DSA DISCOVER

Platform Architecture and Technology Portfolio

for Patient-Specific DSA Characterization and Humoral Risk Assessment

Beyond detection. Discover the biology behind the DSA.

From DSA detection to patient-specific humoral risk profiling

Platform Architecture and Technology Portfolio

Scientific / Strategic working document

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Executive Positioning

DSA DISCOVER is a modular technology platform for characterizing the humoral component of transplant risk. It is intentionally broader than a single quantitative assay: the platform begins with confirmation and quantitative profiling of donor-specific antibody responses and can extend, when needed, into pattern resolution, selective isolation, biologic characterization, longitudinal monitoring, memory analysis, and risk integration.

The core scientific premise is that donor-specific antibody detection identifies alloimmune recognition, but detection alone does not define the magnitude, complexity, or trajectory of that response. Single-point MFI is an assay signal and should not be treated as a direct antibody concentration. Serial titration and dose-response analysis add quantitative dimensions that can be standardized and followed over time. DSA DISCOVER therefore focuses on the question that conventional detection incompletely answers: how much donor-directed humoral pressure is present, what is driving it, and how is it changing?

The platform is designed to operate as a humoral-risk component layer of a broader transplant framework. It does not replace donor/recipient compatibility assessment, biopsy, dd-cfDNA, graft function, molecular mismatch, or other evidence of graft injury. Instead, it provides a quantitative and mechanistic description of the donor-directed antibody response that can be integrated with those other risk domains.



Figure 1. DSA DISCOVER at a Glance: six complementary modules and their clinical value.

1. DSA DISCOVER Architecture

DSA DISCOVER is organized around six modular discovery capabilities**: CONFIRM & QUANTIFY, RESOLVE, ISOLATE, CHARACTERIZE, TRACK & MONITOR, and MEMORY**. The platform typically begins with an existing HLA antibody screening result**. CONFIRM & QUANTIFY** provides an independent HLA-based confirmation of the identified donor-specific reactivity and then extends that result through quantitative titration and dose-response analysis—adding information on apparent potency, maximal response, breadth, and overall quantitative phenotype.

From there, the platform deepens according to the question being asked. **RESOLVE** clarifies complex or overlapping antibody patterns; **ISOLATE** physically separates relevant antibody populations; **CHARACTERIZE** adds functional and molecular context; **TRACK & MONITOR** follows the response longitudinally; and **MEMORY** extends interrogation beyond circulating antibody to the capacity to regenerate a donor-reactive response.

CORE PRINCIPLE
The deeper the question, the deeper the discovery.
Each DSA Discover module adds a new dimension of insight into donor-directed humoral biology—from quantitative phenotype to mechanism, functional context, and longitudinal evolution.

The architecture is intentionally modular rather than a mandatory linear workflow. Routine cases may require only confirmation and quantitative profiling, while difficult, high-risk, or mechanistically important cases can trigger deeper levels of investigation. This creates a common framework for HLA laboratories**,** transplant scientists, and clinical development: start with what is already known, add quantitative resolution, and progressively interrogate the biology when additional information is needed.

Ultimately, the quantitative and biological information generated across DSA DISCOVER can be interpreted together with donor/recipient context and evidence of graft injury, providing a more complete framework for understanding donor-directed humoral risk.

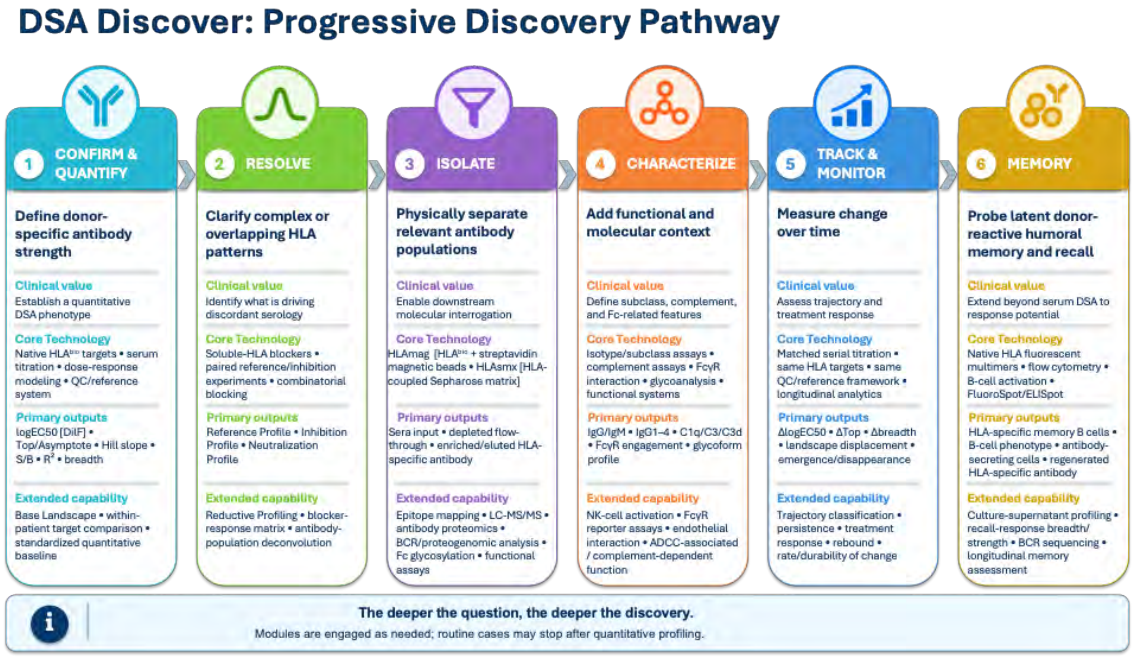


Figure 2. DSA DISCOVER Architecture: a question-driven framework from quantitative state to deeper biological insight.

Clinical-priority alignment

Priority	Core question	Primary DSA DISCOVER contribution
ACCESS	Can the patient receive this donor organ despite DSA, and how substantial is the donor-specific antibody barrier?	Confirm donor relevance; establish the quantitative DSA phenotype; compare donor-specific targets; resolve ambiguous or cross-reactive patterns when needed.
UNDERSTAND	What antibody populations are present, what drives the observed pattern, and what biological properties characterize the response?	Resolve complex reactivity; physically isolate relevant antibody populations; define isotype, subclass, complement, Fc, glycosylation, and functional characteristics where appropriate.
TRACK	Is donor-directed humoral activity increasing, stable, resolving, rebounding, or responding to intervention?	Establish a patient-specific baseline and use standardized serial profiling to define trajectory, magnitude of change, persistence, treatment response, and rebound.
PROTECT	Is the donor-directed humoral response changing in a way that warrants broader evaluation of the graft?	Provide quantitative and longitudinal humoral information that can be interpreted together with graft function, histology, molecular injury markers, and other clinical evidence.

2. Progressive Discovery Modules

DSA DISCOVER is designed as a question-driven, modular platform rather than a mandatory linear workflow. Most investigations begin with CONFIRM & QUANTIFY to establish donor relevance and the quantitative phenotype of the response. Additional modules are applied when the biological or clinical question requires greater resolution.

Routine cases may therefore end after quantitative profiling, while complex serology, unusual donor-reactivity patterns, treatment-response questions, or mechanistic investigations can progressively engage RESOLVE, ISOLATE, CHARACTERIZE, TRACK & MONITOR, or MEMORY.

The deeper the question, the deeper the discovery.

Module	Use when…	Primary output	Position in portfolio
CONFIRM & QUANTIFY	donor relevance and the magnitude of the response need to be established	confirmed HLA-specific reactivity; apparent potency; maximal response; breadth; QC parameters; patient-specific Base Landscape	Core analytical module
RESOLVE	the observed pattern is broad, discordant, cross-reactive, or difficult to explain	Reference, Inhibition, and Neutralization Profiles; Reductive Profiling; blocker-response patterns; antibody-population deconvolution	Advanced pattern resolution
ISOLATE	a defined HLA-reactive antibody population needs to be physically separated or recovered	HLA-depleted and HLA-enriched antibody fractions using HLAmag or HLAsmx; material for downstream molecular and functional interrogation	Advanced discovery and molecular access
CHARACTERIZE	biological properties of a confirmed DSA response need to be defined	isotype/subclass composition; complement-associated activity; Fc-receptor properties; glycosylation and functional phenotype where validated	Modular functional characterization
TRACK & MONITOR	serial comparison, treatment response, persistence, or rebound needs to be evaluated	Trajectory Landscape; ΔlogEC50 / ΔTop / breadth change; meaningful longitudinal change; response classification and treatment-associated trajectory	Recurring longitudinal capability
MEMORY	persistence of donor-reactive humoral memory or recall capacity is the scientific question	HLA-specific memory-B-cell detection; recall-response assays; HLA-specific antibody-secreting cells and regenerated antibody response	Extended research module

COMMERCIAL LOGIC
A practical quantitative core creates the initial entry point. Modular deeper-analysis capabilities expand scientific differentiation, recurring longitudinal use, and advanced translational and biopharma opportunities.

