



Review

Restoring immunosurveillance by dendritic cell vaccines and manipulation of the tumor microenvironment

Angela Vasaturo, Martijn Verdoes, Jolanda de Vries, Ruurd Torensma, Carl G. Figgdr *

Radboud Institute for Molecular Life Sciences, Radboudumc, Department of Tumorimmunology, Geert Grooteplein 26, 6525GA Nijmegen, The Netherlands

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ABSTRACT

Cancer cells evolve from normal cells throughout life and are usually recognized by our immune system and destroyed, a process called immunosurveillance. Unfortunately, in some instances cancer cells paralyze our immune system, resulting in outgrowth and spreading of the tumor. Understanding the complexity of immunomodulation by tumors is important for the development of therapeutical strategies. Nowadays, various approaches have been developed to enhance anti-tumor immune responses and abrogate the immune dampening effect of the tumor and its surrounding environment, including dendritic cell-based vaccines, therapies to counteract myeloid derived suppressor cell function within the tumor and antagonists of inhibitory signaling pathways to overcome 'immune checkpoints'.

The challenge is now to find the right combination of immune based therapies to fully restore immune function and provide a more efficacious and enduring anti-tumor response.

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The role of the immune system in cancer

Cancer immunotherapy received a lot of skepticism due to the fact that tumors grow notwithstanding the presence of an intact immune system. This fact challenged oncologists and immunologists during the past two decades to develop therapies based on the very same immune system to eradicate cancer. Our immune system seems well equipped to recognize and destroy the numerous derailed cells that arise every day, a process called immunosurveillance (Swann and Smyth, 2007). To escape from the control

by the immune system, a developing tumor effectively adopts existing mechanisms that suppress the immune system (Ilkovich and Lopez, 2008). Although immune cells can still be found in expanding tumor tissue, most of them appear to be paralyzed by the tumor and become tolerant or, even worse, participate in dampening of the immune system. Counteracting the dampening effect that the tumor exerts on the immune system is one of the main goals of emerging therapeutical strategies, since it has become clear that restoring an effective immune response aids in recovering from cancer.

Currently, two main strategies are exploited:

- (1) Passive immunotherapy, where antibodies or immune cells (T cells, natural killer (NK) cells) directed against the tumor are infused into the cancer patient (Waldmann, 2003; Schuster et al., 2006). Recently, injection of antibodies that block cell surface receptors sending inhibitory signals to immune cells

Abbreviations: DC, dendritic cell; DTH, delayed type hypersensitivity; MDSC, myeloid-derived suppressor cell; NK, natural killer; TLR, toll-like receptor; Tregs, regulatory T cells; HPV, human papillomavirus.

* Corresponding author.

E-mail address: Carl.Figgdr@radboudumc.nl (C.G. Figgdr).

(so-called ‘checkpoint’ antibodies) have been used to ‘release the (tolerizing) brakes’ of the immune system in the tumor microenvironment (Vasaturo et al., 2013). While this latter strategy effectively prolongs the activated stage of immune cells, it is frequently associated with adverse autoimmune reactions and related toxicity, because it lacks antigen specificity (Pardoll, 2012).

- (2) Active immunization, by administration of anti-cancer vaccines, aimed at boosting the immune system in an antigen specific manner. Besides vaccines composed of tumor fragments or synthetic peptides in combination with adjuvants to stimulate the immune system (Melief and van der Burg, 2008), also cellular vaccines have been exploited during the past decade. These are based on antigen presenting cells, such as dendritic cells (DCs) generated from peripheral blood of cancer patients, which after loading with tumor-associated antigen and activation by adjuvant are reinjected into the same patient to boost T cell reactivity against the tumor. DCs are considered the master regulators in tuning the immune system toward either tolerance or immune activation. They are characterized as the professional antigen presenting cells and as such they are thought to play an important role in reactivating the immune system toward cancer by inducing B and T cells to produce tumor specific antibodies and cytotoxic activity, respectively (Tuettenberg et al., 2007; Lesterhuis et al., 2008; Frankenberger and Schendel, 2012).

