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Review

Germline genetic host factors as predictive biomarkers in immuno-oncology

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In immuno-oncology (IO), the baseline host factors attract significant clinical interest as promising predictive biomarker candidates. Growing evidence from experimental or population-based studies suggests that the host genetic factors contribute to the immunological status of a patient as it plays out at the multiple rate-limiting steps of the cancer immunity cycle. Recent observations suggest that germline genetics may be associated with tumor microenvironment phenotypes, autoimmune toxicities and/or efficacy of immunotherapy regimens and overall cancer survival. Despite these highly intriguing indications, the potential of germline genetic factors as personalized biomarkers of immune-checkpoint inhibition (ICI) remains vastly unexplored. Here, we review the rationale for exploring the germline genetic factors as novel biomarkers predictive of IO outcomes, including ICI efficacy, toxicity and survival, and discuss the comprehensive approaches for the identification of such germline genetic indicators. In addressing the current limitations, we highlight a need for large collaborative consortia in these efforts. We also outline possible avenues for incorporating germline genetic factors into emerging multifactorial tools for a more personalized prediction of ICI outcomes.

Introduction

Immune-checkpoint inhibition (ICI) treatment in melanoma and other solid tumors substantially improved clinical outcomes in treated patients [1–6]. However, given the heterogeneity of ICI efficacy (only 50% of patients currently respond across these treatment modalities) [1–3,5–7], durability of treatment benefit (>50% of responding patients will relapse within 2 years of treatment) (e.g. progression-free survival or overall ICI-related survival) [7,8] and, particularly, significant immune-related adverse events (irAEs) associated with these treatments (>70% of patients will develop severe irAEs, often resulting in treatment termination) [9–11], there is a pressing clinical need to identify reliable and personalized biomarkers predictive of the most beneficial and least toxic outcomes. Regarding the complex nature of ICI-related biology, some of which is still poorly elucidated, prior investigations have focused on tumor properties of ICI-treated patients (tumor mutation burden) [12–14], tumor microenvironment (TME) [15–20] and the composition of peripheral immune cells [21–23]. These studies proposed a number of biological surrogates of ICI efficacy, survival and toxicity [14,20,24–27]. However, only a few have reached clinical applicability to date, and even for these indicators, their relatively low specificity prevents their broader utility as personalized biomarkers. Recently, growing interest has been concentrated on host factors as novel biomarkers of ICI efficacy, toxicity and survival. Host immune homeostasis has been proven to be critical in the determination of ICI success, involving key biological mechanisms controlling host immunity. An emerging determinant of host immune homeostasis is the underlying germline genetic component, which has been shown to impact host immunity; genetic factors explain the large variance in the abundance and activation state of multiple immune cell types, including CD4+ and CD8+ T cells [28–30], immunomodulatory molecules [31,32] and immune-related genes [32,33], likely translating into distinct immune states. Prior studies have demonstrated that inherited genetic variation affects the expression of a number of immune-related genes [34–36]. Hundreds of genetic risk loci for autoimmune and inflammatory diseases have been identified in genome-wide association studies (GWASs) [37], further underscoring the heritable nature of immune variability. Data from The Cancer Genome Atlas (TCGA) and other repositories of genetic information from large populations triggered the hypothesis-driven approaches towards identification of germline factors controlling cancer immune responsiveness [38]. These efforts revealed specific germline/immune signatures, some associated with T-cell tumor infiltrates. Altogether, this clearly outlines a possibility that the germline genetic repertoire may modulate immune-based therapies or ICI-related survival outcomes. This assumption is clinically attractive for two main reasons: (1) the germline variation is highly polymorphic, which substantially increases the potential

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applicability of host germline factors as personalized biomarkers in clinical practice; and (2) germline biomarkers are easily accessible from a simple blood test, potentially replacing other molecular surrogates that require more invasive testing. Despite these clinical innovations, the concept of germline genetics as a novel surrogate of immune responsiveness has yet to be explored comprehensively in immuno-oncology (IO). Prior studies have focused on candidate designs, assessing a handful of genetic variants with the most plausible biological relevance for their association with IO clinical outcomes such as ICI response, toxicity or survival [39,40]. Small-scale variant focus (e.g. variants in CTLA-4, PD-1 and PD-L1); clinical heterogeneity; a lack of independent validation or consideration for other biological, immune or tumor surrogates; and, most importantly, a limited power of sample cohorts were among the major drawbacks preventing the reproducibility of these findings. Due to emerging evidence clearly highlighting germline genetic factors as promising modulators of IO therapies, it is essential that these prior limitations are addressed in adequate and state-of-the art genetic and clinical designs. In this review, we focus on current knowledge related to germline genetic factors in IO, their potential as biomarkers of ICI outcomes, and methodological improvements for their comprehensive identification, and discuss the avenues and potential limitations towards their clinical applicability.

