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Review article

Leveraging high-resolution omics data for predicting responses and adverse events to immune checkpoint inhibitors

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ABSTRACT

A long-standing goal of personalized and precision medicine is to enable accurate prediction of the outcomes of a given treatment regimen for patients harboring a disease. Currently, many clinical trials fail to meet their endpoints due to underlying factors in the patient population that contribute to either poor responses to the drug of interest or to treatment-related adverse events. Identifying these factors beforehand and correcting for them can lead to an increased success of clinical trials. Comprehensive and large-scale data gathering efforts in biomedicine by omics profiling of the healthy and diseased individuals has led to a treasure-trove of host, disease and environmental factors that contribute to the effectiveness of drugs aiming to treat disease. With increasing omics data, artificial intelligence allows an in-depth analysis of big data and offers a wide range of applications for real-world clinical use, including improved patient selection and identification of actionable targets for companion therapeutics for improved translatability across more patients. As a blueprint for complex drug-disease-host interactions, we here discuss the challenges of utilizing omics data for predicting responses and adverse events in cancer immunotherapy with immune checkpoint inhibitors (ICIs). The omics-based methodologies for improving patient outcomes as in the ICI case have also been applied across a wide-range of complex disease settings, exemplifying the use of omics for in-depth disease profiling and clinical use.

1. Introduction

Translating therapeutics for clinical use requires the initiation and successful completion of a clinical development process consisting of a series of clinical trials in order to verify the efficacy and safety of a given treatment. This high-risk process requires an estimated average of 10–15 years and 2 billion dollars for bringing an individual drug to market [1]. In fact, 90% of drugs that advance from a preclinical stage to Phase I trials do not make it to market [2]. Studies investigating the clinical trial data collected between 2016 and 2018 attribute the reasons for these failures to: insufficient clinical efficacy or excessive toxicity (79%), strategic realignment (13%), commercial reasons (7%) and operational or technical shortcomings (1%) [3]. As such, the main factors contributing to the failures in bringing new drugs to the market can be categorized to intrinsic factors, i.e. non-specificity to the molecular targets, poor efficacy or toxicity, and/or extrinsic factors, e.g. improper trial design, attrition rates, incorrect dosage or underlying factors in the treated population. Intrinsic factors can be addressed by improving target selection and drug discovery processes for identification of better drug candidates. The enrolment of patients takes one third of the overall trial duration. However, an eligible patient may not be recruited at the stage of the disease or belong to a specific genotype or sub-phenotype that is targeted by the tested drug. Therefore, the quantification and stratification of patient multi-omics features, in particular the underlying characteristics that contribute to a heterogenous response or toxicity profile in a subgroup of patients, will be helpful for prospectively selecting a subset of the population responding to the drug. If a patient is *a priori* part of the suitable subset, then their participation in the clinical trial will hereby increase the observed efficacy of the tested drug. This has generally been ignored when conducting traditional randomized clinical trials involving large cohorts, as the general assumption overlooks the genetic, environmental or other phenotypic heterogeneity between individuals in the cohort [4]. The patient heterogeneity and corresponding drug response heterogeneity have been exemplified in therapeutics that aim to target complex biological systems such as metabolism, the gut microbiota or the immune system to treat a

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particular disease [5–8], which can lead to a wide range of outcomes for therapies that aim to modulate their functions. For example, individual differences in dietary patterns can have a large impact on the gut microbial composition [9]; the in-born or disease specific errors in genetics can lead to differences in the metabolic network of patient tissues [10, 11]; and the exposures to allergens, pollutants or pathogens can affect patient immune system function [12]. Altogether, the use of multi-omics profiling for the cohort integrated with advanced computational methodologies will provide a potential approach to recruit suitable patients to reduce the high failure rate of clinical trials.

2. Analysis of omics data with machine learning methods

Accessing high-resolution omics data across a large number of individuals and multiple disease settings has revolutionized our understanding of disease. In particular, sequencing for genomics/transcriptomics/metagenomics and mass spectrometry for proteomics/metabolomics/lipidomics have been shown to provide valuable information. These assays can be performed in bulk tissues or fluids, or in conjunction with single cell isolation and imaging techniques for an in-depth look at not only inter-individual differences, but also intra-individual differences between certain cells types or tissues [13–16]. The vast amount of data that can be gathered from these assays will provide an invaluable resource for researchers in identifying the biological features required to confer responses to treatment, as well as minimizing adverse events. These stratified features can then be integrated back into the study design by modifying the appropriate inclusion/exclusion criteria, thereby creating a more defined sub-population tailored to the treatment and potentially increasing the likelihood of a successful trial (Fig. 1). In addition, the classification based on the prognostically significant subgroups and following subgroup analysis can be performed to identify the features associated with the specific subgroup, which can be potential biomarkers for prognosis. Moreover, the prominent features that confer treatment responses or adverse events can also be exploited as potential mechanistic targets for novel therapeutics that could be used in conjunction with the original treatment as combination therapies for an even greater translatability across more individuals (Fig. 1).

