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Towards a better cancer precision medicine: systems biology meets immunotherapy

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Abstract

Systems biology approaches that embrace the complexity of cancer are starting to gain traction in the development of new anticancer therapeutic strategies. In this review we describe how genomic analyses are helping improve our understanding of response to immunotherapy, a front-runner in cancer treatment. We argue that systems-level approaches are needed to help understand the concerted impact of tumor-specific and immune-specific molecular features on clinical outcomes, predict responders and unravel the complexity of tumor ecosystems. This integrated approach will propel immunotherapy into the exciting world of precision medicine.

Keywords

systems biology; immunotherapy; cancer genomics; whole exome sequencing; RNA sequencing; T-cell receptors; neoantigens; HLA typing; immuno-predictive score

Introduction

Genomic abnormalities, subclonal heterogeneity and rapid adaptation are among the key hallmarks of cancer, responsible for tumor initiation, maintenance and progression [1,2]. Genetically unstable tumors have a propensity to rapidly evolve, adapt to new sites and overcome both broadly cytotoxic and targeted therapies. Perhaps the most important consequence of a tumor's capacity to constantly rewire itself under diverse selection pressures is its ability to evade the body's natural defense mechanisms [1,2]. This ability of tumors to adapt, together with the complexity of crosstalks with non-malignant populations in the microenvironment explain some of the challenges encountered in cancer therapeutics development. Indeed the failure rate of pre-clinically promising anticancer molecules is very

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COMPETING INTERESTS

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high at all stages of human clinical trials; very few patients show complete remission and many acquire resistance to therapies [3–5].

Fortunately, approaches that embrace the complexity of cancer, integrate multiple layers of biological activity, model and predict cancer behavior are increasingly being developed and adopted. Such approaches, broadly falling under the umbrella of cancer systems biology, have the potential to guide the development and clinical use of improved and personalized therapies [5–8]. The field of cancer systems biology has greatly benefited from omics technologies that provided valuable insights into the molecular etiologies of cancer. Indeed the emergence of affordable high-throughput sequencing (HTS) techniques that can scan a patient's entire tumor genome and help identify patient-specific actionable targets has ushered the biomedical community into an era of precision medicine [9,10,11].

In the fight against cancer, new weapons are also being invented and perfected. Immunotherapy, the roots of which lie in over a century ago, was recently revived by the success of adoptive cell therapy and monoclonal antibodies, especially those designed to inhibit immune checkpoints [12–14] (Figure 1). Under adequate conditions, it has been suggested that immunotherapy might provide a better alternative with improved clinical outcomes compared to conventional abrasive therapies [15] (Figure 1). Unfortunately, although some patients benefit tremendously from immunotherapy, many others are completely resistant to it [16–20]. Understanding the mechanisms that confer either resistance or long-term benefit from immunotherapy is an important goal. Such mechanisms would likely lead to more effective and less toxic forms of immunotherapy. Since the success or failure of immunotherapy are likely the results of an often complex interplay between tumor and immune mechanisms, it is compelling to hypothesize that systems biology approaches that consolidate molecular, immunological, and omics features, can progressively help understand the determinants of differential clinical responses.

The 'omics' correlates of immunotherapy outcomes

The outcome of immunotherapy treatment depends on ability of a patient's immune system to actively survey, identify and destroy tumor cells. Tumors have a propensity to adopt strategies such as loss of immunogenicity and gain of immunosuppression to escape immune system mediated elimination [2,21,22]. Integrative HTS data analyses have unraveled factors that shape sensitivity to immunotherapy, through modulation of immune-escape and immunogenic mechanisms. Some of these factors are, as discussed below.

Mutation burden

Mutation burden is hypothesized to impact tumor immunogenicity [14]. Retrospective genomic studies in metastatic melanoma, and non-small cell lung cancer (NSCLC) patients treated with either PD1 or CTLA4 antibodies revealed that clinical responders had high median mutation burdens than non-responders [16–18,23]. Metastatic carcinoma patients that harbored mismatch repair (MMR) deficiency, hence usually a high mutation burden, also exhibited longer progression free survival (PFS) after treatment with PD1 antibody [24]. Although mutation burden is positively correlated with immunotherapy outcomes, it performs poorly as a clinical indicator of future response. Indeed certain patients with low

mutation burden do benefit from immunotherapy while certain others with higher mutation burden fail to do so [16–18,23]. Additionally, tumors with traditionally low mutation burden such as gastrointestinal cancers have shown abundant tumor specific T-cell infiltration and hence also thought to be suitable candidates for treatment with immunotherapy [25,26].