Tumor versus immune system: a Yin Yang relationship

It is well known that malfunctioning cells including emerging tumor cells attract immune cells via cytokines (Ilkovitch and Lopez, 2008; Yigit et al., 2010), chemokines (Jordan et al., 2008; Maru et al., 2008; Qin et al., 2009) and alarmins (Chan et al., 2012). The composition and nature of these proteins determine the type of cells that influx into the developing tumor. Besides an influx of effector cytotoxic T cells or NK cells that have a positive influence on the disease (Gooden et al., 2011; Galon et al., 2012), often other less beneficial immune cells end up in the tumor or its environment (Fig. 1). During tumor development, cancer cells clearly steer the immune response to escape from elimination by recruiting the suppressive arm of the immune system, including myeloid-derived suppressor cells, regulatory T cells and macrophages.

Myeloid-derived suppressor cells (MDSCs) are recruited by multiple proinflammatory molecules secreted by cancer or stroma cells to the tumor sites, where they support tumor growth and spreading and hamper immune responses (Sawanobori et al., 2008; Greten et al., 2011; Schlecker et al., 2012). These suppressive cells can efficiently inhibit antitumor T cell responses through a variety of mechanisms. For instance, activated MDSCs produce enzymes that deplete nutrients in the tumor microenvironment required for lymphocyte growth and differentiation. Clinical trials using either inhibitors of such enzymes, such as the indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor INC024360 (NCT01961115) or active immunization against IDO (NCT02077114) are in progress for several tumors (more examples of such clinical trials are reviewed in Wesolowsky et al., 2013). Furthermore, immunosuppressive cytokines that have the potential to inhibit cytotoxic T lymphocytes, as well as activate and expand T regulatory cells (Tregs) are secreted by MDSCs (Kusmartsev et al., 2005; Gabrilovich and Nagaraj, 2009; Nagaraj and Gabrilovich, 2010; Fallarino and Grohmann, 2011; Obermajer et al., 2011; Becker et al., 2013; Steppan et al., 2013). Recent studies have shown that targeting MDSCs and their immunosuppressive function leads to recovery of CD8+ T cell anti-tumor activity, which results in tumor suppression

(Najjar and Finke, 2013; Steppan et al., 2013; Wesolowsky et al., 2013; Yu et al., 2013).

The tumor also exploits other regulatory networks. Several tumors are highly infiltrated by Tregs, which have been the target of several studies (Dannull et al., 2005; Jordan et al., 2008; Golovina and Vonderheide, 2010). However, the presence of Tregs in the tumor is by itself not a sign of active immunosuppression as Tregs must become activated to exert their effect (Lin et al., 2013; Liston and Gray, 2014). While classical Tregs are characterized by expression of FoxP3 (Roncador et al., 2005), CD4, high expression of CD25 and low expression of CD127, the versatility of Tregs is exemplified by a recently discovered FoxP3 negative Treg induced by Interferon β (Liu et al., 2014).

Macrophages also invade the tumor bearing tissue alarmed by signals from the aberrant cells. They are remarkably plastic and, depending on the environmental cues they receive, they can change their phenotype and function from powerful stimulation of inflammatory responses (M1 macrophages) to immunosuppression (M2 macrophages) (Biswas and Mantovani, 2010; Edin et al., 2013).

In most tumors, infiltrated macrophages are considered of the M2 phenotype and their immunosuppressive function is mediated by the secretion of cytokines, chemokines, and proteases, which promote tumor angiogenesis, growth and metastasis. Recently, it was also found that tumor-associated macrophages can interact with cancer stem cells and this interaction leads to tumorigenesis, metastasis and even drug resistance (Hao et al., 2012). Increasing evidence indicates that human tumors may comprise of subpopulations of cells with the ability of self-renewal to drive tumorigenesis, referred to as cancer stem cells. Although initial studies showed increased survival times when cancer stem cells are targeted by immunotherapy (Sun et al., 2010; Hirohashi et al., 2012; Teitz-Tennenbaum et al., 2012; Vik-Mo et al., 2013; Xu et al., 2014), one has to keep in mind that differentiated cancer cells can re-acquire stem cell-like properties (Di et al., 2011) and thus may form a reservoir of new stem cells to replace the ones in the eradicated niche. For example, adoptive transfer of cytotoxic T cells that target specific melanocytic antigens revealed that melanomas can escape from eradication by dedifferentiation (Landsberg et al., 2012).

