Dynamic versus static biomarkers in cancer immune checkpoint blockade - unraveling complexity

W. Joost Lesterhuis^{1*}, Anthony Bosco², Michael J. Millward^{1,3}, Michael Small^{4,5},
Anna K. Nowak^{1,3}, Richard A. Lake¹

¹School of Medicine and Pharmacology and National Centre for Asbestos Related Diseases, University of Western Australia, 5th Floor QQ Block, 6 Verdun Street, Nedlands, Perth WA 6009, Australia.

²Telethon Kids Institute, University of Western Australia, PO Box 855, West Perth WA 6872, Australia

³Department of Medical Oncology, Sir Charles Gairdner Hospital, Hospital Ave, Nedlands, WA 6009, Australia

⁴School of Mathematics and Statistics, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia

⁵CSIRO, Mineral Resources, 26 Dick Perry Ave, Kensington, WA, 6152, Australia

*Corresponding author: willem.lesterhuis@uwa.edu.au

Key words:

Cancer, immune checkpoint, dynamic biomarkers, critical transition, complexity, network biology, CTLA4, PD1, drug discovery

Acknowledgements:

W.J.L. is supported by a John Stocker Fellowship from the Australian Science and Industry Endowment Fund and grants from Cure Cancer/Cancer Australia, R.A.L. by the Insurance Commission of Western Australia. W.J.L., A.K.N. and R.A.L. are supported by project grants and Centre for Research Excellence funding from the National Health and Medical Research Council.

Abstract

Recently, there has been a coordinated effort from academia and the pharmaceutical industry to identify biomarkers that predict response to immune checkpoint blockade in cancer. Although several have been identified, so far no biomarker reliably predicts response in a sufficiently rigorous manner for routine use.

Here, we argue that the therapeutic response to immune checkpoint blockade is a critical state transition of a complex system. Such systems are highly sensitive to initial conditions and critical transitions are notoriously difficult to predict far in advance. However, closer to the tipping point warning signals can be detected. Recent advances in mathematics and network biology are starting to make it possible to identify such warning signals. We propose that these dynamic biomarkers could not only prove useful in distinguishing responding from non-responding patients, but may also allow the identification of new therapeutic targets for combination therapy.

“Even if it were the case that the natural laws had no longer any secret for us, we could still only know the initial situation approximately. [...] It may happen that small differences in the initial conditions produce very great ones in the final phenomena. A small error in the former will produce an enormous error in the latter. Prediction becomes impossible, and we have the fortuitous phenomenon.”

Henri Poincaré, *Science and Method*¹

The particular need for a predictive biomarker for immune checkpoint blockade in cancer

Immune checkpoint blockade has recently shown tremendous success in cancer, with long-term complete tumor regression in a proportion of patients in several cancer types, most notably melanoma^{2,3}, non-small cell lung cancer⁴, kidney cancer⁵ and Hodgkin’s lymphoma⁶. Although melanoma so far seems to be the only cancer to benefit from antibodies that block cytotoxic T lymphocyte-associated antigen 4 (CTLA4)⁷, other cancer types also seem to be amenable to blockade of the programmed cell death protein 1 (PD1) pathway, with response rates between 10 and 25% for bladder⁸, gastric⁹, ovarian¹⁰, head and neck cancer¹¹ and several others. These results have dramatically changed the field of oncology, and for some metastatic solid cancers the word ‘cure’ has now become part of the oncologists’ vocabulary¹².

However, even though these results are very exciting, the majority of patients with metastatic cancers do not respond to immune checkpoint blockade. The almost dichotomous response profile, combined with potentially severe toxicity¹³ and very high costs¹⁴ mean that a reliable predictive biomarker is urgently needed. There are some additional aspects particular to immune checkpoint blockade that shape the requirements of such a biomarker (**Box 1**).

One of these is the phenomenon of pseudoprogression (where tumor volume increases due to infiltrating immune cells and edema, before a subsequent response occurs)¹⁵, which makes it difficult to assess whether to continue treatment in the setting of apparent tumor growth. After all, in most cases this will be due to early treatment failure, rather than prefacing a response.

Another particular aspect of immune checkpoint blockade is a by-product of its response profile: if patients respond to this treatment, they often experience prolonged response durations, while most other systemic treatments in metastatic solid cancers typically only offer the possibility of a modest delay in tumor progression¹⁶. Given the alternative, this places a heavy burden on the negative predictive value of a potential biomarker. For example, if the marker identified a reduction in the probability of response from 25% for the unselected

patient group to 10% for the biomarker-associated group, that would possibly still be too high for patients or their doctors to forsake treatment (**Box 1**).

Lastly, even though immune checkpoint blockade has been developed through a target-based approach, it is not well understood how it actually works. Based on depletion studies in mice it is clear that cytotoxic T cells are of crucial importance¹⁷, but whether they are the only cells of importance remains unclear at this stage with studies showing other cell types may be (either positively or negatively) involved in the effector response, including regulatory T cells¹⁸, macrophages^{18, 19}, helper T cells²⁰⁻²², natural killer cells^{20, 23}, dendritic cells and myeloid-derived suppressor cells^{24, 25}. It is also not fully understood how cytotoxic T cells actually kill cancer cells in vivo following immunotherapy, or how tumor cells could be sensitized to immune destruction^{26, 27}.

A biomarker correlating with an effective response could potentially elucidate some of these aspects of the underlying mechanism of action of immune checkpoint blockade.

Static biomarkers for immune checkpoint blockade

The most clinically advanced biomarkers for response to immune checkpoint blockade include neoantigen expression/mutational load²⁸⁻³⁰, tumor expression of the checkpoint receptor ligands³¹⁻³³ and intratumoral immune infiltrates³⁴. These and other biomarkers are all obtained from pre-treatment patient samples (reviewed in refs.³⁵⁻³⁷).

The unspoken underlying assumption here is that a patient is *destined* to respond or not; if we were able to go back in time and treat that same patient again with the same antibody, he or she would respond identically. But, would this indeed be the case? Interestingly in this respect, tumor-bearing inbred mice show differences in response to checkpoint blockade^{17, 38-40}. Although inbred mice potentially have differences in their T cell receptor repertoires, they are otherwise very similar: their germ-line genomes are identical, they are sex and age-matched, inoculated with a monoclonal cancer cell line (expressing a presumably fixed amount of neoantigens) that reproducibly gives rise to similar-sized tumors, treated with the same antibody at the same dose, and sometimes even housed within the same cage, while still showing differences in response. The difference in response rates is also not primarily determined by size; larger tumors can still respond while smaller tumors escape^{39, 41}. Together, these findings indicate that whatever the difference is between responders and non-responders in the pretreatment setting, it can be very small. And although the pretreatment state is of importance in predicting response as we can conclude from the current correlates

discussed above, it may not encompass all determinants; some decisions are made after the therapy has been administered.

The complexity of an anti-tumor immune response

The immune system contains many switches, thresholds, feed-forward and feedback loops that govern cellular differentiation^{42, 43}, activation or inhibition^{44, 45}, migration and microanatomical distribution^{46, 47}, and immune cell population expansion or contraction^{48, 49}. Similar switches and loops operate on the tumor side, determining whether a cancer cell will die or grow^{26, 50-52}. Lastly, multiple layers of complexity are added by systemic pro- or anti-inflammatory soluble factors^{53, 54}, tissue-specific regulation of cells and genes^{55, 56}, local metabolic effects^{57, 58}, pro- and anti-inflammatory innate immune players⁵⁹, interactions with our external environment⁶⁰, and potentially many other yet unknown factors that all influence the outcome of an immune response and its interaction with target tissue.