Tumor-specific neoantigens

Mutations, while not directly visible to the immune system, drive immunogenicity by manifesting as tumor-specific antigenic peptides, called neoantigens, on a tumor cell's surface [14]. A patient's unique human leukocyte antigen (HLA) type and mutation landscapes can now be computationally inferred from HTS data and further utilized to predict patient-specific neoantigen profiles *in silico* [27–29]. A handful of similarly predicted neoantigens have been validated to elicit a neoantigen-specific autologous T-cell mediated immune response *in vitro* [16,23,30–32]. Functionally, neoantigen burden is positively correlated with mutation burden as well as PFS, as shown in 34 pembrolizumab treated NSCLC patients [23]. Recently, McGranahan *et al.*, provided insights into additional traits of neoantigens [33]. Briefly, they applied a variation of PyClone statistical model to infer clonal (i.e., present in all tumor cells) *versus* subclonal neoantigens in NSCLC patients treated with pembrolizumab and found that patients with longer PFS harbored not only a high mutation load but also a very high proportion of clonal neoantigens [33]. This finding corroborates the notion that clonal neoantigens tend to further enhance anti-tumor immunity compared to non-clonal and less abundant neoantigens [22,33].

Neoantigens as predictive biomarkers

Despite advances in neoantigen characterization, it has been difficult to use neoantigens as predictive biomarkers of immunotherapy response due to several reasons: a) Neoantigen profiles are mostly unique to each patient possibly because the majority of neoantigens originate from passenger mutations [14,16,17,34], b) Neoantigen repertoires might evolve dynamically over time due to genomic instability of the tumors and selection pressures exerted by the immune system; Verdegaal *et al.* for example have shown loss of immunogenic neoantigens in sequential lesions obtained from two melanoma patients [34], c), The accuracy of computational models utilized to construct neoantigen profiles have not been broadly tested; these models may need to be specifically trained on large datasets of tumor isolated neoantigens that are experimentally determined by techniques such as mass spectrometry [35], and d) The neoantigens, albeit predicted in high numbers, might not be visible to the immune system due to disruptions in the antigen processing/presentation machinery. Giannakis *et al.*, for example have shown that colorectal tumors with high neoantigen burden and high immune infiltration (hence favorable anti-tumor immune environment) also harbored immune-evasive mutations, such as those at certain HLA allelic loci and B2M [36]. These mutations might negatively regulate neoantigen presentation on tumor cell's surface, and possibly provide alternate mechanisms of immune escape for immunogenic tumors.

Elucidating immunosuppression

Tumor cells orchestrate a variety of immunosuppressive strategies that lead to dysfunctional T-cell differentiation, T-cell exhaustion, and stromal remodeling to establish an

immunosuppressive environment [37–39]. Recent successes of checkpoint blockade therapies suggest that tumors adopt immunosuppression in part by modulation of the immune-checkpoint pathways. Melanoma patients responding well to CTLA4 antibody indeed have higher median expression of CTLA4 (T-cell inhibitory checkpoint gene) and cytolytic enzymes GZMA and PRF1 (important for T-cell mediated tumor lysis) [17]. Furthermore, differential gene expression analysis in melanoma patients treated with PD1 antibody revealed that non-responders are associated with a transcriptional signature that includes genes required for mesenchymal transition, hypoxia and angiogenesis [18]. Interestingly, the products of these genes promote abnormal tumor vasculature that hinders efficient immune cell infiltration and function, thereby fostering an immunosuppressive environment. Remodeling defective tumor vasculature by normalizing doses of anti-angiogenesis VEGFR2 antibody and its effects on improving efficacy of whole cell vaccine have been demonstrated in breast cancer mouse models [40]. In the future, as gene expression profiles from patients treated with immunotherapy become readily available, understanding and collectively tackling broad-spectrum immunosuppressive mechanisms will likely provide avenues to improve immunotherapy outcomes. Meanwhile, applying innovative unbiased systems approaches on currently available large gene expression cohorts such as The Cancer Genome Atlas (TCGA) data sets can help predict molecular markers associated with immunosuppression. For example, using unsupervised hierarchical clustering based on the expression of a panel of known immune genes, melanoma and bladder tumors could be classified as either T-cell inflamed or T-cell non-inflamed and the classification was validated using immunohistochemistry (IHC) [41,42]. This strategy together with differential gene expression analysis between T-cell inflamed and non-inflamed melanoma tumors enabled Spranger *et al.*, to identify a novel immunosuppressive role of WNT/ β -catenin pathway through the mediation of CD8⁺ T-cell exclusion [41]. In another data driven approach, Rooney *et al.*, defined a simple measure to quantify immune cytolytic activity, calculated as the geometric mean of the RNA expression of cytolytic enzymes GZMA and PRF1 [43]. Their assessment of 18 distinct TCGA tumor types showed that the cytolytic activity varied by tumor-type, and positively correlated with mutation burdens [43]. Factors identified through such strategies need to be systematically evaluated for their plausible impact on immunotherapy outcomes.

