

Practical Implementation of Genetics: New Concepts in Immunogenomics to Predict, Prevent, and Diagnose Drug Hypersensitivity



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Learning objectives:

1. To discuss the specific high-risk genetic variants predisposing different drug hypersensitivity reactions in different patient populations.
2. To understand the role of recently proposed non-human leukocyte antigen (HLA) risk factors in immunopathogenesis of CD8+ T-cell mediated drug hypersensitivity reactions.
3. To describe the utility of acute tissue samples and single-cell sequencing to identify immunodominant drug- and HLA-restricted T-cell receptor (TCR) for advanced immunogenomics approaches to predict, prevent, and diagnose drug hypersensitivity.

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Delayed drug hypersensitivities are CD8⁺ T cell-mediated reactions associated with up to 50% mortality. Human leukocyte antigen (HLA) alleles are known to predispose disease and are specific to drug, reaction, and patient ethnicity. Pretreatment screening is recommended for a handful of the strongest associations to identify and prevent drug use in high-risk patients. However, an incomplete predictive value implicates other HLA-imposed risk factors, and low carriage of many identified HLA-risk alleles combined with the high cost of sequence-based typing has limited economic viability for similar recommendation of screening across drugs and health care systems. For mitigation, an expanding armory of low-cost polymerase chain reaction-based screens is being developed, and HLA-imposed risk factors are being discovered. These include (1) polymorphic variants of metabolic and endoplasmic reticulum aminopeptidase enzymes toward multiallelic screening with

Abbreviations used

ADT- Antibody-derived tag
 APC- Antigen presenting cell
 cDNA- Complementary DNA
 DRESS- Drug reaction with eosinophilia and systemic symptoms
 ER- Endoplasmic reticulum
 mRNA- Messenger ribonucleic acid
 scRNA- Single-cell RNA
 SJS/TEN- Stevens-Johnson syndrome/toxic epidermal necrolysis
 TAP- Tapasin
 TRM- Tissue-resident memory T cell
 UMI- Unique molecular identifier

increased predictivity; (2) regulation by immune checkpoint inhibitors, enabling detolerized animal models of human disease; and (3) immunodominant T cell receptors (TCR) on clonally expanded CD8⁺ T cells. For the latter, HLA risk—restricted TCR provides immunogenomic strategies and samples from a single patient to identify novel HLA-risk associations in underserved minority populations, tissue-relevant effector biomarkers toward earlier diagnosis and treatment, and HLA-TCR—presented immunogenic structures to aid future drug development. © 2022 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2022;10:1689-700)

Key words: Single-cell sequencing; T cell receptor; Human leukocyte antigen; Drug hypersensitivity; Endoplasmic reticulum aminopeptidase; Immunogenomics

INTRODUCTION TO IMMUNOGENOMICS AND DRUG HYPERSENSITIVITY

Immunogenomics incorporates sequencing and genetic engineering platforms to assess how the variance of immune-related genes influences immune function, disease onset, and severity. Although the average human genome differs from reference consensus by 4.1 to 5 million single nucleotide polymorphisms,¹ the HLA complex has been a disease-spanning focus of interindividual immune susceptibility as the most polymorphic locus in the human body. More than 30,000 HLA allelic variants have been described.² With critical function in the presentation of antigen to T cells, different HLA alleles have been associated with altered susceptibility to T cell—mediated disease including delayed drug hypersensitivity reactions (DHRs). CD8⁺ T cell—mediated DHRs often target specific tissues. Severe manifestations include drug-induced liver injury or cutaneous reactions including drug reaction with eosinophilia and systemic symptoms (DRESS) or Stevens-Johnson syndrome/toxic epidermal necrolysis (SJS/TEN), which are associated with up to 9%, 10%, or 50% mortality, respectively.³⁻⁵ Treatment approaches remain limited without high-resolution approaches to dissect and characterize the immunopathogenic T cells driving disease in immunologically heterogeneous tissues. Thus, there is a critical need to identify predictive risk factors for prevention. This was provided by seminal association in 2002 between HLA-B*57:01 and abacavir hypersensitivity syndrome, which developed in up to 8% of patients within the first 6 weeks of treatment, presenting as fever with rash and gastrointestinal and

respiratory symptoms. Importantly, abacavir is a nucleoside reverse-transcriptase inhibitor used to treat HIV with favorable long-term toxicity. However, the onset of hypersensitivity syndrome necessitated patient withdrawal from this efficacious treatment for replacement by less-efficacious regimens.⁶ The complete 100% negative predictive value (NPV) of this association with a 55% positive predictive value (PPV) demonstrated this allele was necessary although insufficient for disease to occur. This then led to the first double-blind, randomized trial of its kind to demonstrate the efficacy of abacavir pretreatment HLA screening to improve drug safety by avoiding its use in HLA-B*57:01—positive patients.⁷ Genome-wide association studies (GWAS) have since identified other strong HLA associations; a few of those are now similarly implementable in the clinic that are specific to drugs, reaction phenotypes, and patient populations with shared genetic ancestry. However, this has driven health disparities with risk discovery limited to only the most prevalent populations, because underrepresented ethnic groups lack the necessary large control cohorts. Moreover, most associations are of lower NPV and PPV, limiting the viability of costly sequence-based HLA typing for clinical recommendations across national cohorts. However, low-cost single-allele screens are being developed and HLA-imposed risk factors are being identified, which propose to increase PPV toward more predictive multi-allelic screening strategies. Moreover advanced immunogenic approaches are being implemented to predict genetic risk in a single patient, to characterize single-cell effector signatures in tissue toward diagnostics and treatments and define HLA-presented moieties to inform structural risk for avoidance in future drug development. We provide an overview of immunopathogenic models of DHR, providing a background for current predisposing genetic risk factors, before elucidating the new immunogenomic concepts and technologies driving advanced prediction, prevention, and treatment strategies. Importantly, these are critically dependent on the clinician-led curation of ethnicity-captured paired tissue samples and international biorepositories.