The unbiased and often hypothesis-free nature of omics efforts mean that one can limit any preconceived biases about the mechanism behind the interplay between drugs, patients and their disease status, and essentially let the data highlight correlations between individual features and a phenotype of interest, which in this case would be a response or an adverse event to the drug. A caveat to the comprehensiveness of omics data is that the size and dimensionality of these data sets can now easily balloon into the petabyte range and spanning several thousand individual features [17–19]. Consequently, appropriate bioinformatic methods to analyze and construct predictive models based on omics are required to draw appropriate conclusions, ensure data integrity, mitigate batch/assay-specific effects and minimize the false positive predictions. In particular, machine learning methods such as deep neural networks are now widely used to learn from omics data [20]. These models, while being extremely powerful tools for discriminating responders from non-responders, or for highlighting the most important features behind an adverse event, still lack the transparency and interpretability compared with the mechanistic models such as Genome-Scale Metabolic Models (GEMs) and Ordinary differential equation (ODE) based models [21–24]. With the advent of new interpretable machine learning models or hybrid models incorporating both structural/mechanistic information along with deep neural networks, we might see increased mechanistic understanding without a loss in predictive performance. As an example, a recent study constructed a biologically interpretable neural network architecture for the prediction of castration resistant prostate cancer utilizing multi-omic features, i.e. mutations, methylation patterns, copy number alterations and gene expression levels [25]. This deep learning approach had higher performance in predicting castration resistant prostate cancer (AUPRC = 0.88, 1033 total cancer samples of which 333 were castration resistant) compared to other machine learning approaches (second-best performing was support vector machines with AUPRC = 0.85), as well as the added benefits of mechanistic interpretability, highlighting the importance of *MDM4* copy number or gene expression increases in patients with castration resistant prostate cancer [25]. Currently, predictive models utilizing omics data and their corresponding identified features for response / adverse events evaluation have the potential to find use in clinical practice and in the development of novel combination therapeutics [26,27].

Combinatorial therapies have shown a greater success rate in the clinic as it employs cancer treatment using more than one approach. Furthermore, combinatorial therapies have the possibility of improving patients drug response and reduce adverse events. In the context of cancer immunotherapy with ICIs, the use of ICIs involve complex host-disease-drug interactions that are highly dependent on the individuals, but we still lack a complete understanding of the important factors necessary to improve patient outcomes (Fig. 2). Moreover, ICIs are frequently used in combination with other therapies, thus making it highly relevant to identify the potential cancer drugs that can be taken with a given ICI for better outcomes [28].

