How to assess response to immune therapy

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Abstract

Immunotherapy is already a mainstay in treating melanoma, lung cancer and renal cancer and shows compelling promise in many different tumors. However, responses to immunotherapy may be present despite apparent initial progression of the tumor. This underlines the importance of defining accurate tumor assessments and response criteria for immunotherapy. The RECIST criteria have therefore been modified to adhere to requirements of immunotherapy. In line with that, this review will focus on the current imaging tools, the statistical evaluations in clinical trials and the biological analysis of the tumor microenvironment that will in the future effectively guide treatment decisions in everyday clinical practice.

Key words: microbiota, PD-L1, pseudo-progression, RECIST, local and peripheral immune response

Introduction

The breakthrough of immunotherapy as an efficient treatment in the fight against multiple cancers challenges the classical response criteria assessments. The release of pre-existing antitumor immunity by impairing inhibitory receptors of the immune checkpoints (e.g. cytotoxic T-lymphocyte antigen 4, CTLA4; programmed cell death protein 1, PD-1 or its ligand PD-L1) have shown very encouraging clinical results in survival in aggressive tumors (i.e. melanoma, lung cancer, renal, urothelial, head and neck carcinoma) [1-3]. The survival of patients is considerably increased in first line treatment for melanoma and non-small cell lung cancer [4-6]. However the evaluation of immune response assessments remains a challenge with the current imaging procedures. In particular tumor shrinkage may be delayed (i.e. 2 to 3 months after treatment initiation) and in few patients an initial increase in tumor size or amount of tumor lesions may be observed [7]. In order to distinguish pseudo-progression from true tumor progression, current standard response criteria may not be adequate to assess response to and progression with immunotherapeutic agents and may cause the failure of active drugs in development. The evaluation of the immune responses (i.e. local and systemic) might complement the clinical and imaging responses. Decrypting the tumor and its microenvironment may guide the definition of 'cancer immunograms' in the quest for effective therapies for an individual patient [8]. Two major questions may challenge the success of the clinical development of immunotherapies: (i) how to best assess the efficacy of immunotherapies, (ii) how to identify patients that may benefit from immunotherapies.

Main innovations

Imaging

Immunotherapies lead to unusual patterns of tumor responses. In the first trials of immunotherapy in metastatic melanoma, some patients developed disease progression followed by partial response or even late complete responses. Since retrospective analysis identified that increased size of lesions was not caused by tumor growth but predominately by immune cell infiltration it was called "pseudo-progression". Such a response is described in melanoma in about 10% of patients.

The initial progression can occur either with an increase in the sum of measures of target lesions and/or unequivo-

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cal increase in non-target disease and/or the appearance of new lesions.

Modified response criteria called irRC (for immune related Response Criteria) were proposed in order to capture that kind of unique pattern of responses [9]. The authors based their criteria on the World Health Organization (WHO) criteria (i.e. bi-dimensional measurement and a total number of lesions of up to 10; 5 lesions per organ). They introduced a new way to evaluate tumor response by adding the measurement of eventual new lesions greater than 5×5 mm to the sum of initial target lesions. They also authorized the addition of measurement of non-target lesions if they reach the size of 10×10 mm. The response was then assessed by the percentage of variation of the new sum compared to baseline (or Nadir) sum, even if the lesions were not the same. These principles are contrary to that of Response Evaluation Criteria In Solid Tumors (RECIST) 1.1 assessment, which favors the definition of representative target lesions rather than assessment of the whole tumor burden. Moreover, by adding each pattern of progression in a single figure, it is impossible to understand the mechanisms of progression compared to pseudo-progression for meta-analyses. Second, the authors defined a principle of re-setting the baseline to the date of suspicion of progression, if the subsequent assessment did not confirm the progression. Moreover, there was no clear definition of radiological confirmed progression versus unconfirmed progression.

New criteria for immunotherapy are awaited, based on RECIST 1.1 criteria, and should solve some of these issues: they should overrule the possibilities of resetting baseline and adding new lesions in the sum of initial target lesions. They would authorize the resumption of therapy in case of progression, until the progression is confirmed by an anticipated scan 4 to 8 weeks later. There will still be issues concerning the outcome of patients while they continue therapy, whereas the likelihood of pseudo-progression would only be of concern in 10% of patients. However, at this time, there is no way to distinguish true progression from pseudo-progression at the first occurrence of a radiological progression, and further work is required to understand the phenomenon and guide the clinicians' decision.

Statistical evaluation

The aim of anticancer drugs is to prolong life, to cure patients or to delay disease progression and recurrence. When evaluating the efficacy of anticancer therapies, overall survival is therefore the primary outcome of interest.

However, the effect of an experimental drug on overall survival is not always observed within the duration of early phase clinical trials. Furthermore, ethical issues could arise: when promising results have been observed from early phase trials, it is difficult to conceive randomized controlled trials without allowing cross-over from standard arm to experimental arm that may however dilute the benefit of the experimental arm.