3. A. CONFIRM & QUANTIFY

Quantitative DSA Profiling — The Core Analytical Module

CONFIRM & QUANTIFY establishes the quantitative baseline of DSA DISCOVER. The module begins with an HLA antibody reactivity identified through existing screening and asks two fundamental questions: is the donor-relevant signal reproducible in an orthogonal HLA-based system, and what is the quantitative phenotype of that response?

Using native soluble/biotinylated HLA targets and controlled serum titration, the module moves DSA assessment beyond a single endpoint signal toward a multidimensional dose-response profile. This provides a standardized framework for comparing individual donor HLA targets within the same patient and for establishing the quantitative reference state used by subsequent DSA DISCOVER modules.

MFI is an assay signal. Titration describes an antibody response.

The key advance is not dilution itself, but reconstruction of the antibody-response relationship across a controlled serum dilution series. Multiple complementary parameters are retained rather than collapsing the response into a single value. These parameters represent assay-derived features of a polyclonal serum response and should be interpreted collectively rather than as direct measurements of molecular affinity or pathogenicity.

The principal question for CONFIRM & QUANTIFY is therefore:

What does the patient’s donor-specific antibody phenotype look like at this time point?

The resulting quantitative phenotype establishes the state of the response. If the patient is subsequently followed longitudinally, this state becomes the baseline for TRACK & MONITOR.

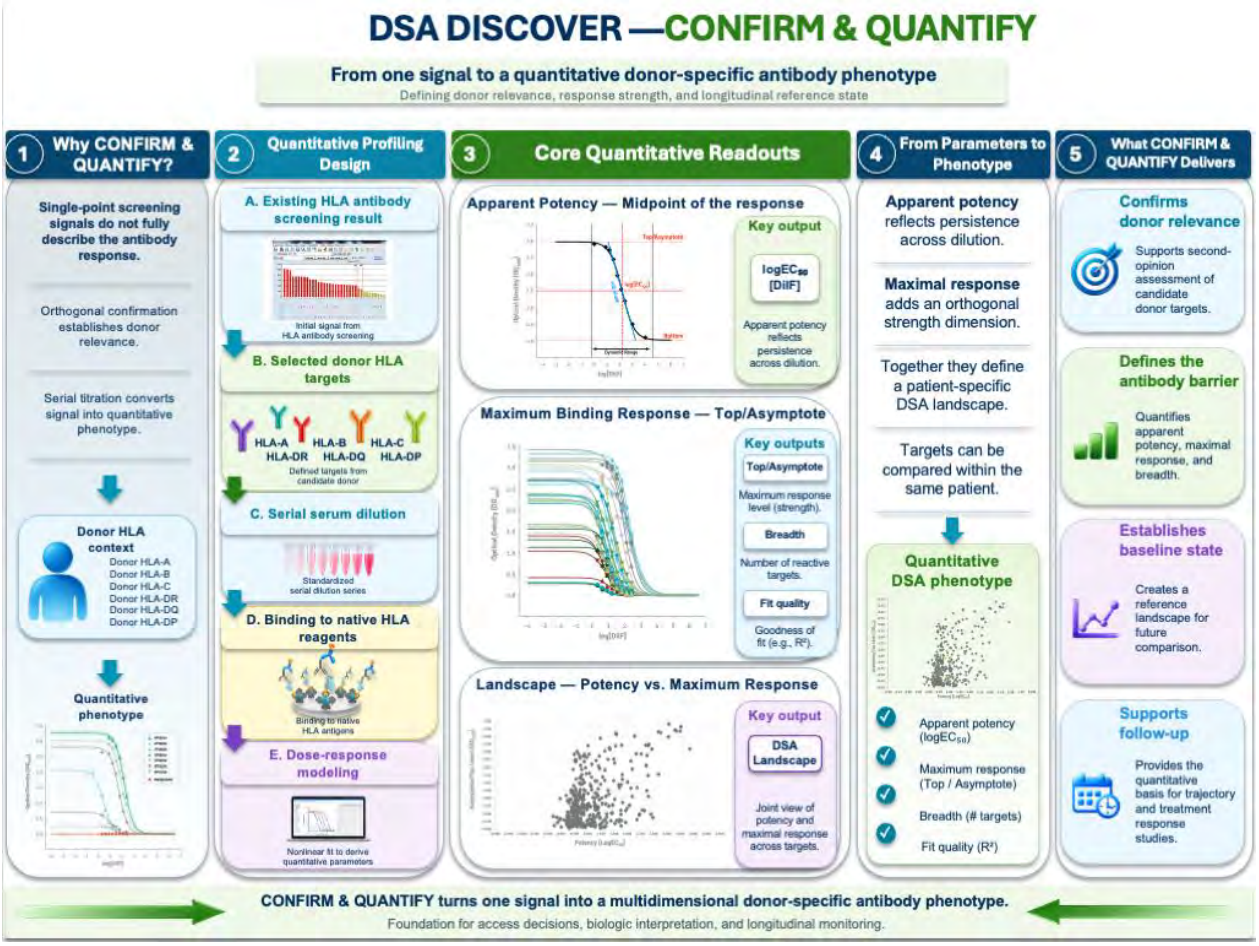


Figure 3. CONFIRM & QUANTIFY: quantitative profiling from screening signal to a patient-specific DSA phenotype.

3.1 Core quantitative parameters

Quantitative DSA profiling generates complementary analytical parameters from the serum dose-response curve. Together, they describe different dimensions of the donor-specific response and provide the quantitative basis for target comparison, patient-specific phenotyping, and subsequent longitudinal analysis.

Parameter	Analytical meaning	Proposed role
$logEC_{50}$ [DilF]	Dilution factor associated with the midpoint of the serum dose-response curve. Integrates antibody abundance and effective binding strength under defined assay conditions.	Apparent DSA potency; not molecular affinity.
Top / Asymptote	Maximum observed assay binding response.	Orthogonal maximal-response dimension.
Hill Slope	Steepness of the transition region.	Curve-shape descriptor; potential heterogeneity indicator.
R^2 / Fit Quality	How well the selected model represents the observed data.	Analytical QC and fit acceptance.
Signal / Background Ratio	Magnitude of target signal relative to background.	Assay discrimination / Analytical positivity.
Donor-target breadth	Number of donor HLA targets with measurable response.	Extent of donor-directed humoral reactivity.

3.2 Patient-Specific DSA Landscape

The Patient-Specific DSA Landscape integrates individual dose-response parameters into a quantitative representation of the donor-directed antibody profile at a defined time point. Apparent potency ($logEC_{50}$ [DilF]) and maximal binding response (Top / Asymptote) form the principal axes of the landscape, allowing each donor-specific HLA target to occupy a position within a common two-dimensional interaction space.

These parameters capture complementary properties of the response. Apparent potency describes how strongly the serum response persists across dilution under defined assay conditions, whereas maximal response describes the achievable magnitude of HLA-specific binding within the assay. Considering both dimensions together distinguishes antibody responses that may appear similar by a single endpoint measurement but differ substantially in their quantitative behavior.

At the patient level, the landscape provides a multidimensional humoral phenotype. It can identify dominant and secondary DSA populations, differences among donor HLA targets, heterogeneity within multi-DSA responses, and overall donor-target breadth. Rather than reducing the response to one number, the landscape preserves clinically and scientifically relevant complexity in an interpretable framework.

The Base Landscape therefore serves five principal functions:

- within-patient comparison of donor HLA targets;
- comparison across patient groups;
- identification of dominant versus secondary DSA populations;
- quantitative phenotyping and grouping patients into clinically meaningful subgroups;
- establishment of the reference state for future longitudinal monitoring.

As larger outcome-linked datasets are generated, the same quantitative coordinate system will also support development of response regions, phenotypic clusters, and multivariable models of humoral risk.

Core question	Is the donor-specific reactivity confirmed, and what is its quantitative phenotype at the current time point?
Clinical / scientific value	Provides second-opinion confirmation of donor-specific reactivity, defines the magnitude and quantitative characteristics of the antibody barrier, compares donor HLA targets within the patient, and establishes a standardized baseline for deeper or longitudinal analysis.
Technology	Native soluble/biotinylated HLA targets; serial serum titration; dose-response fitting; reference and QC framework; patient-specific DSA landscape analytics.
Commercial positioning	Core analytical capability and primary entry point for clinical collaborations, reference testing, standardized analytics, and development of the DSA DISCOVER kit platform.