These characteristics make the immune system and its interplay with other cells and tissues the archetype of a complex or chaotic system⁶¹, which suggests that it is highly sensitive to initial conditions: minuscule differences may go undetected, but may have massive consequences downstream. Yet, despite it being a complex system with decreasing predictability of the behavior of the individual components⁴⁴, the immune system does in most cases respond in a surprisingly predictable and consistent manner, eliminating pathogens without harming normal tissue. In fact, randomness and stochastic variation at the lower level of the immune system are paradoxically integral to a more predictable outcome at the system level⁴⁴. This has for example been shown for T cell activation, where differences in the expression of key regulators have the potential to result in heterogeneous activation within a clone of ostensibly identical cells. This problem is at least partially solved by co-regulation of these same regulators, which limits the heterogeneity of the response⁴⁵. Likewise, although after exposure to a pathogen individual cells follow random cell fate courses over time (non-proliferation, cell division and death), non-protection is highly unlikely, and with a minimum set of rules lymphocyte expansion and contraction during infection follows a predictable course through population averaging⁶²⁻⁶⁴.

Because of these balancing mechanisms the outcome of an immune response is usually consistent, reproducible and adequate. This could lead one to think in a deterministic manner (we can understand and predict the outcome of an immune response if we know all the cellular and molecular constituents of the system), while in fact, it likely is a probabilistic

outcome of a complex system⁶⁵. As a consequence of this interpretation, once in a while we are taken aback by completely unpredictable rapidly spiraling immunological cascades, such as when a minor bacterial exposure in previously completely healthy people results in a fulminant lethal septic shock^{66, 67}, when targeting one single molecule leads to catastrophic cytokine storms in healthy trial subjects within a few hours⁶⁸, or, more positively, when patients with very advanced metastatic cancer show a complete regression within a few weeks after a single administration of immune checkpoint blockade⁶⁹.

The immune checkpoint blockade-responsive tumor displays a critical transition

The almost dichotomous fashion of therapeutic responses to checkpoint blockade, particularly the fast and complete regressions as observed with some of the antibodies in some patients, versus the complete lack of impact in others^{2, 16, 69}, suggests a critical state transition, as is observed in many complex systems in ecology, economics and biology^{70, 71}. These systems undergo slow changes in response to external factors or perturbations, but occasionally they flip to a contrasting state, the likelihood of which increases when the system approaches a tipping point⁷⁰.

Critical transitions can be divided into three phases: a stable initial state, a pretransition state and the new stable state^{72, 73} (**Fig. 1a**). While complex systems are usually very robust to perturbations in the two stable states, they have low resilience and robustness in the pretransition state⁷⁴. Although during that phase the system is still reversible to its initial state, a very minor local perturbation can lead to a self-propagating feed-forward loop that propels the entire system towards the other state, particularly in highly connected networks^{70, 74, 75}. Conversely, appropriate perturbations can also pull the system back towards the initial stable state.

As a consequence, critical transitions are often unpredictable, and the further away from the tipping point, the more difficult it becomes to predict^{70, 76}. In other scientific fields, some mechanisms have been identified that could predict a state transition such as ‘critical slowing down’, the rate at which a system recovers from small perturbations⁷⁷, but it is unclear how universal these warning signs are and how they would apply to the anti-tumor immune response. However, what is clear is that for critical transitions the warning signals may not all lie in the initial state; some biomarkers may be found between the start of treatment and the tipping point (**Fig. 1a**)⁷³. And importantly, while chaotic systems are difficult to predict because small changes will grow rapidly, this also makes them potentially easy to control: a

small change in the initial state (at the right time and in the right direction) can mean that the system will evolve to an entirely different final state^{78, 79}.

The initial state and subsequent events that occur closer to a state transition are of course linked. In the context of immune checkpoint blockade for example, a patient with a broad repertoire of tumor antigen-specific T cells and an inflamed tumor microenvironment may be closer to the tipping point than a patient who does not have these characteristics: subsequent events that may be needed to push the network through the state transition, such as adequate cytokine release and attraction and extravasation of immune effector cells may occur more readily in these patients. Biomarkers in the pretransition state may be the driving factors that tip the complex network towards the response state, thus forming potential effective drug targets⁷³.

What goes on in an immunotherapy-responding tumor?

So how does this work when we artificially try to tweak the system using immune checkpoint blockade? Clinically, the possibilities are rather limited: cure, partial response, mixed response or no response with regards to the cancer, and (auto-immune) side effects or not with regards to the host⁸⁰. The underlying mechanisms resulting in these outcomes, however, could be manifold. Although some primary mechanisms of resistance have been identified (reviewed in ref.⁸¹), it is not known what cellular and molecular programs govern these different outcomes, and whether these programs are heterogeneous between or within each possible clinical outcome group.

Some studies investigating transcriptional responses before^{82, 83} and during⁸⁴⁻⁸⁶ checkpoint blockade in patients have been reported, but so far they have only employed conventional differential expression signatures. A study in 45 melanoma patients treated with anti-PD1 and/or anti-CTLA4 investigated the transcriptomes of peripheral blood T cells and monocytes, comparing pretreatment samples with samples obtained 3 weeks after start of therapy⁸⁴. CTLA4 blockade particularly induced a proliferative signature in transitional memory T cells, while PD1 blockade showed enhanced expression of cytotoxicity and natural killer cell function. Interestingly, there was little overlap between T cell gene signatures in the different patient cohorts⁸⁴. Although peripheral blood is the most convenient and accessible site to obtain serial biomarkers, it is unclear at this stage whether it will allow the identification of a useful biomarker⁸⁷.

A similar study that employed differential expression analysis on whole transcriptome data used tumor tissue from before and 3 weeks after start of ipilimumab, and found that genes associated with T helper 1 type immune responses were increased, while genes involved in cell proliferation decreased, presumably due to T cell infiltration⁸⁵. Similar findings were reported for patients treated with an anti-PD-L1 antibody⁸⁶. A recent study reported results from targeted gene expression analysis of 795 immune or cancer-related genes in melanoma biopsies from 53 patients who were treated with sequential CTLA4 and PD1 blockade, taken at several time points during treatment⁸⁸. This study found a variety of immune-related genes to be differentially upregulated when comparing responders with non-responders. Only a modest overlap in transcriptional signatures between CTLA4 and PD1 blockade groups was observed, confirming previous data in animal models⁸⁹. Some cancer-related genes, such as vascular endothelial growth factor, were downregulated in responders to PD1 blockade, suggesting a potential angiogenic mechanism of therapeutic resistance⁸⁸.

Although these data are very interesting, differential gene expression analysis inherently favors the identification of highly differentially expressed genes, and may not allow differentiation of genes that are expressed as a consequence of the critical transition from ones that are actually driving it⁷⁴. Moreover, the molecular determinants of cellular phenotypes are often themselves not differentially transcribed, rather their increased activity may be dependent on post-transcriptional or post-translational mechanisms^{90, 91}.

