mechanisms acting downstream of TLR ligation have been investigated extensively, much less is known about signalling though other PRR. Teunis Geijtenbeek (Amsterdam, The Netherlands) discussed pathways induced by the C-type lectin Dectin-1 [10]. Dectin-1 is expressed on macrophages and DC and recognises β-1,3-glucans. It is crucial for the initiation of inflammatory responses against fungi. Signalling by Dectin-1 is mediated through novel pathways, involving Syk and NFκB activation leading to cytokine production. The group could show that Syk-independent Dectin-1 cytokine responses were modulated by another signalling pathway. The search for other such pathways triggered by Dectin-1 activation revealed Raf-1 as another adaptor molecule involved. Similar to what the group has previously reported for Raf-1-mediated DC-SIGN signalling [19], Dectin-1 signalling leads to phosphorylation and acetylation of p65 via Raf-1, which lead to the induction of IL-1β, IL-12p40 and other cytokines. Raf-1-mediated signalling therefore dictates cytokine expression, polarizing Dectin-1 induced Th responses towards Th1.

Although substantial progress has been made in deciphering TCR, BCR and TLR signal transduction, the proinflammatory signalling pathways that are activated following engagement of non-TLR PRRs remain poorly defined. Jürgen Ruland (Munich, Germany) presented Card9 as a vital adaptor molecule for innate immunity signalling via non-TLR PRR, complementing the more complex adaptor Card11. Card11 plays an important role in adaptive immunity signalling through ITAMs (immunoreceptor tyrosin-based activation motifs) such as those associated with, for example, the lymphocyte antigen receptors. In order to investigate the functions of Card9, they generated Card9$^{-/-}$ mice which show no obvious defects in adaptive immune responses, but are highly susceptible to fungal infections [20]. As Dectin-1 is the key non-TLR PRR for fungal β-glucan detection [10] they investigated β-glucan-induced downstream signalling of Dectin-1 and found that Card9 is required for NFκB activation, synergizing with Bcl10. They also found that Card9, like Card11, regulates NFκB function by coupling to Bcl10 and Malt1 [13], suggesting that ITAM receptors in cells of both the innate and adaptive arm of the immune response signal through evolutionary conserved pathways involving signalling to the Bcl10–Malt1 complex via distinct CARD-coiled-coil adaptor proteins.

Nikolaus Romani (Innsbruck, Austria) explored the potential of Langerhans cells (LC), skin-resident DC, for immunotherapy. Although this cell type was described already 140 years ago, there are still many open questions concerning their in vivo biology. While Allan et al. reported that antigen presentation by LC does not induce immunity [3], contact hypersensitivity in mice lacking LC yielded conflicting results, depending on the model investigated [5, 29, 30]. However, recently it was shown that these cells can clearly cross-present antigen and induce cytotoxic T cell responses in vivo and that presentation of epicutaneously applied antigen to CD4$^+$ and CD8$^+$ T cells is enhanced by inflammation [45]. In addition, LC are required for the anti-tumour effect observed in a B16.OVA tumour eradication model [44]. In addition, he reported that antigen injected into the skin and targeted to langerin is mainly taken up by LC. This was also true for antigens targeted to DEC-205, although when using such targeting strategies it is important to keep in mind that DEC-205 expression in humans is not as restricted to dendritic cell types as in mice, because B cells and monocytes also express this marker. In view of the fact that LC can be easily targeted in vivo and can efficiently induce potent anti-tumour responses, they might prove highly valuable tools for immunotherapy.

