



When the Gene Editing Guide is the Drug

The rapid evolution of gene therapies and gene editing modalities in particular is challenging how personalised medicines are developed and evaluated. Many rare genetic disorders consist of numerous disease-causing variants, each of which requires a distinct therapeutic intervention. While advances in gene editing are making such treatments increasingly feasible, conventional development pathways are difficult to apply when patient populations are extremely small and individual mutations may be encountered only rarely. Requiring a complete development package, toxicology programme and clinical evidence package for every variant-specific therapy would make novel treatments impractical to develop.

In recognition of this challenge, the US Food & Drug Administration (FDA) issued a draft guidance in February 2026 describing a plausible mechanism framework for individualised therapies targeting specific genetic conditions with known biological cause (docket: FDA-2026-D-1256).¹ Rather than treating every variant-specific therapy as an entirely new product, the framework explores how evidence may be generated and leveraged across therapies that share a common biological cause.

The framework relies on several components: identification of a known causal genetic or molecular lesion; an understanding of untreated disease progression; evidence that the therapy engages with its intended target; and finally, demonstration that clinical outcomes reflect correction of the underlying genetic defect. With this approach, the FDA framework acknowledges that personalised therapies may be developed in settings where the first-in-human study also contributes pivotal evidence supporting effectiveness.

From Named Diseases to Causal Lesions

Gene editing is particularly well suited to the FDA's plausible mechanism framework because modern editing systems are increasingly modular. In this case, the therapy is comprised of a shared editor and delivery chassis plus a patient-specific guide. As such, much of what defines the therapy remains unchanged as the genomic target changes. Under these circumstances, the drug is no longer a unique molecular entity for each genotype. The drug is a platform, while the guide is the variable. This created the opportunity for the first economically coherent route to ultra-rare gene editing, with guide on- and off-target activity (OTA) being the key parameters requiring further investigation.

Rare Diseases as Case Studies

The practical value of this approach is most evident in rare genetic diseases, where hundreds of pathogenic variants may exist across relatively small patient populations. Generating a complete development package for every variant-specific therapy would often be economically and scientifically impractical.

The treatment of an infant with a metabolic disorder caused by severe CPS1 (carbamoyl-phosphate synthetase 1) deficiency using a customised lipid-nanoparticle base editing therapy in 2025 demonstrated the promise of highly personalised genome editing and the limitations of existing development models.² Repeating a full Investigational New Drug (IND) package for the next private allele would have been scientifically redundant and economically fatal. The guidance converts that one-off into policy.

A useful study showcasing how the FDA's framework might operate in practice took place at the University of Pennsylvania and Children's Hospital of Philadelphia programme developing therapies for seven infantile-onset urea-cycle disorders (UCDs).³ These conditions are well suited to the plausible mechanism





framework; the molecular causes are well known, disease progression is well characterised and treatment outcomes can be assessed via measurable biochemical endpoints, i.e., ammonia, glutamine and urea. Rather than developing a standalone therapy for each disease variant, the researchers proposed a customisable prime-editing platform using a common development strategy to help achieve a therapeutic effect across all seven UCDs.

As an initial proof of concept, the team focused on the ASS1 c.1168G>A (p.Gly390Arg) mutation, a recurrent cause of citrullinemia type 1. The proposed product was a two-component prime-editing platform: an AAV8 vector that expresses a patient-specific prime-editing guide RNA (pegRNA) and nicking guide RNA, plus a lipid nanoparticle (LNP) carrying mRNA for a compact prime editor. In this case, transient editor expression limits the off-target window while persistent guide expression allows for repeat LNP dosing. Candidate pegRNAs were first prioritised using the OptiPrime machine-learning platform and subsequently screened experimentally to identify an optimal editing configuration.

In humanised mouse models carrying the variant, the platform achieved efficient correction of the pathogenic allele, with whole-liver editing levels reaching approximately 30–40%, well above the threshold predicted to provide therapeutic benefit (i.e., 5–10%).

This programme provided an experimental basis for pre-IND discussions with the FDA. The proposal focused on an “umbrella-of-umbrellas” clinical trial model under which a shared prime-editing platform, supported by common biodistribution and toxicology data, could be expanded through the addition of new variants for all seven UCDs via IND amendments.³

Framework Strengths and the Gaps That Will Decide the Field

Even though the framework is still a draft and pre-IND advice is nonbinding, its strengths are real. It keeps the statutory effectiveness standard and changes only the permitted form of the evidence. It separates platform risk (biodistribution, immunogenicity, vector toxicity and manufacturing) from sequence risk. It also welcomes cell-based and *in silico* New Approach Methodologies (NAMs) which are defined by the FDA as “innovative testing methods and strategies used to assess the safety, efficacy and quality of FDA-regulated products”.

While the FDA framework provides opportunities to leverage platform-level evidence across multiple therapies, it does not eliminate the need for variant-specific data. Using the UCD study as an example, each guide RNA entered by IND amendment would still require variant-specific optimisation and safety assessment via the characterisation of any unintended insertions or deletions (indels) at both the on-target and off-target sites in the genome. In the framework, off-target method recommendations are explicitly deferred. FDA “intends to provide additional recommendations on nonclinical studies using NGS-based methods... in the near future.” Until then, sponsors are interpolating between this draft and the January 2024 genome-editing guidance, which already asked for two orthogonal nomination methods, one of them unbiased and genome-wide.^{1,4}

Additionally, if IND Phase 1/2 data are to support a biologics license application (BLA), chemistry, manufacturing and controls must look like a commercial product: locked process, inspection-

ready site, validated assays, stability on every individualised lot and a potency method that can be adapted to the next spacer. Research groups cannot meet that bar alone. According to the authors of the UCD study, an abbreviated path to approval “appears to be dependent on a sponsor’s ability to apply the strict requirements ordinarily reserved for late-stage clinical trials to an early-stage clinical trial.”³

The most consequential sentence in the framework is the one that allows a mutation never studied in patients to be added to a BLA based on *in vitro* activity plus an off-target assessment. The intellectual integrity of “plausible mechanism” now depends entirely on what that assessment is.

