

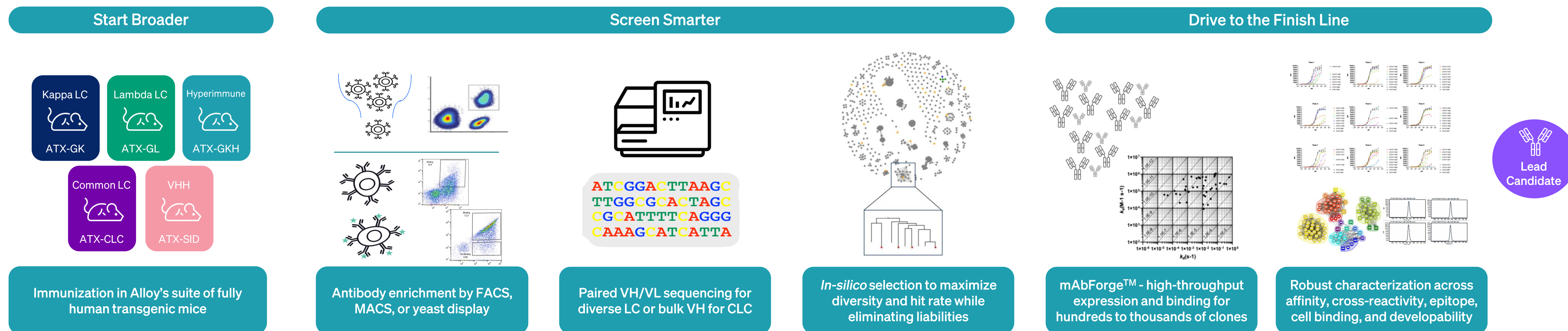
High velocity approach to antibody discovery and optimization using AI-enabled and ML-powered *in silico* workflows combined with high-throughput expression & testing

Simon Friedensohn PhD, Cédric Weber PhD, Dalton Markrush MS, Dominika Petrasova BS, Tyler Tribou MS, Tom Vincent MS, Chew Shun Chang PhD, Melinda Smith BS, Rick Shimkets PhD

Accelerating discovery with *in vivo* platforms and AI/ML workflows

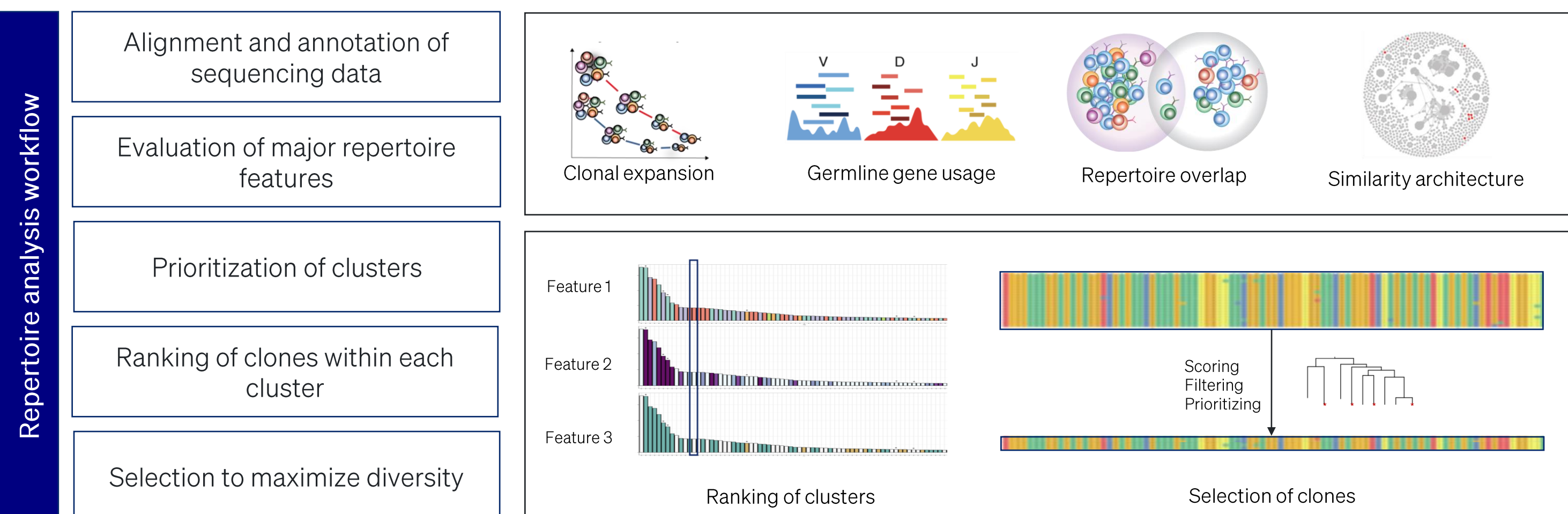
Monoclonal and bispecific antibodies represent a cornerstone of modern therapeutics, addressing critical needs in oncology, autoimmune disease, and beyond through precise targeting of single or dual antigens. The rise of AI-enabled and ML-powered *in silico* tools has potential to revolutionize antibody discovery, enabling structural prediction, *de novo* discovery, and multi-property engineering to accelerate therapeutic development. Despite these advances, most approved and clinical antibodies still originate from *in vivo* platforms, where natural immune selection ensures high affinity, diversity, and developability. At Alloy Therapeutics Inc., we integrate best-in-class *in vivo* discovery using our fully humanized ATX-Gx™ mice with AI-driven workflows for clone selection, developability prediction, and affinity maturation. By combining robust *in vivo* immune repertoires with predictive computational tools, we maximize therapeutic potential while reducing timelines for lead selection and optimization.