Dynamic network biomarkers for response

Network analysis has recently been shown to be an effective approach when trying to tease out key drivers of a biological process, giving structural insight into the connectivity between the players in the tumor microenvironment that correlate with response⁹². Network biology applies graph theory to biological data: genes, proteins, metabolites or other biologically active molecules are visualized as nodes and their interactions as links or ‘edges’⁹³ (**Box 2**). Together, they form complex networks, operating within and between cells, tissues and organs⁹⁴. One of the key advantages of network biology is that it allows the identification of highly connected nodes (‘hubs’)⁹², and bottleneck nodes⁹⁵, which are a priori ideal candidates for biomarker and drug discovery, since the modulation of these hubs allows modulation of a large repertoire of other interacting nodes^{96, 97}. By constructing topological maps of connections between genes and/or gene products, subnetworks (modules) of separate but highly connected pathways with common functions can be distinguished, using high-dimensional molecular data^{98, 99}. This approach was used to for example identify key hubs

and modules that control yeast metabolism¹⁰⁰, inflammatory responses of immune cells¹⁰¹⁻¹⁰³, pathogen sensitivity to antibiotics¹⁰⁴ and epithelial-mesenchymal transition in brain tumors¹⁰⁵. However, recent evidence indicates that when applied to dynamic processes, static network analysis may miss aspects that are important for the function or behavior of the network induced by changing conditions or perturbations^{106, 107}. The more dense a network becomes, the less important individual hubs become, the more resistant the network becomes to perturbations, yet at the price of sudden dramatic ‘global’ cascades, which are extremely hard to predict¹⁰⁸. For example, in marketing through social networks, highly connected hubs are not critical when it comes to communicating large influence cascades¹⁰⁹. Similarly, although it seems logical to assume that taking out the central hubs in a narcotic crime network will disrupt the network, counter intuitively the network actually becomes more efficient when the bosses are removed¹¹⁰. And when cellular transcriptional networks were studied under different conditions over time, different sets of transcription factors were seen to be key drivers at different times, resulting in alternative cellular states: ‘transient hubs’ had the capacity to change interactions in subnetworks that were critical only under certain conditions¹¹¹. These data suggest that it is difficult to predict which nodes are the most important ones for initiating or communicating critical transitions based on static network data only, and that we need dynamic data to identify useful biomarkers in this context.

An interesting novel approach makes use of the fact that key drivers of critical transitions in cellular networks increasingly fluctuate in a strongly correlated manner when approaching the tipping point^{72, 74, 112}. These dynamic network biomarkers are envisaged as the leading subnetworks that first move over the critical point and thus have a strong relationship with the causal molecules, as opposed to differential expressed genes that would usually result from the transition, rather than being the cause of it^{73, 74, 113}. So far, these dynamic network biomarkers have only been applied to predict transitions from a pre-disease state to a disease state, not to predict a therapeutic response. Interestingly, in that setting, the groups of molecules that formed these biomarkers could slightly vary between individuals, presumably due to individual genetic/epigenetic variations⁷² or stochastic variation¹¹⁴. Whether this approach can be used in the context of immune checkpoint blockade remains to be established.

The power of using sequentially sampled patient tissue during treatment to identify novel biomarkers that correlate with response is underscored by a recent study in patients with systemic lupus erythematosus¹¹⁵. The response to specific treatments did not so much correlate with pretreatment signatures, but with changes of these signatures over time, which enabled

patient stratification into distinct groups. The implication is that based on these therapy-induced biomarker changes subsequent additional therapeutic strategies could be selected¹¹⁶. In this case, the dynamic biomarkers were obtained through serial sampling of peripheral blood. In the setting of cancer and immune checkpoint blockade, it is likely that the tumor microenvironment has to be taken into account (**Fig 1b**).

We recently used network analysis of gene expression data from immune checkpoint-blockade responding and non-responding tumors in mice, taken 5-6 days after administration of an anti-CTLA4 antibody which was just before the clinical tipping point, the moment when responding tumors would start to regress while non-responding tumors would keep growing³⁹. This approach identified two response-associated coexpression modules, and nitric oxide synthase 2 (NOS2) as a central hub in the response to CTLA4 blockade in this model (**Fig. 2**). Importantly, by pharmacologically inhibiting or enhancing the biological activity of NOS2 the response rates to anti-CTLA4 were significantly decreased or increased, respectively³⁹. This study provides the first proof of principle that mapping the molecular networks after start of treatment allows identification of novel biomarkers in the context of immune checkpoint blockade (**Fig. 1b**). Importantly, this work suggests that this approach can identify the leading response network and that subsequent informed targeting with (repurposed) drugs can push the system over the tipping point towards a response.

Conclusion: integrating dynamic and static biomarkers in the clinic

Identifying dynamic biomarkers associated with therapeutic response is clearly in its infancy with very little data to date. However, early proof of principle has been established. Importantly, these studies have not only shown that during the effector response novel biomarkers can be identified that would not have been picked up in pretreatment tissue, but also that these biomarkers can be leveraged for novel therapeutic strategies for combination or sequential treatments to improve the efficacy of the initial therapy.

So far, these studies have employed molecular profiling of bulk tissue samples. Given the multicellular complexity of the anti-tumor immune response, there is a limit to what can be learned from these types of analyses. The advent of single cell analysis has made it possible to tease out the molecular players in their cellular context and dissect cell-cell communication in a much clearer manner. This should provide a wealth of information regarding the mechanisms that operate during immunotherapy-induced tumor regression.

Many questions remain. At this moment it is not clear if there is commonality of mechanisms between individual responding patients, or whether there are different routes to successful

tumor regression following checkpoint blockade (**Fig. 3**)¹¹⁷. Are there some patients who will never respond, with tumor/host characteristics that do not allow a therapeutic response even to move towards the tipping point? If so, in that situation a dynamic biomarker would not be useful and pretreatment tissue samples would suffice. In an experimental setting, that would be a host who lacks any cytotoxic T cells¹⁷ for example, but whether there are specific characteristics in the real-world setting that would completely preclude any response is not known.

It is also important to note that although it appears that in some checkpoint blockade responding patients there is a drastic change in system state (a rapid and complete clinical response²), in others the change seems more gradual¹¹⁸. Notwithstanding the different kinetics of these responses between patients, we could still potentially identify individuals that are susceptible to being pushed towards the healthy state with the right treatment by observing the dynamic behavior within the disease state after treatment¹¹⁹.

We envisage that static and dynamic biomarkers could be incorporated into a treatment plan in a complementary manner. If for example, based on a static pretreatment biomarker, a patient was deemed to be potentially responsive to treatment, a dynamic biomarker could allow a subsequent decision shortly after treatment had been initiated (perhaps to continue despite initial progression, based on a positive marker obtained early after treatment initiation, suggesting a diagnosis of pseudoprogression).

Identification of dynamic biomarkers that predict response to immune checkpoint blockade will obviously require serial sampling during therapy. Whilst repeated tumor biopsies are challenging, they are feasible within the context of clinical trials as has been demonstrated in several studies^{34, 84, 85, 88}. It is possible that baseline and serial on-treatment biopsies in initial exploratory studies will identify surrogate biomarkers that could subsequently be accessed using less invasive techniques, such as molecular imaging¹²⁰, fine needle aspiration biopsies, or best case via peripheral blood biomarkers.

The identification of new dynamic biomarkers requires collaboration between mathematicians, computational biologists, cancer immunologists and clinicians. With so many clinical trials of immune checkpoint blockade currently ongoing, there is a very strong rationale to obtain serial patient samples to map the networks underlying successful therapy. If we incorporate dynamic biomarkers into trials it is more likely that we will find a set of biomarkers that will fulfill all the criteria.

Box 1: Why is a biomarker for checkpoint blockade needed and what are its necessary characteristics?

What is so remarkable about immune checkpoint blockade?

- Checkpoint blockade works in a minority of patients for many types of cancers^{2-6, 8-11}.
- But when it works, it often works really well, with prolonged responses for many years^{16, 121}, while in most cancers other treatments typically are effective for only a limited time.
- Responding tumors can first show increased size on imaging before they start to respond ('pseudoprogression')¹⁵.
- It can have severe, life-threatening side effects¹³.
- It is extremely expensive¹⁴.
- Although the targets are defined, the exact mechanism of action of these compounds is incompletely understood.
- Combinations of immune checkpoint blockade may augment response², but the rationale for combination therapies is incompletely understood.