Mechanisms of drug-induced T cell activation

There are three mechanisms by which small—molecular weight drugs drive T cell activation, all of which depend on the formation of a risk HLA—antigen complex with a corresponding TCR. The first to be described was the hapten concept, in which drugs must first bind self-protein to become large (>1,000 Da) non—self-neoepitopes capable of immune recognition (Figure 1, A, i). Antigen-presenting cells (APCs) then engulf and process the drug-hapten into peptides for HLA presentation as a mechanism best described for β -lactam antibiotics.⁸ Next, because the antibiotic sulfamethoxazole can directly stimulate T cells but is not protein-reactive, Pichler⁹ established the pharmacologic interaction concept. Here, the drug antigen binds directly to the HLA or TCR in the absence of APC processing (Figure 1, A, ii). The most recently described is the altered self-peptide repertoire model, which factors in the continuous presentation of tolerized self-peptides by HLA during steady-state immunosurveillance.^{10,11} In the context of HLA-B*57:01, abacavir binds in such way that the HLA antigen-binding cleft is changed, altering the repertoire of endogenously processed self-peptides that can be presented. These previously intolerized HLA-specific antigen complexes are identified as foreign, with a multitude of potential self-

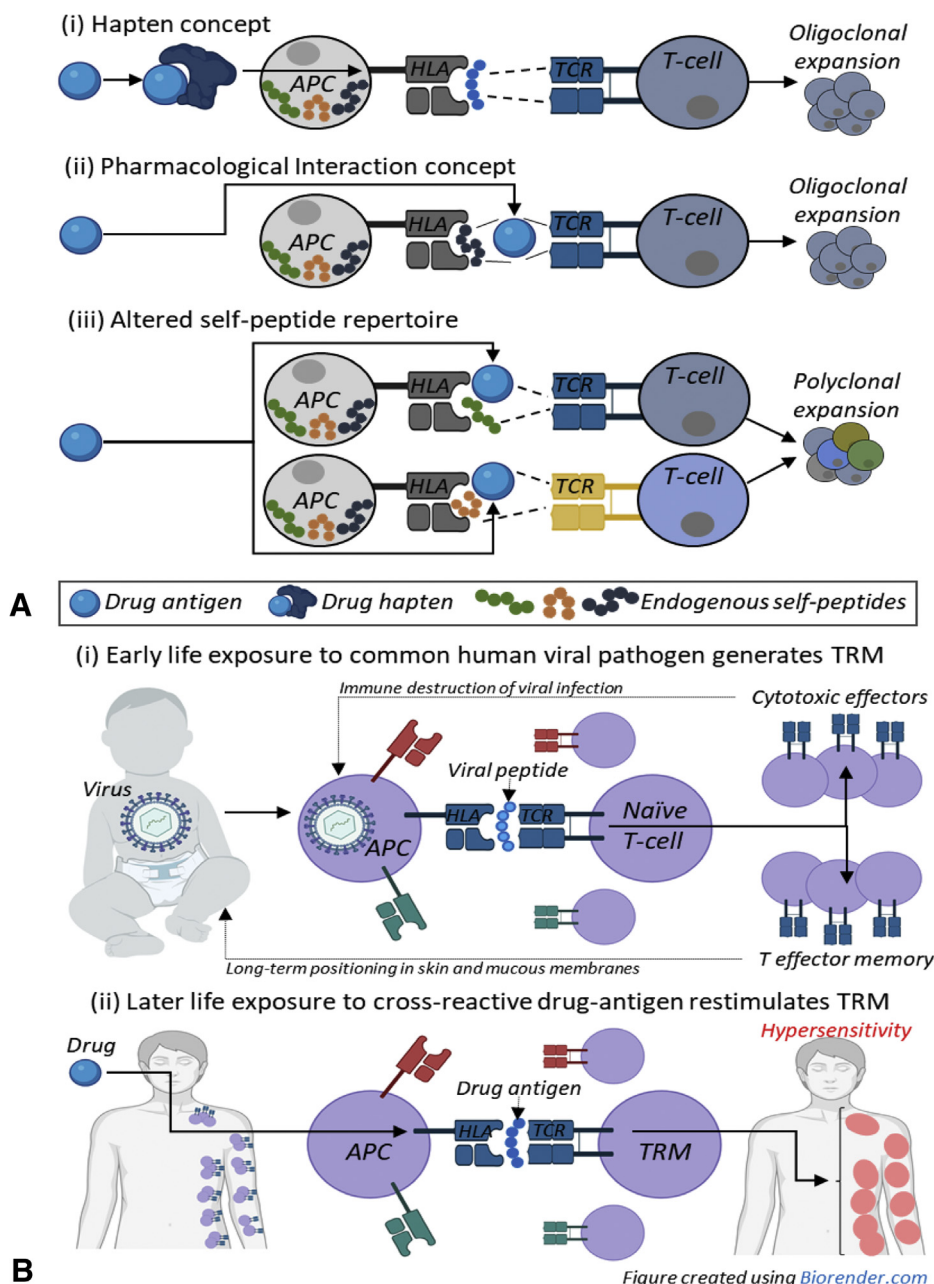


FIGURE 1. Models of drug-induced T cell activation. **(A)** (i) Hapten, (ii) pharmacologic interaction, and (iii) altered self-peptide repertoire models of how drugs activate T cells. **(B)** Heterologous immunity. (i) Patient infected with pathogen that primes naïve T cell to drive tissue-resident memory T-cell (TRM) development. (ii) Drug cross-reacts with viral-primed TRM. APC, antigen presenting cell; TCR, T cell receptor.

peptides driving polyclonal TCR activation opposed to the oligoclonal response observed with more structurally restricted hapten- or pharmacologic interaction-induced activation (Figure 1, A, iii).¹² Importantly, a functional assessment of how each drug activates T cells can be performed *in vitro*, either using postreaction patient-derived memory T cells^{13,14} or through naïve T cell priming platforms developed to assess immunologic risk in healthy drug-unexposed donors.¹⁵⁻¹⁸ Intriguingly, the use of such assays has raised two nonmutually exclusive models for the origins of drug-reactive T cells: a more traditionally

described route in which drug antigens prime naïve T cells,^{17,19,20} and another centered on heterologous immunity in which the drug drives the cross-reactive restimulation of memory T cells primed to previously encountered antigen. For this model, although the initial priming antigen is undefined, abacavir-responsive T cells are observed in all HLA-B*57:01-positive but drug-exposed donors, which implicates broad early-life exposure pathogens such as herpes simplex virus. Yerly et al²¹ reported the reactivity of an HLA-B*57:01-restricted abacavir T cell clone to a herpes simplex virus-1/2-derived