Rationale supporting a role of germline genetics in IO: hereditary basis of host immunity

The assumption that germline genetics affects host immunity has been promoted by intriguing observations suggesting that the composition of peripheral blood cells, cytokine levels and/or serum protein diversity are heritable [28,32,38]. With technological advancements in high-resolution deep immune-phenotyping flow cytometry, many studies [28,30,41–43] reported that immune cell subsets, including the phenotypes of CD8+ and CD4+ T cells (the two primary cytotoxic T-cell compartments stimulated by ICI [44,45]) are under strong genetic influence [46]. In a seminal report, 497 adult female twins (TwinsUK) were profiled by high-resolution deep immunophenotyping flow cytometry for >23 000 robust blood immune cell phenotypes. More than 75% of these traits showed a predominantly heritable influence; most notably, the immune homeostasis of B2, CD4+ and CD8+ cells was largely attributed to the effects of inherited genetics [47]. This study demonstrated that germline genetics has a strong effect on adaptive immune traits, and this finding may have important implications for genetic susceptibilities to autoimmunity (discussed below) and IO. The implementation of GWASs and genome-wide single nucleotide polymorphism (SNP) array technology, coupled with large-scale immune phenotyping, helped to identify common genetic variation associated with specific categorical or quantitative representation of blood cell phenotypes on a large population-based scale. This is exemplified in a large effort [30] analysing 78 000 immune traits in 669 female twins and identifying 300 SNPs (representing 11 genetic loci) that control the phenotypes of 19 functionally important immune subsets in the blood. The most heritable trait among these was the frequency of CD39+ cells within the CD4+ compartment, which is consistent with previous studies showing that CD4+ phenotypes are strongly controlled by germline genetic factors [28]. As CD39+CD4+ T cells are functional T regulatory cells (T-regs) [48], a key subset in the modulation of immune responses in tumorigenesis [49], these findings may be highly relevant for IO; the balance between CD4+ cells and T-reg/CD8+ T cells is an important marker of cancer prognosis and ICI outcomes [20]. The enrichment of T-reg populations in tumor infiltrates has been associated with an immunosuppressive TME attributed to various biological mechanisms, including the secretion of immunosuppressive molecules (e.g. TGF-β and IL-10) that interfere with effector T-cell functions, resulting in reduced ICI efficacy [50,51]. Thus, to boost the efficiency of ICI treatments, the inhibition of immunosuppressive CD39+ T-reg phenotypes has attracted significant attention in IO, particularly by recently introduced antagonists of CD39+ T-reg-induced immunosuppressive adenosine [52]. It is possible that the use of adenosine antagonists in improving ICI efficacy may be enhanced substantially by selective targeting of the patients with germline genetic variation associated with increased production, activation and/or infiltration of specific inhibitory CD39+CD4+ T-cell phenotypes. As such, the evidence of germline heritability of T-regs and other immunosuppressive phenotypes strongly supports further investigations of the germline genetic factors in the context of IO, including tumor–host interactions and utilization of combined and more personalized immune-based therapies.

Germline genetic interaction with TME: a role of germline genetic variation in cancer immune responsiveness