The Bottleneck is the Challenge of Off-target Assessment

Legacy approaches to off-target analysis were largely developed during a period when genome editing was dominated by nuclease-based systems designed to induce double-stranded DNA (DSB) breaks. For example, biochemical methods, such as CHANGE-seq, have proven valuable for identifying candidate off-target sites and informing risk assessment. However, many of these approaches operate outside the cellular environment in which therapies ultimately function and may not fully capture the influence of chromatin structure, epigenetic state or cell-specific DNA repair processes on editing behaviour.

These challenges are also relevant to more complex gene editing modalities including base and prime editors, which introduce alternative off-target mechanisms that legacy approaches were not designed to address, including off-target DNA nicking and bystander DNA deamination.⁵

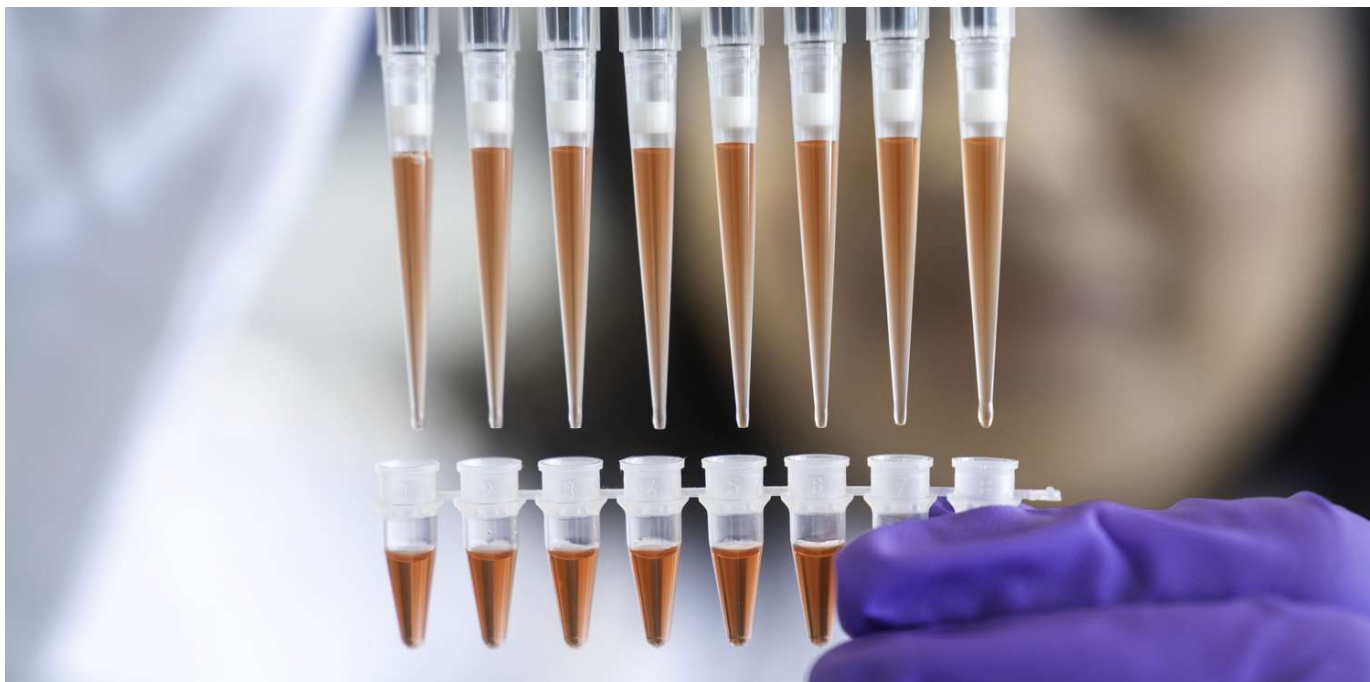
Moving Toward Effective Off-target Assessment for Novel Therapeutic Modalities

Addressing these challenges will require approaches that move beyond indirect measurements and cell-free assays towards the direct, genome-wide characterisation of DNA break formation in biologically relevant cells. As gene therapy programmes become increasingly customisable, the demands on the quality and quantity of off-target data grow. The future of screening must deliver what legacy methods cannot and will be determined by whether it can generate sufficiently comprehensive and quantitative datasets to support guide-specific safety decisions.

While *in silico* approaches remain important for guide design and risk prioritisation, they are inherently limited to sites predicted by existing algorithms. As a result, there is growing emphasis on unbiased, genome-wide methods capable of identifying editing activity irrespective of its genomic location.⁶

Equally valuable is the cellular context in which those measurements are made. Assays that directly detect editing events in intact cells can provide a more representative picture of therapeutic activity than methods based solely on cell-free DNA substrates, capturing the influence of chromatin accessibility, epigenetic state and DNA repair processes on editing outcomes.

The quality of off-target data also depends on the ability to quantify editing events accurately. PCR-dependent workflows can introduce amplification bias that distorts the apparent frequency of editing events, particularly when rare outcomes must be measured against a large background of unedited



molecules. Methods that eliminate amplification bias therefore offer advantages when quantitative comparisons are required across candidate guides or editing platforms. There is also an increased value in workflows being capable of assessing both on-target and off-target activity simultaneously, which provides a more complete picture of editing behaviour and simplifies interpretation of candidate performance.