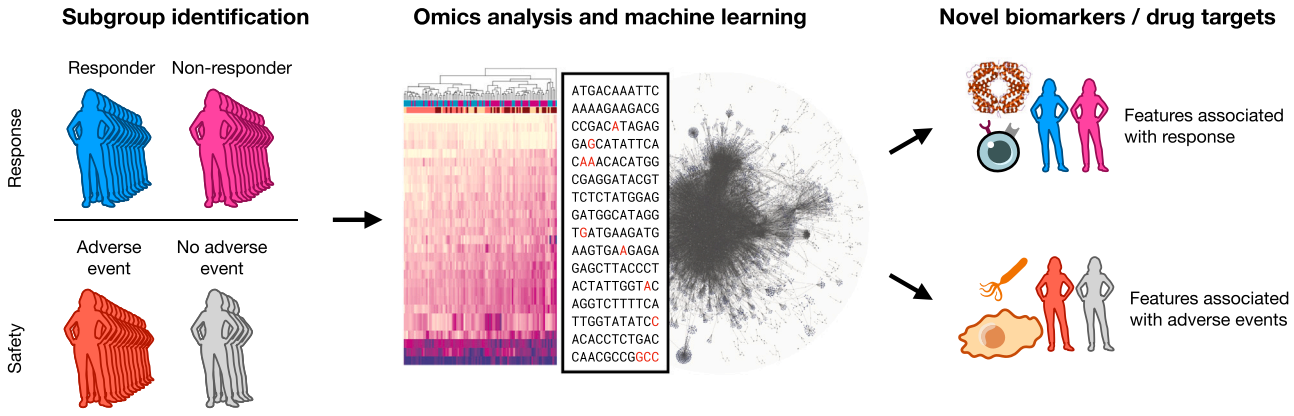

Fig. 1. Advancing drug development with information gathered from omics data. Safety and efficacy are key components of drug development. When running a clinical trial for a drug candidate, subgroups enriched in efficacy/safety can be identified. Analyzing omics data obtained from patients belonging to contrasting subgroups, i.e. responders vs. non-responders or patients experiencing adverse events vs. those that do not, can identify features that are associated with a beneficial drug response and safety profile. These features can be harnessed in the clinic as biomarkers for response or safety, or could be utilized for further studies in order to improve efficacy and safety of a given drug.

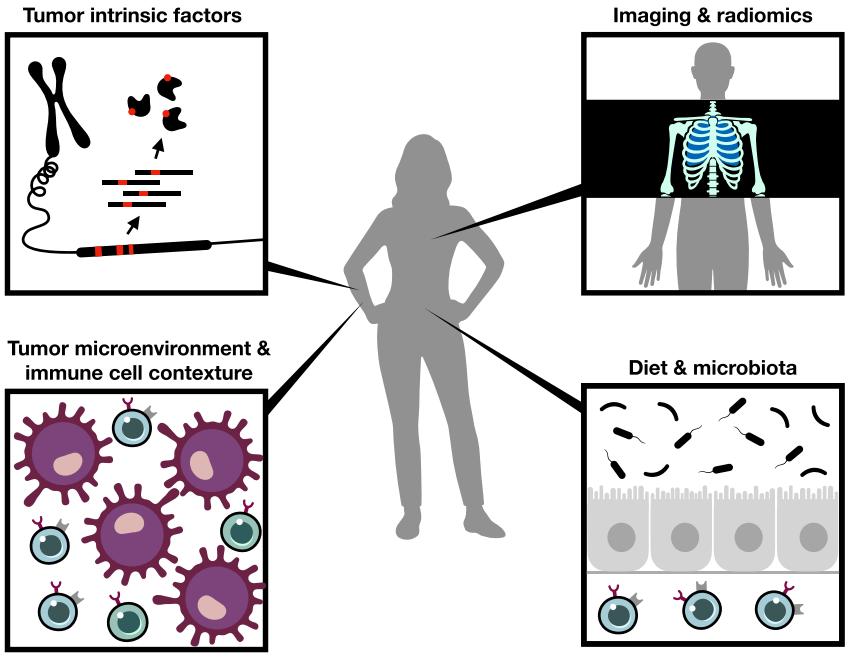

Fig. 2. Drug response and adverse event prediction in cancer immunotherapy with immune checkpoint inhibitors. ICIs target the interplay between the host, tumor and immune system. This requires the consideration of multiple factors in order to comprehensively assess response and safety to ICIs. These factors can be grouped into 4 main categories: Top-left: Tumor intrinsic factors, such as the mutational burden or expression of checkpoint ligands. Bottom-left: Tumor microenvironment and immune cell factors, such as the infiltration of cytotoxic T-cells in the tumor. Top-right: Imaging based radiomics, which are routinely available in the clinic and can be used for input to machine learning algorithms in order to extract visual features of the tumor that are correlated with response or safety. Bottom-right: Diet and the microbiota can directly stimulate immune cells in the gut lining, and subsequently affect whole body immunity which in turn will impact ICI outcomes.