Large pan-cancer 'omics' information has been accumulated through TCGA, including GWAS SNP array data, next-generation sequencing (NGS) data on germline and tumor tissues, corresponding tumor RNA-sequencing (RNA-seq), and other phenotype information such as total tumor lymphocytic infiltrate (TIL) (from genomic and hematoxylin and eosin image data) [53]. Along with the available clinical outcome information, the utilization of these resources allowed for pivotal exploration of the host genetics interacting with putative somatic phenotypes across cancer types. A straightforward approach tested the germline genetic variation that impacts gene expression in tumors [i.e. expression quantitative trait loci (eQTLs)], the variants associated with the expression of genes in their vicinity (*cis*) or gene mapping in different loci of the genome (*trans*). Studies have shown that SNPs reproducibly associated with complex disorders [54] as well as certain pharmacologic phenotypes [55] are significantly enriched for eQTLs relative to frequency-matched control SNPs. Using the integration of TCGA datasets across 24 different human cancers, > 64 000 eQTLs were recently identified as associated with the tumor expression of 18 210 genes (eGenes) [38]. Notably, these genes were enriched for involvement in immune processes, suggesting that tumor expression of immune genes may be shaped by hereditary genetics. Inferring on the abundance of immune cells in TCGA tumor samples using a cellularity deconvolution method [56], the study identified 103 germline genetic variants that were significantly associated with the immune gene signatures of specific immune cell infiltration (31 lymphoid and 72 myeloid signatures). These included T cells, natural killer cells and dendritic cells, suggesting the widespread impact of germline genetics on immune cellularity within the TME [38]. Finally, the two most significant eQTL associations corresponded to two gene loci (ERAP2 and ICOSLG), consistently expressed across all 24 cancer types. The expression levels of these two genes predicted overall survival in patients treated with anti-PD-L1 ICI. In IO, these findings may be of particular interest as both ERAP2 (endoplasmic reticulum aminopeptidase 2) and ICOSLG (the inducible T-cell costimulatory ligand) are involved in antigen processing and presentation on both tumor and antigen-presenting cells, a mechanism that is essential for priming the effector T-cell cytotoxicity [57,58]. It has been suggested that ERAP2 may be an important biological target for increasing the antigen presentation capacity in ICI treatments of solid tumors [59]. The patients with high-expressing germline alleles for these genes may hence potentially benefit from ICI, even in tumors with low tumor mutation burden (TMB). As such, the findings from TCGA clearly provide insight into possible stratification of patients receiving anti-PD-L1 therapy by integrating germline genetic data with TME phenotypes. As the inflammation of tumors is an important factor associated with sensitivity to anti-PD-1/anti-PD-L1 therapies [60,61], the germline genetic factors driving the presence of immune cell infiltration may be important personalized biomarkers in IO. This is further supported by a seminal recent report by the Pan-Cancer Immune Working Group [62] demonstrating a link between certain immunostimulatory tumoral subtypes (i.e. Th17-enriched C3 and IFNγ-dominant C2 subtypes) and favorable cancer prognosis and, potentially, ICI efficacy. A systematic exploration of germline genetic basis predisposing to these signatures may define germline indicators as novel biomarkers of immune cancer

responsiveness, and possibly of IO outcomes.

Germline genetics and ICI outcomes: the candidate studies

With strong evidence that germline genetic factors affect the immune microenvironment in tumors, parallel efforts have tested whether inherited factors are also associated with outcomes of immune-based therapies. Methodologically, the early investigations examined the most plausible biological candidates for their association with cancer survival or therapy response. These include associations of genetic variants in TLR4, P2RX7 and FPR1 with differential outcomes in breast and colon cancer patients treated with adjuvant chemotherapy, likely through the modulation of antitumor immune response [63,64]. Several studies have assessed the contribution of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) polymorphisms [39,40,65] in the context of ICI. Although some significant associations have been reported, these lacked independent validation. Recently, an NGS scan revealed that the germline heterozygous state of human leukocyte antigen (HLA) class I (HLA-I) loci is associated with anti-PD-1 response and post-treatment survival of melanoma and lung cancer patients. This is in line with previous studies reporting that ICI efficacy was dependent on HLA-I immune activity [66]. This effect was enhanced by, but not dependent on, TMB. Furthermore, efficacy of ICI was diminished by somatic loss of HLA-I heterozygosity in the tumor cells, pointing to the role of diverse HLA molecules in effector T-cell selection against certain neoantigens [66]. Importantly, molecular dynamic simulation of HLA-B super-types associated with poor prognosis revealed unique elements that might affect neoantigen recognition by cytotoxic T cells [66]. These results may have important implications not only for predicting the response to ICI based on the germline status of HLA-I, but stratification by HLA-I germline states may predict specific and perhaps personalized neoantigen presentation patterns. This may provide important insight into 'outlier' tumors, such as resistance to ICI observed in tumors with high TMB, versus ICI sensitivity in less mutated 'cold' tumors. This may also be essential for the design of more effective and personalized neoantigen-based therapeutic vaccines that specifically target the 'immunodominant' HLA-I (B locus) neoantigens [66,67].