Dendritic cell vaccines to reinforce the immune response against cancer

After the discovery of the DC and its unique capacity to activate T cells, dendritic cell vaccines were soon predicted as a new weapon to combat cancer (Inaba et al., 1990; Banchereau and Steinman 1998; Dhodapkar et al., 1999; Thurner et al., 1999; Timmerman and Levy, 1999; Steinman and Banchereau, 2007). Also, early studies showed that the unresponsiveness of T cells induced by interleukin 10 could be reversed by dendritic cell vaccination (Chen et al., 2001). Now, 15 years later, it has become apparent that obtaining clear clinical results with dendritic cell vaccines is more difficult than initially foreseen. Initial clinical trials using immature DCs appeared not to result in the optimal vaccine. Maturing DCs with Toll-like receptor (TLR) ligands or cytokine cocktails certainly improved the response in cancer patients (Inaba et al., 2001; de Vries et al., 2003; Tuettenberg et al., 2007; Nakai et al., 2010; Ridolfi et al., 2011). Irrespective of the so far limited clinical responses throughout the years, much was learned about the DCs in order to improve the clinical outcome. In particular, novel immunomonitoring assays were developed to closely follow the immune status in treated patients. Biopsies of delayed type hypersensitivity (DTH) reactions after vaccination appeared to be a prime predictor of an immunological response (de Vries et al., 2005; Aarntzen et al., 2008). More important, such a positive DTH correlated with longer overall survival. Furthermore, the route of administration of the