Although in many cancer immunotherapy studies, dramatic clinical improvements up to complete remission could be observed in individual patients, the benefits for the majority of patients are thus far rather limited. Typically, only those patients in which an anti-tumour immune reaction can be detected, have a reasonable chance of significant improvement, but only in a minority even of such immunological responders can disease progression be halted or even reversed. These findings, although potentially encouraging, highlight the need to further understand all the mechanisms involved to aid vaccine improvement. In this context, not only a well-designed vaccination regime but also the selection of suitable target antigens and adjuvants as well as optimal exploitation of biological mechanisms involved in tumour rejection are important. Volker Lennerz (Mainz, Germany) presented an innovative and ambitious approach for identification of new potential targets for cancer immunotherapy [33]. Patients with stage IV melanoma were immunised with melanoma-loaded DC and their tumour-reactive T cells analysed for recognition of a panel of known tumour antigens. In addition, T cells failing to recognise any of these known antigens were applied to screening of cDNA libraries derived from the respective melanoma and co-transfected with the patients HLA-allele(s) into COS-7 or 293T-cells. Using this approach, Lennerz and co-workers succeeded in identifying several new peptide epitopes derived from known tumour-associated antigens (TAA), only a few of which could have been predicted or were known previously. Furthermore, cDNA library screening resulted in the identification of new TAA, some of which were mutated antigens. The strong individuality of the observed immune responses suggested that monitoring T cell reactivity against known tumour-associated peptides, which is standard practice in many trials, might be insufficient to reliably reflect the whole range of tumour-T cell interactions.

In addition to mutated antigens or antigens overexpressed by the tumour, neo-epitopes generated by altered antigen processing, apoptotic processes or therapeutic interventions, such as radiotherapy, can potentially be exploited for cancer immunotherapy. An important question in this context is whether antigenic peptides from apoptotic and irradiated tumour tissue can be presented to CTL. This question was addressed by Jacques Neefjes (Leiden, The Netherlands) with a beautiful system established in his lab [36]. Using immunofluorescence and photobleaching, he showed that MHC I peptides can be transferred through gap junctions not only between normal, healthy cells and APC, but also between apoptotic cells and APC, so that both cells can present novel apoptosis-dependent MHC I antigens. By eluting MHC I peptides from irradiated and non-irradiated cells, he could also show that irradiation-induced mTOR activation and subsequent protein neosynthesis results in presentation of epitopes not present before irradiation. Furthermore, in a mouse colon adenocarcinoma model, irradiation or transfer of tumour-specific T cells alone had little or no effect, while a combination of the

two not only reduced tumour outgrowth but also resulted in complete tumour regression in more than 60% of treated mice, demonstrating that irradiation can be used to boost anti-tumour responses [38].

An alternative approach, presented by David Schrama (Würzburg, Germany), relies on targetting the tumour microenvironment required for tumour growth and sustenance. Among the tumour stroma-associated antigens (TSAA), three (FAPα, S100A and survivin) were selected for further investigation. FAPα is overexpressed in cancer-associated fibroblasts in more than 90% of common cancers and its overexpression is correlated with enhanced tumour growth, invasion and metastasis, probably due to reduced dependency on exogenous growth factors [27]. High levels of S100A4 in the tumour stroma are associated with poor prognosis. Using a reverse immunology approach, a number of potential HLA-A2-restricted T cell epitopes from FAPα were identified, optimised and tested for spontaneous immunoreactivity in melanoma patients. Those HLA-A2-restricted FAPα peptides that were recognised by patients were also able to stimulate CTL responses in vitro and induce immune responses in HLA-A2/K^b transgenic mice. The same approach was also used to identify HLA-A1-restricted epitopes from S100A4 to which spontaneous T cell responses could be detected in melanoma patients. Whether these epitopes could be suitable for immunotherapy remains to be investigated. T cell epitope identification of the third TSAA presented—survivin—has already led to initiation of a clinical trial (SuMo-Sec-01). One advantage of survivin as a target for immunotherapy is that it is not only expressed by the tumour but also by the microenvironment. Thus, vaccination strategies based on survivin might elicit responses against the tumour as well as the tumour stroma, thereby potentiating anti-tumour effects of the vaccine. As a further advantage, many patients already show pre-existing T cell responses against survivin. SuMo-Sec-01 is still ongoing, but first preliminary results are encouraging.