Following from these distinctive characteristics, specific criteria for a useful biomarker include:

- It needs a very high negative predictive value (i.e. the biomarker should at least point out who will certainly not respond).
- Although a single pre-treatment biomarker would be ideal, a biomarker that could be used early in treatment would still be of great value: response prediction during the first (or second) cycle would allow identification of patients who may benefit from continuing or not; pseudoprogression would be readily identified; financial burden and potential toxicity would be limited with only 1-2 administrations of the antibody, and it may identify new targets for intervention^{39, 117}.
- An ideal biomarker would be non-invasive – i.e. repeated biopsies would not be required.
- An ideal biomarker would be valid and reliable in different cancer populations.

Box 2: The application of network science to biomedicine

Network theory represents complex systems as graphs, where the agents are represented as *nodes* (**a**), and their relations as links (*edges*), to analyze the structure and by inference the behavior of these complex systems.

An early example of this approach is the “Bridges of Königsberg” problem. The city Königsberg (now Kaliningrad) consisted of several islands and two riverbanks, connected to each other by seven bridges. The mathematical problem posed by the people of the town was to create a walk through the city that would cross each bridge only once. Leonard Euler solved the problem in 1736: by positing the bridges as edges and the various landmasses as nodes, he showed that it was impossible to create a path through the city that would cross each bridge only once¹²². In the last two decades the field has grown exponentially and the application of graph theory to complex networks has resulted in a much improved understanding of the structure and behavior of complex systems such as power grids, the world wide web, ecosystems, social behavior, infectious disease transmission, financial markets and cell biology¹²³.

In biomedical applications of network theory, the nodes are biomolecular entities (e.g. genes or proteins), and the edges are a representation of their relationships (e.g. coexpression or physical interaction)⁹². This approach allows the identification of (groups of) molecules that are associated with a certain phenotype or clinical trait. For example, network analysis of high dimensional molecular data can identify subnetworks of highly interconnected genes (‘modules’) that are important for specific cellular processes¹⁰⁰, or highly connected individual nodes (i.e. nodes with many edges, often referred to as *hubs*, the red node in **a**; or nodes with a central role in connecting other parts of a network although they do not have a lot of edges, referred to as *bottleneck nodes*, the yellow node in **a**) that drive disease processes, such as brain cancer invasion¹⁰⁵ or breast cancer oncogenesis¹²⁴.

Figures and Legends

Figure 1: Dynamic biomarkers in immune checkpoint blockade

a) The successful therapeutic response to immune checkpoint blockade follows the pattern of a critical transition of a complex system^{70, 71}. This is characterized by three phases, an initial stable state, a pretransition state (where the system can still revert back to the initial state) followed by the transition and a new stable state¹¹³. Static pre-treatment biomarkers are obtained at the beginning in the initial state, while dynamic biomarkers are taken on the way towards the response, including the pretransition state (after start of treatment)^{72, 75}. **b)** One way of identifying dynamic biomarkers is by taking biopsies at multiple time points in responding versus non-responding subjects and using a network biology approach⁹² to map the molecular networks that are associated with effective tumor regression following immune checkpoint blockade³⁹. This has the potential to identify hub gene products that are driving the response. Not only can these hubs be used as powerful biomarkers for response, but potentially also as drug targets: by pharmacologically targeting these hubs the response to immune checkpoint blockade can be significantly increased³⁹.

Figure 2: Identifying biomarkers for response in responsive versus non-responsive tumors. This figure illustrates the approach described in ref [39]. **a)** Tumors from inbred mice that respond differently to anti-CTLA4 were sampled at the point of a clinical response. **b)** Transcriptomic analysis of responding, non-responding and control tumors was undertaken. **c)** Network analysis was used to identify differentially activated coexpression subnetworks and their hubs associated with response. **d)** Drugs that phenocopy the response-associated network were shown to increase the response rate to anti-CTLA4.

Figure 3 - The rugged landscape of possible responses to immune checkpoint blockade: what routes are followed towards complete tumor regression? Each ball represents the network in the tissue at the beginning of treatment. After treatment there is a moment where a tipping point occurs in responding patients and the cancerous tissue regresses till it becomes normal healthy tissue. Several hypothetical scenarios/routes of response are illustrated.

Patient 1: this patient is intrinsically resistant; the response will never initiate. The tissue will remain in its cancerous state (at least for this treatment; the landscape may look different for another treatment –including another checkpoint antibody- for this patient). It is unclear at this stage whether such patients exist in the context of immune checkpoint blockade.

Patient 2: this patient has the right prerequisites to initiate the response. Whether it will succeed depends on many events along the way, as described in the text.

Patient 3: in this patient the tissue network is very close to the tipping point, a small change to the network induced by immune checkpoint blockade can result in a critical transition. In addition, there are no hurdles on the way to the tipping point that could prevent the transition from occurring; the patient is always going to respond. Although this is a view that can sometimes be heard in discussions on particular patient groups, it is questionable whether it is true. Even in the best starting situation (high neoantigen load, broad TCR repertoire, inflamed microenvironment, young patients, as is for example seen in Lynch syndrome-associated colorectal cancer), the response rate is still not close to 100%¹²⁵. And in identical inbred mice using checkpoint blockade sensitive tumors, dichotomous responses are still observed³⁹. As discussed in the text, the stochastic nature of the adaptive immune response does imply that at the outset we can never be fully certain of the outcome.

Patient 4: this patient is similar to patient 2, except that the response route at some point converges with that of patient 3. This is just to highlight the possibility that patients may have similar response patterns despite distinct starting situations. It is not known how much heterogeneity exists between responding patients: could differential mechanisms operate in successfully rejected tumors (before and after the tipping point)?

References

1. Poincarre, H. Science and Method. (T. Nelson, London; 1914).
2. Wolchok, J.D. et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* **369**, 122-133 (2013).
3. Robert, C. et al. Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. *New Engl J Med* **364**, 2517-2526 (2011).
4. Garon, E.B. et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* **372**, 2018-2028 (2015).
5. Motzer, R.J. et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med* **373**, 1803-1813 (2015).
6. Ansell, S.M. et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* **372**, 311-319 (2015).
7. Hodi, F.S. et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *New Engl J Med* **363**, 711-723 (2010).
8. Rosenberg, J.E. et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* **387**, 1909-1920 (2016).
9. Muro, K. et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* **17**, 717-726 (2016).
10. Hamanishi, J. et al. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. *J Clin Oncol* **33**, 4015-4022 (2015).
11. Seiwert, T.Y. et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol* **17**, 956-965 (2016).
12. Eggermont, A.M., Kroemer, G. & Zitvogel, L. Immunotherapy and the concept of a clinical cure. *European journal of cancer* **49**, 2965-2967 (2013).
13. Michot, J.M. et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *European journal of cancer* **54**, 139-148 (2016).
14. Zafar, S.Y. Financial Toxicity of Cancer Care: It's Time to Intervene. *J Natl Cancer Inst* **108** (2016).
15. Cohen, J.V. et al. Melanoma Brain Metastasis Pseudoprogression after Pembrolizumab Treatment. *Cancer Immunol Res* **4**, 179-182 (2016).
16. Ribas, A. et al. New challenges in endpoints for drug development in advanced melanoma. *Clin Cancer Res* **18**, 336-341 (2012).
17. Suttmoller, R.P. et al. Synergism of cytotoxic T lymphocyte-associated antigen 4 blockade and depletion of CD25(+) regulatory T cells in antitumor therapy reveals alternative pathways for suppression of autoreactive cytotoxic T lymphocyte responses. *J Exp Med* **194**, 823-832 (2001).
18. Simpson, T.R. et al. Fc-dependent depletion of tumor-infiltrating regulatory T cells co-defines the efficacy of anti-CTLA-4 therapy against melanoma. *J Exp Med* **210**, 1695-1710 (2013).
19. Zhu, Y. et al. CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. *Cancer Res* **74**, 5057-5069 (2014).
20. van Elsas, A. et al. Elucidating the autoimmune and antitumor effector mechanisms of a treatment based on cytotoxic T lymphocyte antigen-4 blockade in combination with