High velocity workflow for *in vivo* human antibody discovery using ATX-Gx mice



In silico clone selection to maximize diversity and hit rate

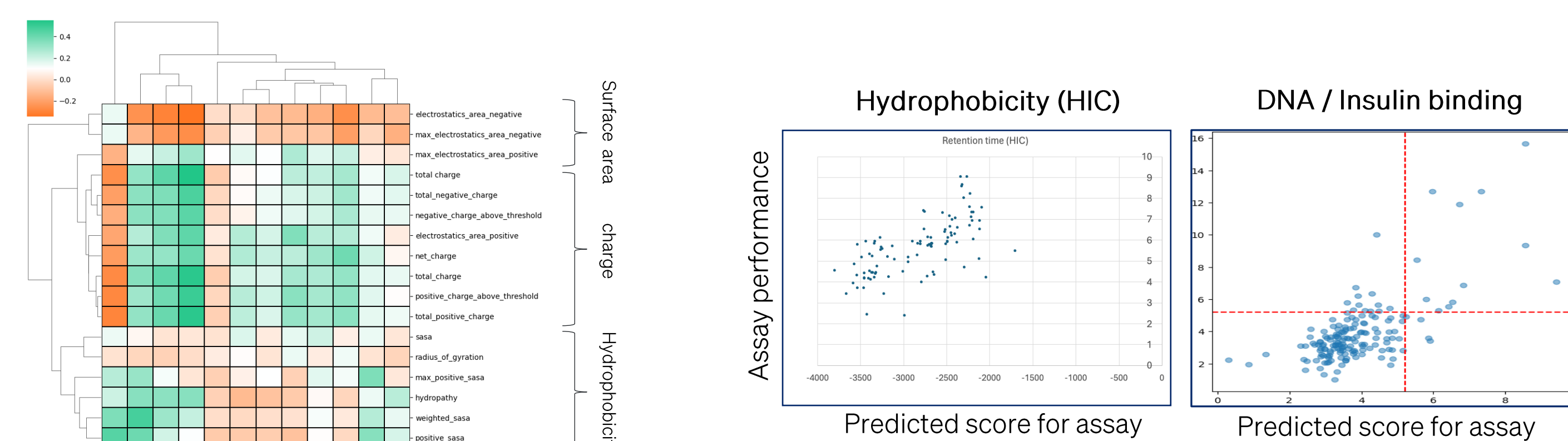
Unlike hybridoma or single B cell workflows that can be dominated by a small number of expanded clonotypes, Alloy's fully integrated, *in silico* clone selection workflow optimally samples and selects diversity from the entire repertoire.