Germline genetics and ICI outcomes: genetic risk to autoimmunity

Recent GWASs have identified >1000 SNPs associated with >60 different autoimmune conditions [68,69]. Interestingly, the association patterns showed considerable genetic pleiotropy – the enrichment of autoimmunity risk variants often overlapped between different autoimmune traits, and vice versa, suggesting the complex nature of genetic susceptibility to these diseases [68,70–73]. Remarkably, among the genetic variants associated with specific immune-cell phenotypes, such as Th cells [74], CD4+ or CD8+ T cells [75,76], there was a consistent enrichment for autoimmunity risk variants. In fact, a comprehensive analysis estimated that >75% of T-cell-specific phenotypic variation is explained by cis-acting eQTLs, significantly enriched for autoimmune risk loci [77]. This may have an important implication in IO as the activity and balance of CD4+ and CD8+ T cells is instrumental in the success of ICI [20]. If the process of regulation of these cells is controlled by underlying autoimmune variants, it is highly plausible that the germline status of these variants in patients treated by ICI would modulate the treatment outcome. Also, autoimmunity is most prevalent among irAEs associated with ICI, including autoimmune manifestations in gastrointestinal, endocrine and other sites. It is possible that the germline susceptibility to these autoimmune conditions may stratify patients by risk to the same autoimmune irAEs before the ICI treatment is administered. Of note, while the autoimmune risk alleles confer only a small risk effect (relative risk ranging between 1.1 and 1.5), these effects will likely be enhanced substantially upon ICI, with possible actionable clinical applicability as predictive biomarkers (a concept detailed below). This is exemplified in our recent study that tested 25 risk variants, each associated with multiple autoimmune conditions in recent GWASs, for their effect on ICI efficacy. We found that genetic variation in the IL-2/IL-21 region (a risk locus for allergy, colitis and type 1 diabetes) was associated with increased anti-PD-1 response, with a significance surpassing multiple testing adjustments [odds ratio (OR) 0.26, 95% confidence interval 0.12–0.53; P=0.0002]. Our study provides the first indications that autoimmune genetic susceptibility may modulate ICI efficacy, suggesting that systematic testing of autoimmune risk loci can reveal personalized biomarkers of ICI outcome [78]. We have recently found a significant association between an eQTL in the IL-10/BATF3 locus on 1q32 and favorable melanoma survival, showing a clinically relevant effect size that complements other established clinicopathological prognostic markers [79]. High BATF3 expression is associated with the differentiation of CD8α^{+} dendritic cells and Th17 cells [80,81], key players in tumor-immune responsiveness. Unlike an established paradigm of immuno-suppressive mechanism, IL-10 has been shown to play an important role in antitumor immune surveillance inducing the expression of pro-inflammatory IFNγ cytokine [82]. Interestingly, the detected eQTL in the IL-10/BATF3 locus from our study [79] is also a well-established variant associated in recent GWASs with the risk of multiple autoimmune conditions [83], strongly suggesting that the baseline genetic risk of autoimmunity may be favoring longer overall survival in immunogenic tumors, and possibly IO treatment outcomes.

Genome-wide approaches in IO: GWASs

The genome-wide DNA microarray technologies used in GWASs offer an affordable platform for testing the association of common genetic variation across the genome with the phenotypes of interest, traditionally with disease risk. However, there is clinical skepticism regarding GWASs in case–control genetic risk studies, stemming from the low effect size observed for the vast majority of identified associations with common genetic variants. Historically, this has been a major drawback in clinical applicability of this technology in oncology and other complex disease designs. However, recent advances in understanding the common genetic variation prompted an intriguing hypothesis that the role of common genetic variation in the pharmacogenomics context will likely be substantially enhanced. It is highly possible that, unlike case–control disease risk design, the genetic variants significantly associated with, for example, therapy resistance will not undergo the same negative selection pressure as disease-risk alleles [84]. This means that these variants, while exerting a significant biological effect associated with, for example, a specific therapy response, may likely be in high population frequency, and as such reliably 'testable' in a GWAS array design. In our recent study, we clearly demonstrated that the effect of autoimmune risk variants (IL-2/IL-21/PTPN2) associated with anti-CTLA-4 and anti-PD-1 resistance is significantly larger (OR>3) compared with the modest risk effects of these variants observed in autoimmune risk GWASs (OR 1.1–1.5) [78]. The increased penetrance of these autoimmune risk variants observed in ICI-treated patients is likely due to the combined synergistic interaction between baseline (modest) autoimmune risk and ICI-induced immune stimulation [69]. The most significant association surpassing multiple testing correction in this study was observed for anti-PD-1 efficacy and rs17388568, a variant mapping to IL-2/IL-21, two key cytokines modulating tumor immune responsiveness through enhancement of CD8+ T-cell activities and increased intratumoral T-cell infiltration [85,86]. Additional support comes from our recent biomarker study showing that the specific, functionally relevant, autoimmune risk variants in interleukin pathways may be associated with improved melanoma survival, with the effect size reaching clinical applicability, independent of the current prognostic melanoma predictors [79]. All of these data indicate that GWASs focused on common variants in the setting of clinical end points, such as therapy efficacy or patient survival, may not only reveal the significant associations with clinically actionable effects, but also point to possible novel drug targets for improved