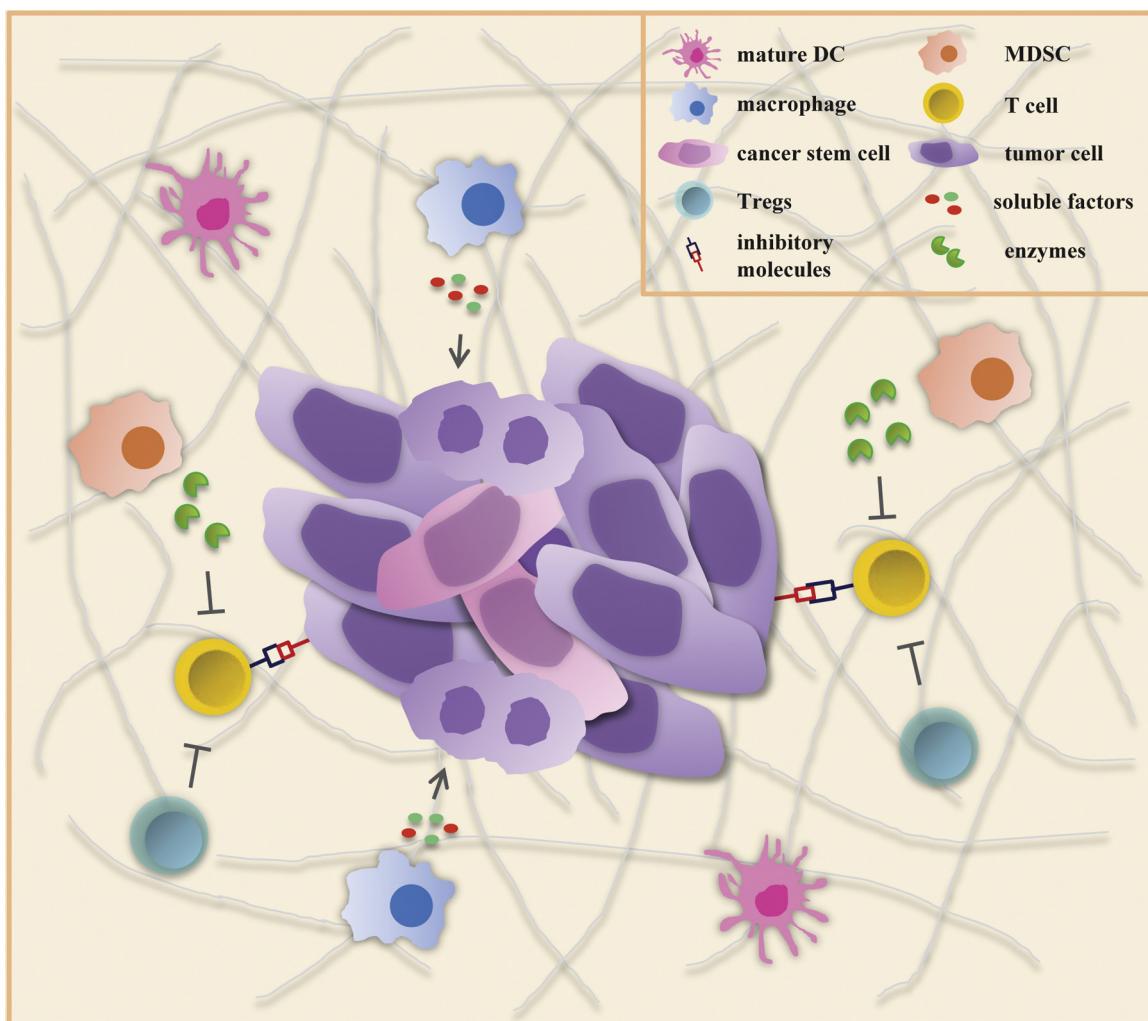


Fig. 1. The immunosuppressive tumor microenvironment. During tumor development, cancer cells attract suppressive immune cells: MDSCs and Tregs inhibit antitumor T cells responses and macrophages induce tumor cell proliferation and spreading establishing an immunosuppressive microenvironment.

dendritic cell vaccine appeared to modulate the immune response (Lesterhuis et al., 2011). In addition, several other parameters, such as lymph node homing, showed to be critical for a clinical response (Figdor et al., 2004; Aarntzen et al., 2013). One of the reasons for the so far limited clinical effects may be that the *in vitro* differentiated and matured monocyte derived DCs become exhausted because of the long culture time (7–9 days). Therefore, as an alternative approach, the use of natural occurring DC subsets is currently explored instead of monocyte derived DCs. Natural occurring DCs circulating in the peripheral blood can be divided into plasmacytoid DCs and myeloid DCs. The latter are typically subdivided into CD1c+, CD141+ and CD16+ DCs (it should be noted that the latter cell type is by some considered a subset of monocytes (MacDonald et al., 2002; Ziegler-Heitbrock et al., 2010) each with their own characteristics. The first clinical data obtained with plasmacytoid DCs indeed show a better outcome compared to standard therapies (Tel et al., 2013) affirming the use of natural occurring DCs as vaccine. Although all of these improvements of the dendritic cell vaccine were helpful, still too few patients benefitted from the vaccines. There are several reasons that can explain the limited clinical outcome yet. Since dendritic cell vaccination is an experimental approach, only those patients that have no other options left enter the clinical trials. It is to be expected that patients in an earlier stage of their disease and thus a better functioning immune system will benefit more from the dendritic cell vaccine. For the fitter patient

the better outcome is to be expected because of the higher number of circulating DCs found in those people (Suchanek et al., 2010). Notwithstanding their poor condition, substantial clinical benefit with longer survival has been shown for individual patients with no other treatment options. Whatever origin or subset of DCs used, the success of anti-cancer dendritic cell vaccination is highly dependent on the availability of an effective tumor-associated antigen. Most dendritic cell vaccines thus far are targeted against self-antigens, which are overexpressed by the tumor. These are not ideal, since the tumor cells will have to express and present these antigens to the immune system above a certain threshold to overcome tolerance, which is typically installed for self-antigens. By contrast, virally induced cancers, such as human papillomavirus (HPV) induced cervical cancer can be treated by vaccines against viral, non-self neoantigens, such as the protein E7 (Kenter et al., 2009). Other examples of neoantigens arise from mutations in self-proteins. Lynch syndrome is a genetic condition in which patients have a high risk to develop colon cancer. A clinical trial is currently ongoing, in attempt to prophylactically vaccinate such patients against frameshift-derived neoantigens which are expressed during the development of colon cancer (NCT01885702). Neoantigens can also arise from somatic mutations within the tumor. These can be identified by exome sequencing of the genes expressed by the tumor and could be at the basis of truly personalized anti-cancer vaccines (Robbins et al., 2013; van Rooij et al., 2013).