Immune architecture and dynamics in cancer: implications for immunotherapy

Prognostic significance of tumor infiltrating immune cells

Tumor infiltrating lymphocytes (TILs) impact anti-tumor responses, and TIL features, such as their type, location and density hold prognostic relevance in patients treated with conventional therapies across numerous tumor types [44,45]. Denkert *et al.*, proposed TIL levels to be the best independent predictor of complete response to neoadjuvant chemotherapy in breast cancer patients; this observation was made using a multivariate logistic regression model including various immuno-histopathology parameters collected from 1,058 pre-treated breast cancer biopsies [45]. Traditionally known as prognostic biomarkers of conventional therapies, TIL levels are now emerging as prognostic biomarkers of checkpoint blockade immunotherapy. Recently, Tumeh *et al.* reported that metastatic

melanoma patients who responded well to PD1 antibody had a higher pre-existing cytotoxic T-lymphocyte (CTL) density, which increased further post immunotherapy [20]. Additionally, their stepwise logistic regression model identified CTL density at the invasive tumor margin as the best predictor of radiographic tumor shrinkage after PD1 antibody treatment [20].

Relevance of immune cell composition in anti-tumor immunity

TIL's cellular composition is predictive of their ability to mount an anti-tumor response. To demonstrate this, Oved *et al.*, characterized constituent immune cell-types in 91 metastatic melanoma TILs using flow cytometry, and measured individual TIL reactivity using interferon- γ release assay. Combining these data using an integrative approach, they reported that TIL reactivity was best predicted by relative abundance of various cell-types, and not by any single cell-type, and that TIL reactivity could be restored *in vitro* by selectively manipulating relative cell-type fractions [46]. Traditional cell sorting approaches, such as flow cytometry, are confined to a narrow range of known phenotypic markers. As alternatives, integrative computational models have been developed to deconvolute cell-type heterogeneity from gene expression data [47,48]. For example, the CIBERSORT method uses support vector regression together with reference cell-type expression signatures derived from purified tissues to estimate relative cell-type abundances based on gene expression profiles of tumors [47]. "Virtual" cell sorting approaches have an added advantage over traditional approaches in terms of their applicability to frozen and paraffin-embedded samples. In another deconvolution approach, Spitzer *et al.*, constructed mass cytometry data driven force-directed graphs to cluster immune-cell populations based on their phenotypic similarities; the resulting graphs were representative of canonical immune cell organizations in various tissue types [49]. Such methods applied to the tumor microenvironment would likely enable identification of deviations from immune homeostasis, which are inherent to tumors.

Dynamic modeling of tumor-immune interactions

The dynamic crosstalk between tumor and various immune compartments regulate tumor progression; Mlecnik *et al.* for example showed changes in immune infiltrates especially local CTL depletion prior to onset of metastasis in primary colorectal tumors [50]. Understanding dynamic tumor-immune interactions becomes especially relevant in immunotherapy because the efficacy of this line of therapy depends on each patient's immune state, which constantly evolves. Murine models mimicking human TME have been conventionally used to study dynamic interactions between various immune compartments [51,52]. Systems-level mathematical modeling, such as Ordinary Differential Equations (ODE), have also provided avenues to simulate intricate tumor-immune dynamics [53–55]. For example, Kronik *et al.*, built a personalized ODE model based on the immune-, tumor- and pharmaco- kinetics of 15 prostate cancer patients in phase 2 clinical trials for an allogeneic whole-cell vaccine. This model, once trained on patient's initial PSA kinetics, accurately predicted sequential PSA level changes in 80% of patients. Additionally, Kronik *et al.*, also suggested personalized immunotherapy regimens for patients by simulating the effect of variations in vaccine's timing and dosage on response [55]. Of note, mathematical models frequently require making simplifying assumptions such as exponential tumor

growth that might not consistently hold true. Nonetheless, such models can elegantly capture some important aspects of the temporal dynamics of TME, generate testable predictions and guide improvements in personalized immunotherapy.

