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The good, the (not so) bad and the ugly of immune homeostasis in melanoma

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Abstract

Within the immune system multiple mechanisms balance the need for efficient pathogen recognition and destruction with the prevention of tissue damage by excessive, inappropriate or even self-targeting (auto)immune reactions. This immune homeostasis is a tightly regulated system which fails during tumour development, often due to the hijacking of its essential self-regulatory mechanisms by cancer cells and facilitated not only by tumour intrinsic properties, but also by the microbiome, host genetics, and other factors. In certain ways many cancers can therefore be considered a failure of immune control rather than a disease of the tissue of origin, as the acquisition of potentially oncogenic traits through mutation occurs constantly in most tissues during proliferation. Normally, aberrant cells are well-controlled by cell intrinsic (repair or apoptosis) and extrinsic (immune) mechanisms. However, occasionally oncogenic cells survive and escape control. Melanoma is one of the first cancer types where treatments aimed at restoring and enhancing an immune response to regain control over the tumour have been used with various success rates. With the advent of “modern” immunotherapeutics like anti-CTLA-4 or anti-PD-1 antibodies that both target negative immune-regulatory pathways on immune cells resulting in durable responses in a proportion of patients, the importance of the interplay between the immune system and cancer has been established beyond doubt.

INTRODUCTION

Immune homeostasis refers to the delicate and finely regulated balance of appropriate immune activation and suppression in tissues and organs, driven by a myriad of cellular players and chemical factors. Each of these factors contributes to an immune system that can successfully recognize and fight infections and prevent cells from escaping their proliferative controls, whilst retaining appropriate and timely down-regulation of immune reactions, and providing tolerance against self-antigens to prevent autoimmunity. In health, the immune system is constantly stimulated and dampened but overall maintains a relative steady-state, acting similar to buffer systems which can balance and equal out opposing inputs over a wide dynamic range. Failure of this mechanism can result in the clinical appearance of cancer,

where either the detection of aberrant cells or the required clearance of such cells is disturbed. Intriguingly, both an immune system that is insufficiently dampened leading to chronic inflammation, and one that cannot recognize or destroy cells with acquired oncogenic properties, can lead to the development of cancer. Moreover, as cancer and the immune system constantly shape each other, co-developing and evolving together, the selective pressure imposed by the immune system can paradoxically counteract its own success. This may occur by (i) selecting for tumour variants that are less immunogenic and (ii) creating and inducing feedback loops that lead to the down-regulation or blockade of the immune response. With recent advances in anti-cancer treatments based on enhancing or restoring the ability of the immune system to destroy cancer cells, more and more of the key players that determine the nature of the cancer-immune interaction and the net outcome (tumour control or escape) are being discovered. In this review we will summarize different factors and mechanisms that play a role in regulating immune homeostasis in the context of the prototypical tumour for cancer immunotherapy, melanoma. We attempt to convey the concept that immune homeostasis is not a specific and clearly definable state, but rather a multilevel system that can be heavily influenced by immunotherapy.

THE THREE “Es” OF CANCER IMMUNOEDITING

Cancer development in the context of an immune system was first described in terms of the three “Es”: Elimination (the good), Equilibrium (the not so bad), and Escape (the ugly) in 2004 by Dunn et al.¹, and has since become dogma. This description of immune interaction shaping tumour progression continues to be refined and developed. Elimination describes immune eradication of cells with oncogenic potential prior to their establishment and further growth, a process at which the immune system is extremely efficient but that is poorly defined and inherently challenging to study. However, it seems to be clear that antigen recognition on tumour cells by effector CD8⁺ T cells (Teffs) plays a major role in tumour cell clearance², assisted and complemented by various other cell types of the innate and adaptive immune system that all contribute to immune homeostasis in the relevant tissue³.

Occasionally, initial clearance of an oncogenic cell fails, and a tumour becomes established. These early and locally restricted cancers are frequently slow growing and may be partially controlled by the immune system. During this so-called equilibrium phase, when cancer cells that escaped elimination are kept under control by the immune system, a new form of immune homeostasis (equilibrium or potentially senescence) manifests (Figure 1), with successful destruction of tumour cells on one side and tumour escape from immune attacks

on the other, resulting in a slow growth of tumour mass that may go undetected over decades⁴. This imperfect balance is maintained within the tumour microenvironment (TME), representing a complex interplay of direct and indirect interactions between tumour and immune cells, and between immune cell subsets (Figure 2). Clinical examples for this immune-cancer equilibrium can be gained from reports in the literature describing transplantation of melanoma to immunosuppressed solid organ transplant recipients, with cancer emerging in the recipient several decades after the organ donor was declared cancer-free⁵.