Cancer immunotherapy

Many current vaccination trials focus on late stage malignancies. Part of the reason why only few patients benefit from these treatments is the accumulation of immune defects during cancer progression. Accumulation of suppressive cell types as well as effector T cell apoptosis or deficiencies contribute to immunological ignorance with respect to the tumour [14, 39]. Earlier interventions to prevent recurrence after removal of the tumour or to slow down progression might therefore be more efficient in prolonging survival of patients. Licia Rivoltini (Milano, Italy) reported on two such current trials, one in early stage (IIb–III) melanoma patients with a high risk of relapse and the other in prostate carcinoma patients with rising PSA levels after conventional therapy. Vaccination with a modified antigenic HLA-A*0201-binding peptide mixture (MelanA/Gp100/NY ESO-1/survivin for melanoma, survivin, PMSA-1 and -2 for prostate carcinoma) emulsified in Montanide® complemented by low-dose cyclophosphamide for downmodulation of Treg elicited significant increases in the frequency of $CD8^+$ T cells specific for all the vaccine antigens, in peripheral blood and draining lymph nodes of a large proportion of patients. In the melanoma trial, in which vaccination is given in the absence of detectable disease, more patients will need to be recruited to evaluate whether a significant clinical benefit can be achieved. Nevertheless, what has become apparent so far from these studies is that already at early stages Treg are expanded and that low-dose cyclophosphamide may not be the most effective way of counteracting suppression, despite some reports in the literature [17]. In addition, T cells primed with modified peptides often do not recognise the tumour in a reproducible fashion. However, although memory formation for vaccine antigens seemed to be relatively inefficient, the prostate cancer vaccination appeared to be able to control PSA levels in those patients developing an immune response. It might therefore be an alternative to conventional hormone therapy. The use of adjuvants other than Montanide® could possibly enhance memory formation and further improve the vaccine.

Two phase I/II clinical trials for the treatment of renal cell carcinoma (RCC) which had been performed in Tübingen were presented by Peter Brossart (Bonn, Germany). In the first, 20 patients were treated with DC pulsed with MUC1 peptide and the pan-DR-binding peptide PADRE to stimulate additional $CD4^+$ T cell responses [54]. In six patients, vaccination induced regression of the metastatic sites, and in four additional patients, disease stabilisation could be achieved. Similarly positive results were observed in the second trial, where DC were loaded with RNA for the RCC antigens CEA, MUC1, survivin, MAGE-1 and the influenza matrix protein IMP as control antigen. Very recently two novel treatments for RCC became available. Sorafenib and sunitinib are receptor tyrosine kinase inhibitors which function by interfering with angiogenesis and tumour growth and survival [37]. While sunitinib does not alter any of the cellular characteristics investigated in vitro, sorafenib interferes with DC maturation, migration, stimulatory capacity and TLR-mediated signalling in vitro and CTL induction in vivo in mice. Sunitinib, but not sorafenib, on the other hand, reduces the percentage of Treg in treated mice [23]. Sorafenib therefore seems unsuitable for combination with immunotherapy.

Two unconventional approaches, which are still at a very early stage of development, were presented by Aladar

Szalay and Gunther Hartmann. With the use of recombinant bacteria carrying fusion gene cassettes comprising GFP and luciferase, Aladar Szalay (Würzburg, Germany) provided an interesting approach to monitor the fate of intravenously injected bacteria. As they showed in previous studies, systemically administered, light-emitting bacteria selectively gain entry to and replicate in solid tumours [55]. This represents a new tool for non-invasive monitoring of tumour locations or metastases in living animals. It could be shown that the majority of bacteria are found in the central necrotic regions of tumours. An alternative approach could be the use of a new recombinant vaccinia virus, GLV-1h68, which was constructed by inserting three expression cassettes, [*Renilla* luciferase–green fluorescent protein (RUC–GFP) fusion, β-galactosidase, and β-glucuronidase] into the viral genome [56]. This recombinant virus not only possesses enhanced tumour-targeting specificity, allowing detection of tumours as small as 1/50 mm^3 in live animals in real time but also caused pronounced tumour regression. This system therefore combines the concepts of oncolytic virus-mediated tumour diagnosis and therapy. In xenograft models of human breast or pancreatic tumour, intravenously injected GLV-1h68 virus led to complete tumour eradication in all mice tested. In an ovarian tumour xenograft model, the combination of GLV-1h68 and chemotherapy also resulted in complete tumour regression within 60 days. Transcriptional profiling of regressing tumours revealed gene expression signatures consistent with immune defence activation, including IFN-stimulated genes (*STAT-1* and *IRF-7*), cytokines, chemokines, and innate immune effector function. These findings suggest that viral oncolysis may synergise with immune mechanisms to induce tumour eradication, providing a novel perspective for the design of future cancer immunotherapies.