Proprietary models to predict developability and filter for liabilities

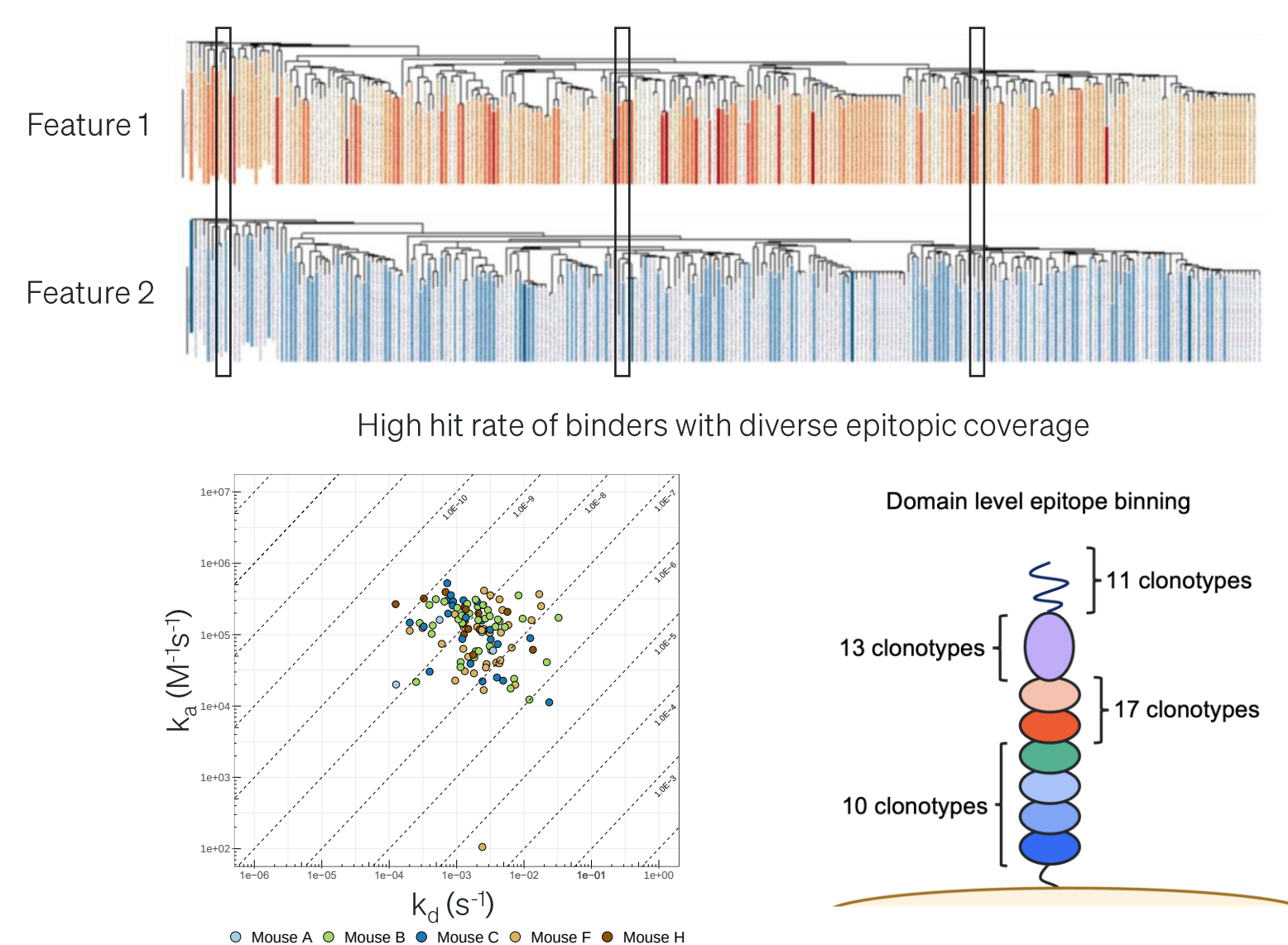
Extracted sequence and structural features are correlated with biophysical measurements for each tested antibody.