This may explain the quest for surrogate endpoints of overall survival that should fulfil several criteria, introduced by Prentice in 1989 [10], to be validated in a given cancer location and for a given treatment:

- surrogate endpoint is a predictor of overall survival,
- surrogate endpoint should capture fully the effect of treatment on overall survival.

Since this original definition, criteria to validate surrogate markers have been extended (Figure 1) [11]. Of note progression occurs earlier than death and is on the causal pathway between cancer and death for different cancer diseases. Progression-free survival has been validated as a surrogate outcome for some treatments in some cancers [12]. Once progression-free survival has been validated as a surrogate for overall survival, it could be used as a primary endpoint in clinical trials. In that context, cross-over at progression could be allowed in randomized clinical trials and no bias or dilution effect may be triggered by the intention-to-treat analysis.

In the presence of pseudo-progression, the increase in tumor size would be usually considered as a progression. However this may reflect neither an increase in cancer cell number nor a treatment failure.

One statistical issue is to properly model the response to treatment from repeated tumor size measurements in the context of pseudo-progression. When a progression occurs under immunotherapy, it is not possible to determine if it is a "real" progression or a pseudo-progression and a clinical decision is taken: to continue or to interrupt the treatment. Complex statistical models could be used to deal with the existence of pseudo-progression. Actually, pseudo-progression could be seen as a hidden state since at the time of imaging it is not possible to determine if the observed increase in tumor size is a progression or not. Hidden Markov models have been introduced in the context of hidden state. They would allow the computation of transition rates from pseudo-progression to confirmed progression or to response. Since transition rates could be computed, factors that influence the transition rates could be identified. These factors would be markers of progression or response.

Since immunotherapy has been postulated to act not directly on tumor size but by modulating the tumor environment, alternative surrogate endpoints such as objective response rates have been proposed as potential accurate surrogates (Figure 1); their evaluation is pending.
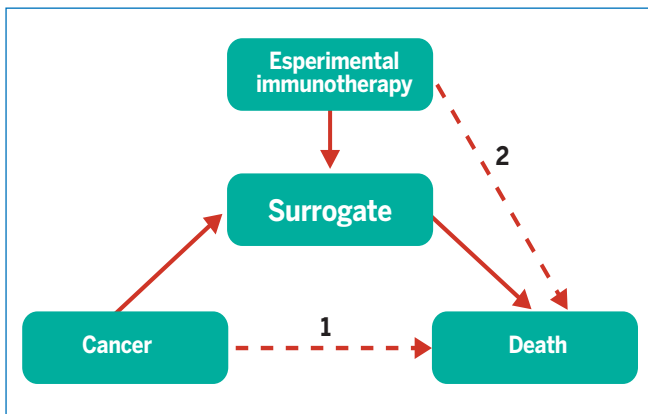
Fig. 1. A surrogate marker may be valid if the effect of cancer on death (1) and of treatment on death (2) could be identified (modified from: [13]).

Tumor microenvironment assessment

The tumor biology may help to predict and assess the response to immunotherapy either by eliciting the local or peripheral immune response. To assess the innate and adaptive immune response (tumor cell infiltration, pharmacodynamic biomarkers of immunotherapy, including cytokine profiles, biomarkers of tumor aggressiveness constituting an immunological "barrier") sequential samples (i.e. tumor, blood and stool) may be collected before and after treatment with immunotherapies. Current major tumor (e.g. the immunogenic cell death induction, the PD-L1 expression, the amount of neoantigens) and immune features (e.g. the immune infiltration, cytokine profile, immune activation by the microbiota) of interest will be developed in the following chapter.

Extensive immunohistochemical (IHC) assessment of relevant immunomarkers at baseline and after treatment may help to evaluate the local immune response. Different aspects may be explored using IHC: (i) immunogenic cell death [i.e. calreticulin (and phosphorylated eif2α)] [14], HMGB1 [15], LC3B [16], and Mx1 [17], (ii) the T-cell infiltrates [i.e. CD3, CD8, Foxp3, and CD8/Foxp3 ratio (Teff/Treg ratio)], the antigen-presenting cells (APCs): CD68, CD163 (immunosuppressive APCs); DC-LAMP, CD1a (dendritic-cell APCs) and natural killer cells (NK) (anti-NKp46) and (iii) the programmed cell-death ligand 1 (PD-L1) protein.

Some assays may be guided by laser microdissection of specific areas in frozen tumor tissues. Moreover flow cytometry analyses on fresh tissue sections of tumor-infiltrating lymphocytes (TILs) using specific fluorochrome-labeled antibodies will help to quantify, in the CD45+ fraction of cells, the percentages of the different cell subtypes. The immunogenic cell death of tumor cells is known to be induced by different anti-cancer agents (e.g. oxaliplatin, doxorubicin, radiotherapy) and triggering the adaptive immune response by releasing damage associated molecular patterns [i.e. calreticulin, ATP, high-mobility group protein box-1 (HMGB1) release and type I interferon production] [17-21].