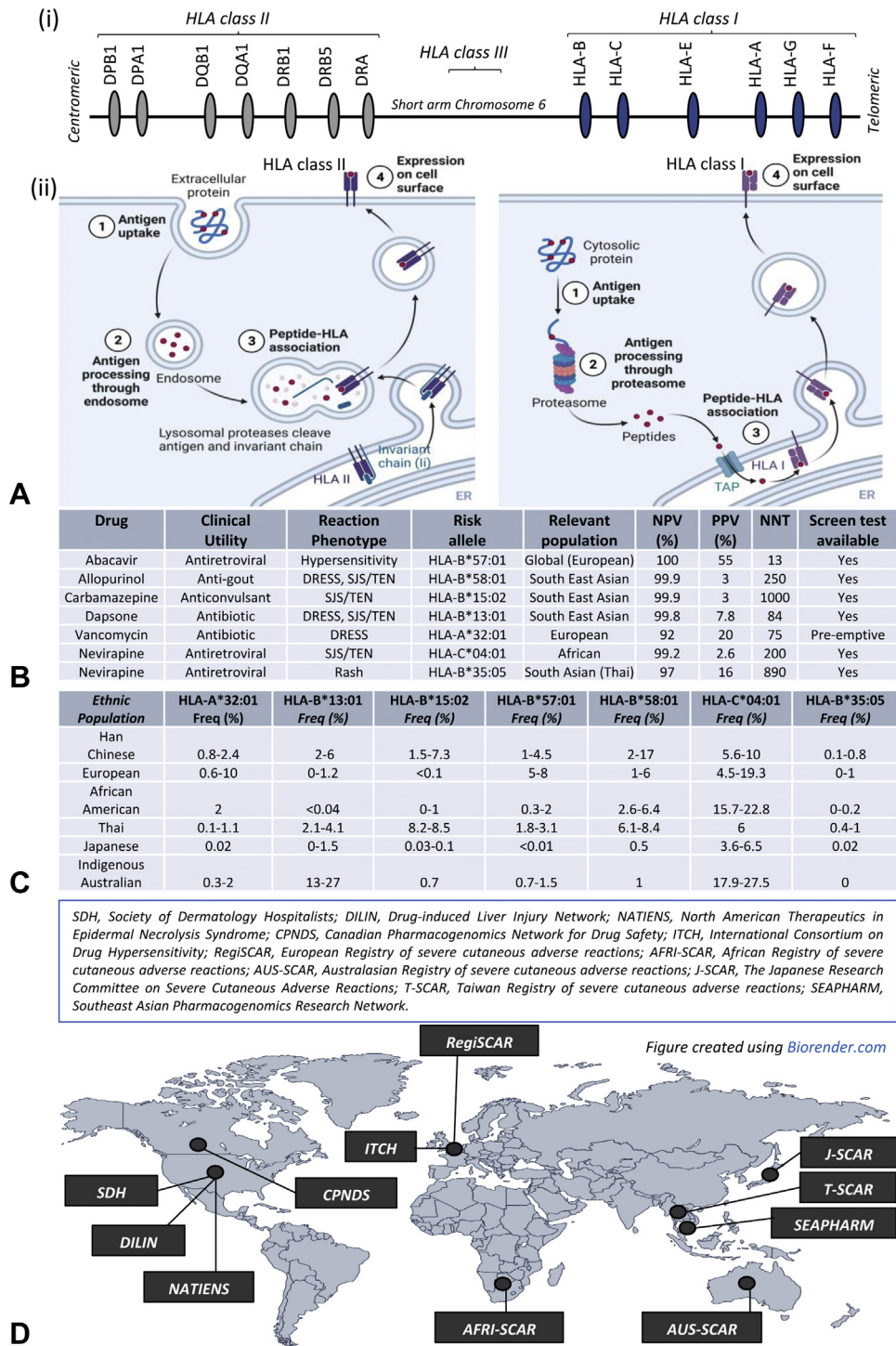


FIGURE 2. Human leukocyte antigen risk and drug hypersensitivity. (A) Schematic of HLA (i) locus organization and (ii) peptide antigen loading pathways. (B) Key HLA class I risk alleles with implemented, potential, or preemptive screening assays. (C) Allele frequency (%) of key HLA risk alleles in representative ethnic populations. (D) Map showing location of drug hypersensitivity-focused clinical repositories and consortia. *DRESS*, drug reaction with eosinophilia and systemic symptoms; *ER*, endoplasmic reticulum; *NNT*, number needed to treat; *NPV*, negative predictive value; *PPV*, positive predictive value; *SJS/TEN*, Stevens-Johnson syndrome/toxic epidermal necrolysis; *TAP*, tapasin.

peptide sequence. This model is postulated to be the reason for the tissue-specific targeting of disease, because virally primed memory T cells (Figure 1, B, i) migrate to and reside as CD103-

expressing tissue resident memory T cells (TRMs) in the skin and mucous membranes until proposed cross-reactive drug exposure (Figure 1, B, ii). However, this remains a developing

hypothesis, and whereas the accumulation of activated TRMs (CD45RO⁺CD69⁺CD103⁺) was recently reported in skin biopsies after intradermal drug challenge in patients with different DHRs, similar characterization during acute disease is warranted to pinpoint the origins of response-driving populations.²²⁻²⁴