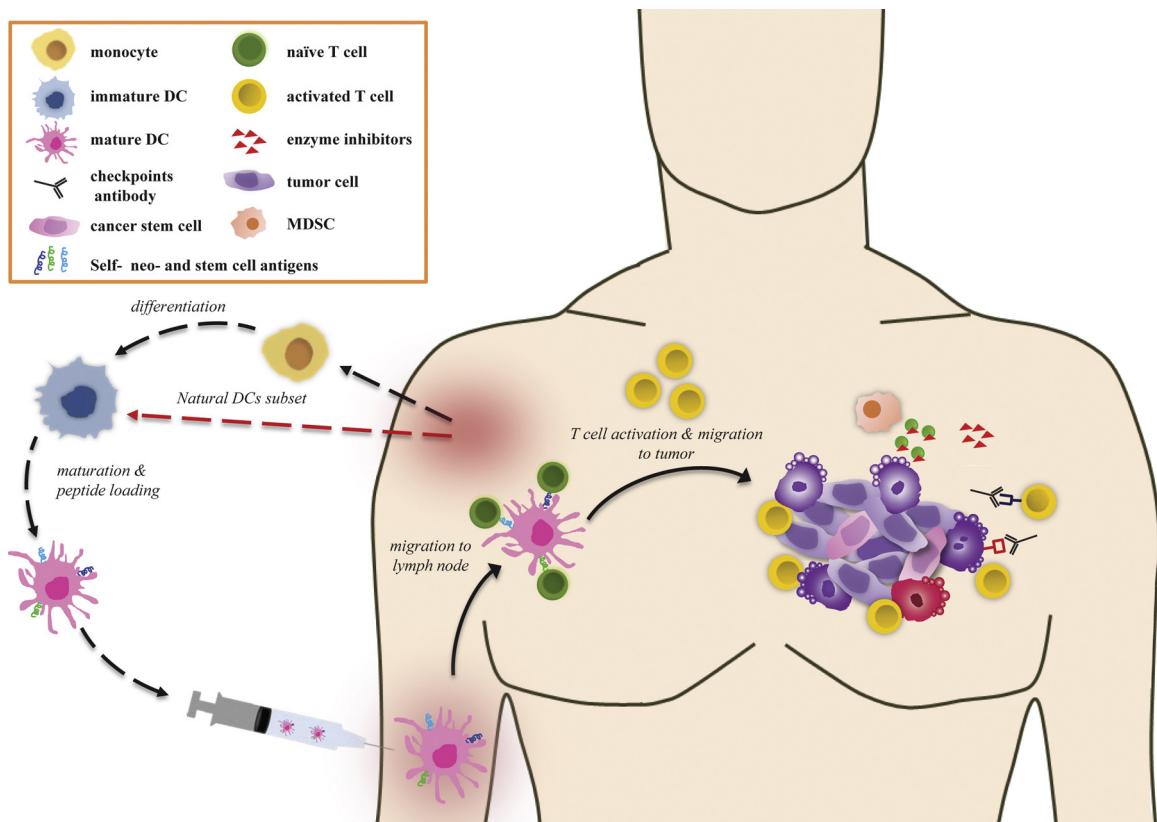


Fig. 2. Combining DC vaccination with immune checkpoint antibodies or enzyme inhibitors. DC vaccination of cancer patients leads to the induction of tumor specific T cells that migrate to the tumor microenvironment. A better anti-tumor response is to be expected when DC vaccination is combined with drugs that take away the immunosuppressive brakes that prevent tumor cell eradication.

Dendritic cell vaccines based on antigens specifically expressed by cancer stem cells, will wipe out this rare self-renewing cancer cell population, which is thought to be at the basis of disease recurrence. In this regard the embryonic stem cell antigen Oct4A might be an attractive target, since it was shown to be expressed by for example ovarian cancer stem cells. It has been demonstrated that healthy people possess immunological memory against this antigen (Dhodapkar et al., 2010; Dhodapkar 2010; Di et al., 2013), which, in ovarian carcinoma patients, is kept at bay by the immunosuppressive mechanisms exerted by the tumor.

In a heterogeneous tumor cell population not every tumor cell expresses the same tumor antigens (Boiko et al., 2010). This heterogeneity has profound implications on therapeutical responses, irrespective of the type of vaccine used. Thus, DC vaccines should ideally contain a mix of tumor-associated (neo)antigens to optimize the eradication of an heterogeneous tumor population (Phuphanich et al., 2013). Support for this approach comes from the success of dendritic cells loaded with total tumor cell lysates (Burgdorf et al., 2006; Pellegatta et al., 2006; Ali et al., 2009; Trepakas et al., 2010; Chiang et al., 2011; Kandalaft et al., 2013). Multiple clinical trials with DC vaccines targeting different cancers have been carried out with the goal of strengthening T-cell activation (Cranmer et al., 2004; Lesterhuis et al., 2004). The different strategies used to improve the T cell-stimulatory capacity of DCs, including different methods of generating DCs, different antigen-loading techniques, maturation status of the cells and route of administration are summarized in Table 1.