Proprietary, ML trained models are used to predict developability for each sequence and de-prioritize clones with liabilities.



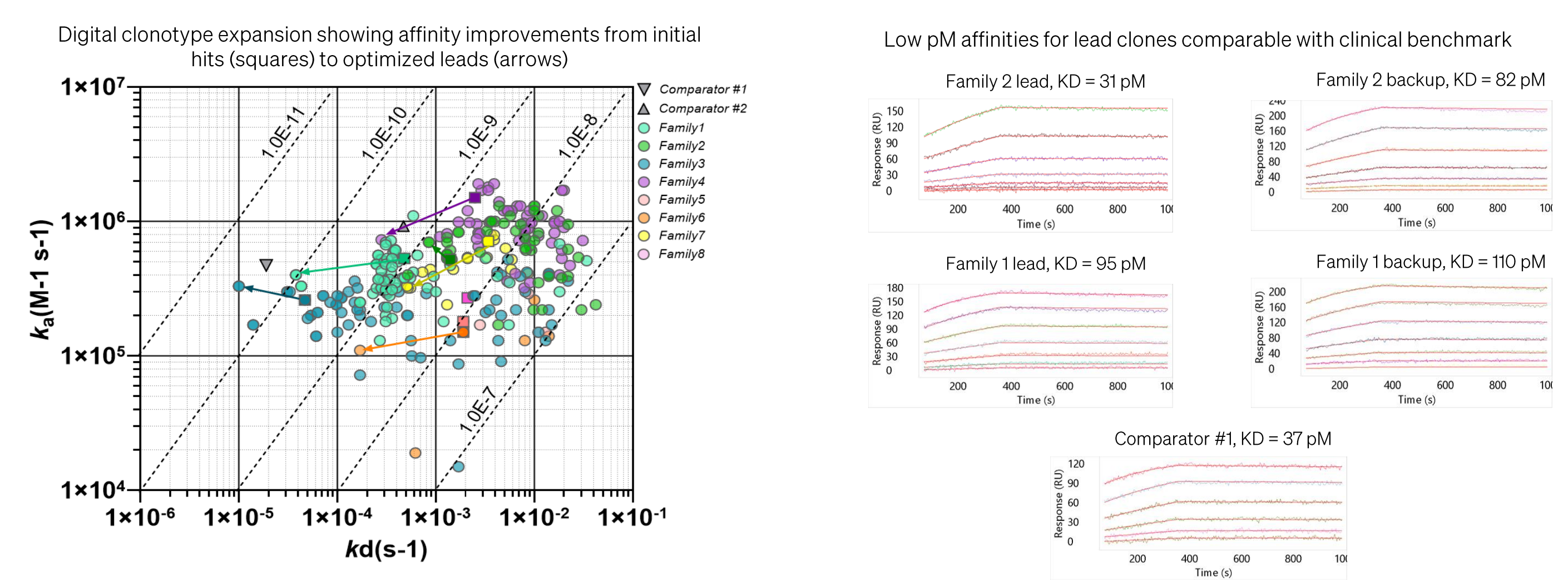
Diverse sequence and epitopic coverage from ATX-CLC campaign

- ATX-CLC-VK4 mice were immunized with a recombinant protein antigen and bulk VH sequencing performed on non-enriched B cell repertoire
- In silico* clone selection was applied to the *in vivo* antibody repertoire, identifying matured, expanded sequences across diverse clusters and phylogenetic trees
- 306 clones from *in silico* selection were expressed and tested by mAbForge, identifying 113 binders across 72 clonotypes for a 37% hit rate
- Identified binders show high epitopic diversity with binding sites across all domains of the target protein