What triggers the escape of a tumour from equilibrium is not well defined, and may be simply a result of the constant evolution of the tumour in the presence of immunological pressure. Here, the immune system can be excluded from the tumour⁶, unable to recognize the tumour⁷, or controlled by immunosuppressive mechanisms⁸. This can lead to the establishment of metastatic tumours and clinically evident late-stage disease. Immune escape can be disrupted or reverted using treatments aimed at either bringing cancer immune homeostasis back into balance (stable disease), or at skewing it in favour of the immune system (objective/complete responses). The major factors contributing to tumour escape and resulting tumour progression range from complex immunological and tumour-intrinsic to diverse host factors, many of which are amenable to treatment⁹. Many excellent reviews have highlighted and explained these factors in detail, and we will only briefly mention some of the major players here¹⁰⁻¹⁴.

FACTORS CONTRIBUTING TO CANCER IMMUNE ESCAPE

Immune factors

The crucial anti-tumour activity of Teffs can be efficiently controlled by regulatory CD4⁺ T cells (Tregs), a T cell subset with a wide range of immunosuppressive properties. Tregs can frequently be found within tumours, often following the influx of specific Teffs to dampen their activity and to establish a localized immune homeostasis. Their numerous presence in a TME is often indicative of immune escape and poor prognosis: specifically, these cells are able to induce a pro-tumoural microenvironment by secreting suppressive cytokines, and by inducing lysis of Teffs and dendritic cells (DCs), pro-tumoural metabolic effects, and tumour angiogenesis¹⁵. As such, the Teff (beneficial):Treg (adverse) ratio can be used to measure whether immune homeostasis is being tipped in a pro-tumoural or anti-tumoural manner¹⁶. Recently, regulatory B cells (Bregs) - a relatively new and understudied B cell subtype - have also been reported to infiltrate tumours, and can similarly promote immune escape by

producing suppressive cytokines and may even facilitate the recruitment of Tregs¹⁷. Other tumour-promoting and immunosuppressive cells include tumour-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs). TAMs are known to promote tumour proliferation, metastasis and angiogenesis, as well as to inhibit anti-tumour T cell responses¹⁸. MDSCs can equally suppress anti-tumour T cell responses, and are often elevated and indicative of a poor prognosis in melanoma¹⁹.

Helper CD4⁺ T cell (Th) subtypes including type 17 Th (Th17) and follicular Th (Tfh) often provide specific stimuli to other immune cell types in a context-specific manner and can have both pro- and anti-tumoural activity, depending on developmental and environmental cues²⁰. Most immune cells have positive and negative regulatory pathways that can be engaged to control their activity. The most well-known negative checkpoints include cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1). While CTLA-4 is an inhibitor of early T-cell activation, PD-1 contributes to T-cell exhaustion in peripheral tissues; both are commonly adaptively upregulated as a consequence of IFN signalling within the inflamed TME²¹. Upregulation of these molecules can result in immune inactivation, once engaged by their respective binding partners that are often expressed on tumour cells themselves²².

Which of the numerous immune cell subpopulations matter the most to an individual patient's clinical course is the subject of intense ongoing study. Currently, the greatest emphasis is placed on the Teff:Treg ratio and the relative balance of key checkpoint molecules such as CTLA-4 and PD-1. However, in any complex system such as the tumour-immune microenvironment, it is possible that our understanding of critical flow control points is biased towards molecular and cellular targets for which effective clinical- or research-grade therapies are available at any given time. Furthermore, immune adaptations are dynamic and occur in response to parallel changes within target cancer cells, thus both must be considered simultaneously to contextualise the relevant immunomodulatory factors.

Tumour factors

During tumour development genomic aberrations can accumulate within tumour cells and be particularly responsible for immune evasion or treatment resistance. The WNT/ β -catenin pathway is a melanoma cell intrinsic pathway which, when activated, results in the exclusion of T cells from the tumour, and thus cancer immune evasion: this pathway drives the exclusion of CD103⁺ DCs from the TME, which are critical mediators for antigen presentation and lead to the attraction of T cells⁶. Inactivating mutations in IFN γ pathway

genes can lead to an inability to induce HLA on tumour cells, a mechanism that typically compromises immune recognition by interfering with the normal presentation of immunogenic antigens, rendering tumour cells less visible to the immune system²³. Inactivation of the p53 pathway causes skewing of the TME leading to immune suppression and evasion, largely via hyperactivation of NF- κ B (tumourigenesis) and STAT3 (development of MDSC, tumourigenesis)²⁴. Frequently in melanoma, PTEN loss results in PI3K-dependent activation of immunosuppressive cytokines and decreased tumour-infiltrating lymphocytes (TILs)²⁵.