The PD-L1 expression that is measured by IHC assays on tumor cells [6] and tumor infiltrating lymphocytes [22, 23] may help to predict which patients are more likely to respond to immune checkpoint blockers (ICB) [i.e. anti-programmed cell death-1 (PD-1)/PD-L1 antibodies].

Immune infiltration is reputed to be prognostic in many different tumors [24-26], and the tumor antigen-specific cytotoxicity of the immune infiltration may be determined by tumor TNF-α expression [27]. Furthermore, an immunoscore, evaluating T-cell functions as determined by Dr Jérôme Galon and colleagues, is a prognostic and a potential predictive tool for the response to immunotherapy [28]. The immunoscore may be determined by means of quantitative polymerase chain reaction (qPCR) on baseline and post-treatment tumor samples, using probe sets to detect (i) pro-Th1 [e.g. T-bet (Tbx1), Cxcl9, Cxcl10, Cxcl11, Ccl5, IFNγ], (ii) pro-Th2 (e.g. GATA3, STAT5, STAT6, IL-2, IL-4, IL-5, IL-13), (iii) pro-T regulator (i.e. IL-10, TGFβ, Foxp3) and (iiii) gene products associated with dendritic cell differentiation (e.g. Flt3L, Batf3, Csf1, Csf2).

Moreover the amount of tumor neoantigens that are formed as a consequence of a hypermutated status and subsequent DNA damage may be evaluated [29]. The neoantigen repertoire may reflect the genetic instability in different human cancers, e.g. in gynecological cancers (i.e. breast cancer mutation BRCA and the polymerase e POLE mutation) [30, 31] in colorectal cancers (i.e. microsatellite instability MSI status) [32] and in lung cancer (i.e. smoking status) [33, 34]. Tumor neoantigens enhance broad specific T-cell reactivity and may therefore increase the clinical activity of immunotherapies (e.g. ICB) [35].

Furthermore, tumor aggressiveness and immune barriers may be evaluated on tissue sections. Several chemokines (i.e. CCL5) expressed by lymphocytes at the invasive margin of metastasis are reputed to elicit pro-tumoral effects (i.e. tumor proliferation and invasion), as the corresponding receptors (i.e. CCR5) are expressed by tumor cells [36]. Moreover the tumor endothelium is of particular interest in colorectal cancer (CRC) and ovarian cancers as (i) it may constitute a barrier for T lymphocytes and (ii) vascular endothelial growth factor (VEGF) is a key therapeutic target. Fas ligand (FasL) activating the extrinsic apoptotic pathway, may be involved in the down-regulation of CD8+ effector cells but not T regulator cells. Indeed tumor endothelial cells that express FasL are able to kill TILs. Of note CRC and ovarian cancers have the most FasL expression [37]. VEGF receptor

(VEGFR) and Fasl expression in tumor endothelial cells is evaluable by IHC.

The peripheral immune response may be evaluated by flow cytometry analysis of peripheral blood mononuclear cells (PBMC). The percentages of naive and effector/memory T cells and T regulators may be evaluated. Moreover the T-cell activity against telomerases can be assessed to evaluate T cell responses directed against shared tumor antigens [38].

Finally gut dysbiosis may be responsible for defects in the activation of the adaptive immune responses. In line with that, the efficacy of ipilimumab in patients with metastatic melanoma may correlate not only with the amount of neoantigens but, in particular, with patients showing a prerequisite adaptive immune response directed against specific neoantigens that show a strong homology with certain bacteria (e.g. *Burkholderia pseudomallei* antigen) [29]. Furthermore, monitoring the peripheral CD4+ T-cell memory responses against distinct species of bacteria and the changes of the gut microbiome over time using pyrosequencing of rRNA gene amplicons on stools have been shown to be associated with patient outcomes in some clinical settings (e.g. *Bacteroides fragilis* and *B. thetaiotaomicron* and *Barnesiella intestinihominis* for ipilimumab responses in melanoma, *Enterococcus hirae* and *B. intestinihominis* for metronomic cyclophosphamide in lung cancer, and *Burkholderia cepacia* for chemotherapy in lung cancer) [39-41]. Stools are collected before and after therapy to process metagenomics and metatranscriptomics of feces that may be of particular interest in assessing the response to immunotherapies [42, 43].

Future challenges

The EORTC guidelines for the evaluation of immune therapy activity by imaging are awaited. They will be based on currently used RECIST criteria and oriented for everyday practice. Furthermore, an extensive assessment of the phenotypic and functional dynamics of tumor immune infiltrates, at baseline and post-treatment samples, may efficiently guide treatment decisions in the near future. Thus systematic biopsies, blood and stool collections are warranted. The collaboration between radiologists, statisticians, researchers, surgeons and oncologists will be the cornerstone in that success story.

Acknowledgments

Conflicts of Interest

The Authors declare there are no conflicts of interest in relation to this article.

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