Human leukocyte antigen and current state of risk implementation

Human leukocyte antigen loci are divided into classes I through III (Figure 2, A, i). Whereas class III is nonclassical and represents complement, TNF, and heat shock proteins, HLA class I encompasses HLA-A, HLA-B, and HLA-C genes with protein expressed on all nucleated cells to present endogenous peptides to cytotoxic T cells (Figure 2, A, ii) for a physiologic role in the clearance of virus-infected cells. In contrast, HLA class II includes HLA-DP, HLA-DQ, and HLA-DR genes, in which protein is more selectively expressed on APC for the presentation of exogenous peptides (Figure 2, A, ii) to T helper cells in response to extracellular infections. Since the discovery of an association between HLA-B*57:01 and abacavir hypersensitivity, several other strong HLA associations with complete NPV have been described and clinically implemented for ethnic populations of high allelic carriage (Figure 2, B). The association of HLA-B*15:02 with hypersensitivity to the antiepileptic drug carbamazepine and preemptive screening were first conducted in a few countries, especially Taiwan. Taiwan, Hong Kong, Thailand, and Singapore now endorse HLA-B*15:02 pre-prescription screening.²⁵⁻²⁷ Moreover, prospective screening of HLA-B*13:01 is proven to reduce the incidence of dapsone hypersensitivity in Chinese²⁸ and Thai²⁹ populations, and screening for HLA-B*58:01 associated with hypersensitivity to the anti-gout drug allopurinol has been selectively implemented in Southeast Asia.^{30,31} However, routine HLA testing is not yet similarly advised by the European Medicines Agency or the US Food and Drug Administration.³² A recent evaluation of a Canadian populace showed that HLA-B*58:01 screening was underused in countries outside East Asia, in which less than half of allopurinol-exposed patients of high-risk East Asian ancestry who were tested³³ raised the need for greater clinical awareness. It is clear that risk alleles are specific not only to drugs, but also to the ethnic population and life-threatening reaction phenotype. This is exemplified by the association between HLA-A*31:01 and carbamazepine-induced DRESS, but not SJS/TEN, in both European and Chinese populations,³⁴ and associations between HLA-B*15:02 and HLA-B*57:01 with carbamazepine-induced SJS/TEN in Chinese and European populations, respectively.^{35,36} New risk alleles continue to be discovered, including strong associations for high-utility antibiotics. These include HLA-A*32:01 and vancomycin-DRESS in Europeans in 2019,³⁷ and HLA-B*13:01 and HLA-B*15:02 with co-trimoxazole-induced severe cutaneous adverse reactions in Asian populations in 2020 and 2021.^{38,39} A diversity of alleles are associated with diverse drugs, as extensively reviewed,^{40,41} so it is intriguing that the same risk alleles are shared by structural diverse antigens. This is epitomized by the association of B*35:01 with liver injury caused by *Polygonum multiflorum* in 2019,⁴² green tea in 2020,⁴³ and co-trimoxazole in 2021.⁴⁴ Shared binding is interesting for identifying alternate risk alleles for the same drug across ethnicities, as demonstrated by the similar risk for HLA-C*04:01-induced hypersensitivity across HLA-C*04 variants with a shared F pocket motif, including alleles prevalent in

Hispanic (C*05:01) and African American (C*18:01) populations.⁴⁵ However, few risk alleles outside those discussed have translated to the clinic. This is because of an incomplete NPV and considerable diversity in allelic carriage among different ethnic populations (Figure 2, C), which implicate other undefined HLA-risk alleles, as well as a low PPV, which implicates undefined non-HLA genetic risk factors. In recent years, this has driven two strategies: the development of low-cost, single HLA-allele screening assays and the genomic investigation of HLA-imposed risk factors to reduce the number of patients needed for testing to prevent one case (NNT).

Critical need 1: development of economically viable screening assays for clinical implementation

DNA sequence-based HLA typing is the reference standard, providing discrimination of most HLA class I alleles through focused amplification of exons 2 and 3 at a resolution able to identify novel allelic variants. However, with advances in next-generation sequencing (NGS), bioinformatic algorithm-informed computational tools have been developed to match NGS fragments to different HLA allele variants with high accuracy. These include HLA-HD, OptiType, HLAforest, HLAreporter, and PHLAT, which are capable of HLA prediction and imputation using either whole-exome sequencing or whole-genome sequencing.⁴⁶ Because the cost of NGS is decreasing, prediction and imputation of HLA may serve as an alternative future method for HLA genotyping.⁴⁷ Nonetheless, sequencing methods remain time-consuming, dependent upon specialist expertise and equipment, and costly; HLA-A*31:01 typing is reported to be 300 GBP.⁴⁸ This is particularly true for alleles with incomplete NPV and PPV and with expression restricted to particular populations, which complicates implementation in countries with a diverse multiethnic populace. However, the converse strategy of testing only patients from high-carriage populations will leave other patients at risk. For example, nonselective screening in US populations identified more than twice the at-risk patients for HLA-B*15:02—restricted carbamazepine hypersensitivity compared with screening Asian patients alone.⁴⁹ To facilitate broader risk screening, multigene panel tests have been developed encompassing high-risk alleles to provide extended risk genotype information. One such panel costing 50 GBP and inclusive of HLA-A*31:01, HLA-B*15:02, HLA-B*57:01, HLA-B*58:01, HLA-B (158T), and HLA-DQB1 (126Q) is economically viable across alleles with 0.0069 increased quality-adjusted life-year gain and a total cost savings of US \$491, but not for individual drugs including carbamazepine with HLA-B*15:02, and allopurinol with HLA-B*58:01.⁵⁰ Importantly, for these well-defined highly-predictive alleles, RT-PCR-based assays have been developed, including HLA-B*58:01⁵¹ or HLA-B*57:01/HLA-B*58:01,⁵² and HLA-B*15:02⁵³ or HLA-B*15:02/HLA-A*31:01.⁵⁴ Reverse transcription-PCR is both rapid and cheap, requiring only an initial sequence-specific design for highly sensitive target-allele amplification within a few hours, and it is a highly established technique suited to most on-site clinical laboratories. Moreover, multiple genes can be targeted through multiplexed assays, enabling the simultaneous assessment of risk and housekeeping control genes to prevent false negatives.⁵⁵ Thus, expansion of the single-allele screening tool armamentarium is required to provide similar strategies across drugs and populations and drive clinical validation studies. For example, a single-allele screening assay