Inter- and intra-tumour heterogeneity is now well-described in melanoma, and confers a challenge to immune cells in several ways. Most melanomas express tumour-specific immunogenic antigens, including cancer-testis antigens and melanoma differentiation antigens, but the pattern and frequency of expression often changes during disease progression²⁶, epithelial-mesenchymal-like transition and BRAF activation²⁷, requiring constant re-adjustment of the immune response. While HLA Class I can be selectively down-regulated or entirely lost either regionally or temporally by tumour cells²⁸, even immune-induced inflammation can result in proteasome-dependent changes to antigen processing and HLA-presented epitopes²⁹ that lead to a mismatch between T cell repertoire and tumour-presented antigens. Additionally, melanomas can secrete immunosuppressive cytokines and chemokines, including TGF- β and IL-6³⁰, while simultaneously failing to produce immune-attracting factors like CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10, all of which have been shown to be responsible for the recruitment of Tregs and associated effective anti-tumour immunity³¹. All cells, including tumours, also secrete exosomes containing DNA, RNA, and proteins. These have been implicated in modulating both pro- and anti-tumour effects, including tumour antigen presentation, impairment of DC function, establishment of a pre-metastatic niche and induction of Tregs³².

Changes in tumour metabolism can also result in unfavourable conditions that exclude immune cells from the TME or impair their activity. Tumour hypoxia directly hinders immune cell infiltration, and results in adenosine accumulation, which in turn binds immune cell receptors to inhibit pro-inflammatory activity, and enriches for immune-regulatory cells³³. The hypoxic TME may also drive tumours to generate ATP from glucose via the Warburg effect, which although an inefficient process compared with oxidative phosphorylation, both facilitates tumour cell survival and completely depletes the TME of glucose, thus depriving activated T cells of an essential nutrient to proliferate³⁴. Up-

regulation of indoleamine 2,3-dioxygenase (IDO) in cancer cells serves a similar immunosuppressive function by depleting the TME of tryptophan, another essential nutrient for T cell proliferation, potentially leading to immunotherapy failure³⁵.

Other factors

Patient characteristics such as age and gender can equally influence cancer immunity. Older melanoma patients have reduced T cell trafficking, communication, and differentiation, which contributes towards reduced overall survival³⁶. Males have a higher melanoma incidence, which in part may be explained by females having higher levels of tumour antigen-specific T cells³⁷. However, although conflicting reports exist, hormonal changes and immunosuppression that occur in females during pregnancy have been associated with highly aggressive forms of melanoma³⁸. Host genetics, such as perforin mutations, can also make individuals more susceptible to developing certain types of cancer³⁹ as the immune system is deficient in a critical tool used to maintain homeostasis.

One of the newest additions to the host factors known to influence the inter-relationship between cancer cells and the immune system is the human microbiome, and specifically the gut microbiome. Our understanding of the influences exerted by the microbiome on the host immune system, and specifically its ability to modulate the anti-tumour immune response, is still in its infancy. Much can be inferred from the extensive evidence linking dysregulation of the gut microbiome (termed “dysbiosis”) to a wide range of critical inflammatory diseases, including inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis⁴⁰. In the cancer setting, dysbiosis has an established role in moderating development of graft-versus-host disease⁴¹ and survival outcomes⁴² following haematopoietic stem cell transplantation. Similarly, the risk of developing colitis following CTLA-4 blockade was associated with lower abundance of *Bacteroidetes* organisms and under-representation of distinct microbial biochemical pathways⁴³. What is not clear at this stage is whether dysbiosis *necessarily* precedes cancer development, and the qualitative and quantitative effects cancer can exert on microbiota.

Ultimately, cancer immune escape may occur due to one or several of the above factors. In melanoma, adaptive IFN-driven cellular (Treg) and molecular (inhibitory checkpoint molecule upregulation) mechanisms of immunosuppression have been well-described, but do not explain all observable states of the tumour-immune microenvironment. It is clear that the ability to successfully disrupt or reverse immune imbalance using treatment will depend on the nature of this homeostatic escape and on how the TME has been moulded over time.