4. B. RESOLVE

DSA Pattern Resolution

Patient sera are inherently polyclonal, and a broad HLA reactivity pattern may arise from very different underlying antibody structures: a single dominant cross-reactive population, several independent antibody populations, antibodies directed against shared structural epitopes, or mixtures of major and minor reactivities. RESOLVE is designed for situations in which the observed screening or quantitative profile identifies what is reactive but does not sufficiently explain what is driving the pattern.

The module uses soluble HLA as a defined perturbation reagent. A patient sample is analyzed in parallel under two conditions: an untreated condition that preserves the original serologic pattern and a matched condition in which one or more soluble HLA molecules are added before measurement. The soluble HLA competes for, binds, and functionally removes the corresponding antibody fraction from the measurable pool. The resulting change in the downstream HLA profile provides a direct experimental readout of which reactivities are linked to that blocker. This parallel reference-versus-blocked design was the core principle of the earlier neutralization studies and was used to simplify complex patterns into interpretable component profiles.

Reference, Inhibition, and Neutralization Profiles

The analytical framework is built around three related profiles:

Reference Profile (RP).

The untreated serum is measured without soluble HLA blocker. This preserves the full observable reactivity pattern and establishes the baseline against which the perturbation experiment is compared.

Inhibition Profile (IP).

The same serum is pre-incubated with a defined soluble HLA molecule and then measured under otherwise matched conditions. Antibody populations captured or competitively occupied by the soluble HLA are reduced in the subsequent readout.

Neutralization Profile (NP).

The neutralization profile is the differential profile generated from the two paired measurements: [NP = RP – IP]. Rather than viewing inhibition only as a loss of signal, subtraction converts the blocking event into a positive map of the antibody reactivities removed by the soluble HLA blocker. This makes the affected HLA pattern easier to visualize and interpret.

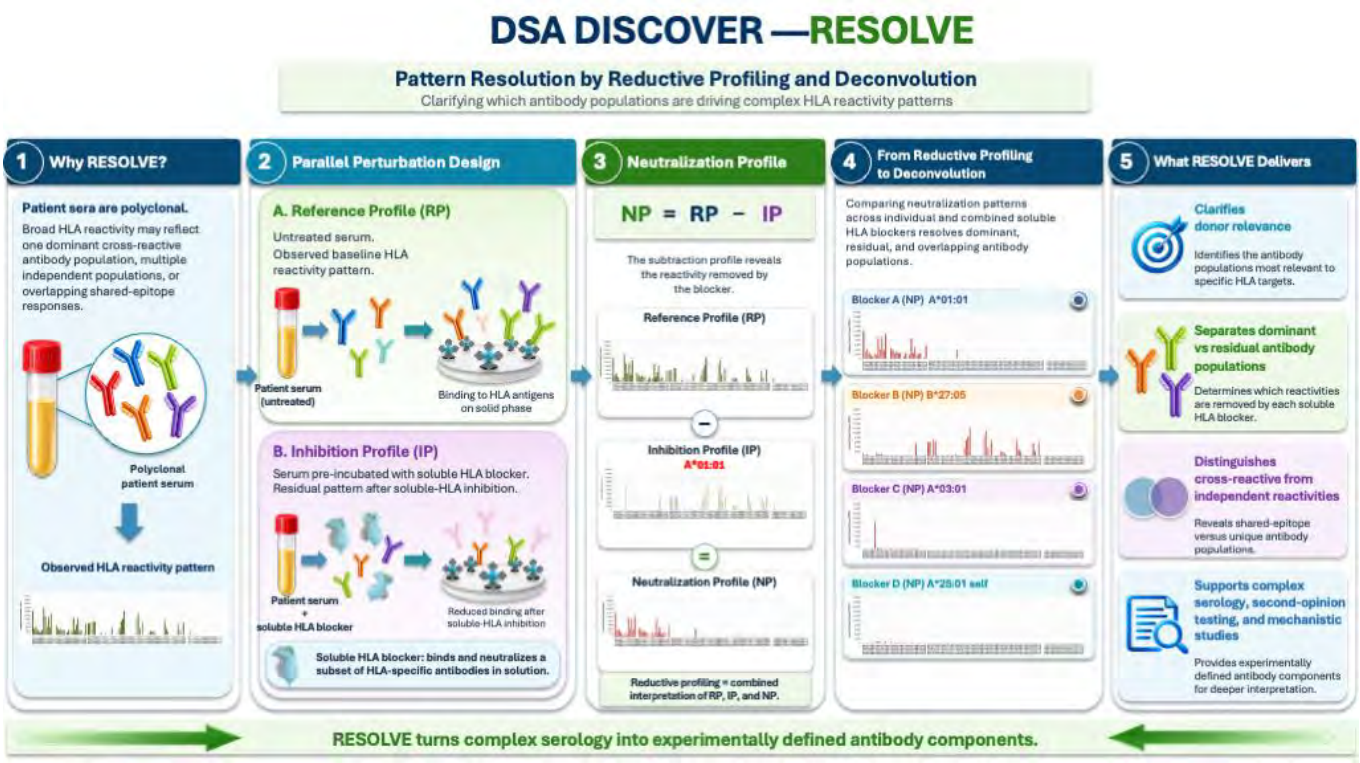


Figure 4. RESOLVE: pattern resolution by reductive profiling and deconvolution.

Reductive Profiling

Reductive Profiling is the complete experimental and analytical process comprising the Reference Profile, Inhibition Profile, and derived Neutralization Profile. The term reflects the purpose of the method: a complex polyclonal serologic pattern is progressively reduced into simpler, experimentally defined components.

The important feature is that the method does not assign specificity solely from the magnitude of an individual HLA signal. Instead, it asks how the entire profile changes when a defined HLA interaction is removed from the system. A blocker that suppresses only one or a few targets suggests a relatively restricted antibody relationship. A blocker that removes a broad group of otherwise distinct HLA signals indicates that those reactivities may be connected through a shared antibody population or structurally related epitope recognition. Earlier studies demonstrated that individual soluble HLA molecules could eliminate broad sets of reactivities, revealing relationships that were not obvious from the unperturbed profile alone.

This makes Reductive Profiling particularly useful for distinguishing between several biologically different explanations for an apparently broad DSA pattern:

- one dominant cross-reactive antibody population recognizing an epitope shared across multiple HLA molecules;
- multiple independent antibody populations contributing separately to the profile;
- nested or overlapping cross-reactive groups, in which one blocker removes only part of a broader pattern;
- dominant plus minor populations, where the major response can be removed experimentally and residual reactivities become visible.

From Reductive Profiling to Deconvolution

Deconvolution is the higher-order interpretation obtained when the reductive experiment is repeated with multiple individual HLA blockers. Each blocker generates a characteristic neutralization pattern. When these patterns are compared across the same patient sample, they form a matrix of blocker → affected reactivities. Shared reductions across several targets indicate connected antibody recognition, whereas reactivities that remain after one blocker but disappear after another identify separable antibody populations.

Combinatorial blocking is especially powerful. If HLA-A removes one portion of the pattern, HLA-B removes another, and the combination of A+B removes essentially the complete profile, the data support at least two experimentally distinguishable antibody components. Conversely, if a single blocker reproduces nearly the full reduction achieved by a larger blocker combination, that HLA may represent a dominant structural driver of the observed cross-reactive pattern. The earlier neutralization work used multiple soluble-HLA combinations in precisely this way to assign broad cross-reactive profiles to a smaller number of underlying immunizing or structurally related HLA interactions.

Deconvolution therefore shifts the analysis from “Which beads are positive?” to “Which antibody populations are responsible for those signals, and which HLA structures connect them?”

Scientific and Clinical Utility

Within DSA DISCOVER, RESOLVE provides an experimental bridge between quantitative serology and deeper mechanistic characterization. It can be used to:

- determine whether a broad profile is driven by one dominant cross-reactive population or several independent populations;
- test whether a donor-specific HLA is part of a larger cross-reactive antibody network;
- identify shared-pattern relationships among HLA alleles that may not be apparent from screening intensity alone;
- separate dominant from residual antibody reactivities;
- compare the relative contribution of several candidate HLA blockers;
- generate simplified antibody components for subsequent quantitative, structural, or mechanistic investigation.

The method is particularly valuable in complex serology, discordant cases, unusual donor targets, broad sensitization, second-opinion testing, and mechanistic studies where understanding the architecture of the response is more informative than simply counting positive HLA signals.