Table 1
Summary of prior studies investigating the association between germline variants and immune-checkpoint inhibition (ICI) clinical outcomes.

Cancer (metastatic)	Cohort size	Treatment drug	ICI outcomes	Germline target	Effect size (95% CI); *P*-value	Reference
Multiple	1535	Multiple	Post-ICI OS	HLA-I (homozygous in at least one locus of HLA-I versus heterozygous at all loci)	1.38 (1.11–1.70); *P*=0.003	[66]
Melanoma	152	Anti-CTLA-4	Efficacy	CTLA-4 promoter region	3.39 (1.62–7.10); *P*=0.002 (rs4553808) 2.89 (1.23–6.83); *P*=0.02 (rs11571327) 0.39 (0.18–0.82); *P*=0.009 (rs231775)	[39]
Melanoma	14	Anti-CTLA-4	Efficacy/post-ICI OS	CTLA-4	12.5 (0.8–18.6); *P*=0.04 (rs11571316) >6.8 (0.5–12.3); *P*<0.07 (rs11571317) <0.006 (rs3087243; OS log-rank test)	[40]
Melanoma	121	Anti-CTLA-4	Efficacy	FcγR-activating receptor	*P*=0.016 (Fisher's exact test)	[111]
Melanoma	19	Multiple	Efficacy	CDKN2A	*P*=0.03 (binomial test)	[112]
Melanoma	169	Anti-PD-1	Efficacy	IL-2/IL-21	0.26 (0.12–0.53); *P*=0.0002	[78]
Melanoma	213	Anti-CTLA-4	Efficacy	PTPN2	2.79 (1.36–5.73); *P*=0.005	[78]
Glioblastoma	One case report	Anti-PD-1	Efficacy	POLE	Patient experienced an objective radiographic response	[113]
Multiple	154	Anti-PD-1/anti-PD-L1	Efficacy/toxicity	Variants in miRNA 3′UTR and promoter regions	Specificity for response=89% (random forests) Specificity for toxicity= 76% (random forests)	[114]

CTLA-4, cytotoxic T-lymphocyte-associated protein 4; HLA, human leukocyte antigen; IL, interleukin; CI, confidence interval; OS, overall survival.

combinatorial treatment modalities. In fact, IL-21 in combination with anti-PD-1 is currently being evaluated in an ongoing clinical trial in solid tumors [87]. In IO, the use of germline GWAS design has been largely successful in linking the germline genetics with an immune-component TME [38], as described above. Currently, no large GWAS effort has been reported for ICI outcomes, despite an urgent need for such analysis. The lessons learned from GWASs clearly point to the need for a large population ascertainment for the successful discoveries of germline associations; however, such cohorts have not been available in the IO setting. It has been established that >2500 cases and an equal number of controls are needed to achieve statistical power of 80% for the discovery of moderate-to-high penetrant loci with an effect size >2. Thus, a large collaborative effort, pooling the ICI-treated patient resources in a multi-institutional framework, is critical to meet this statistical requirement. Recently, an international consortium has been established to conduct the first comprehensive GWAS on metastatic melanoma ICI efficacy, toxicity and ICI-associated survival, with a goal of pooling >10 000 patients treated across different ICI modalities [88]. While still in the initial phase, some data have already been generated, particularly for anti-CTLA-4 treatment efficacy. Significant associations have been noted for anti-CTLA-4 resistance with established autoimmune risk loci (unpublished data), reaching the statistical threshold of GWAS level of significance ($P<10E-8$). Remarkably, however, while these loci showed only low-penetrant association effects in original autoimmunity risk GWASs, the observed effect size for these variants in the ICI study are much larger (relative risk of 4–5), clearly implying their 'actionable' clinical potential.