Thus, tailoring therapeutic interventions to individual tumours is vital to produce improved and durable patient outcomes, and will likely warrant repeated interrogation (e.g.: through serial biopsies and immune characterisation) over time.

DISRUPTING CANCER IMMUNE ESCAPE

The ultimate goal of therapeutic interventions is to convert tumours from an escape phase (progressive disease), into a new long-lasting equilibrium (stable disease) or elimination phase (partial/complete response) (Figure 3). Although a durable conversion is intended, new tumour escape can occur in a prompt or delayed manner which is often patient and treatment-specific, and can even occur heterogeneously across different metastatic sites within the same patient.

Current therapies in melanoma include surgery, chemotherapy, radiotherapy, targeted therapy with kinase inhibitors, and more recently immunotherapy with checkpoint inhibitors such as CTLA-4, PD-1 and PD-L1. These immunotherapeutics have resulted in previously unobserved objective and durable responses in a subset of melanoma patients, with anti-PD-1 therapy outperforming anti-CTLA-4 therapy in objective response rates, and lower therapy-induced immune-related adverse events (irAEs)²². Mechanistically, the blockade of these checkpoint molecules that are pivotal in maintaining physiological immune homeostasis enhances immune responses via different cellular mechanisms. PD-1 blockade induces the expansion of tumour-infiltrating exhausted-like CD8⁺ T cell subsets, and CTLA-4 blockade induces expansion of ICOS⁺ Th1-like CD4⁺ T cell subsets⁴⁴ and may deplete Tregs via antibody-mediated cellular cytotoxicity (ADCC). Both treatments can therefore be viewed as attempts to break tumour-induced immune tolerance, and the frequent co-appearance of treatment-related adverse events demonstrates the mechanistic overlap between tumour-induced immune dampening and self-regulatory immune pathways that promote homeostasis. As with most therapeutic approaches, resistance to checkpoint blockade can also occur, and has been recently associated with defects in essential immune cell pathways such as IFN-receptor signalling or antigen processing⁴⁵, similar to those that contribute to tumour establishment and escape from equilibrium in the first instance.

Much progress has been made in an experimental/clinical trial setting with regards to immunotherapeutic approaches, which include novel checkpoint molecules, induction of cellular effector functions, metabolic targets, myeloid cell-derived cytokines, chemokines and factors, and adjuvants. Immunologically poised tumours appear more likely to benefit from immune checkpoint blockade therapy or other immunotherapies; these are characterized by

having a high mutational burden, an increased IFN γ signature, a low Treg/CD3⁺ ratio, a robust general antibody response, high clonality, an increased Teff/Treg ratio, increased Teffs, decreased suppressor cells and high expression of immune checkpoint molecules on tumours and TILs (e.g.: PD-L1)⁴⁶. Intuitively, immunologically unresponsive tumours behave conversely, and may be more likely to benefit at first from either alternative or additional treatments, including oncolytic viruses or vascular disrupting agents. In addition, targeting metabolic immune checkpoints using IDO inhibitors⁴⁷ or adenosine receptor antagonists⁴⁸ may also alter the TME in an immunologically favourable manner. The use of combination or sequential treatments is generally more effective than single agent use. To achieve maximum therapeutic benefit from immunotherapeutics, and in turn establish a new equilibrium or elimination state, multiple treatment adjustments may be necessary over time to address the constantly changing cancer-immune dynamics and the substantial intratumoural genomic and immune heterogeneity⁴⁹. For example, it may be essential to concurrently enhance antigen presentation, induce cancer cell death and induce the activation of cellular effector functions, whilst reversing immunosuppression¹³.

Organotypic differences in immune homeostasis

Often, mixed clinical responses can be observed in patients, with different inter- or intra-organ lesions responding (elimination), remaining stable (equilibrium) and progressing (escape) simultaneously⁵⁰. Therefore, all phases of immune homeostasis can be observed within single patients during treatment, pointing to intrinsic tumour, organ and immune microenvironment-specific differences between different metastases⁵¹. It is well accepted that advanced melanoma patients with brain metastases generally have a dismal prognosis, with limited benefit to therapeutic interventions other than surgery or radiotherapy. This can be at least partially explained by a very unique microenvironment, distinguished by the presence of a functional blood-brain barrier that limits the passage of molecular and cellular elements⁵². Similarly, non-visceral metastases are associated with a better survival relative to visceral metastases⁵³, and metastases in certain organs like adrenal glands and lungs can be more responsive to treatment than others^{51, 54}. These differences could be explained by the presence of organotypic immunity, which we define as differences in immune cell composition and recruitment across different organs with metastatic disease. For instance, the presence of non-circulating tissue-resident memory (Trm) T cells in soft tissues and bone may play an important organotypic role in the magnitude⁵⁵ and durability⁵⁶ of anti-tumour immune responses. We also anticipate that the recruitment of non-resident anti-tumour immune cells

is restricted in certain organs, either due to physical barriers or to inadequate chemokine-mediated migration. Alternatively, the presence of unique immune-cell compositions at anatomically distinct metastatic sites may require different interventions to affect a positive immune outcome.