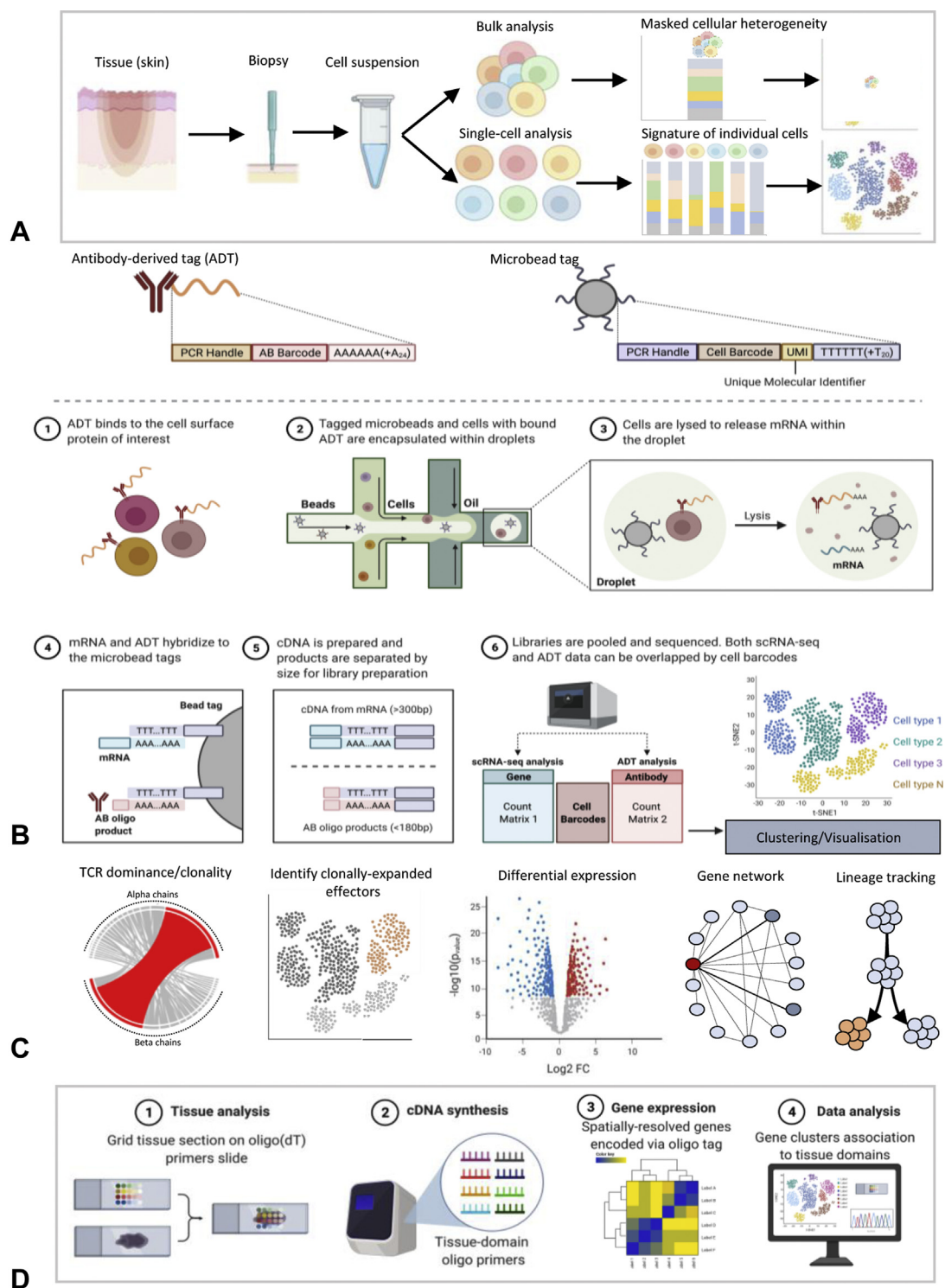


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FIGURE 3. Single-cell sequencing to explore immunopathogenesis. Schematic of **(A)** the resolution provided by single-cell compared with bulk sequencing methods, **(B)** single-cell RNA (scRNA)-TCR cellular indexing of transcriptome and epitopes sequencing methodology, **(C)** utility of single-cell data to explore dominant TCR and identify the clonally expanded effector population, differential gene expression signatures, and network and lineage analysis, and **(D)** spatial transcriptomics. *ADT*, antibody-derived tag; *cDNA*, complementary DNA; *mRNA*, messenger ribonucleic acid; *UMI*, unique molecular identifier.

would provide the cost-effective validation of recently described associations between life-threatening green tea—, *P. multiflorum*—, and co-trimoxazole-induced liver injury and HLA-B*35:01.^{41–43} A clear example demonstrating how this need can be met is the recent development of an HLA-A*32:01 RT-PCR screen for the risk for DRESS associated with vancomycin.³⁶ Although not yet translating guidelines, the 100% sensitivity and specificity for HLA-A*32:01 detection with as little as 10 ng of DNA⁵⁶ and short turnaround may be appropriate to enable immediate treatment and post-prescription screening, with therapy altered in patients who are subsequently identified as positive.

Critical need 2: non-HLA risk factors toward multiallelic screening or predictive models of disease

Whereas HLA screening provides an immediate avenue for DHR prevention, the incomplete PPV of all associations to date indicates other patient-specific risk factors that align with CD8⁺-driven immunopathogenic disease. These now span genetic and environmental risk, proposing advanced gene-engineered models of disease or multiallelic screening.

GENETIC VARIATION IN THE HLA-PRESENTED IMMUNOPEPTIDOME

The only non-HLA risk factor recommended for preventative DHR screening remains cytochrome (CYP)2C9, an enzyme involved in metabolizing phenytoin.⁵⁷ In particular, the *3 variant of CYP2C9 has a 90% reduced functional capacity, limiting clearance of the immunogenic parent drug. Although individuals with this profile are not prohibited from treatment, the 2020 Clinical Pharmacogenetics Implementation Consortium guideline is for a 25% or 50% reduction of the starting dose in HLA-B risk-restricted patients, defined as intermediate or poor CYP2C9 metabolizers, respectively.⁵⁸ For other drugs, multiple enzymes may have a role, in which polymorphic variants of both N-acetyl transferase 2^{59,60} and glutathione S-transferase⁶¹ are associated with but not replicated in high-risk patients for the onset of sulfamethoxazole hypersensitivity. There remain limited replicated metabolizing enzyme associations with DHR, implicating only a minor role in predisposing disease, a conclusion supported by similar quantification of drug–protein conjugates piperacillin-allergic and tolerant patients.⁶² However, the structure of the HLA-presented immunogenic antigen remains undefined with a proposed influence of self- and/or drug-derived peptides. Critically, recent GWAS indicate a genetic impact of intracellular peptide processing pathways to diversify the HLA-loaded immunopeptidome. Briefly, in a sub-Saharan African cohort, Carr et al⁶³ reported a minor association between HLA-C*04:01–restricted nevirapine rash and a specific endoplasmic reticulum aminopeptidase (ERAP) allotype. Endoplasmic reticulum aminopeptidase is an enzyme epistatically associated with HLA and is involved in processing peptides to the correct length for loading onto HLA class I molecules. Hypoactive variants are previously described in the context of viral infection, which undertrims peptides to form longer, less immunogenic moieties and impair HLA-restricted clearance of hepatitis C infection.⁶⁴ In support, a recent targeted analysis used sequence-based ERAP typing to report that risk HLA-B*57:01–expressing patients with hypoactive ERAP1 allotypes were more likely to tolerate abacavir.⁶⁵ Although by itself HLA typing for abacavir hypersensitivity is highly successful, the initial