T-cell receptor repertoires profiling

Intratumoral T-cell receptor (TCR) diversity (i.e., number of distinct TCRs) and clonality (i.e., frequency of particular TCRs) are emerging as important correlates of anti-tumor response. Evidence suggests that neoantigens drive TCR diversity [56,16,23,30–32]. Besides, TCR diversity and clonality may impact clinical outcomes of the administered therapy. For example selective expansion of TCR clones (high clonality) was associated with poor survival in diffuse large B-cell lymphomas (DLBCL) patients receiving combination chemotherapy [57]. Conversely, high clonality was associated with improved survival in melanoma patients receiving anti-PD1 immunotherapy [20,57]. Furthermore, administered therapy may also remodel the TCR profiles. For example, a preference towards polyclonal TCR expansion was observed in melanoma and breast cancer patients after treatment with anti-CTLA4 immunotherapy [19,58]. Moving forward, deep sequencing and single cell sequencing technologies will continually enable more precise quantification and characterization of TCR repertoires across diverse tumor types [58]. Importantly, this will enable identification of pre-existing TCR repertoire patterns possibly predictive of patient's susceptibility to specific type of immunotherapy.

Establishing an Integrative Immuno-predictive score

Recently, Colli *et al.*, built a receiver operating characteristic curves (ROC) model to predict response to immunotherapy exclusively based on mutation burdens, [59]. Such univariate approaches are however unlikely to recapitulate all multifaceted and complex aspects of anti-tumor immunity and will likely fail to be broadly predictive. Blank *et al.*, recently conceptualized a cancer immunogram that is aimed towards establishing comprehensive patient-specific immune profiles, highlighting several possible determinants of immunotherapy response [60]. Besides, additional features may also need to be identified and explored for their significance as clinical response predictors. As an example, copy number alterations especially those of PDL1/PDL2 have now been shown to negatively correlate with PFS in Classical Hodgkin Lymphoma and NSCLC patients [61,62]. Moving forward, there is a need to combine these immune profiles into an intuitive interpreter of clinical response. On that note, we envision an integrative systems approach leading to a data-driven multivariate immuno-predictive score, which would integrate both tumor-specific and immune-specific factors so as to determine the likelihood of response to immunotherapy in a patient (Figure 2).

Conclusion

A variety of mechanisms and regulatory networks controlling immunogenicity and immune-suppression are responsible for differential immunotherapy outcomes. The complexity of these mechanisms and networks once embraced will lead to systems-level, integrative approach that combines both tumor and immune molecular features, captures the interactions between tumor and non-tumor cells. This will in turn lead to patient-specific

immunotherapy response predictors that will pave the way for a precision approach to immunotherapy.

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ABBREVIATIONS

CTL	Cytotoxic T-lymphocyte
HLA	Human leukocyte antigen
HTS	High-throughput sequencing
IHC	immunohistochemistry
MMR	Mismatch repair
NSCLC	Non-small cell lung cancer
TCGA	The Cancer Genome Atlas
TCR	T-cell receptor
TIL	Tumor infiltrating lymphocytes
TME	Tumor's microenvironment

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Figure 1. Chronological view of the history of immunotherapy based therapeutics

BiTE: Bispecific T cell Engager; FDA: Food and Drug Administration, USA; HBV:

Hepatitis B Virus; mAb: Monoclonal Antibody; IMiDs: Immunomodulating Drugs; OS:

Overall Survival; PFS: Progression Free Survival; * FDA approval not applicable;

**Checkpoint mABs in clinical trials stage III

Figure 2. Establishing an integrative immuno-predictive score

Whole exome sequencing (WES) and RNA sequencing (RNA seq) analyses provide key insights into the TME specifically regarding drivers of immunogenicity (mutation and neoantigen burdens) and immunosuppression (immune-gene expression, cell types in TME, T-cell receptor repertoires). These features can be combined using a systems-level approach into an immuno-predictive score, which would serve as a personalized predictor to guide treatment options for patients identified as suitable for immunotherapy.