Core question	What antibody populations and HLA relationships are driving the observed reactivity pattern?
Clinical / scientific value	Experimentally separates cross-reactive from independent antibody components, clarifies donor relevance, identifies dominant and residual populations, and reveals shared HLA recognition patterns.
Technology	Paired Reference and Inhibition Profiles using soluble HLA blockers; derived Neutralization Profiles; Reductive Profiling; Combinatorial Deconvolution analytics.
Commercial positioning	Advanced problem-solving module for complex serology, second-opinion testing, reference-laboratory investigations, and mechanistic collaborations.

5. C. ISOLATE

Physical Isolation and Recovery of HLA-Specific Antibody Populations

ISOLATE converts an HLA reactivity pattern into experimentally accessible antibody material. Whereas RESOLVE uses soluble HLA perturbation to determine which antibody populations contribute to a complex serologic pattern, ISOLATE physically captures, removes, and recovers defined HLA-reactive antibody fractions for direct molecular investigation.

The core principle is HLA-specific affinity capture. Native soluble HLA molecules are immobilized on a solid support and exposed to serum or another antibody-containing sample. Antibodies recognizing the immobilized HLA are retained, while non-binding antibodies remain in the flow-through. Following washing, the HLA-reactive population can be recovered by controlled elution and subsequently re-analyzed as an enriched antibody fraction.

This creates two complementary experimental materials: an HLA-depleted fraction, representing the residual antibody repertoire after removal of the target population, and an HLA-enriched/eluted fraction, representing antibodies physically associated with the selected HLA target. Comparison of the original sample, depleted fraction, and recovered fraction provides a direct means to validate specificity, test deconvolution models, and investigate the molecular properties of individual donor-reactive antibody populations.

5.1 Two Complementary Isolation Formats

DSA DISCOVER can use two HLA-capture formats that serve different experimental scales and objectives.

Platform	Architecture	Primary role	Typical output
HLAmag	Streptavidin-coated magnetic beads loaded with biotinylated native HLA (HLA^{bio})	Rapid, small-scale and parallel HLA-specific capture	Depleted sample plus bead-bound / recovered HLA-specific antibody fraction
HLAsmx	Native soluble HLA immobilized on a Sepharose matrix and used in column or suspension format	Preparative isolation, serial fractionation and higher-capacity antibody recovery	Flow-through, wash and purified HLA-specific elution fractions

HLAmag — Magnetic HLA Affinity Capture

HLAmag uses streptavidin-coated magnetic particles as a modular capture surface for HLA^{bio}. The magnetic format allows the HLA specificity of the capture reagent to be changed simply by loading a different biotinylated HLA molecule, making it particularly attractive for small-volume samples, parallel allele comparisons, rapid adsorption experiments, and multi-condition studies. The magnetic-bead development work establishes streptavidin magnetic beads as the underlying capture surface for biotinylated HLA and includes antibody-removal and elution development.

The major advantage of HLAmag is experimental flexibility. Multiple donor or candidate HLA specificities can be evaluated in parallel using defined bead quantities, and sequential capture rounds can be applied when depletion of a specific antibody population is required. This makes HLAmag particularly suitable for screening-scale isolation, confirmation of RESOLVE findings, comparative adsorption experiments, and generation of smaller HLA-specific antibody fractions for downstream analysis.

HLAsmx — Preparative HLA-Sepharose Separation

HLAsmx uses native soluble HLA immobilized onto a Sepharose matrix. This format is designed for more preparative applications in which antibody-containing samples are passed through an HLA-specific matrix, followed by controlled washing and elution of the bound antibody fraction.

Historical studies demonstrated that HLA-coupled Sepharose matrices can selectively deplete anti-HLA antibodies, recover antibody following elution, and be operated through repeated load–wash–elute cycles. The matrix was also shown to have measurable capacity and reproducible column performance.

HLAsmx becomes particularly powerful when matrices are used sequentially. A first HLA column removes one component of a polyclonal serum; the flow-through can then be applied to a second HLA matrix, and successive rounds progressively separate the original reactivity into simpler fractions.

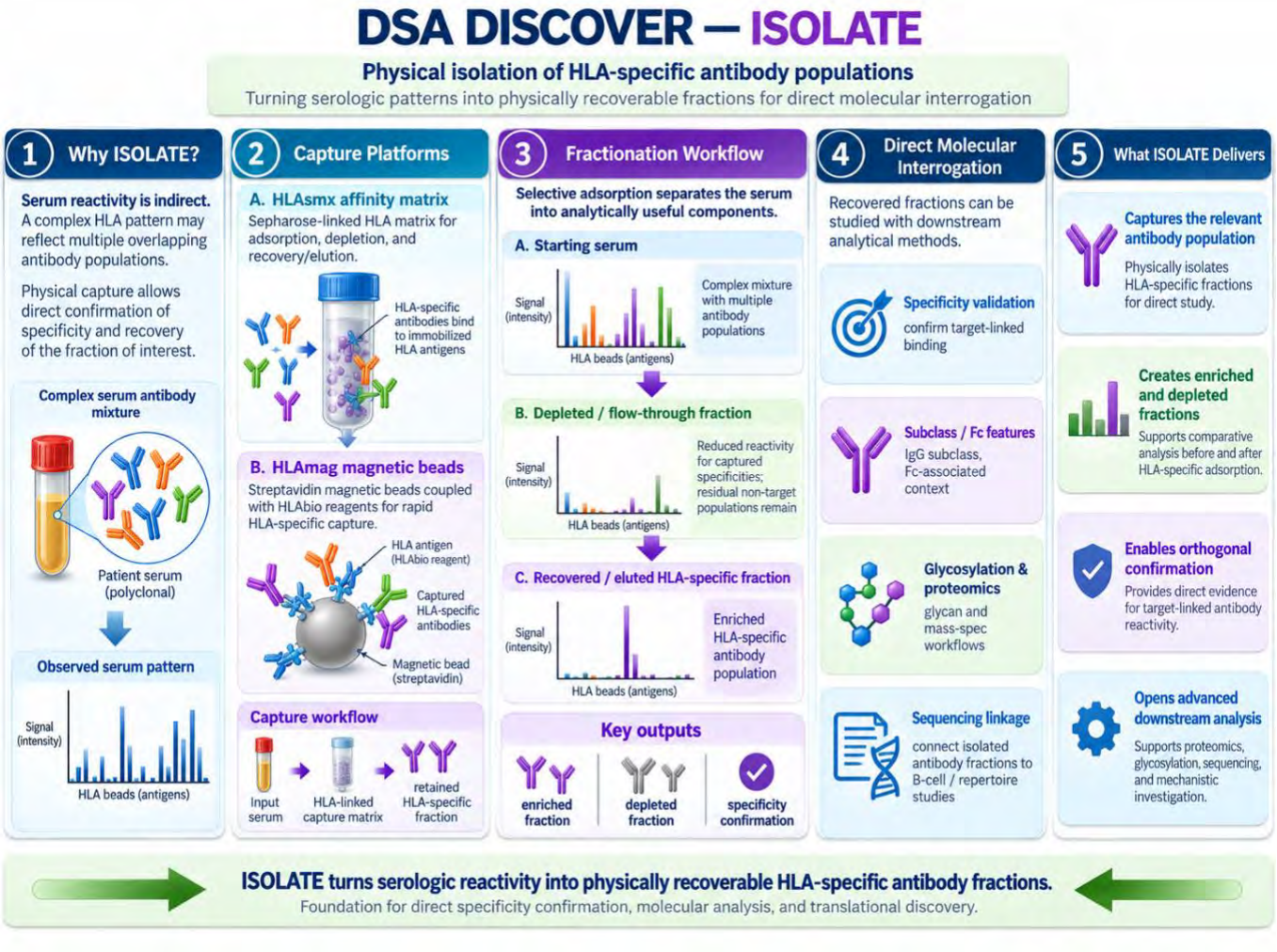


Figure 5. ISOLATE: HLA-specific affinity capture, fractionation, and molecular interrogation.

5.2 From Serologic Pattern to Molecular Material

The scientific significance of ISOLATE is that it converts an inferred antibody population into material that can actually be measured.

A typical workflow begins with a target identified through CONFIRM & QUANTIFY or RESOLVE. A selected HLA molecule is immobilized on HLAmag or HLAsmx, and the patient sample is exposed to that matrix. The captured population is then separated from the residual serum, washed, recovered, and reassessed for HLA reactivity. The enrichment step can be validated by comparing the input, flow-through, and eluate against the original HLA reactivity profile.

Serial HLAsmx or HLAmag capture can progressively fractionate a polyclonal response and create defined materials for subsequent molecular analysis.