Finally, off-target assessment needs to inform programme decisions during guide selection and lead optimisation, rather than just as part of an IND package. Approaches that can be implemented in-house and generate actionable results within days are becoming more valuable as developers evaluate larger numbers of candidate guides while maintaining control of proprietary data and development timelines. Methods that address these requirements, such as INDUCE-seq, can provide the genome-wide, cell-based evidence needed to support guide-specific safety assessments under the FDA's plausible mechanism framework.⁶ They do not replace the need for orthogonal nomination methods, integration analyses, toxicology studies or long-term follow-up, but rather complement the broader body of evidence required to support clinical development.

Make the Pathway Executable

The FDA's plausible mechanism framework is the first regulatory document that treats modular genome editing as modular, offering a potential route to bringing personalised therapies to patients whose needs would often be neglected by conventional development programmes. That is its strength. Its weakness is that it still needs evidence equal to the claim it is making.

The UCDs study demonstrates that platform-level knowledge may be leveraged across programmes, but confidence in guide-specific safety remains essential. As personalised gene therapies expand, technologies capable of generating accurate, genome-wide assessments of editing outcomes in biologically relevant cells are likely to become increasingly important. A PCR-free, cell-based map of DSBs, paired with a nick-adapted readout of base-editor engagement, is the natural contents of that file.

Combined with computational approaches that help prioritise candidates, such methods could provide the evidence needed to support efficient therapeutic development that makes the plausible mechanism framework executable.

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Simon Reed

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Defined Raw Materials – Robust Biomanufacturing

A recombinant and animal-origin-free raw material strategy for antibody, vaccine and cell & gene therapy manufacturing.

The Manufacturing Imperative

Biomanufacturing is converging on a common requirement: greater productivity with more control. In monoclonal-antibody and cell-culture vaccine processes, that means intensified, serum-free platforms that boost yield and ensure supply chain security. In cell and gene therapy, it means retaining phenotype, vector quality and process consistency as technologies under development are being scaled and transferred. Across modalities, well-characterised and high-quality raw materials are moving from an operational preference to a strategic must-have.

The Regulatory Landscape

This evolution aligns with convergent global expectations. FDA's 2024 draft CGT guidance highlights adventitious-agent risk, lot-to-lot consistency, identity and material qualification; it advises manufacturers to consider materials free of human- or animal-derived proteins, such as recombinant proteins. EMA states that, when applicable, animal reagents should be avoided and replaced with non-animal-derived reagents of defined composition. ICH Q5A(R2) likewise advises avoiding human- and animal-derived raw materials, including serum and porcine trypsin, whenever possible. In Japan, PMDA expects qualification and specifications for media, additives and reagents and advises avoiding heterogeneous serum wherever possible. In China, NMPA has made ICH Q5D - derivation and characterisation of cell substrates, applicable to relevant studies. Overall, the pattern is global, and the aim is for GMP manufacturers to have risk-based CMC strategies for their processes through defined composition, lot-to-lot consistency, traceability, regulatory-aligned documentation and supply chain security.

Designed for the Transition

Novo Nordisk Pharmatech supports this transition with two animal-origin-free recombinant products:

Recombinant Insulin (Insulin Human AF), produced by recombinant microbial expression in yeast, serves as a serum-free growth-factor supplement in CHO, HEK293 and Sf9 cultures to boost productivity of monoclonal antibodies (mAbs), vaccines, induced pluripotent stem cells (iPSCs) for cell & gene therapy and viral vectors.

TrypsinNex® is a recombinant porcine-sequence trypsin expressed in *E. coli* for controlled cell dissociation, cell-culture-based virus production and biopharma diagnostics. Its high purity and beta-trypsin content paired with extensive GMP documentation make enzyme performance and qualification more predictable.

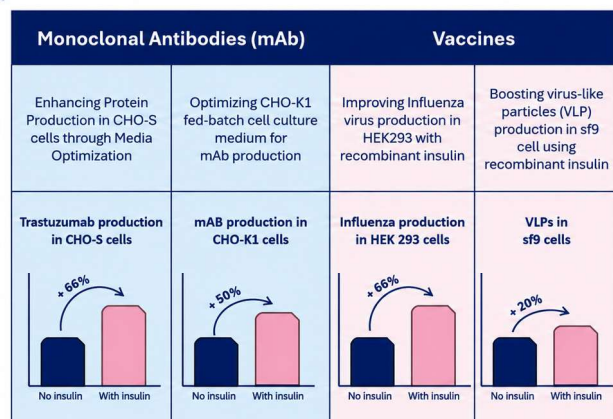
APPLICATION EVIDENCE ACROSS THE FIELD

Recombinant Insulin

Monoclonal antibodies production: This defined, animal-origin-free raw material can be tailored as a growth factor supplement in cell culture media across different biomanufacturing platforms. In CHO-S cultures, supplementation of three chemically defined media improved Trastuzumab productivity in every medium, with the greatest increase – 1.7-fold – at 1 mg/L of insulin in the best-performing condition. In CHO-K1 fed-batch culture, 2 mg/L of insulin in basal medium increased maximum cell density 1.2-fold and IgG production 1.5-fold; adding insulin to the feed likewise improved IgG productivity 1.5-fold without a discernible change in cell proliferation (Figure 1, A).