Genome-wide approaches in IO: NGS in discovering common and rare germline variation as IO biomarker

As the cost of NGS is gradually decreasing, a parallel comprehensive assessment of both common and rare variants contributing to ICI outcomes becomes feasible. However, the data complexity is among the major hurdles of NGS technologies, and the standard single SNP tests may not be the most appropriate. The use of gene-burden tests, such as the Sequence Kernel Association Test [89,90], may reduce this problem by analysing both common and rare variants in the regions of interest. The cost of these technologies is still prohibitive to allow for testing a large number of patients on a population scale. Whole-exome sequencing (WXS) offers a relatively cost-effective alternative; however, it targets only exonic regions (representing ~1% of the genome). Nevertheless, the expectations that coding genetic variation may exert causal biological effects with imminent clinical applicability is another notable advantage in favor of WXS.

Whole-genome sequencing (WGS) offers the most comprehensive platform for the discovery of germline genomic determinants of ICI. However, the use of WGS in large population scans is less realistic due to the high cost and analytical complexity. Other platforms from biological investigations will need to be implemented in prioritization of relevant genomic loci in order to reduce the computational challenges of WGS data analysis (as discussed below). Nevertheless, there has been an imminent pharmacogenomic interest in NGS-based exploration of germline genetic variation in the therapeutic context, including IO, by institutional [91,92] or industrial entities [93]. Despite these major promises, the NGS technologies in the area of germline genetics in IO are yet to be fully explored as feasible discovery platforms in the context of larger patient populations.

Clinical applicability of inherited genetic factors as clinically relevant biomarkers of ICI

The germline genetic variants in IO may serve as putative predictive biomarkers with personalized potential; the genetic variation may be polymorphic or private (rare in the population) and can therefore represent a predictive 'fingerprint' specific to individual patients. Moreover, germline variants can be assessed in a non-invasive blood test by a low-cost genotyping assay, and can therefore be feasibly applied in clinical stratification of a large number of patients prior to treatment in a robust high-throughput setting. A particular example of germline ICI biomarkers in current clinical practice is the status of mismatch repair deficiency in colon cancer [94,95]; the germline mutations in mismatch repair pathways, usually manifested by microsatellite instability, result in high TMB, and hence increased neo-epitope presentation and better immune tumor recognition upon ICI, particularly in anti-PD-1 treatments. Testing the mismatch repair deficiency before treatment may stratify the patients who are most likely to benefit from ICI therapies. However, this strategy is only applicable for ~5% of colorectal cancer