The earlier deconvolution studies showed that recovered fractions could retain the reactivity characteristics of the originating antibody population and that serial HLA-specific separation could simplify complex serum profiles into experimentally defined components.

5.3 Molecular Interrogation of the Isolated Antibody Fraction

The recovered HLA-specific antibody fraction becomes a starting material for a much broader set of analyses that cannot be performed with the same specificity on unfractionated serum.

HLA specificity and epitope mapping.

The eluate can be retested against HLA panels to determine the complete cross-reactive profile associated with the isolated population. Competitive binding, neutralization, allele panels, mutational HLA variants, or structurally related HLA molecules can then be used to refine the recognized epitope space.

Isotype and subclass composition.

The isolated material can be analyzed for IgG versus IgM and for IgG1–4 composition. Because the analysis is performed on an HLA-enriched fraction rather than total serum immunoglobulin, subclass information becomes directly associated with the donor-reactive antibody population.

Mass-spectrometric antibody characterization.

Recovered antibodies can support intact-mass, subunit, peptide, and LC-MS/MS analyses. Clonotypic peptide signatures may provide information on antibody heterogeneity and the number of dominant molecular species within an apparently polyclonal response.

Antibody sequence interrogation.

Proteomic sequencing can be extended by combining LC-MS/MS-derived clonotypic peptides with matched B-cell receptor repertoire sequencing from available PBMC or B-cell material.

Fc glycosylation profiling.

Antigen-specific enrichment creates the opportunity to study glycosylation of the HLA-specific IgG fraction rather than bulk serum IgG. Glycopeptide LC-MS or related methods can examine features such as fucosylation, galactosylation, sialylation, and bisecting GlcNAc. This is particularly attractive because Fc glycosylation can influence Fc-receptor engagement, complement interactions, and antibody effector behavior.

Functional interrogation.

Isolated fractions can be compared for complement-associated activity, Fcγ-receptor binding, NK-cell activation, endothelial-cell interaction, or other mechanistic readouts. Importantly, the isolated population can be evaluated alongside the depleted serum fraction, providing a direct experimental framework for asking whether removal of a defined DSA population materially alters a functional response.

Core question	Can the antibody population of interest be physically separated, recovered, and interrogated directly?
Clinical / scientific value	Converts inferred HLA reactivity into defined antibody fractions, enabling direct confirmation of specificity, physical deconvolution of polyclonal responses, and molecular investigation of donor-reactive antibodies.
Technology	HLAmag: HLA^{bio}-loaded streptavidin magnetic beads for rapid small-scale capture and depletion. HLAsmx: HLA-coupled Sepharose matrices for preparative adsorption, serial fractionation, and antibody recovery. Flow-through, wash, and eluate fractions support downstream analysis.
Downstream applications	HLA specificity and epitope mapping; Ig isotype/subclass analysis; LC-MS/MS and antibody proteomics; proteogenomic sequence assignment; Fc glycosylation analysis; complement/Fc-receptor studies; functional assays.
Commercial positioning	Advanced discovery and translational module for mechanistic studies, biopharma collaborations, biomarker development, complex case investigation, and molecular characterization of HLA-specific antibody populations.

6. D. CHARACTERIZE

Functional and Molecular Context of the DSA Response

CHARACTERIZE extends a quantitatively defined, donor-relevant antibody response from “how much antibody activity is present?” to “what type of antibody response is present, and what biological properties may modify its behavior?”

Two patients can have quantitatively similar DSA profiles yet differ substantially in antibody composition and potential effector mechanisms. The response may differ in isotype, IgG subclass distribution, Fc glycosylation, complement engagement, Fc-receptor interaction, and cellular effector activity. CHARACTERIZE adds these dimensions to the underlying quantitative phenotype rather than replacing it.

The preferred analytical sequence is therefore deliberate: first establish that the signal is technically robust and donor relevant, then quantify the response, and only then add molecular or functional characterization when the clinical or scientific question requires greater biological resolution.

The central principle is:

Quantity defines the antibody pressure; characterization describes how that antibody population may behave.

These features should be interpreted as modifiers of the donor-specific antibody phenotype, not as stand-alone measures of pathogenicity. Their meaning depends on the amount of antibody present, its HLA specificity, epitope accessibility, polyclonal composition, and the biological environment in which the antibody interacts with the graft.

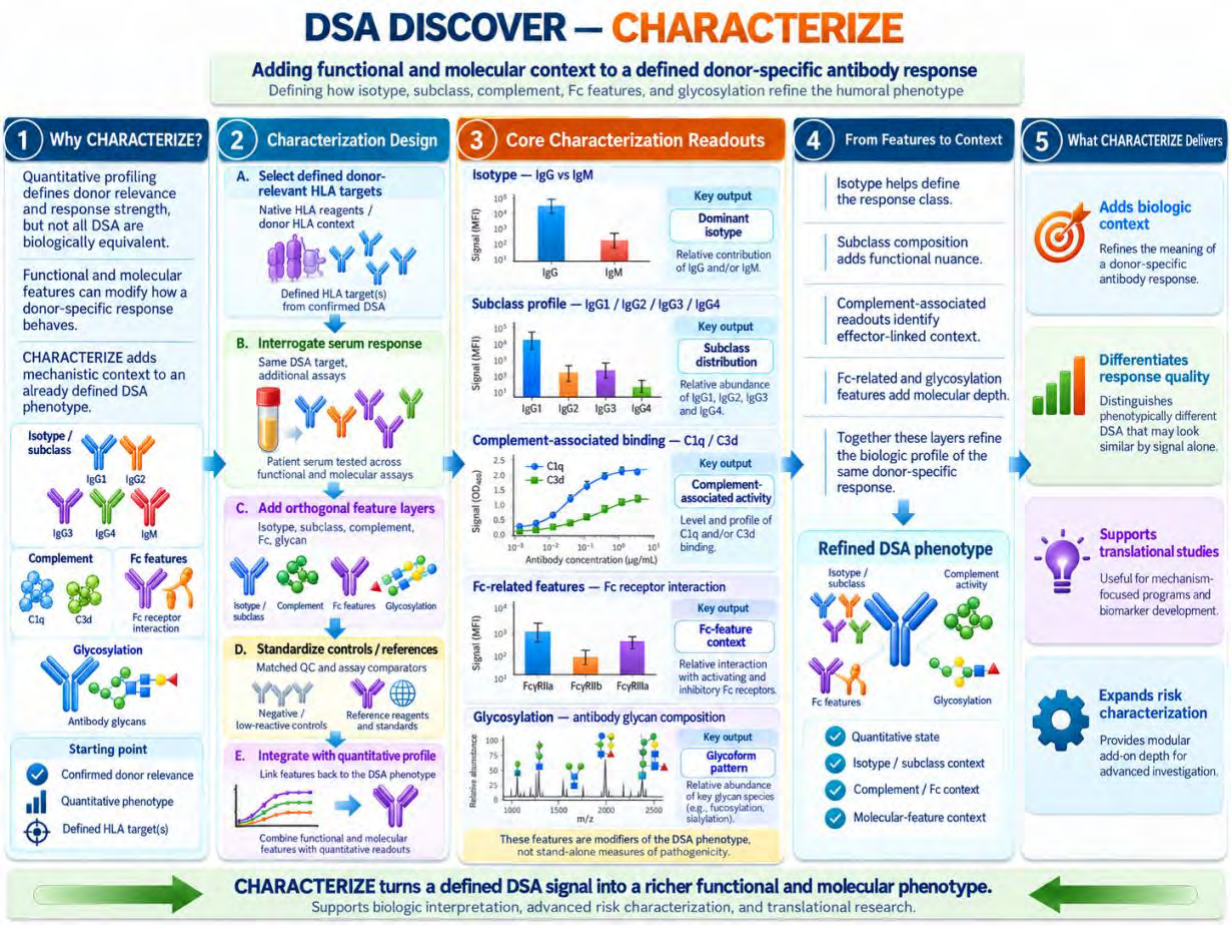


Figure 6. CHARACTERIZE: functional and molecular characterization of the donor-specific antibody response.

6.1 Isotype and IgG Subclass Composition

CHARACTERIZE first defines the immunoglobulin composition of the donor-specific response. HLA-reactive antibody may be predominantly IgG, IgM, or mixed, and the IgG component can be further resolved into IgG1–4. Because subclasses differ in Fc-receptor interaction, complement engagement, and effector potential, their relative contribution can add biologic context to otherwise similar quantitative DSA profiles.

The goal is to move beyond simple subclass positivity toward a composition profile linked to the quantitative DSA phenotype—for example, the relative contribution of IgG1, IgG2, IgG3, and IgG4 to the measurable HLA-specific response. This enables comparison across donor targets, longitudinal time points, and treatment states.