Vaccine manufacturing: In HEK293SF-3F6 cells cultured in chemically defined medium and infected with influenza H1N1, recombinant insulin added at infection increased hemagglutinin

A



B

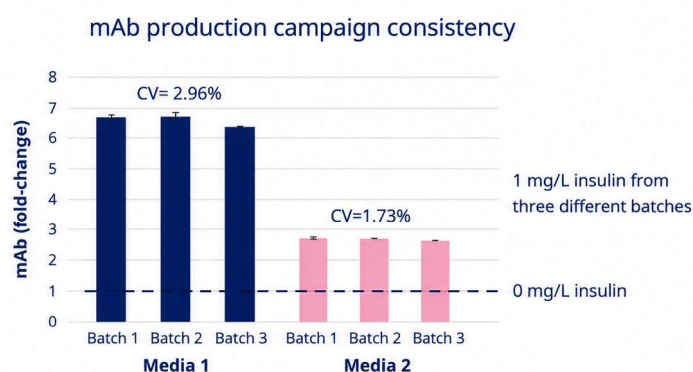


Figure 1: (A) Studies overview of enhanced monoclonal antibodies, viral vaccine and VLPs production by supplementation of recombinant insulin to the cell culture media. (B) Recombinant insulin batch-to-batch consistency ensures reliable production of mAbs



(HA) concentration at 46 hours post-infection, reaching up to 1.7-fold at 25-100 mg/L, without significantly changing cell density. The higher output was associated with further Akt phosphorylation during infection. In an Sf9/baculovirus model in chemically defined medium producing HIV-1 Gag VLPs, 1 mg/L insulin increased viable cell density and VLP output by 1.2-fold, reaching 5.6×10^{10} VLP/mL; particle size remained within the expected 100-200 nm range (Figure 1, A). These model-specific results illustrate how a recombinant insulin input can support transparent formulation design and adjustable process control for vaccine-relevant production, while product-specific process development, qualification and validation remain essential.

Overall, the highlighted results strengthen the value proposition of supplementation of the cell culture media with recombinant insulin: an animal-origin-free, chemically defined raw material produced under GMP standards to match the needs of biomanufacturers for predictable, reliable and consistent processes (Figure 1, B).

TrypsiNNex®

Across several studies, TrypsiNNex® shows how a high-purity, animal-origin-free recombinant trypsin can bring control to upstream steps that strongly influence manufacturing consistency, cell passaging and cell culture-based virus production.

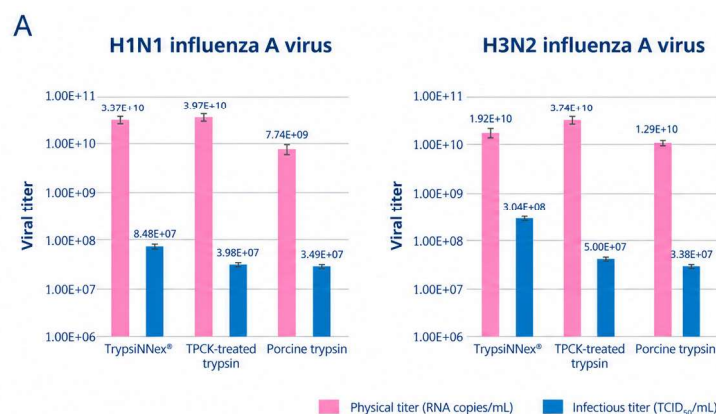


Figure 2: (A) TrypsiNNex® benchmarked in MDCK-produced influenza virus against alternative trypsin products. Virus yields based on viral RNA copies/mL (physical titer; pink bars) are similar. Functional titre (TCID₅₀/mL; blue bars) indicates that the viral particles had higher infectivity with TrypsiNNex®. (B) All manufactured batches of TrypsiNNex® contain a consistently high amount of beta-trypsin, supporting process efficiency and predictability.

Cell dissociation: In a study spanning HEK293, MDCK, MRC5, BM-MSC, UC-MSC and AD-MSC cultures, it performed comparably to commonly used animal-derived trypsin and trypsin-like enzymes, while maintaining high post-dissociation viability. For example, HEK293 cells were efficiently detached with 200 U/mL after five minutes, with more than 90% viability. The study also defined cell-line-specific concentration and incubation windows for 2D and 3D cultures, supporting a more systematic transfer of passaging protocols across cell-therapy and production platforms. TrypsiNNex® supports effective dissociation without the presence of EDTA, also in Vero cells (Figure 2, A).

Viral vaccine manufacturing: In MDCK-SIAT1 influenza production, DOE work established distinct conditions for H1N1 and H3N2. At optimised strain-specific settings, TrypsiNNex® matched physical titers observed with animal-derived trypsin and delivered the highest functional, infectious titer in the tested comparison.

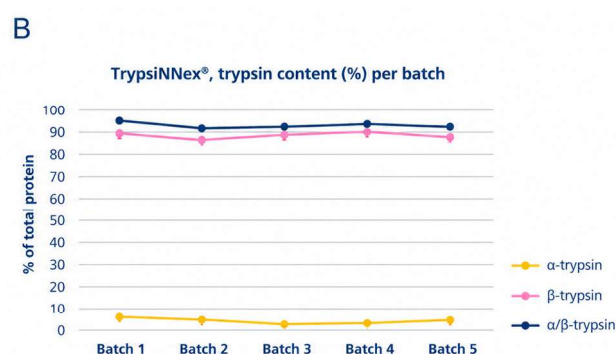
Together, these findings illustrate the value of a high-purity recombinant enzyme: predictable, tunable dissociation and viral-maturation performance without animal-origin input. With fully defined specifications and high batch-to-batch consistency, it can strengthen raw-material control and reproducibility in tightly regulated processes (Figure 2, B).

Key Message

Defined, animal-origin-free inputs allow biopharma manufacturers to tune media and enzymatic conditions individually, preserving flexibility while supporting a more transparent and scalable path from research to GMP-ready manufacture.

Novo Nordisk

Novo Nordisk Pharmatech A/S is a Danish life-science company headquartered in Koege, Denmark and is 100% owned by Novo Nordisk A/S. Its core activity is the development and supply of high-quality ingredients and active pharmaceutical ingredients (APIs). Founded in 1949 as FeF Chemicals, the company has more than 75 years of manufacturing and process expertise. Its portfolio includes Recombinant Insulin, Benzalkonium Chloride (BKC) and enzymes such as TrypsiNNex®, with its broader technology strategy covering expression, modification, purification and formulation. NNPR operates across the value chain and enables pharmaceutical and biopharmaceutical manufacturers globally.