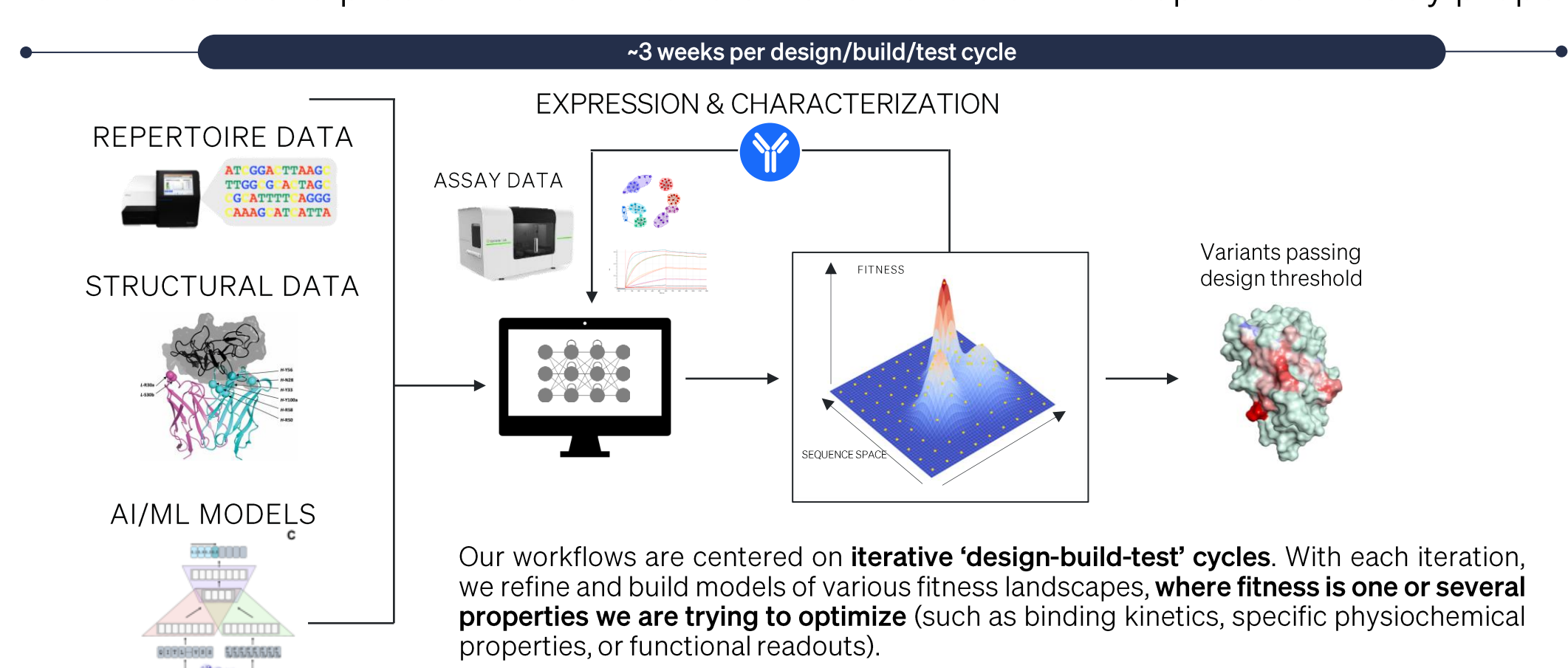
Affinity maturation by AI-guided clonotype expansion

Utilizing Alloy's sequence-first antibody discovery workflow, functional clonotypes from initial characterization can be rapidly optimized by mining additional variants from the immune repertoire data, enabling selection of diverse, high-affinity hits.