3. Immune checkpoint inhibitors in cancer therapy

One of the defining hallmarks of cancer is the ability of tumor cells to evade surveillance and subsequent destruction by the immune system [29]. Therapies that aim to prime the immune system to start attacking the cancer are known as immunotherapies and have revolutionized the way to treat many different types of cancers [30]. Immunotherapies that target the interactions between negative-costimulatory receptors on the surface of certain immune cell classes (T-cells in particular) and their ligands are known as ICIs, and have found widespread clinical use in the treatment of metastatic cancers [31]. Cancer cells actively bind to these receptors in order to exhaust neighboring T cells, which limits their effector function and turns the T cell into a state of dysfunction, thereby maintaining immune tolerance. ICIs targeting the programmed cell death 1 protein (PD-1) and its ligand (PD-L1), alone or in combination with the cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) are widely used in the treatment of several types of late-stage cancers, with new agents being introduced all the time [32]. As of October 2022, nine ICIs have been approved for the treatment of 18 different cancer types, with nearly half of all metastatic cancer patients given the opportunity to receive an ICI regiment as part of their treatment [31]. While ICIs have been successful in creating a durable response in late-stage cancers, the majority of patients do not respond to ICIs [33]. Furthermore, immunotherapy can result in both acute and chronic immune related adverse-effects (irAEs), most often when administering combination treatments targeting both PD-1 and CTLA-4 [34]. These can range from common and relatively mild ones, such as rashes and vitiligo experienced in ~50% of patients with melanoma [35], to fatalities most commonly occurring due to ICI-induced colitis [36]. As such, identifying which factors can distinguish responders from non-responders as well as factors associated with irAEs are necessary for improving patient outcomes and extend ICI applicability to malignancies with poor or no efficacy.

4. Predictors of responses and adverse events in ICI therapies

4.1. Cancer immune features as biomarkers of responses

A starting point for identifying predictive biomarkers for ICI response came from quantifying the expressed ligands of immune cell checkpoint receptors in the tumor. For ICIs targeting the PD-1/PD-L1 axis, quantifying cancer cell expression of PD-L1 had mixed clinical utilization partly due to the difficulties in establishing pathological concordance for quantifying PD-L1, as well as the observation that subsets of patients with tumors containing low or no expression of PD-L1 could still respond and benefit from ICI treatment [37–40]. Several studies had showed the potential to predict patient response to ICI treatment with the expression of other checkpoints (i.e. CTLA4, PD-L2) that can be accessed in cancer patients before ICI treatment [41], and recent studies explored the utility of dynamic profiles of serial immune biomarkers and genomic features, which may provide a non-invasive and time-efficient approach for ICI efficacy prediction [41,42].

4.2. Genomics based biomarkers of responses

Another observation is that ICIs has been mostly confined to epithelial cancers with relatively high tumor mutational burden (TMB), in part owing to the large number of potential tumor-specific neoantigens that can be generated and subsequently recognized by ICI-primed T-cells [43]. The landmark KEYNOTE-158 study prompted therefore accelerated FDA approval of ICIs for solid tumors with a high TMB (at least 10 mutations per megabase), as quantified by the targeted DNA sequencing assay [44]. These aforementioned biomarkers for ICI responses might have utilized complex biological assays, as in the mutational burden case, but the extracted features are relatively simple and the targeted nature of these assays do not actually profile entire -omes for a complete look into the biological processes of the tumor.

Genomic studies employing whole-genome-sequencing or exome sequencing of tumors undergoing ICI therapy have therefore been conducted to further elucidate which specific genetic mutations or genome-wide mutational signatures drive a response to ICI (Fig. 2). A study that surveyed exome sequencing data from more than 1500 patient receiving ICI therapy across multiple cancer types, uncovered that the homozygosity in the genetic locus encoding the major histocompatility complex class I (MHC-I), which is part of the antigen presentation machinery, is associated with a 38% increased hazard of death in anti-CTLA-4 or anti-PD-1 therapy [45]. Variation in the antigen presentation machinery could increase the chance of peptides harboring tumor neoantigens being bound and presented to induce the immune responses. Defects in tumor interferon signaling, which plays a critical role in immune cell

mediated killing of cancer cells, has also been associated with an unfavorable response to ICI, especially in melanoma [46,47].