Spontaneous changes in immunity

Abscopal effects have been reported in melanoma, where untreated distant lesions can spontaneously regress (partially or completely) following the local treatment (e.g.: with intralesional therapy or radiotherapy) of isolated sites of disease, arguing for the beneficial skewing of immune homeostasis towards an anti-tumour elimination phase by local therapy, which becomes generalised by mobile immune elements⁵⁷. This immune-mediated effect has been seen in radiotherapy-treated patients, where non-irradiated lesions at distant metastatic sites regress, and can be accompanied by systemically-detectable increases in cancer antigen-specific antibody responses, T cell activation (ICOS^{high} CD4⁺ T cells) and HLA-DR expression⁵⁸. As such, novel studies investigating the synergistic effects of concurrent radiotherapy and immunotherapy are underway, with early reports suggesting improved objective response and overall survival rates⁵⁹.

Gut microbiome effects on therapeutic efficacy

An association between gut microbial composition and the efficacy of cytotoxic chemotherapy has been demonstrated in pre-clinical models, in which specific modulation of Th17 responses plays a central role, similarly to what has been observed in non-malignant inflammatory disorders⁴⁰. In murine experiments, Viaud and colleagues demonstrated that compositional shifts in gut microbiota induced by cyclophosphamide treatment resulted in the activation of specific “pathogenic” Th17 cells⁶⁰ within draining lymph nodes that were indispensable for the anti-cancer effect of cyclophosphamide in several tumour models⁶¹. Interestingly, chemotherapy-induced changes to the gut-lumen barrier facilitated translocation of specific Gram-positive organisms into draining lymph nodes, whereupon Th1 and pathogenic Th17 differentiation occurred.

More recently, roles for specific microbial taxa and microbiome-level compositional factors have been implicated in the efficacy of immunotherapy⁶²⁻⁶⁴. Whilst the precise mechanisms underlying these effects on treatment outcome require further investigation, one clear point of commonality is that therapy-induced changes in the gut microbiota alter the ongoing microbiota-immune interactions in such a way that anti-tumour immune responses are

favoured. In the case of anti-CTLA-4 treatment, relative enrichment of immunogenic *Bacteroides* species (*B. thetaiotaomicron*, *B. fragilis*) facilitated local and nodal CD11b⁺ DC mobilisation, leading ultimately to more generalised activation of Th1 responses⁶⁴. In a murine model of anti-PD-L1 treatment for melanoma, improved DC maturation and function was similarly involved in the systemically-enhanced T cell and anti-tumour responses conferred by *Bifidobacterium*⁶³. In melanoma patients, compositional differences in the faecal microbiota typified by enrichment of members of the *Ruminococcaceae* family were shown to increase the likelihood of response to PD-1 blockade⁶². Equally important are clinical data suggesting that commensal microflora play a role in the development of immunotherapy-related toxicities, such as the high-grade colitis seen frequently in the setting of CTLA-4 blockade⁴³. The potential for a modifiable factor such as the gut microbiome to influence both efficacy and toxicity warrants particular focus on the autoimmune and inflammatory processes underlying immunotherapy-related adverse events.

The unwanted induction of autoimmunity

The treatment-induced immunological conversion of tumours can be further complicated by the onset of irAEs that clinically mimic classic autoimmunity, and can lead to early treatment cessation or requirement of immunosuppressive medication that poses a theoretical but difficult to quantify risk to treatment efficacy⁶⁵. These irAEs are interpreted as a break in immune tolerance towards self-antigens, occur frequently in immunotherapy-treated patients and can be viewed as indicators of a successful, albeit excessive, immune activation. Importantly, however, this does not strictly imply *on-target* (i.e.: anti-tumour) immune activation. A recent study in melanoma patients treated with anti-PD-1 antibodies showed that the onset of an irAE was correlated with an increased overall survival, with the largest clinical benefit seen in patients with three or more irAEs, and of particular interest, those with cutaneous irAEs⁶⁶. In addition, this overall survival benefit was not lost in patients treated with systemic steroids for irAEs, arguing that immunosuppressive medication may not entirely compromise an ongoing anti-tumour immune response. However, it remains to be proven whether individual patient genetics and susceptibility to autoimmunity may reliably predict improved response rates to treatment⁶⁷, and what exactly defines and regulates the relationship of autoimmunity and cancer in immune homeostasis⁶⁸.