6.2 Complement and Fc-Related Effector Properties

Complement-associated measurements add a functional dimension to a quantified DSA response. Readouts may include C1q binding, C3/C3d deposition, or other complement-associated signals where technically validated. These results are most meaningful when interpreted together with antibody abundance, subclass composition, epitope geometry, and assay conditions rather than as isolated binary markers.

CHARACTERIZE can also extend to Fcγ-receptor engagement, including selected FcγRI, FcγRII, or FcγRIII interactions. These measurements provide an intermediate mechanistic layer between HLA binding and downstream cellular activity and may help explain why quantitatively similar antibody populations differ in biological behavior.

6.3 Fc Glycosylation and Molecular Phenotype

Fc glycosylation can further modify antibody-effector interactions. Relevant features may include fucosylation, galactosylation, sialylation, bisecting GlcNAc, and broader glycoform distributions.

A particularly powerful opportunity arises when CHARACTERIZE is combined with ISOLATE. HLA-specific antibodies recovered through HLAmag or HLAsmx can be subjected to glycopeptide or intact-protein mass spectrometry, enabling molecular characterization of the donor-reactive antibody fraction rather than bulk serum IgG. This creates a direct progression from DSA identification and quantification to isolation and molecular Fc profiling.

6.4 Functional Antibody Activity

At the deepest level, CHARACTERIZE can extend into functional assays that test whether a defined HLA-specific antibody population can engage downstream effector pathways. Potential applications include NK-cell activation, FcγR-dependent reporter assays, monocyte/macrophage activation, endothelial-cell interaction, ADCC-associated readouts, and complement-dependent functional assays.

These experiments are especially informative when the antibody input has already been quantitatively defined and, where appropriate, physically enriched through ISOLATE. This reduces the complexity of unfractionated serum and improves the ability to associate a functional response with a specific donor-reactive antibody population.

6.5 Integrated and Longitudinal Characterization

The value of CHARACTERIZE is not any single marker, but the ability to build a multidimensional DSA phenotype by combining quantitative strength with isotype, subclass, complement, Fc characteristics, glycosylation, and functional activity.

Where feasible, characterization should move from qualitative positivity toward relative composition and normalized measures. Examples include subclass-specific response relative to total HLA-specific IgG or complement-associated activity relative to the underlying antibody response. This can help distinguish a simple increase in antibody quantity from a true change in biological composition or effector potential.

Longitudinally, CHARACTERIZE can determine whether the nature of the response changes even when its overall quantity does not—for example through subclass redistribution, altered complement activity, changing Fc glycosylation, or evolving functional behavior. This makes the module particularly relevant to mechanistic studies, treatment-response programs, biomarker development, and longitudinal immune profiling.

Core question	What molecular and functional properties characterize the donor-specific antibody response, and how might those properties modify its biological behavior?
Clinical / scientific value	Adds mechanistic depth to an already confirmed and quantitatively defined DSA phenotype by defining antibody composition, Fc-associated properties, complement engagement, and functional activity.
Technology	IgG/IgM isotype profiling; IgG1–4 subclass composition; complement-associated assays; Fcγ-receptor interaction; Fc glycosylation and proteomic analysis; cellular functional assays where technically validated.
Analytical positioning	Characterization parameters are interpreted relative to the underlying quantitative DSA phenotype, allowing distinction between changes in antibody abundance and changes in biological composition or effector potential.
Commercial positioning	Modular advanced-characterization platform for translational research, therapeutic-development programs, biomarker discovery, longitudinal immune profiling, and mechanistic investigation of donor-specific antibody responses.

7. E. TRACK & MONITOR

Longitudinal DSA Surveillance

TRACK & MONITOR adds time to the quantitative phenotype established by CONFIRM & QUANTIFY. Rather than generating a new type of biochemical measurement, it applies the same standardized DSA DISCOVER framework repeatedly so that each patient can be compared against their own prior state. The same patient, donor HLA targets, assay chemistry, titration design, reference system, QC criteria, analytical parameters, and interpretation framework are reproduced across serial measurements.

This controlled within-patient design is particularly powerful because each patient becomes their own reference. Rather than asking whether an absolute value is “high” or “low” relative to unrelated individuals, the central question becomes: Has this patient’s donor-specific humoral response materially changed from its established baseline and is that movement greater than the expected analytical variability?

Serial quantitative testing can identify responses that are emerging, increasing, stable, fluctuating, persistent, decreasing, resolving, disappearing, or rebounding. They can track changes in apparent potency, maximal binding response, donor-target breadth, curve behavior, and position within the DSA Landscape. Together, these parameters transform DSA from a static laboratory observation into a measurable biological trajectory. The emphasis is therefore not simply on repeated detection, but on the direction, magnitude, and durability of quantitative change.

A static DSA profile describes a state. A quantitative trajectory describes a process.

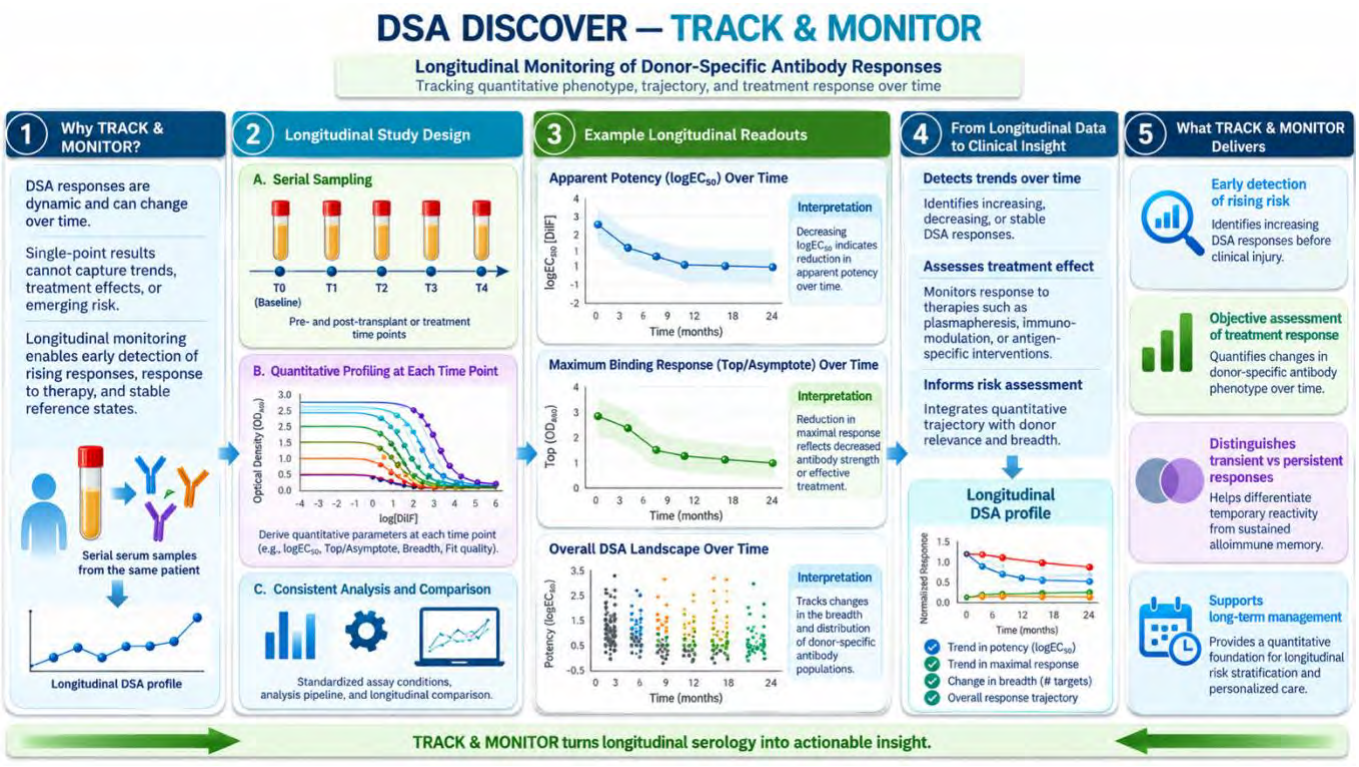


Figure 7. TRACK & MONITOR: longitudinal profiling of DSA trajectory and treatment response.

7.1 From Base Landscape to Trajectory Landscape

The Base Landscape generated in CONFIRM & QUANTIFY defines the patient's reference state. TRACK & MONITOR connects successive measurements of the same DSA within that quantitative space, producing a Trajectory Landscape.