4.3. Gene expression-based biomarkers of responses

As ICI treatments involves priming of the host immunity towards a cancer, the magnitude of a response will depend not only on tumor-intrinsic factors, e.g. mutational burden or gene expression programs, but also on the interplay between cancer cells and host immunity/metabolism. Factors of ICI response that lie proximal to the tumor include the degree of immune infiltration [48], which has been profiled by assays ranging in complexity from simple cell type counting, e.g. through histological or flow cytometry methods, to bulk and single-cell transcriptomics. The latter provides a full picture of the expression programs within individual cell types [49]. Single cell gene expression studies of tumor samples from multiple patients undergoing ICI have now been performed and provide not only information about the cancer cells, but also any other cell type that is present in the tumor microenvironment [50]. In metastatic melanoma, bulk transcriptomics from 473 patient tumors along with single cell transcriptomics from 33 patient tumors identified a gene expression program in cancer cells responsible for T-cell exclusion and nonresponse to ICI and consisted of genes involved in antigen presentation, interferon signaling, response to the complement system and immune modulation [50]. By mining publicly available cell line transcriptomics and drug perturbation data sets, the researchers were able to extract cancer cell lines associated with this resistance program and subsequently identify drugs that preferentially kill these cells. This analysis discovered the CDK4/6 inhibitor abemaciclib as a drug that could preferentially target the T-cell exclusion program, and murine experiments verified this finding *in vivo*, with mice receiving a combination of abemaciclib and anti-PD-1 therapy experiencing reduced tumor growth and longer survival than anti-PD-1 monotherapy alone. This study highlights the potential of omics for dissecting the tumor-immune landscape and enabling the identification of novel drug candidates for boosting ICI response rates. Currently, abemaciclib is being investigated in clinical trials as a combination therapy with ICI for various cancer types (e.g., NCT02791334, NCT04751929, NCT04627064).

4.4. Radiomics based biomarkers of responses

Imaging of patients to assess the response over the course of ICI therapy is standard practice [51]. These images, collected routinely using for instance computerized tomography (CT) or positron emission tomography (PET), allows for the complete visualization of the primary tumor and secondary metastatic sites [52]. While standard radiological assessment of these images is performed (Fig. 2), e.g. to classify stage and tumor subtypes, radiomic approaches aim to extract higher-level features of the image data, based on the presumption that genomic and molecular features in the tumor that contribute to ICI response will affect the tumor physiology and therefore be detectable from imaging data [53]. A large study assessing CT scans of 1055 primary and metastatic lesions obtained from 203 metastatic melanoma and non-small cell lung cancer patients was able to extract features from the imaged lesions and used them to train machine learning models for ICI response at the lesion or patient level [52]. Response prediction performance at the lesion level was superior in non-small cell lung cancer (test AUC-ROC = 0.83 for pulmonary metastatic lesions, 42 responding and 31 non-responding lesions obtained from 25 patients) compared to melanoma (test AUC-ROC = 0.64 for lymph node lesions, 17 responding and 43 non-responding lesions obtained from 22 patients), but combining these into patient response predictions yielded a test AUC-ROC of 0.76 (70 patients) across both cancer types [54].

4.5. The association between gut microbiota and ICIs responses

Distal and environmental effects can also influence host immunity, and consequently also impact the outcomes of ICI. Of particular interest has been the gut microbiota, due to its inherent link to host immunity and potentials for modulation through diet, prebiotics/probiotic supplements and fecal microbiota transplant (FMT). It is now well established, from a multitude of studies, that specific bacterial species and metabolic processes in the gut can directly influence ICI response [55–64]. Fecal metagenomic sequencing (MGS) studies in melanoma patients undergoing ICI has identified bacterial species belonging to the *Faecalibacterium*, *Akkermansia* and *Bifidobacteria* genera as especially important predictors for positive responses. Although the exact mechanisms behind the immunomodulatory effects of these microbes are still unclear, several studies have shown the important roles of the microbial production of immunomodulatory metabolites (e.g. short-chain fatty acids [65,66], TMAO [67] and bile acids [68]), direct induction of beneficial immune cells lining the intestine in a MHC-I [69] or Toll-like receptor [70] type manner, and bacterial epitopes cross-reacting with ones found in cancer [71].

Meta-analyses of pooled MGS data sets obtained from different studies have been used to construct machine learning models based on host microbiota features (e.g. microbial composition, microbial genes, and microbial metabolic pathways) [60–62], with the best performing models achieving AUC-ROC values of around 0.7 [61,62] across different data sets. An issue with utilizing the microbiota for responses prediction is the large heterogeneity between patient populations in terms of microbial composition. No predominant species can therefore be used as consistent biomarkers across studies. Therefore, future work will trend to identify a more consistent microbial feature set that can be generalized across patient populations, for instance by moving from species point of view towards a more functional one. Our previous work on establishing predictive models across patient cohorts also suggested an increased importance of functional features compared to the individual species [60]. However, it is worth noting that MGS reveals the microbial composition, but not actually capture the metabolic activities of microbes. Therefore, it would require analyses of fecal metatranscriptomics/proteomics or metabolomics for the dynamic profiling in the context of ICI.