- a B16 melanoma vaccine: comparison of prophylaxis and therapy. *J Exp Med* **194**, 481-489 (2001).
21. Lesterhuis, W.J. et al. Synergistic effect of CTLA-4 blockade and cancer chemotherapy in the induction of anti-tumor immunity. *PloS one* **8**, e61895 (2013).
 22. Santegoets, S.J. et al. T cell profiling reveals high CD4+CTLA-4 + T cell frequency as dominant predictor for survival after prostate GVAX/ipilimumab treatment. *Cancer Immunol Immunother* **62**, 245-256 (2013).
 23. Armand, P. et al. Programmed Death-1 Blockade With Pembrolizumab in Patients With Classical Hodgkin Lymphoma After Brentuximab Vedotin Failure. *J Clin Oncol* (2016).
 24. Santegoets, S.J. et al. Myeloid derived suppressor and dendritic cell subsets are related to clinical outcome in prostate cancer patients treated with prostate GVAX and ipilimumab. *J Immunother Cancer* **2**, 31 (2014).
 25. Pico de Coana, Y. et al. Ipilimumab treatment results in an early decrease in the frequency of circulating granulocytic myeloid-derived suppressor cells as well as their Arginase1 production. *Cancer Immunol Res* **1**, 158-162 (2013).
 26. Martinez-Lostao, L., Anel, A. & Pardo, J. How Do Cytotoxic Lymphocytes Kill Cancer Cells? *Clin Cancer Res* **21**, 5047-5056 (2015).
 27. Halle, S. et al. In Vivo Killing Capacity of Cytotoxic T Cells Is Limited and Involves Dynamic Interactions and T Cell Cooperativity. *Immunity* **44**, 233-245 (2016).
 28. McGranahan, N. et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* **351**, 1463-1469 (2016).
 29. Van Allen, E.M. et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* **350**, 207-211 (2015).
 30. Rizvi, N.A. et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* **348**, 124-128 (2015).
 31. Larkin, J. et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med* **373**, 23-34 (2015).
 32. Topalian, S.L. et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* **366**, 2443-2454 (2012).
 33. Borghaei, H. et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *N Engl J Med* **373**, 1627-1639 (2015).
 34. Tumeh, P.C. et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568-571 (2014).
 35. Blank, C.U., Haanen, J.B., Ribas, A. & Schumacher, T.N. CANCER IMMUNOLOGY. The "cancer immunogram". *Science* **352**, 658-660 (2016).
 36. Sacher, A.G. & Gandhi, L. Biomarkers for the Clinical Use of PD-1/PD-L1 Inhibitors in Non-Small-Cell Lung Cancer: A Review. *JAMA Oncol* (2016).
 37. Topalian, S.L., Taube, J.M., Anders, R.A. & Pardoll, D.M. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat Rev Cancer* **16**, 275-287 (2016).
 38. van Elsas, A., Hurwitz, A.A. & Allison, J.P. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. *J Exp Med* **190**, 355-366 (1999).
 39. Lesterhuis, W.J. et al. Network analysis of immunotherapy-induced regressing tumours identifies novel synergistic drug combinations. *Sci Rep* **5**, 12298 (2015).
 40. Grosso, J.F. & Jure-Kunkel, M.N. CTLA-4 blockade in tumor models: an overview of preclinical and translational research. *Cancer immunity* **13**, 5 (2013).

41. Woo, S.R. et al. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* **72**, 917-927 (2012).
42. Koues, O.I. et al. Distinct Gene Regulatory Pathways for Human Innate versus Adaptive Lymphoid Cells. *Cell* **165**, 1134-1146 (2016).
43. Hasbold, J., Corcoran, L.M., Tarlinton, D.M., Tangye, S.G. & Hodgkin, P.D. Evidence from the generation of immunoglobulin G-secreting cells that stochastic mechanisms regulate lymphocyte differentiation. *Nat Immunol* **5**, 55-63 (2004).
44. Germain, R.N. The art of the probable: system control in the adaptive immune system. *Science* **293**, 240-245 (2001).
45. Feinerman, O., Veiga, J., Dorfman, J.R., Germain, R.N. & Altan-Bonnet, G. Variability and robustness in T cell activation from regulated heterogeneity in protein levels. *Science* **321**, 1081-1084 (2008).
46. Sallusto, F. et al. Switch in chemokine receptor expression upon TCR stimulation reveals novel homing potential for recently activated T cells. *Eur J Immunol* **29**, 2037-2045 (1999).
47. Gerner, M.Y., Torabi-Parizi, P. & Germain, R.N. Strategically localized dendritic cells promote rapid T cell responses to lymph-borne particulate antigens. *Immunity* **42**, 172-185 (2015).
48. Duffy, K.R. & Hodgkin, P.D. Intracellular competition for fates in the immune system. *Trends Cell Biol* **22**, 457-464 (2012).
49. Duffy, K.R. et al. Activation-induced B cell fates are selected by intracellular stochastic competition. *Science* **335**, 338-341 (2012).
50. Ashkenazi, A. & Salvesen, G. Regulated cell death: signaling and mechanisms. *Annu Rev Cell Dev Biol* **30**, 337-356 (2014).
51. Janes, K.A. et al. A systems model of signaling identifies a molecular basis set for cytokine-induced apoptosis. *Science* **310**, 1646-1653 (2005).
52. Zaretsky, J.M. et al. Mutations Associated with Acquired Resistance to PD-1 Blockade in Melanoma. *N Engl J Med* (2016).
53. Pepys, M.B. & Hirschfield, G.M. C-reactive protein: a critical update. *J Clin Invest* **111**, 1805-1812 (2003).
54. Dinarello, C.A. Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. *Chest* **112**, 321S-329S (1997).
55. Matzinger, P. & Kamala, T. Tissue-based class control: the other side of tolerance. *Nat Rev Immunol* **11**, 221-230 (2011).
56. Amit, I., Winter, D.R. & Jung, S. The role of the local environment and epigenetics in shaping macrophage identity and their effect on tissue homeostasis. *Nat Immunol* **17**, 18-25 (2016).
57. Doedens, A.L. et al. Hypoxia-inducible factors enhance the effector responses of CD8(+) T cells to persistent antigen. *Nat Immunol* **14**, 1173-1182 (2013).
58. Buck, M.D., O'Sullivan, D. & Pearce, E.L. T cell metabolism drives immunity. *J Exp Med* **212**, 1345-1360 (2015).
59. Woo, S.R., Corrales, L. & Gajewski, T.F. Innate immune recognition of cancer. *Annu Rev Immunol* **33**, 445-474 (2015).
60. Sivan, A. et al. Commensal Bifidobacterium promotes antitumor immunity and facilitates anti-PD-L1 efficacy. *Science* **350**, 1084-1089 (2015).
61. Holland, J.H. Studying complex adaptive systems. *J Syst Sci Complexity* **19**, 1-8 (2006).