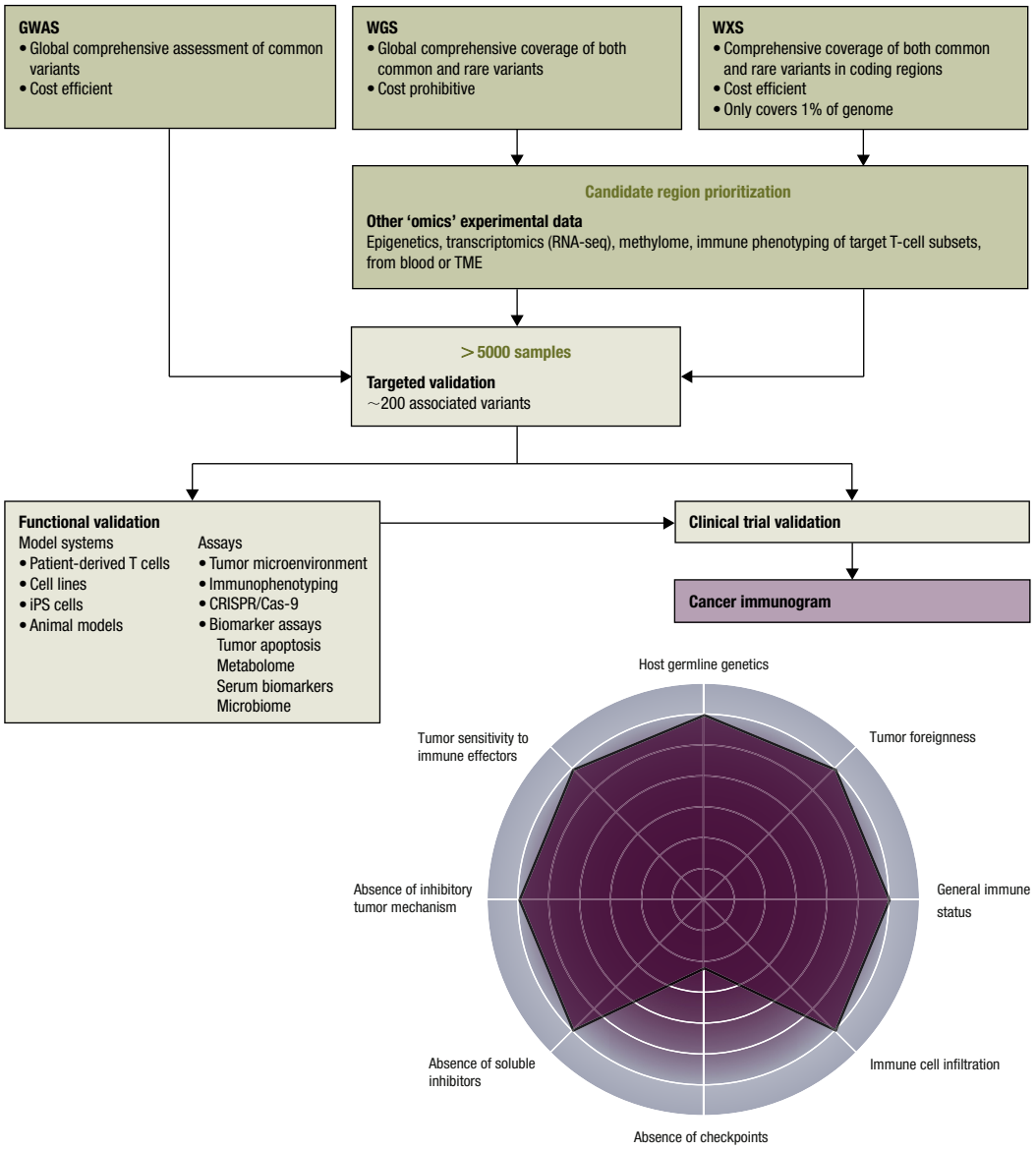

Figure 1. Proposed strategies for the discovery, validation and clinical implementation of germline genetic biomarkers of immune-checkpoint inhibition (ICI) outcomes. A genome-wide analysis in the setting of a large collaborative consortium that pools patients' resources and harmonized clinical information identifies ICI-associated germline variation using genome-wide association studies, whole-genome sequencing or whole-exome sequencing platforms. Multi-omic data are integrated to further refine and prioritize genomic loci for targeted validation in an independent patient cohort. Functional and clinical validation is performed on a subset of germline variants that are reproducibly associated with ICI clinical outcomes in both discovery and validation patient cohorts. The validated host germline genetic markers can subsequently be integrated with other ICI biomarkers to generate personalized ICI prediction models (cancer immunograms). Further clinical tests in prospective populations can be designed (likely in the framework of a prospective clinical trial) to translate the germline genetic biomarkers of immuno-oncology outcomes into routine clinical practice following the biomarker development pipelines established previously [115,116].

(CRC) patients with high-risk germline mismatch repair mutations, although recently, the microsatellite instability of CRC, in general, has been shown to be a sufficient indicator of ICI benefits [96]. In contrast, it is expected that the common germline genetic variants as predictors of IO outcomes from genome-wide scans will expand the predictive applicability to the general patient population. From the preliminary data described above, it is likely that numerous loci would be associated with ICI efficacy, toxicity or survival, showing similar genetic pleiotropy as observed in autoimmune risk susceptibilities (e.g. many different loci, likely in distinct pathways, would contribute to the same treatment regimen efficacy, same toxicity site or survival). Therefore, the application of polygenic scores (PS) testing multiple variants simultaneously will be essential in these designs; PS have proven to be extremely powerful for increasing the effect size over five-fold, compared with the predictive power of individual variants alone [97]. The clinical applicability of IO germline biomarkers will likely be driven by developing germline polygenic signatures that will further facilitate the integration of ICI-related host genetic factors with previously proposed ICI biomarkers, for example by implementing the 'clumping and thresholding' method, applied in prior GWASs [97–99]. With the availability of molecular and clinical information from tumors and peripheral blood (e.g. TMB, TIL, RNA-seq, PD-L1 status and immunoscores), the identified IO-associated germline variants (defined as cumulative PS) can be integrated with other proposed ICI biomarkers in machine-learning modalities, as applied recently [100,101]. For example, the ongoing clinical trial for non-small cell lung carcinoma shows substantially improved predictive power for ICI response by combining expression levels of IFNγ and immunohistochemistry of tumor PD-L1 expression, which is superior

compared with the predictive value of each of these markers individually [102]. The combination of multifactorial ICI biomarkers may also be performed in the clinical framework of 'cancer immunograms', currently presented for a growing number of immunogenic cancers [103–105], some of which are already reaching possible clinical utilization [106]. Adding the germline genetic information into immunograms will allow for a holistic perspective of the tumor-immune landscape, thus improving the optimal selection of personalized IO therapy at an individual patient level by accounting for unique combinations of all relevant ICI predictors.