4.6. Multi-omics based biomarkers of responses

Identifying the most important tumor-intrinsic factors for ICIs across cancer types remains a challenging feat. Individual studies are often fragmented across widely different malignancies, ICIs, patient demographics, and evaluation criteria. The integrative approach that combines multi-omics data, including genomics, transcriptomics, proteomics, metabolomics, and/or metagenomics, has been developed to predict the responses in cancer patients with ICI treatments [72–74]. Meta-analyses of publicly available omics data obtained from ICI studies have therefore been conducted in order to increase study power and use the large sample sizes to construct predictive models for ICI response. One of the largest meta-analysis performed consisted of >1000 whole-exomes/transcriptomes obtained from ICI-treated cancer patients spanning six different cancer types [75]. Individual data sets were then subjected to standardized bioinformatic processing, normalization and response evaluation in order to retrieve germline mutations, somatic mutations, mutational signatures, copy number alterations, HLA typing and gene expression levels, which could then be standardized and used as input features for a machine learning model (XGBoost) for predicting ICI response. The final trained model scored clonal TMB, *CXCL9* expression, ultraviolet (UV) associated mutational signature, APOBEC mutational signature, and tobacco mutational signature as the top 5 most important features. The machine learning based predictor significantly outperformed ($p < 0.05$, DeLong test for AUC comparisons) mutational burden as a lone predictor on three different test cohorts

(AUC-ROC = 0.86 *vs*. 0.68 for the KEYNOTE-028 study of multiple tumor types [n = 76] [76], AUC-ROC = 0.66 *vs*. 0.58 for the University Hospital Essen study of melanoma [n = 121] [77] and AUC-ROC = 0.70 *vs*. 0.62 for the Samsung MC study of non-small cell lung cancer [n = 144] [78]). Furthermore, the importance of the UV, APOBEC and tobacco associated mutational signatures for model prediction suggests the need to employ whole-exome/genome sequencing assays or large-scale targeted sequencing panels for the prediction of ICI response, as these signatures require the sequences of large/multiple sections of the tumor in order to accurately compute.

4.7. Omics based biomarkers of adverse events

Omics-based adverse events prediction in ICI has been comparatively less studied, possibly due to the difficulty of harmonizing widely disparate irAEs across malignancies, choice and dose dependency of the ICI used, different organ systems being affected, varying time to irAEs, and a wide spectrum of frequencies and severity [34]. The safety profile of the most commonly used ICIs has been relatively well established, with patients undergoing ICI therapy targeting CTLA-4, either alone or in combination with PD-1. The patients with combinatory ICIs therapies experienced higher frequencies of any-grade irAE (up to 60%) than those on PD-1 monotherapy alone (5–20%), with a similar distribution when limiting to grade 3 or higher irAEs (55% *vs*. 6% for PD-1 vs. monotherapy alone, respectively) [34]. Therefore, most studies have focused on improving the safety profile of CTLA-4 and PD-1 + CTLA-4 ICI therapies and have also been limited to investigating single immune related factors, including T-cell receptor diversity [79], autoimmune antibody generation and clonal expansion of cytotoxic T-cells [80]. A study aiming to identify tumor-intrinsic factors contributing to irAEs was recently performed in order to address this knowledge gap [81]. By accessing adverse event reports from the FDA Adverse Event Reporting System, subsetting for irAEs from cancer patients undergoing PD-1/PD-L1 ICI and linking these with omics data from The Cancer Genome Atlas (TCGA), the omics-based signatures were associated with the presence/absence of irAEs. This analysis identified cytolytic activity, interferon-gamma signaling, PD-1 expression, TCR diversity, M1 macrophage abundance, cytotoxic T-cell abundance and B-cell abundance as features that were significantly positively correlated with the presence of irAEs (Note that M1 macrophage/cytotoxic T-cell/B-cell abundance are predicted abundances as determined through immune cell deconvolution from bulk tumor transcriptomics data in the TCGA). The correlation analysis with individual features of mRNA, miRNA, lncRNA and protein expressions revealed that mRNA expression of lymphocyte cytosolic protein 1 (*LCP1*) and adenosine diphosphate dependent glucokinase (*ADPGK*) are strongly associated with the presence of irAEs, both of which are involved in the activation of T-cells. Constructing a bivariate predictive model using the expression of these two genes could predict irAEs in an independent cohort with an AUC-ROC of 0.8 (28 patients, of which 14 experienced any-grade irAEs). The fact that the expression of genes involved in T-cell activation are enriched in patients with irAEs could point towards cross-reactivity of antigens that are present in both tumors and healthy tissue.