62. Subramanian, V.G., Duffy, K.R., Turner, M.L. & Hodgkin, P.D. Determining the expected variability of immune responses using the cyton model. *J Math Biol* **56**, 861-892 (2008).
63. Hodgkin, P.D., Dowling, M.R. & Duffy, K.R. Why the immune system takes its chances with randomness. *Nat Rev Immunol* **14**, 711 (2014).
64. Buchholz, V.R., Schumacher, T.N. & Busch, D.H. T Cell Fate at the Single-Cell Level. *Annu Rev Immunol* **34**, 65-92 (2016).
65. Hodgkin, P.D. A probabilistic view of immunology: drawing parallels with physics. *Immunol Cell Biol* **85**, 295-299 (2007).
66. Angus, D.C. & van der Poll, T. Severe sepsis and septic shock. *N Engl J Med* **369**, 840-851 (2013).
67. Stevens, D.L. The flesh-eating bacterium: what's next? *J Infect Dis* **179 Suppl 2**, S366-374 (1999).
68. Suntharalingam, G. et al. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N Engl J Med* **355**, 1018-1028 (2006).
69. Chapman, P.B., D'Angelo, S.P. & Wolchok, J.D. Rapid eradication of a bulky melanoma mass with one dose of immunotherapy. *N Engl J Med* **372**, 2073-2074 (2015).
70. Scheffer, M. et al. Anticipating critical transitions. *Science* **338**, 344-348 (2012).
71. Scheffer, M. et al. Early-warning signals for critical transitions. *Nature* **461**, 53-59 (2009).
72. Chen, L., Liu, R., Liu, Z.P., Li, M. & Aihara, K. Detecting early-warning signals for sudden deterioration of complex diseases by dynamical network biomarkers. *Sci Rep* **2**, 342 (2012).
73. Liu, R., Aihara, K. & Chen, L. Dynamical network biomarkers for identifying critical transitions and their driving networks of biologic processes. *Quant Biol* **1**, 105-114 (2013).
74. Liu, R. et al. Identifying critical transitions and their leading biomolecular networks in complex diseases. *Sci Rep* **2**, 813 (2012).
75. Liu, R., Chen, P., Aihara, K. & Chen, L. Identifying early-warning signals of critical transitions with strong noise by dynamical network markers. *Sci Rep* **5**, 17501 (2015).
76. Wu, F.X., Wu, L., Wang, J., Liu, J. & Chen, L. Transittability of complex networks and its applications to regulatory biomolecular networks. *Sci Rep* **4**, 4819 (2014).
77. Veraart, A.J. et al. Recovery rates reflect distance to a tipping point in a living system. *Nature* **481**, 357-359 (2012).
78. Boccaletti, S., Grebogi, C., Lai, Y.C., Mancini, H. & Maza, D. The control of chaos: theory and applications. *Physics Rep* **329**, 103-197 (2000).
79. Ott, E., Grebogi, C. & Yorke, J.A. Controlling chaos. *Phys Rev Lett* **64**, 1196-1199 (1990).
80. Page, D.B., Postow, M.A., Callahan, M.K., Allison, J.P. & Wolchok, J.D. Immune modulation in cancer with antibodies. *Annu Rev Med* **65**, 185-202 (2014).
81. Pitt, J.M. et al. Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors. *Immunity* **44**, 1255-1269 (2016).
82. Hugo, W. et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* **165**, 35-44 (2016).
83. Saenger, Y. et al. Blood mRNA expression profiling predicts survival in patients treated with tremelimumab. *Clin Cancer Res* **20**, 3310-3318 (2014).
84. Das, R. et al. Combination therapy with anti-CTLA-4 and anti-PD-1 leads to distinct immunologic changes in vivo. *J Immunol* **194**, 950-959 (2015).

85. Ji, R.R. et al. An immune-active tumor microenvironment favors clinical response to ipilimumab. *Cancer immunology, immunotherapy : CII* **61**, 1019-1031 (2012).
86. Herbst, R.S. et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* **515**, 563-567 (2014).
87. Hegde, P.S., Karanikas, V. & Evers, S. The Where, the When, and the How of Immune Monitoring for Cancer Immunotherapies in the Era of Checkpoint Inhibition. *Clin Cancer Res* **22**, 1865-1874 (2016).
88. Chen, P.L. et al. Analysis of Immune Signatures in Longitudinal Tumor Samples Yields Insight into Biomarkers of Response and Mechanisms of Resistance to Immune Checkpoint Blockade. *Cancer Discov* (2016).
89. Gubin, M.M. et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. *Nature* **515**, 577-581 (2014).
90. Hudson, N.J., Dalrymple, B.P. & Reverter, A. Beyond differential expression: the quest for causal mutations and effector molecules. *BMC Genomics* **13**, 356 (2012).
91. Alvarez, M.J. et al. Functional characterization of somatic mutations in cancer using network-based inference of protein activity. *Nat Genet* (2016).
92. Barabasi, A.L. & Oltvai, Z.N. Network biology: understanding the cell's functional organization. *Nature reviews. Genetics* **5**, 101-113 (2004).
93. Vidal, M., Cusick, M.E. & Barabasi, A.L. Interactome networks and human disease. *Cell* **144**, 986-998 (2011).
94. Talukdar, H.A. et al. Cross-Tissue Regulatory Gene Networks in Coronary Artery Disease. *Cell Syst* **2**, 196-208 (2016).
95. Yu, H., Kim, P.M., Sprecher, E., Trifonov, V. & Gerstein, M. The importance of bottlenecks in protein networks: correlation with gene essentiality and expression dynamics. *PLoS Comput Biol* **3**, e59 (2007).
96. Albert, R., Jeong, H. & Barabasi, A.L. Error and attack tolerance of complex networks. *Nature* **406**, 378-382 (2000).
97. Jeong, H., Mason, S.P., Barabasi, A.L. & Oltvai, Z.N. Lethality and centrality in protein networks. *Nature* **411**, 41-42 (2001).
98. Hartwell, L.H., Hopfield, J.J., Leibler, S. & Murray, A.W. From molecular to modular cell biology. *Nature* **402**, C47-52 (1999).
99. Segal, E., Friedman, N., Koller, D. & Regev, A. A module map showing conditional activity of expression modules in cancer. *Nature genetics* **36**, 1090-1098 (2004).
100. Costanzo, M. et al. The genetic landscape of a cell. *Science* **327**, 425-431 (2010).
101. Amit, I. et al. Unbiased reconstruction of a mammalian transcriptional network mediating pathogen responses. *Science* **326**, 257-263 (2009).
102. Bosco, A., Ehteshami, S., Panyala, S. & Martinez, F.D. Interferon regulatory factor 7 is a major hub connecting interferon-mediated responses in virus-induced asthma exacerbations in vivo. *J Allergy Clin Immunol* **129**, 88-94 (2012).
103. Bosco, A., McKenna, K.L., Firth, M.J., Sly, P.D. & Holt, P.G. A network modeling approach to analysis of the Th2 memory responses underlying human atopic disease. *Journal of immunology* **182**, 6011-6021 (2009).
104. Kohanski, M.A., Dwyer, D.J., Wierzbowski, J., Cottarel, G. & Collins, J.J. Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. *Cell* **135**, 679-690 (2008).
105. Carro, M.S. et al. The transcriptional network for mesenchymal transformation of brain tumours. *Nature* **463**, 318-325 (2010).
106. Quax, R., Apolloni, A. & Sloot, P.M. The diminishing role of hubs in dynamical processes on complex networks. *J R Soc Interface* **10**, 20130568 (2013).