Current methodological impediments and solutions in germline genetic studies of ICI

Despite accumulating evidence supporting the contribution of germline genetics to host immunity, knowledge on host genetic factors as predictive biomarkers of ICI clinical outcomes is limited. Few reported associations with ICI efficacy or survival have been independently cross-validated or moved forward to clinical utilization (summarized in Table 1). Also, to date, no systematic scan has been reported on genetic variation as a surrogate of IO outcomes at the genome-wide level. The main reason for these research gaps is a lack of sufficiently powered ICI patient cohorts. We estimate that >5000 patients will be needed to achieve statistical power of ~80% for the discovery of low-penetrant germline loci associated with ICI outcomes. This clearly indicates the need for a large international collaboration pooling patient resources. While an ICI-focused germline consortium is currently being formed, an important component will be the harmonization of clinical data, particularly for patients outside of clinical trials, due to expected clinical heterogeneity of this information (particularly for ICI toxicity, heterogeneity of ICI treatments, etc.) across centers. Subtle differences in adjustments for clinical factors will have a contributing effect on germline genetic association tests in this context, so appropriate considerations must be put in place to address these confounding factors.

Genome-wide approaches will be instrumental in the comprehensive discoveries of germline contributions to ICI. In a standardized way, the collaborative consortia should follow the pattern of GWAS design, eventually complemented by NGS technologies, which are still relatively costly for the scope of larger populations. A solution to this will be the prioritization of genomic regions for more targeted assessment, based on other layers of genetic, epigenetic, proteomic or immune-based evidence. This will also address the functional impact of identified genetic variants, as many will map in non-coding regions; understanding how they affect gene regulation and immune function will otherwise be challenging. While some of the non-coding associations have been explained by a regulatory effect on transcriptional regulation of the proximal genes (e.g. eQTLs), there is a need for more thorough functional assessment of GWAS signals. The implementation of other 'data imputation'-based methods, such as transcription-wide association studies (TWASs), will address some of these impediments, capitalizing on molecular and transcriptomics data from large immune cell populations, extracted from other publically available repositories (e.g. MuTHER [107] and GTEx [108]). This is particularly important for large collaborative efforts, such as the recently established melanoma IO germline consortium, in which the patient cohorts pooled retrospectively from standard-of-care clinical settings will have limited availability of other biological specimens (e.g. tumors) for functional investigations. The preliminary analyses from the ongoing study of the melanoma IO germline consortium implementing a TWAS have revealed transcriptional networks in cytokine-related pathways enriched by germline genetic susceptibilities associated with ICI efficacy and toxicity (unpublished data). With a more comprehensive picture of germline genetics associated with ICI, it is also difficult to disentangle the causal relationships between SNPs, intermediate immune phenotypes, other candidate biomarker surrogates (e.g. TMB, cytokine levels) and outcomes (toxicity, efficacy, survival). Computational methods relying on Mendelian randomization are being developed to address this problem [109,110], and while this will require a large sample size, it will be possible within large collaborations such as the newly established melanoma IO germline consortium. Standardization in terms of platforms, experimental conditions and bioinformatics pipelines is critical for the success of this type of investigation. As such, the putative biomarkers discovered in the genome-wide designs (that include training and validation phase) may need to be tested further in prospective clinical trials (metastatic or adjuvant) before developing clinical tests (see Figure 1).

In summary, the compelling evidence in support of germline host factors as IO biomarkers opens up attractive avenues for personalized prediction of ICI clinical outcomes. Addressing the past impediments, large consortia are currently being established in different tumor types to elucidate a role of germline genetic factors in modulation of ICI toxicity, efficacy and survival. With the availability of large publicly available data already accumulated from tumors, immune cells, TME and many 'omics' platforms, the comprehensive functional mapping of germline genetic underpinning of IO clinical end points may be possible in the near future. Integrating other tumor-specific and host factors with germline genetic information will be essential to develop robust ICI predictive algorithms. In addition to biomarker improvement, these may eventually pave a path towards the discovery of novel biological targets for improved IO therapies that will minimize toxicities while maximizing clinical benefits at an individual patient level.

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