finding with nevirapine suggests the importance of such variation across drugs. Thus, studies are warranted to improve PPV and NNT using ERAP, but also other highly polymorphic HLA-regulating loci such as killer cell–like immunoglobulin receptors linked to an HLA-imposed predisposition to other immune-mediated disease.⁶⁶ Discovery of these multiallelic associations will remain dependent on large allergic and tolerant patient cohorts but will improve risk prediction for currently defined but lowly predictive HLA risk alleles toward cost-effective and implementable screening strategies.

DYNAMIC REGULATION PROPOSES INTRAINDIVIDUAL SUSCEPTIBILITY

Whereas the stable interaction of HLA-antigen-TCR is the dependent signal for T cell activation, a second signal provided by co-activating and co-inhibitory signaling pathways regulates the T cell activation threshold, with the summative outcome skewing toward activation (allergy) or anergy (tolerance), respectively. Blocking co-inhibitory checkpoints including programmed death-1 and cytotoxic lymphocyte antigen 4 has been shown to increase the drug-specific response of T cells after naive priming,^{15,67} most recently with sulfasalazine.¹⁴ Furthermore, blocking these pathways using immune checkpoint inhibitors to bolster antitumor T cell response in cancer patients has been associated with increased rates of hypersensitivity with subsequently administered drugs. Studies reported liver injury in patients treated with ipilimumab and dacarbazine, severe rash with vemurafenib after treatment with nivolumab, and a higher proportion of patients developing sulfasalazine hypersensitivity after treatment with pembrolizumab.^{68,69} Immune-mediated adverse effects during immune checkpoint therapy are well-documented but often attributed to enhanced reactivity to self-antigen and autoimmunity, yet these studies demonstrate similarly heightened immunogenic risk with drugs.^{70,71} Moreover, polymorphic variants of these regulatory receptors are associated with predisposition to T cell–mediated autoimmune disease^{72,73} but are not yet similarly reported for risk for DHR. Nonetheless, these studies point to the likelihood that these regulatory elements drive both genetic and therapeutic inter- and intraindividual DHR risk. Future multiomic investigations will elucidate environmental risk pressures on cell regulation and drive increased clinical awareness of potentially increased drug immunogenicity during and after immune checkpoint inhibitor treatment.^{74,75}

Genetic variation in HLA risk–restricted TCR repertoire

There is theoretical 2×10^{19} diversity of TCR $\alpha\beta$,⁷⁶ albeit with a more restricted individual human repertoire estimated at 2×10^7 .⁷⁷ This variety is driven during thymic development and somatic rearrangement of the variable (V) and joining (J) or V, J, and constant (C) domains of respective α and β chain loci that form the HLA antigen–interacting heterodimer TCR protein. Further diversity in the antigen-binding complementary determining region (CDR)3 is driven by random nucleotide additions and deletions.⁷⁸ Application of next-generation sequencing with TCR-targeted primers has enabled the fine characterization of exact TRBV, TRBJ, and β CDR3 sequences. This was used by Chung et al⁷⁹ to show preferential private TCR β chain use in blisters from HLA-B*58:01–restricted allopurinol SJS/TEN patients. In 2021, Villani et al²³ similarly showed that CD8⁺ T cell effectors of patients with diverse drug-induced TEN had