107. Tanaka, G., Morino, K. & Aihara, K. Dynamical robustness in complex networks: the crucial role of low-degree nodes. *Sci Rep* **2**, 232 (2012).
108. Watts, D.J. A simple model of global cascades on random networks. *Proc Natl Acad Sci U S A* **99**, 5766-5771 (2002).
109. Watts, D.J. & Dodds, P.S. Influentials, Networks, and Public Opinion Formation. *J Consumer Res* **34**, 441-458 (2007).
110. Duijn, P.A., Kashirin, V. & Sloot, P.M. The relative ineffectiveness of criminal network disruption. *Sci Rep* **4**, 4238 (2014).
111. Luscombe, N.M. et al. Genomic analysis of regulatory network dynamics reveals large topological changes. *Nature* **431**, 308-312 (2004).
112. Shi, J., Li, T. & Chen, L. Towards a critical transition theory under different temporal scales and noise strengths. *Phys Rev E* **93**, 032137 (2016).
113. Liu, R., Wang, X., Aihara, K. & Chen, L. Early diagnosis of complex diseases by molecular biomarkers, network biomarkers, and dynamical network biomarkers. *Med Res Rev* **34**, 455-478 (2014).
114. Raj, A. & van Oudenaarden, A. Nature, nurture, or chance: stochastic gene expression and its consequences. *Cell* **135**, 216-226 (2008).
115. Banchereau, R. et al. Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. *Cell* **165**, 1548-1550 (2016).
116. Jourde-Chiche, N., Chiche, L. & Chaussabel, D. Introducing a New Dimension to Molecular Disease Classifications. *Trends Mol Med* **22**, 451-453 (2016).
117. Lesterhuis, W.J., Bosco, A. & Lake, R.A. Comment on "drug discovery: turning the titanic". *Science translational medicine* **6**, 229le222 (2014).
118. Gettinger, S.N. et al. Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. *J Clin Oncol* **33**, 2004-2012 (2015).
119. Tanaka, G., Hirata, Y., Goldenberg, S.L., Bruchovsky, N. & Aihara, K. Mathematical modelling of prostate cancer growth and its application to hormone therapy. *Philos Trans A Math Phys Eng Sci* **368**, 5029-5044 (2010).
120. Ehlerding, E.B., England, C.G., McNeel, D.G. & Cai, W. Molecular Imaging of Immunotherapy Targets in Cancer. *J Nucl Med* **57**, 1487-1492 (2016).
121. Schadendorf, D. et al. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. *J Clin Oncol* **33**, 1889-1894 (2015).
122. Euler, L. Solutio Problematis ad Geometriam Situs Pertinentis. *Commentarii Academiae Scientiarum Imperialis Petropolitanae* **8**, 128-140 (1736).
123. Watts, D.J. & Strogatz, S.H. Collective dynamics of 'small-world' networks. *Nature* **393**, 440-442 (1998).
124. Taylor, I.W. et al. Dynamic modularity in protein interaction networks predicts breast cancer outcome. *Nat Biotechnol* **27**, 199-204 (2009).
125. Le, D.T. et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* **372**, 2509-2520 (2015).

Competing interests statement

W.J.L., A.B. and R.A.L. hold a patent on a method for the identification of immunotherapy-drug combinations using a network approach.

Glossary

Immune checkpoints

Stimulatory or inhibitory pathways that regulate the scale of adaptive immune responses.

Cytotoxic T lymphocyte-associated antigen 4 (CTLA4)

An inhibitory immune checkpoint molecule, which is upregulated on effector T cells after activation where it competes for ligand with a stimulatory receptor CD28.

Programmed cell death protein 1 (PD1)

An inhibitory immune checkpoint receptor expressed on activated lymphocytes and highly expressed on exhausted T lymphocytes. Ligation results in an impairment of proliferation, cytokine production, cytolytic function, and survival of T cells.

Biomarker

A biomarker is a measurable indicator of normal biological processes, pathogenic processes, or biological responses to a therapeutic intervention. In oncology biomarkers are often used as predictors of disease outcome, such as prognosis or response to treatment.

T cell receptor repertoire

T cell receptors are formed by the random recombination of a set of germ line encoded elements with mechanisms to enhance junctional diversity. Thus genetically identical individuals can create unique T cell receptor repertoires.

Cytotoxic T cells

A subset of T cells, expressing CD8, that have the capacity to directly kill target cells, such as cancer cells or virus infected cells, after recognizing antigenic peptides on that cell.

Regulatory T cells (Treg)

A subset of CD4+ T cells that inhibit immune responses; they can down modulate the induction of a response and can limit the proliferation of effector T cells.

Complex system

A complex system is characterized by emergent behavior. The system itself may consist of a large number very simple parts interacting in simple ways, but with a large number of such interactions. The observed behavior of the entire system is not what one would immediately expect from the individual components.

Chaotic system

A chaotic system is one that exhibits irregular and apparently random variation, but is in fact completely described by a small number of deterministic equations. Chaotic systems are characterized by sensitive dependence on initial conditions (a small change in the initial state leads to a large change in outcome), extreme mixing (different initial conditions will eventually become arbitrarily close, for a short time), and, exhibiting bounded, deterministic, but aperiodic behavior.

Critical (state) transition

The behavior of complex systems typically changes smoothly (gradually) with changes in the system parameters. However, at a critical state transition a small perturbation to the system parameters will result in a sudden and dramatic change in the systems behavior.

Tipping point

A tipping point is the (biological) state at which a critical state transition occurs.

Graph Theory

The mathematical study of graphs, abstract structures used to model pairwise relations between members of a set. A graph in this context is made up of nodes that are connected by edges. Mathematical graph theory tends to focus on analysis of symmetry and structure, while in physics the same objects are called networks and the emphasis is on structures with a very large number of nodes and the description of the statistical interaction between them.

Nodes ('hubs')

Many complex systems are characterized by complex networks. A complex network is represented by a series of nodes connected by edges. One can visualize these nodes and edges as points (nodes) connected by line segments (edges). The edges determine which nodes are connected to which other nodes. Nodes that have a relatively large number of edges are called hubs.

Bottleneck nodes

A bottleneck node is a node with a central role in connecting other parts of a network. A large number of paths between random pairs of nodes will pass through a bottleneck node.

Transient hubs

Transient hubs are hubs that rise to prominence only for a short time. In a time varying network, a transient hub will have a large number of edges only for some of the time.

Subnetworks

A subnetwork is any subset of the nodes in a network and the edges connecting nodes within that subset. It is a piece of a network.

Dynamic biomarkers

Predictive biomarkers (i.e. associated with response to treatment) that are obtained after treatment has been initiated.

Static biomarkers

Predictive biomarkers obtained at a single time point.

Highlighted references

2. Wolchok, J.D. et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 369, 122-133 (2013).
 3. Robert, C. et al. Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. *New Engl J Med* 364, 2517-2526 (2011).
 4. Garon, E.B. et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 372, 2018-2028 (2015).
 5. Motzer, R.J. et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. *N Engl J Med* 373, 1803-1813 (2015).
 6. Ansell, S.M. et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med* 372, 311-319 (2015).
 7. Hodi, F.S. et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *New Engl J Med* 363, 711-723 (2010).
 8. Rosenberg, J.E. et al. Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 387, 1909-1920 (2016).
 9. Muro, K. et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 17, 717-726 (2016).
 10. Hamanishi, J. et al. Safety and Antitumor Activity of Anti-PD-1 Antibody, Nivolumab, in Patients With Platinum-Resistant Ovarian Cancer. *J Clin Oncol* 33, 4015-4022 (2015).
 11. Seiwert, T.Y. et al. Safety and clinical activity of pembrolizumab for treatment of recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-012): an open-label, multicentre, phase 1b trial. *Lancet Oncol* 17, 956-965 (2016).
- Refs. 2-11 are reports of clinical trials demonstrating the tremendous efficacy of immune checkpoint blockade in some cancer patients, and the relative inefficacy in others.**

28. McGranahan, N. et al. Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 351, 1463-1469 (2016).
29. Van Allen, E.M. et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. *Science* 350, 207-211 (2015).
30. Rizvi, N.A. et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 348, 124-128 (2015).

Refs 28-30 report on the correlation between pretreatment biomarkers and the response to immune checkpoint blockade.

39. Lesterhuis, W.J. et al. Network analysis of immunotherapy-induced regressing tumours identifies novel synergistic drug combinations. *Sci Rep* 5, 12298 (2015).