The design of combinatorial RNA oligonucleotides against melanoma was presented by Gunther Hartmann (Bonn, Germany). The rationale of this approach is to combine both gene-silencing and immunostimulatory function in one RNA molecule in order to develop new tools for effective treatment of cancer. Previously, the group had demonstrated that the 5′-triphosphate end of RNA generated by viral polymerases is responsible for RIG-I-mediated detection of viral RNA molecules resulting in interferon-α production [28]. This detection could be abrogated by capping of the 5′-triphosphate end or by nucleoside modification of RNA. These processes occur physiologically during posttranscriptional RNA processing in eukaryotes, thus providing a means to discriminate between viral and self RNA and initiate antiviral immune responses, for example, through TLR signalling or RIG-I. A synthetic combinatorial RNA molecule (3p-2.2) was designed, comprising free 5′-triphosphates as RIG-I ligands, and a TLR7 ligand motif in the sense strand, while the antisense strand functions as siRNA against bcl-2. The therapeutic activity of this combinatorial RNA molecule was assessed in a B16 melanoma model, where the TLR7-plus RIG-I-dependent immunological activity could be detected in vivo, along with gene silencing of bcl-2 and induction of apoptosis in melanoma cells. C57/BL6 mice engrafted with B16 melanoma cells and treated with three intravenous injections of 3p-2.2 show a significant reduction in tumour size. These results demonstrate that combinatorial RNA oligonucleotides could be an interesting option for combining immunostimulatory and gene-silencing properties in one molecule suited for, but not limited to, treatment of cancer.

Overall, results from the clinical trials and pre-clinical studies presented in this meeting were encouraging. The major problem, however, is clearly the low percentage of patients in which significant improvements can currently be reliably achieved [34]. Optimisation both of vaccines and vaccination protocols should hopefully lead to the highly desired breakthrough in the future, allowing more patients to benefit from immunotherapy. Carl Figdor (Nijmegen, The Netherlands) pointed out the need for more proof-of-principle studies and improved monitoring schemes to achieve this goal. Such a new monitoring parameter could be the presence of vaccine-related TAA-specific T cells in delayed-type-hypersensitivity skin biopsies, because this correlates well with favourable clinical outcome [11]. One important issue addressed by his group is the question of the best administration route for DC-based vaccines. While intranodal injection of the vaccine might be expected to yield the best results, they found that DC loaded with MHC I peptides [12] with or without MHC II peptides or, alternatively, mRNA from defined melanoma antigens (tyrosinase and gp100) migrated to adjacent lymph nodes to present the antigen there only in some patients. Moreover, intranodal injection is technically difficult, presenting a further obstacle. Interestingly, it turned out that survival of patients injected intradermally is actually increased compared to the intranodally injected group. This might be due to some sort of selection process imposed upon DC as they have to actively migrate, possibly resulting in a better quality of DC reaching the lymph node. However, as for the intranodally injected DC, little migration to adjacent lymph nodes was observed. Stimulation of the injection site can improve the migration rate, but in the ongoing study the effects observed so far are not significant. Targeting the model antigen keyhole limpet haemocyanin to APC using a humanised antibody against DC-SIGN, on the other hand, clearly improves immune responses [46, 47]. A new trial using plasmacytoid DC as vaccine should provide further insights into the potential of these cells for immunotherapy.

In summary, immunotherapy, especially cancer immunotherapy, nowadays already offers benefits for at least

some patients and at the same time holds a huge promise for the future. While there is still much room for development, especially with regard to the route of antigen delivery and targeting, choice of adjuvants and antigens, and controlling immune dysfunction and counter-regulatory factors in patients, there are many reasons to be optimistic about current and future successes.

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