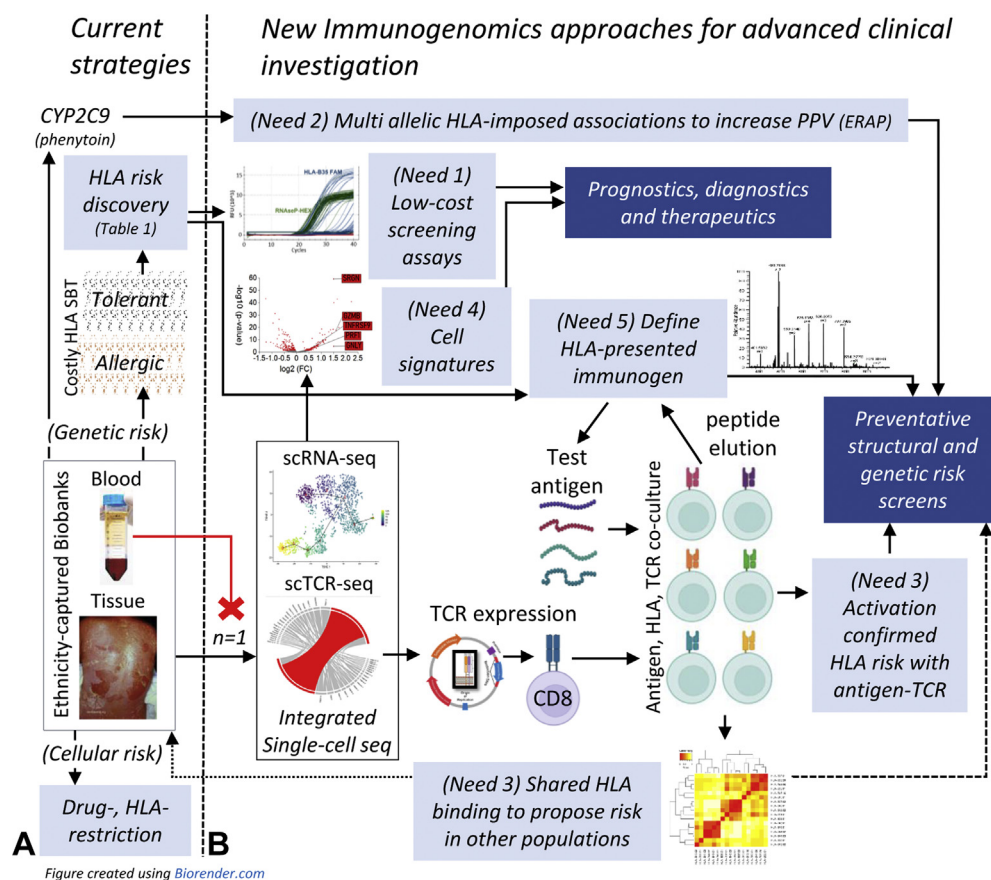


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FIGURE 4. Immunogenomics approaches. (A) Current. Large cohorts for HLA risk discovery by genome-wide association study. (B) New. (Need 1) Low-cost PCR-based HLA screens. (Need 2) Multi-allelic screening. (Need 3) TCR-HLA-engineered co-culture systems to propose and confirm HLA risk. (Need 4) Single-cell sequencing identification of clonally expanded effector population signatures. (Need 5) Mass spectrometry-linked risk HLA elution to identify risk HLA-presented immunogenic antigen(s). ERAP, endoplasmic reticulum aminopeptidase.

highly restricted TCR use, in which the top five clonotypes accounted for up to 90% of all detectable TRBV, indicating the relevance of immunodominant TCR across drugs. However, the β chain incompletely defines antigen specificity, which is also influenced by the corresponding α variable chain.^{80,81} Single-cell (sc)TCR sequencing captures both the α and β sequences. This was used by Pan et al⁸² for blisters of patients with HLA-B*15:02-restricted carbamazepine-induced SJS/TEN to find a TCR α CDR3 VFDNTDKLI/ TCR β CDR3 ASSLAGELF clonotype shared and dominantly expanded across patients. Critically, this clonotype was not dominantly expanded in peripheral blood, which highlights the need to assess affected tissue. It was not similarly observed in risk HLA-expressing drug-tolerant patients, indicating disease dependence on the expression of both a risk HLA and TCR. Thus, although novel allelic associations within as yet undescribed risk-associated genes will likely remain discovered by GWAS, the field now recognizes the use of single-cell multimodal omics⁸³ to detect the dominant TCR. These data can subsequently be used as a strategy to uncover novel HLA-risk associations in underrepresented ethnic groups, for novel biomarkers of disease for diagnosis and treatment, and for the immunogenic risk HLA-TCR-presented structures driving disease, as discussed subsequently.

Critical need 3: a precision, single patient-informed risk prediction strategy using ethnicity-recorded biorepositories

A major advantage of single-cell sequencing is the provision of the full sequence of a TCR $\alpha\beta$, supplying the opportunity for synthetic expression to test HLA and antigen restriction *in vitro*. Briefly, Pan et al⁸² linked TCR α /TCR β expression constructs of the expanded TCR clonotype to a mouse constant domain and transfected into a murine 5KC hybridoma. TCR-transfected cells were then co-cultured with HLA class I-deficient lymphoblastoid C1R APC or those expressing HLA-B*15:02 and drug for 48 hours, before an assessment of activation via cytokine secretion. They confirmed that the clonotype of interest was HLA-B*15:02- and carbamazepine-restricted. Although HLA-B*15:02 is a well-established risk for carbamazepine hypersensitivity, this study provides important insight into how scTCR-seq applied to patient samples of underrepresented ethnicities could both propose and validate HLA risk in a single patient. Upon observing previously undefined HLA allele-restricted drug-induced response, this personalized immunogenomic approach could translate to informing risk more generally across ethnicity-matched population. Appropriate ethnicity-

matched samples can be selected from the growing number of diverse international registries for DHR pairing ethnicity-recorded patient metadata with clinical samples (DNA, RNA, blood, and tissue) outlined in Figure 2, D. The benefit of these clinical repositories is epitomized by the identification of the first HLA risk allele relevant to DHR prevention in diverse underrepresented Australian Indigenous populations.⁸⁴ Briefly, Somogyi et al⁸⁴ identified three indigenous Australian patients with phenytoin-induced DRESS, who shared the expression of HLA-B*56:02. This allele has up 19% expression in these populations but 0% in Europeans,⁸⁴ highlighting diverse pharmacogenetic variation and a pathway to determine risk in understudied populations to mitigate knowledge and health disparities. Whereas this study proposed HLA-B*56:02—restricted risk, single-cell sequencing on acute tissue will identify and enable the synthetic expression of the dominant TCR for testing against autologous HLA-expressing APC. Such an approach will confirm and validate this risk allele for extension across these underrepresented populations.

Critical need 4: single-cell sequencing-informed, tissue-relevant effector biomarkers for diagnostics and targeted treatments

There remains no consensus treatment guideline during the acute management of SJS/TEN owing to a lack of clinical trials to support therapeutic intervention. In recent years, cyclosporine has demonstrated some observational success to reduce mortality,⁸⁵ and the use of etanercept improves time to reepithelization by reducing concentrations of the major cytotoxic mediator granulysin,⁸⁶ yet the effect on mortality was not statistically significant. This highlights the need to discover novel biomarkers to develop the first targeted treatments. However, this has been limited by a lack of appropriate methods for high-resolution and unbiased characterization of immunologically heterogeneous populations within diseased tissue and the signature of clonally expanded effector T cells. Compared with bulk sequencing, single-cell methods provide this resolution (Figure 3, A). The advent of droplet-based microfluidics devices has enabled a fully integrated, multifocal, single-cell pipeline for RNA-TCR-cellular indexing of transcriptome and epitopes sequencing⁸⁷ for unbiased transcriptome, functional TCR, and surface protein expression of each cell (Figure 3, B). Analysis of these big-data sets requires initial bioinformatics for the unsupervised clustering of cells by a similar transcriptomic signature, with subsequent alignment to reference databases providing an initial inference of cell type. However, these are becoming increasingly standardized practices with the frequent release of new tools to aid users in downstream analyses.⁸⁸⁻⁹¹ To date, single-cell application to investigate DHR remains limited, but it includes comparative scRNA-TCR-seq study of abacavir-positive patch test with drug-unexposed skin¹¹ and for identifying novel treatment strategies in a patient with standard therapy-refractory DRESS. In the latter 2020 study, Kim et al⁹² compared the transcriptomic profile of single cells in skin and blood samples from this patient to identify upregulation of the JAK-signal transducer and activator of transcription pathway. This led the clinical team, who had exhausted all classical treatment options, to administer JAK1 and 3-inhibitor tofacitinib for effective disease control. This is the first demonstrated example of single-cell sequencing to identify an unconventional but effective treatment for severe cutaneous adverse reactions during active uncontrolled