In this study, network analysis of transcriptomic data from immune checkpoint blockade responding and non-responding tumours in a mouse cancer model pinpointed response-associated molecular modules and hubs that could be targeted to improve the response rate.

45. Feinerman, O., Veiga, J., Dorfman, J.R., Germain, R.N. & Altan-Bonnet, G. Variability and robustness in T cell activation from regulated heterogeneity in protein levels. *Science* 321, 1081-1084 (2008).

Ref. 45 provides an example of how randomness at a cellular level is managed to result in controlled variability at a population level: the level of expression of two signalling proteins that drive T cell activation in a switch-like fashion is normally distributed across the population of cells, which has the potential to result in marked variability in

activation between T cell clones, but because these molecules are co-regulated, the diversity of the overall T cell response is ultimately limited.

44. Germain, R.N. The art of the probable: system control in the adaptive immune system. *Science* **293**, 240-245 (2001).

63. Hodgkin, P.D., Dowling, M.R. & Duffy, K.R. Why the immune system takes its chances with randomness. *Nat Rev Immunol* **14**, 711 (2014).

65. Hodgkin, P.D. A probabilistic view of immunology: drawing parallels with physics. *Immunol Cell Biol* **85**, 295-299 (2007).

Ref. 65, and also ref. 63 and 44 provide insightful perspectives on how the immune system functions on a -generally reproducible and robust- systems level and how this is shaped on a lower level through random and probabilistic events.

70. Scheffer, M. et al. Anticipating critical transitions. *Science* **338**, 344-348 (2012).

71. Scheffer, M. et al. Early-warning signals for critical transitions. *Nature* **461**, 53-59 (2009).

Refs 70 and 71 provide an excellent overview of critical transitions in many complex systems in nature and society.

72. Chen, L., Liu, R., Liu, Z.P., Li, M. & Aihara, K. Detecting early-warning signals for sudden deterioration of complex diseases by dynamical network biomarkers. *Sci Rep* **2**, 342 (2012).

73. Liu, R., Aihara, K. & Chen, L. Dynamical network biomarkers for identifying critical transitions and their driving networks of biologic processes. *Quant Biol* **1**, 105-114 (2013).

74. Liu, R. et al. Identifying critical transitions and their leading biomolecular networks in complex diseases. *Sci Rep* **2**, 813 (2012).

75. Liu, R., Chen, P., Aihara, K. & Chen, L. Identifying early-warning signals of critical transitions with strong noise by dynamical network markers. *Sci Rep* **5**, 17501 (2015).

Refs. 72-75 report on the development of mathematical models to identify dynamical network biomarkers that can predict a critical transition from a healthy to a disease state.

79. Ott, E., Grebogi, C. & Yorke, J.A. Controlling chaos. *Phys Rev Lett* **64**, 1196-1199 (1990).

This seminal paper introduces the idea of exploiting chaos to drive a chaotic system to an arbitrary desired state. The method introduced here has since become known as OGY control. In essence, one applies minute but well timed perturbations to the system to steer the trajectory toward the desired outcome.

92. Barabasi, A.L. & Oltvai, Z.N. Network biology: understanding the cell's functional organization. *Nature reviews. Genetics* **5**, 101-113 (2004).

93. Vidal, M., Cusick, M.E. & Barabasi, A.L. Interactome networks and human disease. *Cell* **144**, 986-998 (2011).

Refs. 92 and 93 review the principles behind and the application of network science in the field of biology.

106. Quax, R., Apolloni, A. & Sloot, P.M. The diminishing role of hubs in dynamical processes on complex networks. *J R Soc Interface* **10**, 20130568 (2013).

107. Tanaka, G., Morino, K. & Aihara, K. Dynamical robustness in complex networks: the crucial role of low-degree nodes. *Sci Rep* **2**, 232 (2012).

108. Watts, D.J. A simple model of global cascades on random networks. *Proc Natl Acad Sci U S A* 99, 5766-5771 (2002).

109. Watts, D.J. & Dodds, P.S. Influentials, Networks, and Public Opinion Formation. *J Consumer Res* 34, 441-458 (2007).

110. Duijn, P.A., Kashirin, V. & Sloot, P.M. The relative ineffectiveness of criminal network disruption. *Sci Rep* 4, 4238 (2014).

111. Luscombe, N.M. et al. Genomic analysis of regulatory network dynamics reveals large topological changes. *Nature* 431, 308-312 (2004).

Refs. 106-111 provide examples of the limitations of using network analysis of static data for the interpretation of dynamical processes in complex systems.

112. Shi, J., Li, T. & Chen, L. Towards a critical transition theory under different temporal scales and noise strengths. *Phys Rev E* 93, 032137 (2016).

Ref. 112 provides a mathematical framework from which to develop a critical transition theory as a function of three time scales - the time scale of instability in the system characterization, the dynamical time scale of state transition within the stable system, and the time scale associated with averaging of system behavior (and hence the effect of noise). Rather than providing a complete solution to the problem of critical transition, this paper provides a road map and minimal model for understanding such transitions in both the large and small noise case, and for intrinsic and extrinsic transitions.

Short biosketches of the authors

Dr. W. Joost Lesterhuis, MD (medical oncology), PhD (tumor immunology), graduated in medical oncology in 2010 at the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands and is now a research fellow and group leader at the School of Medicine and Pharmacology, University of Western Australia, Perth, Australia. Dr. Lesterhuis' research focuses on immune checkpoint blockade in cancer, particularly on identifying new combination therapies in preclinical models using systems biology approaches and translating those into clinical trials.

Dr. Anthony Bosco obtained his PhD from the University of Western Australia in 2007 and subsequently held post-doctoral research positions at the Telethon Kids Institute, Perth, Australia and the Arizona Respiratory Center and BIO5 Institute, University of Arizona, Tucson, AZ. He currently is Team Leader Systems Immunology at the Telethon Kids Institute. Dr. Bosco's research focuses on reverse engineering of immune response networks from genomic profiling data.

Prof. Michael Millward is a medical oncologist at Sir Charles Gairdner Hospital and Professor of Clinical Cancer Research within the School of Medicine and Pharmacology, University of Western Australia, with research and clinical interests in melanoma, thoracic oncology, and early Phase clinical trials. He has published widely on clinical and translational aspects of melanoma and lung cancer, and lead many early phase trials of immunotherapy treatments. Prof. Millward was the President of the Australasian Lung Cancer Trials Group until August 2012.

Prof. Michael Small is the CSIRO-UWA Chair in Complex Engineering Systems in the School of Mathematics and Statistics at the University of Western Australia. His primary interest is in characterization and modeling of complex systems and using this technology to understand both natural and engineered systems. His research largely focuses on complex networks, nonlinear dynamics and chaos, and collective behavior. He is editor of *Chaos: An Interdisciplinary Journal of Nonlinear Science* and of *International Journal of Bifurcations and Chaos*.

Prof. Anna K. Nowak is a medical oncologist at Sir Charles Gairdner Hospital and Professor within the School of Medicine and Pharmacology, University of Western Australia, with research and clinical interests in malignant mesothelioma and neuro-oncology. She was amongst the earliest researchers demonstrating that chemotherapy and immunotherapy could be synergistic treatment modalities in cancer treatment, rather than antagonistic. Prof. Nowak has lead a number of clinical trials of chemo-immunotherapy in mesothelioma.

Prof. Richard A. Lake is a molecular immunologist at the School of Medicine and Pharmacology, University of Western Australia where he is deputy director of the National Centre for Asbestos Related Diseases. His primary research interest is tumor immunology, both in preclinical models and patient trials, with particular focus on T cell-directed immunotherapy. Prof. Lake was one of the pioneers of the field of combination chemo-immunotherapy.