João Monteiro

With a PhD in Infection Immunology, João has supported customers from preclinical development through to GMP manufacturing. His experience includes roles as a Field

Application Scientist and Clinical Account Manager, combining scientific expertise with customer-focused engagement.



Chantale Julien

With over 20 years of experience in the pharmaceutical industry, Chantale's role as a Senior Global Product Manager encompasses managing several product portfolios, providing technical and scientific support, strategic market positioning and adherence to GXP requirements.



From Cell Culture to Human-relevant Models

Every generation of mammalian cell culture has solved one biological challenge while revealing the next. The earliest researchers sought to keep living cells alive outside the body, opening a new window into biology and medicine. As these techniques matured, demand grew for scalable culture systems capable of producing cells for vaccines, biologics and regenerative medicine. Yet increasing cell numbers was only part of the challenge, as attention also turned towards recreating the physical, biochemical and mechanical cues that shape cellular behaviour within human tissues. This pursuit drove the development of microcarriers for scalable cell expansion, hydrogels for recreating the cellular microenvironment and microfluidic systems for introducing dynamic culture conditions, laying the foundation for more human-relevant *in vitro* models with the potential to reduce reliance on animal models.

maintaining cells over extended periods. Improvements in sterile technique, specialised culture media, incubator technology and cell line development transformed cell culture from a specialised method into a routine laboratory tool.¹ This progress accelerated discoveries in developmental biology, immunology, cancer research and molecular medicine. Cultured cells also became increasingly important for biotechnology.

By the late twentieth century, mammalian cell culture had become indispensable across academia and industry. It became central to vaccine production, recombinant protein expression, monoclonal antibody manufacturing and, more recently, cell and gene therapies.² As these applications expanded, so did the demand for larger quantities of cells. Conventional culture methods, however, struggled to keep pace with production requirements. The next challenge was scale: how could hundreds of millions, or even billions of cells be produced using tools designed for small laboratory experiments?

The Beginning: Learning to Culture Living Cells

Modern biotechnology owes much of its success to the ability to maintain mammalian cells outside the body. Before reliable culture techniques were established, biological processes were studied mainly in whole organisms or fixed tissue samples. This limited the ability to examine cellular behaviour under controlled conditions. Mammalian cell culture changed that by allowing living cells to be observed and manipulated directly. It soon became a foundational tool across the life sciences.

The transition was not straightforward. Early work was constrained by sterility, media formulation and the difficulty of

Cell Culture Moves Beyond the Laboratory

As mammalian cell culture became central to biotechnology, the need for larger-scale cell production grew. Emerging fields such as regenerative medicine, cell therapy and cellular agriculture required production platforms capable of generating more cells than conventional laboratory methods could support. However, many adherent cells continued to be cultured using tissue culture flasks and multilayer vessels. While effective for planar culture, scaling these systems by increasing vessel numbers became labour-intensive, space-consuming and difficult to standardise as production increased. Reproducibility between batches also became more difficult to maintain as cultures scaled.

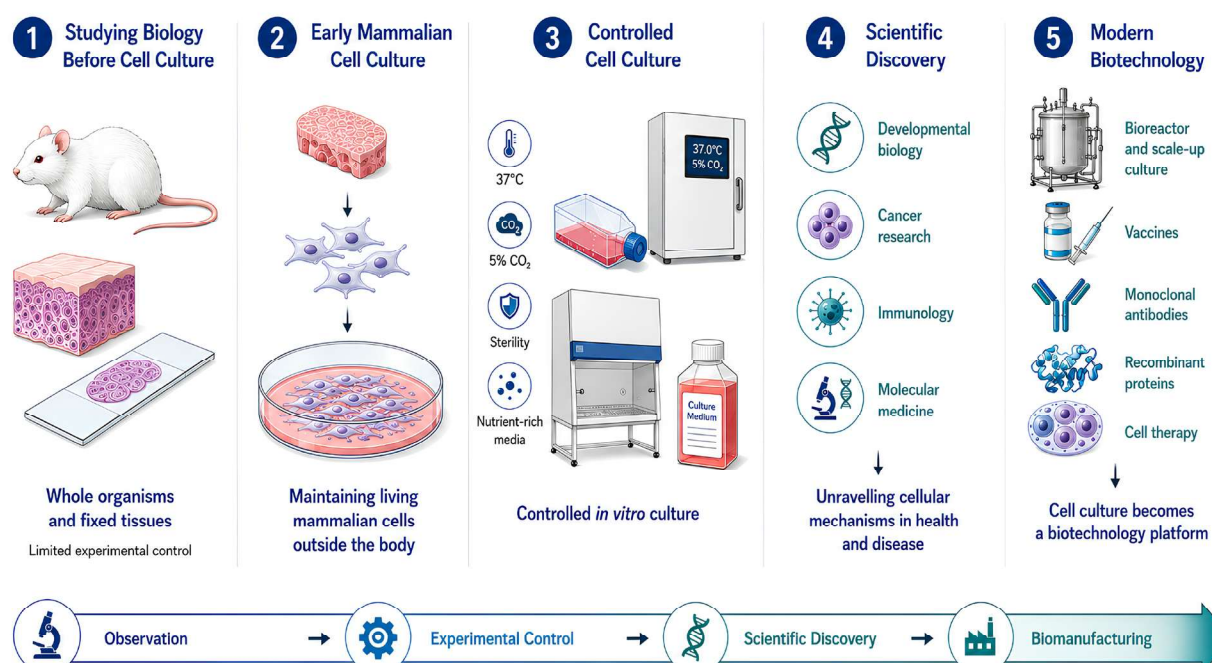


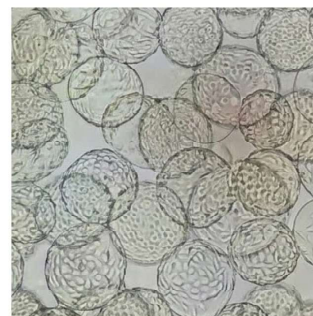
Figure 1. Conceptual overview of the evolution of mammalian cell culture, highlighting its progression from observation and experimental control to biological discovery and biomanufacturing.