FUTURE PERSPECTIVE

The intricate mechanisms at play between the immune system and tumours, and how these contribute to tumour elimination and escape are still under investigation. As more and more targetable immune checkpoints are identified, combining multiple different immune activating drugs has become a very real and tempting prospect. The combination of CTLA-4 and PD-1 blockade has demonstrated promise in this approach. However, a recent study showed that excessive adverse effects can arise when combining an immune activating ligand (OX40L) with an inhibitory molecule (anti-PD-1)⁶⁹, ultimately leading to the early termination of a clinical trial. As such, combination treatments may not be as straightforward as initially anticipated, and warrant careful pre-clinical testing to ensure that such concerted efforts to push the cancer-immune balance in one direction are at least safe, if not efficacious. Besides from the question of which traditional and novel agents are best combined with (other) immunomodulatory drugs, the most suitable treatment duration has not yet been defined⁷⁰. One important issue to address in ongoing translational studies is whether the intensity and duration of immune activation can be optimised to a 'middle ground' state of selective pressure in order to prevent the development of treatment-resistant subclones and reflexive immune dampening.

As a relative newcomer to the field, a role for the gut microbiota in immunotherapy for melanoma is emerging, and speculation exists regarding the relevance of skin microflora to melanomagenesis, but inadequate data yet exist to arrive at any firm conclusion. Studies are needed to gauge how the microbiome affects immunosurveillance of nascent neoplasia, and whether this is in any way modifiable, as this will be critical to the public health applications of microbiome-based interventions for cancer prevention. Furthermore, to date, studies of the microbiota in cancer have focused almost exclusively on bacterial populations. However, bacteria do not comprise the sum total of microflora. Viruses have a well-established and widely-relevant causal relationship with multiple cancers and classically invoke cellular immune responses that could overlap mechanistically with anti-tumour immunity. Additionally, the commensal mycobacteria are also known to play a significant role in mucosal immune homeostasis⁷¹, but have not yet been studied in the context of cancers such as melanoma.

Despite all of these open questions and challenges, cancer therapy has gained a completely new arsenal of treatments, with immunomodulatory agents leading to tumour shrinkage and durable responses including no clinical evidence of residual disease. Whether this is a reflection of a cure - complete eradication of all tumour cells - remains questionable,

although early anti-CTLA-4 trials may suggest this⁷²⁻⁷⁴. In most cases, ongoing changes in immune parameters that may result in disease relapses many years after achieving complete remissions^{75, 76} are indicative of the (temporary) establishment of a new immune equilibrium that includes immunological memory and the constant control of actively dividing tumour cells throughout the body⁷⁵. Understanding how to trigger, and more importantly maintain, the level of immune control in the majority of patients, while being able to recognize the early signs of a necessary intervention (biomarker discovery), will be the biggest challenge in translational oncoimmunology over the next decades. This equilibrium, if maintained indefinitely, would transform melanoma into a chronic asymptomatic disease.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1. **Phases of the physiological and pathological immune homeostasis.**

Figure 2. **Immune and tumour factors contributing to cancer immune escape.** In the equilibrium phase, a balance exists between tumour and immune cells, which simultaneously prevents tumour outgrowth and clearance. A number of the pro- (red) and anti- (green) tumoural immune cell subtypes and chemokines/cytokines, or factors that can act in both ways (black), are shown here and are described in the text.

Figure 3. **Disruption of the cancer immune escape using immunotherapy.** **1** - Once tumour cells escape equilibrium they become clinically apparent. **2** – Immunotherapy can break the tumour-induced and the natural tolerance, and may result in the appearance of immune-related adverse events (irAEs). Successful treatment can either establish a 2nd equilibrium that controls tumour growth, or completely eliminate the cancer. **3** – This 2nd equilibrium that is now kept in a new balance by different immunosuppressive and activating factors compared to the pre-treatment equilibrium may persist indefinitely, or when disturbed, result in another escape that may benefit from a subsequent therapeutic intervention.

Immune Homeostasis

Cancer Immune Homeostasis

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