Future perspective

Last year the highly ranked journal *Science* selected Immunotherapy as the breakthrough of the year 2013. This is

the result of hard work of the immunological community worldwide. After many years of research, immunologists can now offer various approaches to combat life threatening diseases like cancer. Not only different types of immunological approaches have been developed, but we also have obtained a much more detailed insight of the tumor microenvironment and how a tumor interacts with the immune system throughout the various stages of development. It has become clear that if we want to exploit the immune system to cure cancer, we have to act at multiple levels. In the first place, boosting the immune system by adoptive transfer of antigen specific T cells (Darcy et al., 2014; Hinrichs and Rosenberg, 2014), by vaccines including DC vaccines, or by antibodies targeting immune checkpoints (Weber, 2010; Vasaturo et al., 2013) is extremely important. Although the latter lack antigen specificity and are thereby associated with considerable autoimmune side effects, therapeutic strategies have been optimized to deal with some of those to keep toxicity within limits. Of course the more specific the immune intervention the better. This means that rather than differentiation antigens, which are so far frequently used for vaccination and T cell therapy, real (patient specific) tumor antigens are preferable. This would lead to a much more focused and patient personalized approach (Hacohen et al., 2013).

Secondly, aside from boosting the effector arm of the immune system, one would like to combat the immunosuppressive microenvironment. One way to achieve this is by the use of inhibitors of enzymes that degrade tryptophan and arginine to stimulate T cell infiltration. Direct targeting of surface receptors of immune inhibitory Tregs or MDSC remains difficult because no specific surface markers are available. Of course checkpoint antibodies can be applied to also block co-inhibitory molecules and their ligands on these cells and on tumor cells themselves.

Table 1

Strategies to improve T cell-stimulatory capacity of dendritic cells for cancer vaccine.

Dc maturation	Mature DCs, immature DCs	McIlroy and Gregoire (2003) and Schaft et al. (2005)
Dc subsets	Monocyte-derived DCs, plasmacytoid DCs, myeloid DCs	Romani et al. (1996) and Tel et al. (2013)
Dc antigen loading	DCs pulsed with peptides, whole-tumor lysates, cDNA or mRNA encoding tumor antigens, or fused with tumor cells	Van Tendeloo et al. (2001), Wierecky et al. (2006), Ovali et al. (2007), and Rosenblatt et al. (2011)
Route of administration	In vivo delivery of antigen Intra-dermal or intra-nodal DCs administration	Tacken et al. (2007) Bedrosian et al. (2003), Figdor et al. (2004), and Lesterhuis et al. (2011)

Thirdly, we believe that much more effort must be spent on identifying patients that will be susceptible to immunotherapy. Initial studies show that in depth assessment of how immune cells infiltrate and are distributed in the tumor microenvironment (Fridman et al., 2012; Bindea et al., 2014), expression of transcriptome derived biomarkers (Ulloa-Montoya et al., 2013) but also DTH monitoring, all relate with the outcome of immunotherapy. With all new immunotherapeutic trials currently underway, we expect a wealth of information on new potential biomarkers expressed by the tumor or in the blood of a patient that have prognostic or even predictive value.

Finally, with the various immunological approaches available to date and still in the pipeline, we need to think thoroughly on combining different forms of immunotherapy and other cancer treatments (Fig. 2). One interesting approach would be combining anti-checkpoint antibody therapy with DC based vaccination or adoptive T cell transfer. Crucial and challenging is the timing of the different treatment schedules. First vaccination to boost antigen specific and subsequent checkpoint antibody treatment? Or both at the same time? These and related questions will dominate the field for the upcoming years. Another potential caveat is to discriminate which of the therapies given to a patient is effective and which not. There is a clear tendency that nowadays patients are well informed about potentially effective treatments and 'shop around' from different treatment options. Given the fact that immune related effects sometimes only appear several months after treatment the chance is very likely that such a patient is already on another study, which certainly complicates interpretation of the clinical results.

In summary, major efforts by numerous immunologists have now set the stage for immunotherapy. The future is bright but at the same time complicated: we still have a long road to go.

Conflict of interest

The authors state that there is no conflict of interest.

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