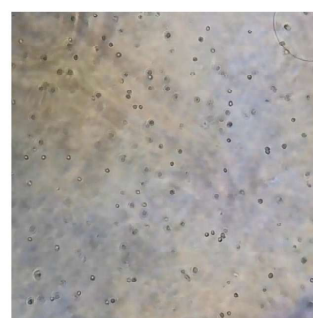
Scaling Adherent Cell Culture with Microcarriers

Representative Images

Dissolvable Microcarriers



Representative Dissolvable Microcarriers



Recovered Cells Following Dissolution and Harvest

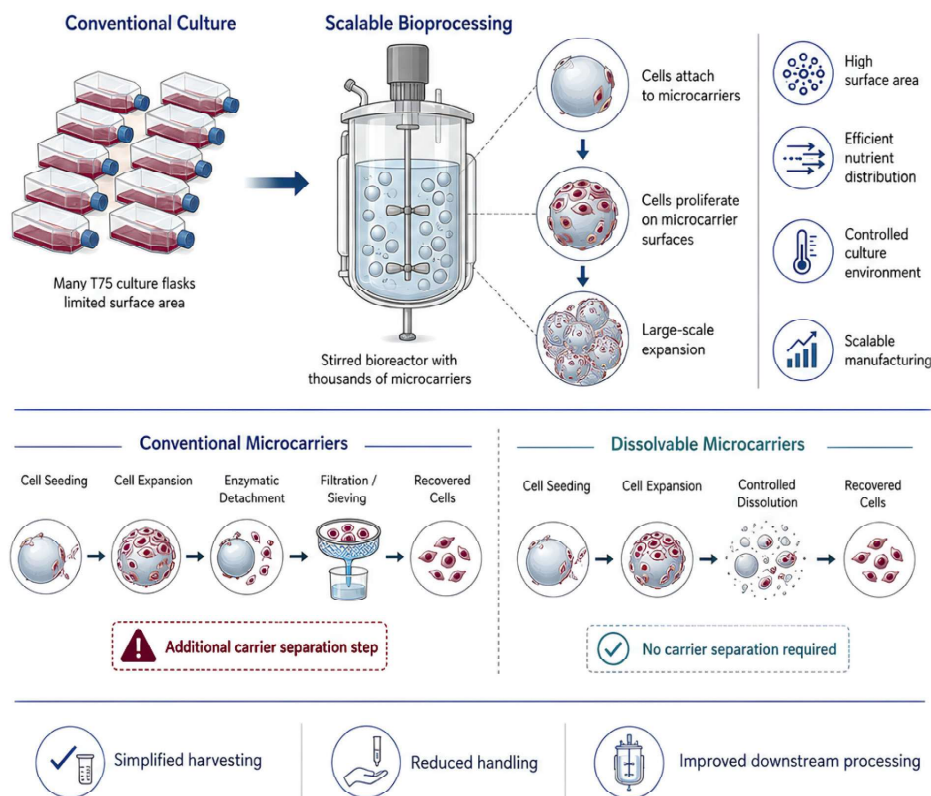


Figure 2. Microcarriers enable scalable expansion of adherent cells in stirred bioreactors, while dissolvable microcarriers can simplify cell harvest. Representative images show dissolvable microcarriers (top) and recovered cells following harvest (bottom).

These limitations marked a turning point in the evolution of mammalian cell culture. Rather than simply asking how cells could be grown, attention shifted towards how they could be expanded at scale. This brought together expertise from cell biology, biomaterials and bioprocess engineering to develop technologies designed specifically for cell expansion. Among the most influential solutions to emerge from this convergence was the microcarrier. Microcarriers remain widely used for scaling adherent cell culture in research and biomanufacturing.

Solving the Scale Problem: Microcarriers

Microcarriers were developed to address the challenge of scaling adherent mammalian cell culture. Unlike suspension cells, which grow freely in liquid media, adherent cells require a surface on which they can attach, spread and proliferate. Traditional tissue culture plastic provided this surface, but adding more flasks to scale production quickly became impractical. As cell-based manufacturing expanded, laboratories needed a way to generate greater numbers of cells without substantially increasing space, labour or infrastructure. Microcarriers provided a practical solution to this challenge.

The concept was remarkably simple. Instead of growing cells on large flat surfaces, scientists introduced microscopic particles that remained suspended in stirred bioreactors while providing surfaces for cell attachment. Millions of these particles could generate a large culture area within a small volume. This transformed adherent cell culture from a labour-intensive laboratory technique into a scalable bioprocess. Microcarriers became a cornerstone of large-scale cell manufacturing.

Conventional microcarriers, however, often require additional separation steps during cell harvest, increasing process complexity and potentially reducing cell recovery.³ This limitation has driven the development of biodegradable and dissolvable microcarrier technologies that simplify downstream processing. Microcarrier properties such as stiffness, surface charge and particle size can influence cell attachment and expansion performance.³⁵ These findings highlight the growing importance of material design in cell manufacturing. But even if cells could now be expanded efficiently at scale, were they behaving as they would within the human body?