Movement can occur along several dimensions:

- change in apparent potency;
- change in maximal response;
- gain or loss of donor-target breadth;
- emergence or disappearance of individual DSA;
- divergence of different DSA populations within the same patient.

This is particularly important in patients with multiple DSA, because individual antibody populations may behave independently. One response may strengthen while another remains stable or resolves.

The trajectory therefore preserves information that is difficult to capture with repeated positive/negative classifications or isolated endpoint measurements.

7.2 Quantifying Meaningful Change

Longitudinal interpretation requires more than demonstrating that two numerical values differ. The analytical objective is to determine whether observed movement exceeds expected assay variability and therefore represents a credible change in the underlying humoral response.

Potential longitudinal metrics include:

- ΔlogEC50 [DilF]
- ΔTop / Asymptote
- change in donor-target breadth
- emergence or disappearance of DSA
- magnitude and direction of landscape displacement
- rate and persistence of change

This framework distinguishes random assay movement from a reproducible change in donor-directed humoral activity.

7.3 Clinical and Translational Applications

TRACK & MONITOR is most relevant where the evolution of the response matters more than a single measurement.

Key applications include:

Pre-existing DSA / transplantation across DSA

Establish the pre-transplant antibody phenotype and determine whether donor-directed responses remain stable, intensify, decline, or rebound after transplantation.

De novo DSA

Determine whether a newly detected response is transient, persistent, progressively strengthening, or expanding to additional donor targets.

Desensitization and antibody-removal therapy

Measure the magnitude and durability of DSA reduction and identify subsequent rebound.

AMR-directed intervention

Follow quantitative changes in the humoral component alongside graft-function, histologic, molecular, or other injury markers.

Therapeutic development and clinical trials

Provide quantitative pharmacodynamic endpoints such as depth of DSA suppression, durability of response, time to rebound, and differential behavior among individual donor-specific antibodies.

7.4 Monitoring Across the DSA DISCOVER Platform

TRACK & MONITOR can also extend beyond antibody quantity.

Once a quantitative baseline has been established, serial investigation may incorporate outputs from other modules, including changes in:

- cross-reactive pattern structure from RESOLVE;
- IgG subclass or complement-associated activity from CHARACTERIZE;
- isolated antibody populations from ISOLATE;
- memory-associated responses from MEMORY.

This creates an important distinction between:

Quantitative evolution — how much the DSA response changes;

and

Biological evolution — whether the composition or functional properties of that response also change.

TRACK & MONITOR therefore provides the temporal framework through which multiple DSA DISCOVER measurements can be compared.

Core question	How is the donor-specific humoral response changing over time, and does that change exceed the patient's established analytical baseline?
Clinical / scientific value	Converts a quantitative DSA baseline into a patient-specific trajectory, defining direction, magnitude, persistence, treatment response, and rebound of donor-directed humoral activity.
Technology	Standardized repeat titration; matched donor HLA targets and QC/reference framework; longitudinal logEC_{50}, Top, breadth, and landscape analysis; trajectory and change analytics.
Key applications	Pre-existing DSA surveillance, dnDSA follow-up, transplantation across DSA, desensitization, AMR-directed treatment, pharmacodynamic studies, and therapeutic-development trials.
Commercial positioning	Recurring-use monitoring capability for longitudinal clinical services, treatment-response programs, clinical trials, and future automated trajectory analytics.

8. F. MEMORY

HLA-Specific Humoral Memory

MEMORY extends DSA DISCOVER beyond circulating antibody to the cellular reservoir capable of regenerating a donor-reactive humoral response. Circulating DSA primarily reflects antibody being produced by antibody-secreting cells and long-lived plasma cells. Memory B cells represent a different compartment: they may persist without producing substantial circulating antibody but can rapidly expand and differentiate following renewed antigen exposure.

This distinction creates an important biological question:

Is donor-reactive humoral memory present even when circulating DSA is weak, declining, or no longer detectable?

MEMORY is therefore designed to interrogate the latent capacity for recall, complementing the serum-based measurements generated by the other DSA DISCOVER modules.

The module can approach this problem through two complementary strategies: direct identification of HLA-specific memory B cells and functional recall assays in which memory B cells are activated and their resulting antibody response is measured.

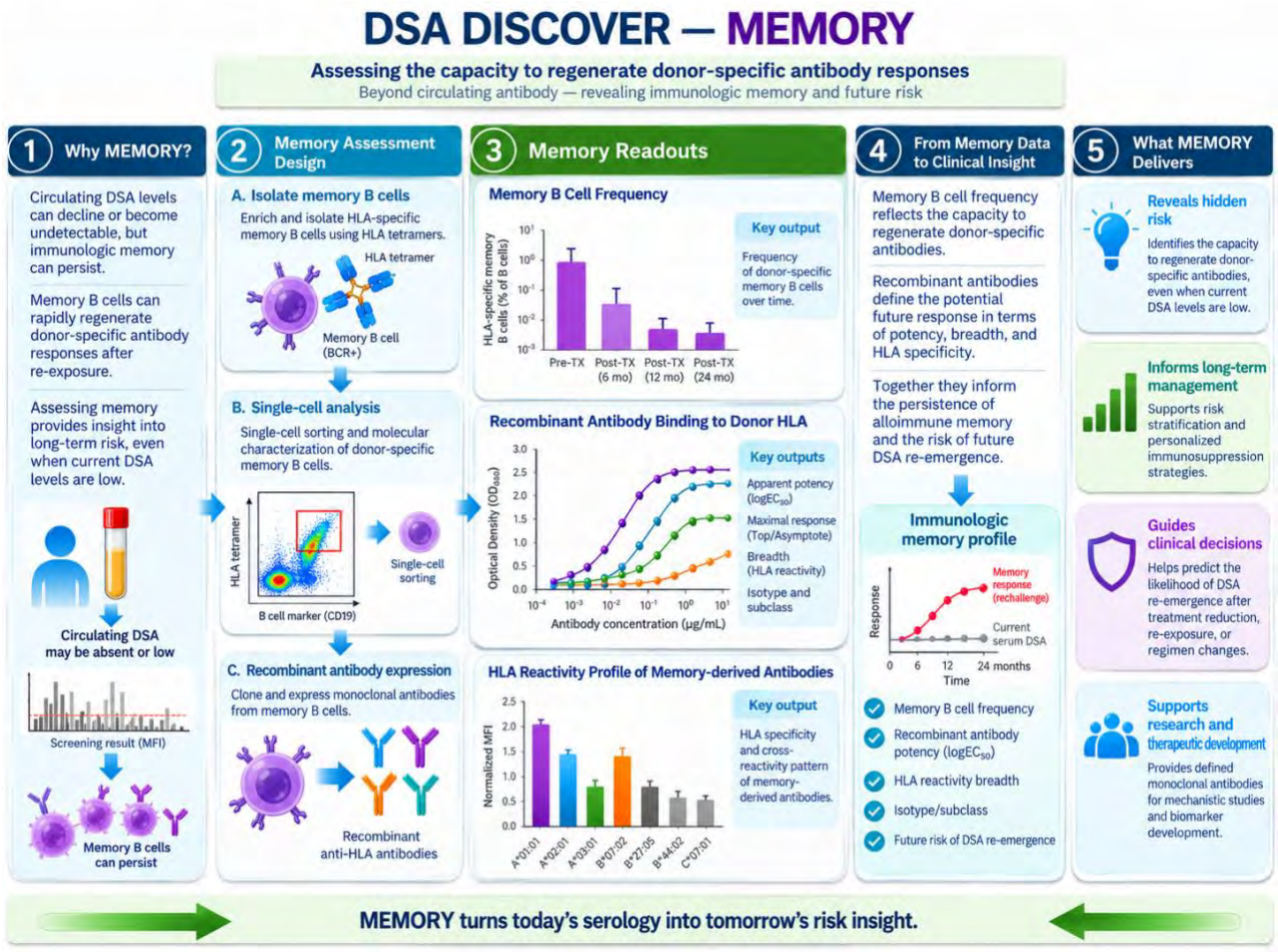


Figure 8. MEMORY: assessment of HLA-specific humoral memory and recall potential.

8.1 Direct Detection of HLA-Specific Memory B Cells

Biotinylated native HLA molecules can be assembled into fluorescent multimeric probes and used to identify B cells whose surface B-cell receptors recognize a defined HLA target.

Potential formats include:

- fluorescent HLA tetramers or multimers;
- dual-color HLA probes for increased specificity;
- simultaneous interrogation of donor and non-donor HLA targets;
- flow-cytometric enumeration and phenotyping of HLA-reactive B-cell populations.