disease. Most recently, Hertzman et al⁹³ compared punch biopsy from unaffected and positive delayed intradermal test skin to understand the T cell immunopathogenesis of rare corticosteroid-induced systemic contact dermatitis. These initial studies show the clinical utility of single-cell sequencing for the immunogenomic elucidation of cellular immunopathogenesis at unparalleled resolution. In addition, integrated assays provide future scope to use the dominantly expanded TCR to define drug-reactive effector signatures, all cell and gene interactions within the broader cell atlas, and lineage tracking to inform dynamic response (Figure 3, C). These pipelines are paired with transposase-accessible chromatin with sequencing^{94,95} to assess epigenetic modifications, and spatial transcriptomic methods for the localization of signatures of interest in histologic tissue sections (Figure 3, D).⁹⁶ Analyses of the as-yet undefined single-cell signatures of clonally expanded effector populations will be required across drug-induced reactions to provide novel reaction-specific biomarkers toward diagnostics and targeted treatments.

Critical need 5: strategies to define and screen HLA risk—presented immunogenic structures

Patient T cells can be stimulated through *in vitro* co-culture with culprit drugs or their synthetically stable metabolites, most recently tolvaptan,¹² dapsone,¹³ vancomycin,⁹⁷ and clozapine.⁹⁸ Moreover, testing HLA-restricted patient T cells against comparative drugs or structural analogs can elucidate cross-reactivities to ensure safe ongoing treatment.^{97,99,100} However, the actual HLA-presented immunogenic structures driving T cell activation remain undefined with the potential influence of diverse drug-derived- or self-peptides and diverse TCR. Understanding the antigenic structure is possible through the immunogenomic interpretation of risk HLA and TCR, with several approaches recently implemented to understand these structures. Initial mass spectrometry-focused investigations eluted peptides from HLA-B*57:01 with and without abacavir treatment, demonstrating an altered self-peptide repertoire as a new model of T cell activation.^{9,10} Abacavir not only modifies the peptide repertoire expressed at the cell surface, it alters the intracellular tapasin-mediated loading of peptide complexes.¹⁰¹ With a correlation between immunogenicity and stable HLA—peptide complex formation, methods have been developed to generate immunopeptidome thermostability curves able to train artificial network models to predict most immunogenic peptides.¹⁰² Human leukocyte antigen elution methods have been applied to characterize naturally processed flucloxacillin-modified peptides similarly.^{103,104} Because HLA presentation alone does not drive T cell activation, these position 4—restricted flucloxacillin-modified peptides were then used to immunize risk-associated HLA-B*57:01—expressing transgenic mice. The authors then demonstrated T cell immunogenicity by monitoring IFN- γ secretion from subsequently extracted and antigen-reexposed splenocytes.¹⁰⁴ However, the protein origins of such hapten complexes remain unknown, and the *in vitro* assays that are described are limited by the restricted protein repertoire of *in vitro* co-cultures or the focus on high-abundance model protein. To navigate this issue, the field requires strategies for high-throughput peptidome library screening approaches incorporating both risk HLA-expressing APC and patient blister—inferred immunodominant TCR to identify the full array of antigenic sequences driving T cell activation.¹⁰⁵

Conclusion

From a current clinical perspective (Figure 4, A), risk HLA typing is implementable for select high-risk drugs in high-risk populations to predict and prevent the onset of DHR. However, the cost of sequence-based typing and a low PPV continues to hinder clinical implementation for most alleles and drugs. The only screening-recommended non-HLA risk factor is low-function CYP2C9 variants before treatment with phenytoin. To enable further implementation for HLA allele screening, an extended armamentarium of low-cost PCR-based screens is needed with the advantage of a short turnaround suited to equipment and expertise available in most on-site clinical laboratories (Figure 4, B, need 1). Moreover, recent GWAS studies highlighted HLA-imposed risk factors including ERAP, which must be similarly assessed across drugs for their potential to increase PPV and reduce NNT toward cost effective multiallelic screening (Figure 4, B, need 2). However, two of the most significant discoveries of the past few years are immunodominant risk HLA- and drug-antigen-restricted TCR. These provide novel immunogenomic avenues to answer key remaining critical questions, but with discovery dependent on clinician-led curation of disease-relevant biorepositories pairing clinical metadata, patient ethnicity, HLA type, and relevant tissue samples from acute disease. Detailed clinical reporting to capture HLA type and patient ethnicity as well as the submission of tissue-relevant acute clinical samples to these global biorepositories must be considered part of the practical guide to treatment. This in turn will provide advanced immunogenomic data-driven diagnosis and treatment strategies for the patient at hand and enable future application to deliver population-personalized prediction and prevention strategies, as described. In their most accessible application, these biorepositories enable patient clustering to identify shared alleles, as demonstrated by Somogyi et al⁸⁴ to propose the risk for HLA-B*56:02 for developing phenytoin-DRESS in Indigenous Australian populations. In their most complex application, repositories enable state-of-the-art immunogenomics approaches to combine single-cell sequencing and *in vitro* co-culture platforms to identify and then functionally validate HLA-restricted drug-TCR risk in tissue from a single patient, with extended relevance across the ethnicity-matched population (Figure 4, B, need 3). Afterward, the tissue-relevant signatures of clonally expanded effector T cell populations provide biomarkers for diagnosis and targeted treatments (Figure 4, B, need 4) and the HLA-TCR-presented immunogenic drug-and/or self-derived moiety pathways for future structural risk prevention (Figure 4, B, need 5).

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