Recreating the Cellular Environment: Hydrogels

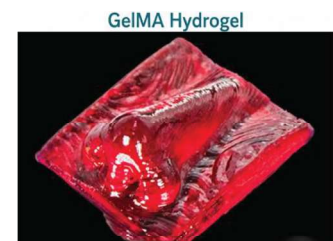
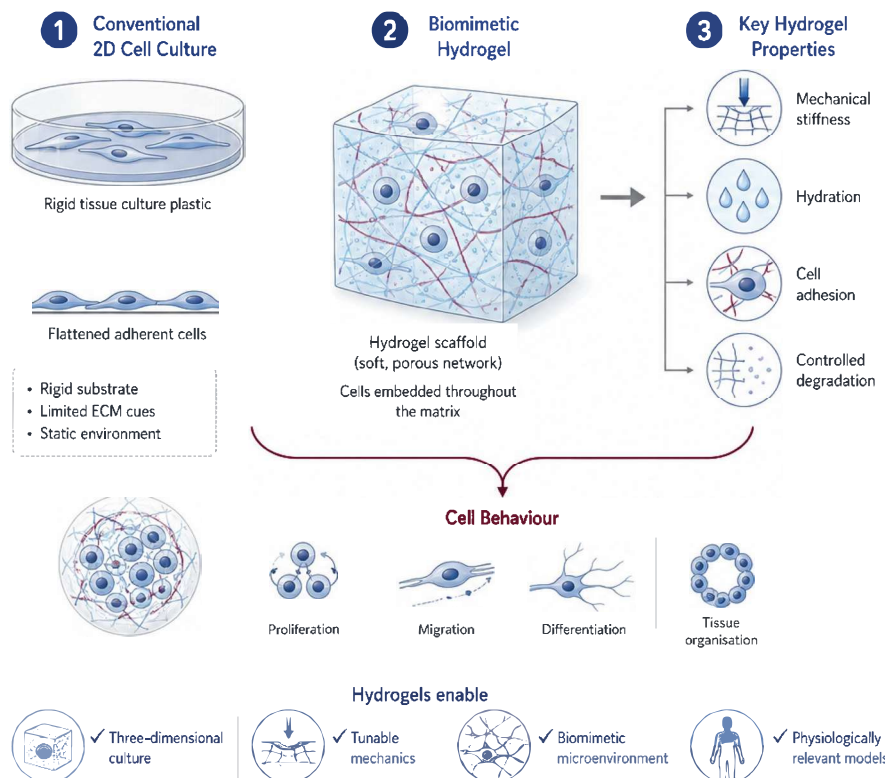
Expanding cells at scale did not necessarily create better biological models. While conventional culture systems enabled efficient cell expansion, they still lacked many of the biological and mechanical cues found within living tissues. It became clear that recreating the cellular environment was as important as expanding the cells themselves. Attention therefore shifted towards the microenvironment surrounding each cell. The search for greater biological relevance ultimately led to the extracellular matrix.

Inside the body, cells exist within the extracellular matrix (ECM), a dynamic environment that regulates cellular behaviour through biochemical signals, mechanical properties and cell-matrix interactions. The ECM therefore became a key focus for recreating tissue-specific conditions in culture. Hydrogels emerged as a promising solution because their highly hydrated, three-dimensional polymer networks can be engineered to better resemble native tissues.⁶ Hydrogels are now used across

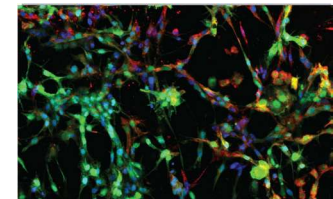


Biomimetic Hydrogels Recreate the Cellular Microenvironment

Representative Images



HUVEC Culture in GelMA



hBMSC Culture in GelMA

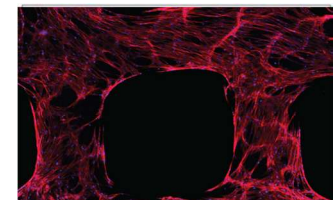


Figure 3. Biomimetic hydrogels support physiologically relevant cell–matrix interactions and tissue engineering applications. Representative images show a photocrosslinked GelMA hydrogel (top), human umbilical vein endothelial cells (HUVECs) cultured within GelMA-G90 (middle), and human bone marrow-derived mesenchymal stromal cells (hBMSCs) cultured on GelMA-G90 (bottom).

tissue engineering, regenerative medicine, organoid culture and three-dimensional bioprinting. Their tunability makes them valuable for creating more physiologically relevant *in vitro* models.

Importantly, hydrogels can do more than provide structural support. Their physical and biochemical properties can be tuned to influence cellular behaviour. This allows key features of the cellular microenvironment to be studied under controlled conditions. However, hydrogels alone do not inherently reproduce the dynamic flow and transport conditions found within living tissues. Recreating these features required a way to introduce controlled movement and flow.

Introducing Dynamic Culture: Microfluidics

If hydrogels helped recreate the structure of living tissues, microfluidics introduced another defining feature: flow. In the body, cells are continually exposed to moving nutrients, signalling molecules and mechanical forces that shape their behaviour. Microfluidic systems reproduce aspects of these conditions by directing small volumes of fluid through precisely defined channels. Flow rate, shear stress and chemical gradients can therefore be controlled in ways that are difficult to achieve in static culture. This makes microfluidics particularly useful for studying how cells respond to dynamic environments.

Microfluidic technologies can be applied across very different biological scales. At the single-cell level, they have been used to isolate, retrieve and characterise rare cells, including circulating tumour cells.^{7,8} At the tissue-model level, organ-on-chip systems use controlled fluid flow to recreate selected features of organ-specific environments. One lung-on-a-chip model, for example, maintained airway epithelial cells at an air–liquid

interface and reproduced important functional characteristics, supporting its use in toxicological testing and pulmonary drug delivery studies.⁹ Together, these examples highlight the versatility of microfluidics, from handling individual cells to creating more physiologically relevant tissue models under precisely controlled conditions.