The role of T-cells in mediating the irAE induced by ICIs have been examined closer in a study that performed state of the art single cell or bulk omics analyses of immune cells obtained from 71 blood samples of metastatic melanoma patients undergoing checkpoint immunotherapy and experiencing irAEs [82]. This data set allowed for the dissection of distinct immune cell populations, with CD4 + effector memory cells being enriched in samples from patients with severe irAEs. Moreover, targeted sequencing of the T-cell receptor indicated that high receptor diversity is also strongly correlated with severe irAEs. By utilizing computational tools to extract immune cell abundances and T-cell receptor sequences from samples subjected to bulk transcriptomics, a logistic regression model for severe irAE (grade 3 or higher) was trained and validated with a AUC-ROC of 0.90 (27 patients, of which 12 experienced grade 3 or higher IrAEs).

Factors for determining irAEs, while promising, are still at an early stage and would need to be prospectively validated as a method for identifying at-risk patients for ICI induced irAEs. Furthermore, designing drugs that target genes potentially responsible for irAEs, e.g. *LCP1* and *ADPGK*, could potentially reduce toxicity towards the host at the risk of hampering the ability of immune cells to attack the cancer.

5. Modulating the host microbiota for improved efficacy and safety of ICIs

As described above, immunotherapy has shown successful treatment outcomes in multiple cancers, while a high number of patients do not respond to the treatment. Therefore, strategies to enhance the efficacy of immunotherapy is probably part of the solution to improve their effectiveness. Several approaches have been developed to enhance the efficacy of ICI, including rational ICIs combinations, combination of ICIs and radiotherapy, combinations of cancer vaccines and ICIs, modulation of immune metabolism and mudulation of gut microbiota [83–88]. Since the combinations of cancer therapies have been extensively discussed [87], here we focused on how microbiota engineering contributed to improved ICI efficacies.

One way that can infer the host microbiota towards a more ICI favorable state is to assess the patient's dietary intake and optimize the dietary patterns. A recent study assessing the metagenomes and dietary habits of 128 melanoma patients identified the correlation between dietary fiber intake and the improved progression-free survival on ICI therapy [89]. Unexpectedly, intake of over-the-counter probiotic supplements in these melanoma patients was negatively correlated with ICI response, which clearly highlights the need to better distinguish rationally designed ICI specific probiotics from general probiotic supplements [89]. While both of these findings were also validated in murine models, which potentially implies a causal connection between diet, probiotics and ICIs responses, a recent meta-analysis of previous ICI studies have instead found positive correlations between probiotics intake and improved survival [90].

Intervention studies aiming to alter the host microbiota towards a responder state by introducing exogenous microbes have recently been conducted. Two studies that employed FMT from metastatic melanoma ICI responders into patients with anti-PD-1-refractory metastatic melanoma have shown positive results, with a subset of patients gaining a clinical response and considerable remodeling of the tumor microenvironment [91,92]. FMT comes with its limitations, especially the risk of accidental transmission of pathogens. Due to this risk, the FDA has announced several safety alerts about patients developing FMT-related complication, including two deaths.

Probiotic interventions are potentially a more attractive option for modulating non-responder microbiomes due to their defined nature, albeit with the risk of losing the complex ecological dynamics of a fecal sample microbiota. A randomized phase 1 trial had investigated the combination of anti-PD-1 +anti-CTLA-4 ICI therapy in 30 patients of treatment-naïve metastatic renal carcinoma with or without the supplementation of *Clostridium butyricum*, a bifidogenic and butyrate-producing bacterium [93]. Although the primary endpoint of enhancing the abundance of *Bifidobacterium spp*. was not met, patients that received the probiotic experienced a significantly longer progression-free survival than those with ICI therapy alone (12.7 months vs. 2.5 months) [93].