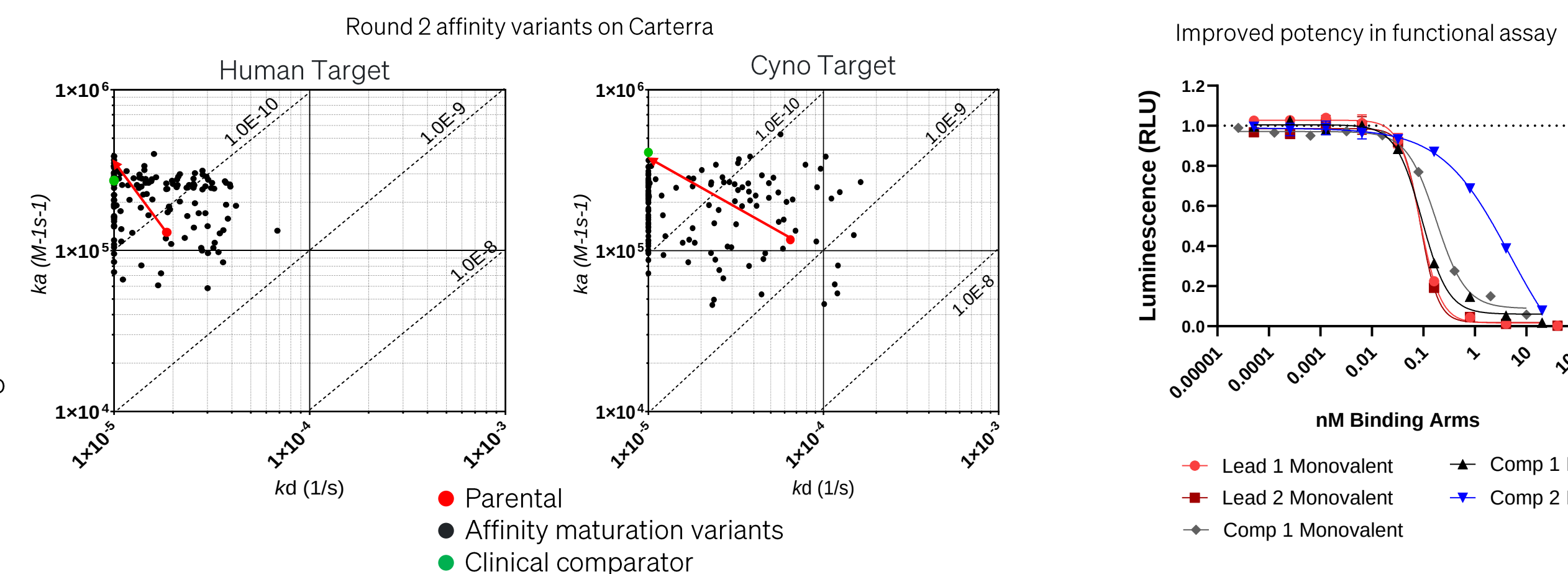
Antibody optimization using *in silico* design and mAbForge

Alloy uses combined *in silico* and wet lab technologies in iterative design-build-test cycles to rapidly optimize antibodies across a range of parameters including affinity, specificity, pH sensitivity, and developability. Initial diversity is computationally designed leveraging data from immune repertoires, structural modeling, and generative AI prediction algorithms. Hundreds to thousands of variants can then be expressed and tested by mAbForge or billions of sequences displayed in phage or yeast libraries. After each round of optimization, data is used to refine models and predict the next round of variants to dial in the optimal antibody properties.



Antibody affinity maturation

- Panel of antibody variants computationally designed based on *in vivo* repertoire analysis and generative AI inverse folding models
- Rapid expression and testing of ~180 variants per round by mAbForge with iterative design after each round
- After 2 rounds, antibody affinities had improved to <100 pM (LOD for Carterra) and functional potency was better than clinical comparators



Alloy Therapeutics platforms and services

Alloy democratizes access to foundational drug discovery tools, technologies, services, and company creation capabilities for partners including academia, small-medium sized biotech, and large pharma. We bring experience across multiple modalities including antibodies, bispecifics, TCRm, genetic medicines, and cell therapies. Our ATX-Gx suite of transgenic, humanized mouse strains continue to be a leading industry platform for antibody discovery. Alloy Discovery Services integrates proprietary *in vivo*, *in vitro*, and *in silico* discovery, engineering, and optimization workflows for smarter and faster monoclonal discovery, bispecific discovery, and TCRm discovery (Keyway™). mAbForge, accessible either through an active ATX-Gx license or in a discovery campaign with Alloy Discovery Services, can deliver expression and binding data on hundreds of clones in as fast as 3 weeks.