The resulting HLA-binding cells can be further characterized using conventional B-cell markers to distinguish naïve, class-switched memory, unswitched memory, activated, or other B-cell phenotypes, depending on the scientific question.

The strength of the DSA DISCOVER approach is the availability of defined native HLA reagents that can serve as the antigen-specific recognition component.

This creates a direct cellular readout:

How many B cells capable of recognizing this donor HLA remain in the patient?

8.2 Functional Memory — Recall and Antibody Regeneration

Direct enumeration identifies HLA-binding B cells, but it does not establish whether those cells can generate an antibody response. A complementary approach therefore asks whether the memory compartment is functionally competent.

Peripheral B cells can be stimulated ex vivo using polyclonal activation conditions that drive memory B cells toward antibody-secreting cells. The resulting recall response can then be interrogated using complementary HLA-specific readouts. ELISpot or FluoroSpot assays can enumerate activated B cells that differentiate into antibody-secreting cells directed against a defined HLA target. In parallel, culture supernatants can be collected and tested against native HLA reagents using DSA DISCOVER analytical formats to characterize the specificity, breadth, and relative strength of the regenerated antibody response. This enables evaluation of the specificity, breadth, and potentially quantitative characteristics of the recalled antibody response.

This approach changes the question from:

“Is antibody present in the serum?”

to:

“Can the patient’s memory compartment regenerate antibody against this donor HLA?”

That distinction may become particularly informative when serum antibody has fallen below detection but donor-reactive memory persists.

8.3 Connecting Humoral Memory to Clinical DSA Behavior

Circulating DSA provides a snapshot of the antibody response that is measurable at a given time point. HLA-specific memory B cells represent a different biological compartment: the capacity to regenerate that response after renewed antigen exposure or immune stimulation. These two measurements may therefore provide complementary information.

A patient may have declining or undetectable circulating DSA while donor-reactive memory B cells remain. Conversely, persistent serum antibody may reflect continued antibody production without necessarily indicating the size or activity of the memory compartment. MEMORY is intended to investigate this relationship and determine whether cellular humoral memory provides additional context beyond serum antibody alone.

Serum DSA describes what is circulating. MEMORY asks what can return.

This distinction may be particularly relevant in several clinical settings:

- Historical or resolved DSA: determining whether donor-reactive memory persists after circulating antibody becomes weak or undetectable.
- De novo DSA: investigating whether emergence of circulating antibody is accompanied by expansion of detectable donor-specific memory.
- Post-treatment monitoring: determining whether therapies reduce only circulating antibody or also affect the B-cell populations capable of regenerating it.
- DSA rebound: assessing whether persistent memory may contribute to recurrence of donor-specific antibody after an initial treatment-associated decline.

This creates an important extension of TRACK & MONITOR. Longitudinal serum measurements describe the expressed humoral response, whereas MEMORY can investigate the underlying capacity for recall. Together, these measurements may help distinguish temporary suppression of circulating antibody from a more durable change in donor-reactive humoral immunity.

As clinical datasets develop, the key translational question will be whether the presence, frequency, or functional activity of HLA-specific memory B cells helps explain differences in DSA persistence, recurrence, treatment durability, or rebound. Until those relationships are established, MEMORY should be positioned as a research and translational biomarker rather than an independent clinical risk predictor.

8.4 Longitudinal Humoral Memory

The memory compartment may evolve differently from circulating DSA. Serum antibody may decline or become undetectable after therapy while donor-reactive memory B cells and a measurable recall response persist, creating a distinction between serologic resolution and immunologic resolution. These should not be assumed to be equivalent.

This provides a natural connection with TRACK & MONITOR: serum trajectories describe how the expressed antibody response changes over time, while MEMORY evaluates whether the capacity to regenerate that response persists, contracts, or re-emerges. Longitudinal assessment may therefore help distinguish transient suppression of circulating DSA from a more durable change in donor-reactive humoral immunity.

TRACK follows what the humoral response is doing.
MEMORY asks what the immune system still remembers.

8.5 Potential Applications

The most compelling initial applications are research settings in which serum DSA alone may not describe the complete history or future potential of the humoral response.

Potential applications include:

- patients with previous sensitization but no currently detectable DSA;
- persistence of donor-reactive immunity after apparent DSA resolution;
- transplantation across historical DSA;
- investigation of de novo DSA development;
- assessment before and after B-cell- or plasma-cell-directed therapy;
- mechanisms underlying DSA rebound;
- comparison of persistent versus resolving antibody responses;
- donor-specific B-cell repertoire and clonal-lineage studies;
- development of cellular biomarkers for humoral immune memory.

A particularly interesting therapeutic-development question is whether an intervention suppresses only the circulating antibody compartment or also modifies the memory B-cell reservoir from which future responses can arise.

MEMORY adds the cellular history and recall potential of the donor-specific response to the DSA DISCOVER phenotype.

Core question	Does the patient retain HLA-specific humoral memory capable of regenerating a donor-reactive antibody response?
Clinical / scientific value	Extends DSA assessment beyond circulating antibody by identifying donor-reactive memory B cells and testing the functional capacity to regenerate HLA-specific antibody responses.
Technology	Fluorescent native-HLA tetramers/multimers; flow-cytometric B-cell identification and phenotyping; memory-B-cell activation; HLA-specific FluoroSpot/ELISpot; activated-culture supernatant profiling; BCR sequencing.
Key applications	Historical sensitization, resolved or weak DSA, DSA rebound, treatment-response studies, donor-specific B-cell repertoire analysis, and mechanistic studies of humoral immune memory.
Commercial positioning	Extended research platform for mechanistic studies, therapeutic-development programs, cellular biomarker discovery, and future expansion of DSA DISCOVER beyond serum antibody.

9. Technology Backbone and Portfolio Coherence

The breadth of DSA DISCOVER is enabled by a common soluble-HLA reagent engine. The same native HLA platform can be configured differently according to the biological question: as an immobilized target for quantitative binding, a soluble competitor or neutralizer for pattern resolution, a matrix ligand for selective isolation, or a fluorescent probe for B-cell interrogation. This provides technical continuity across modules and creates a coherent product family rather than a collection of unrelated assays.

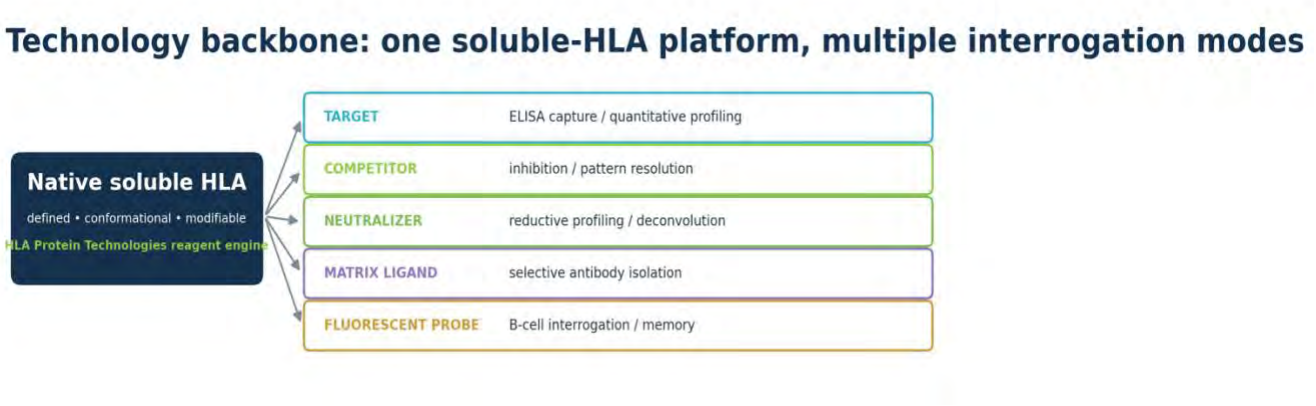


Figure 9. Common soluble-HLA reagent backbone supporting multiple DSA DISCOVER interrogation modes.

Scientific and Commercial Positioning

The immediate commercial center of gravity is CONFIRM & QUANTIFY together with TRACK & MONITOR: these modules address concrete HLA-laboratory needs, generate standardized longitudinal data, and align directly with the current clinical-investigation strategy. RESOLVE provides an important second-opinion and difficult-case differentiator. ISOLATE and CHARACTERIZE create higher-depth discovery and partnering opportunities, while MEMORY represents a longer-term portfolio extension.

PORTFOLIO MESSAGE
A practical quantitative core. Deeper modules when the case demands them. One reagent platform connecting confirmation, mechanism, trajectory, and future humoral-memory analysis.

10. Selected References

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