Recently, microbial correlates for ICI induced irAEs have also been investigated. As mentioned earlier, ICI induced colitis has been reported as the main source for anti-CTLA-4 related deaths (70%) [36]. An early study aiming to identify stool biomarkers for ICI induced colitis performed metagenomic profiling of 34 prospectively enrolled melanoma patients and reported higher abundances of bacteria belonging to the Bacteroidetes phylum in patients that did not experience colitis [94]. However, the 16 S rRNA sequencing used to profile the microbiota here

does not have enough resolution to distinguish microbes at lower taxonomic resolutions. In order to obtain species/strains level associations, as well as functional and genetic ones, MGS based studies of the microbiome related to irAEs have now been performed [61,95]. One study examined MGS data from a cohort of 77 advanced melanoma patients undergoing PD-1 + CTLA-4 blockade with approximately half of the patients experiencing severe or higher grade irAEs. Abundances of *Bacteroides intestinalis* and *Intestinibacter bartlettii* were found to be correlated with grade 3 or higher irAEs, confirming and expanding upon the findings of the aforementioned 16 S rRNA-seq based study. These findings were validated in antibiotic treated mice followed by gavage with *B. intestinalis*, which resulted in increased ileal damage compared to non-gavaged mice. Another recent study also examined correlations between microbes, assayed using MGS, and irAEs resulting from anti-PD-1 ICI [61]. This analysis identified *Lachnospiraceae* spp. and *Streptococcoccus* spp., with *Streptococcoccus* spp. being associated with lower progression-free survival, as well as increased incidence of irAEs across multiple organs, whereas *Lachnospiraceae* spp. was associated with both response to ICI and incidence of irAEs. This suggests varying outcomes of the immunostimulatory effect of gut microbes on the host, with some leading to an increased immunity towards both cancer and healthy tissue, whereas other might only be contributing to autoimmunity without any cancer-specific targeting. The results from studies on how the gut microbiota can affect the emergence of irAEs are still in the preclinical phase and will therefore require further validation in human intervention trials to see if they can provide a reduced ICI toxicity profile without impacting response rates.

6. Upcoming challenges in multi-omics analysis

Omics technologies have emerged as powerful tools for dissecting the heterogeneity among individual patients, including inflammatory bowel disease [96], diabetes [97], cardiovascular disease [98] and as highlighted in the ICI context [61,75,95,99]. However, there are some challenges that must be investigated in order to improve the responses to ICIs and reduce irAEs. One issue is the increasing scale and variation in assay type, handling protocols, experimental design and data analysis, which ask how to ensure reproducibility and translatability across studies. This will be of extreme importance to computational biologists aiming to conduct decentralized research by pooling data from multiple sources. As exemplified in ICI response prediction models, predictive performance on unseen data remains limited, especially when collected from external cohorts. Moreover, standards for reporting of metadata have been lacking which can introduce missing values or bias when harmonizing data sets. For example, a deceivingly simple metric as drug response will depend on the response metric used (e.g. progression-free survival, overall-survival, biomarker values, clinical evaluations) as well as the timing of the evaluation across the course of treatment [100]. As different investigators might adhere to their preferred reporting standard of choice, establishing improved consensus standards for reporting will have to be introduced.

While studies continuously tend to include larger number of patients as well as profiling multi-omics at the same time in order to tackle inter-patient heterogeneity, the appreciation of intra-patient variability is another avenue that will have to be further explored in future studies. This can range from utilizing multiple samples collected from the same individual over the course of treatment in order to assess biomarker stability over time and identify the most suitable temporal range for drug interventions, as well as utilizing single cell and spatial omics methods for identifying cell type subsets that might experience differential responses to a drug.

These challenges raised the requirements to overcome them in the development of omics-based biomarkers and assays. So, we can best take advantage of them in order to further our comprehension of disease biology and design better therapeutics to maximize personalized approaches towards the eradication of disease. Furthermore, as evidenced by publications and published datasets in recent years, there is still a big gap between evidence for clinical trials and evidence for real clinical usage. Beyond the validation of predictive models in the trial settings, the clinical usability of these developed predictive models for ICIs responses and irAEs in the real-world setting will be another big challenge that needed to be addressed for scalable applications. One needs to consider the practical implementation of these companion diagnostics. For instance, the required assays need to be conveniently available in all hospitals globally that are treating patients either at the site or via a diagnostic service. This is a challenge even with standard genomic diagnostics in oncology. The cost also needs to be tolerable to the payers (private insurance or public), which would require the development of improved omics assays at a lower cost.

Statement

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CRediT authorship contribution statement

Angelo Limeta: Conceptualization, Investigation, Visualization, Writing – original draft preparation. **Francesco Gatto**: Supervision, Writing – review & editing. **Markus J. Herrgård:** Supervision, Writing – review & editing. **Boyang Ji:** Conceptualization, Supervision, Writing – review & editing. **Jens Nielsen:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare no competing interests.

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