Microfluidics also creates a natural bridge between the technologies discussed earlier. Biomaterials can provide structure and biochemical cues, while controlled flow introduces transport and mechanical forces that are central to living tissues. These elements can be combined within a single culture system rather than studied separately. This allows cell behaviour to be examined under conditions that capture several features of the tissue environment at once. At this point, the story of cell culture becomes less about individual technologies and more about how they work together.

Bringing Everything Together

Looking back over the evolution of mammalian cell culture, a clear pattern emerges. What began as an effort to keep cells alive outside the body gradually expanded into a broader effort to recreate the conditions in which they naturally function. Microcarriers addressed the challenge of scale, hydrogels helped recreate aspects of the extracellular matrix and microfluidics introduced dynamic culture conditions. Together, these technologies have transformed what can be achieved in the laboratory. Each has contributed a different piece of the puzzle required to build more representative biological models.

Perhaps the most important lesson is that no single technology can faithfully reproduce human biology. Scalable cell expansion,



Integrating Dynamic Culture with Biomaterials

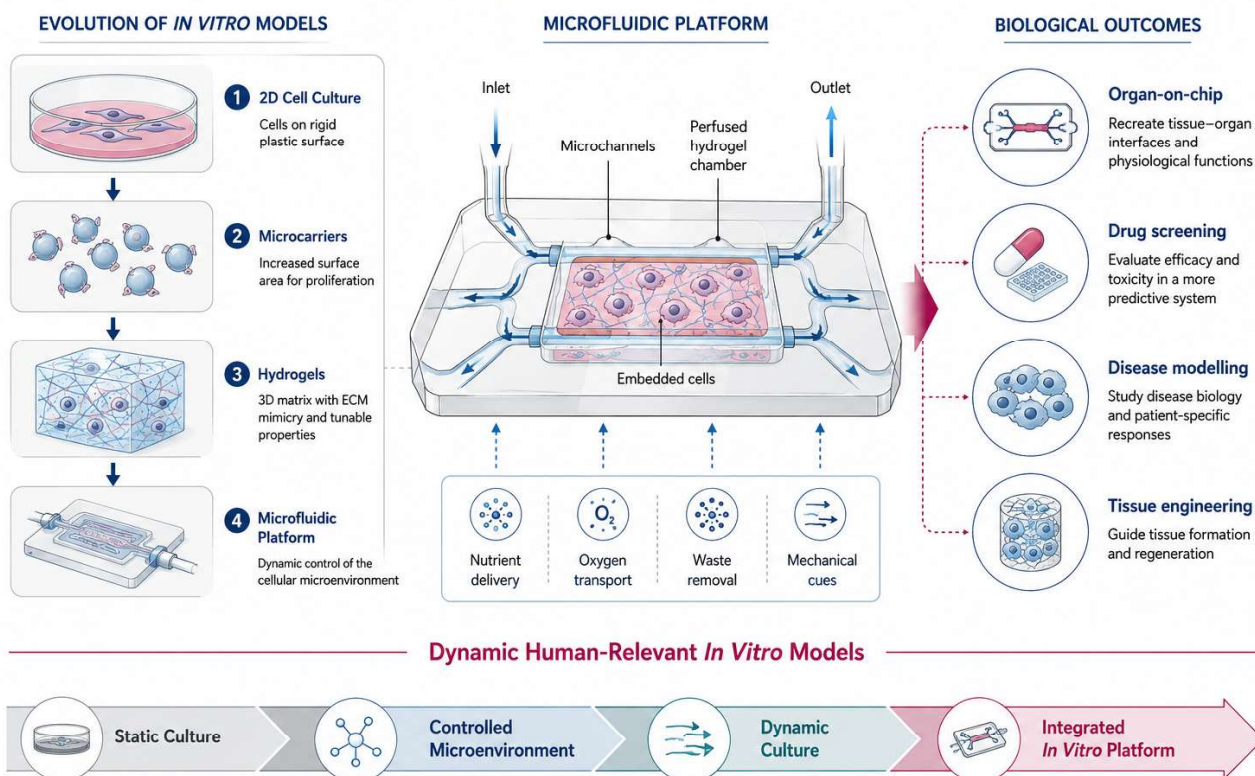


Figure 4. Integration of biomaterials and microfluidics to create dynamic and physiologically relevant *in vitro* models.

biomaterials and dynamic culture systems each address different aspects of the cellular environment. Increasingly, these complementary approaches are being combined to support applications ranging from organoids and organ-on-chip systems to regenerative medicine and drug discovery.⁹ This convergence is helping create more representative and predictive experimental models. Ultimately, the future of mammalian cell culture will depend on how effectively these technologies can work together.

Looking Ahead

The future of mammalian cell culture is being shaped by the need for laboratory models that better predict human biology. Across pharmaceutical development, regenerative medicine and biotechnology, cell biology, biomaterials, engineering and computational approaches are being brought together to create more physiologically relevant and reproducible *in vitro*

systems. Organoids, organ-on-chip technologies and engineered biomaterials are taking these platforms beyond proof-of-concept towards practical applications in drug discovery, disease modelling and personalised medicine. This reflects a broader change in how biological models are designed and applied. Rather than relying on a single technology, the field is combining complementary approaches to capture more of the complexity of human tissues.

The history of mammalian cell culture is a story of continuous refinement. What began as an effort to keep cells alive outside the body has evolved into a multidisciplinary field that combines biology, engineering and materials science to recreate increasingly representative models of human tissues. Each generation of technology has built upon the last, steadily narrowing the gap between conventional laboratory culture and the complexity of living systems. Yet the underlying goal remains





From Cell Expansion to Human-Relevant Models

Integrating scalable cell expansion, biomimetic materials and dynamic culture to support human-relevant *in vitro* models for drug discovery, disease modelling and regenerative medicine.

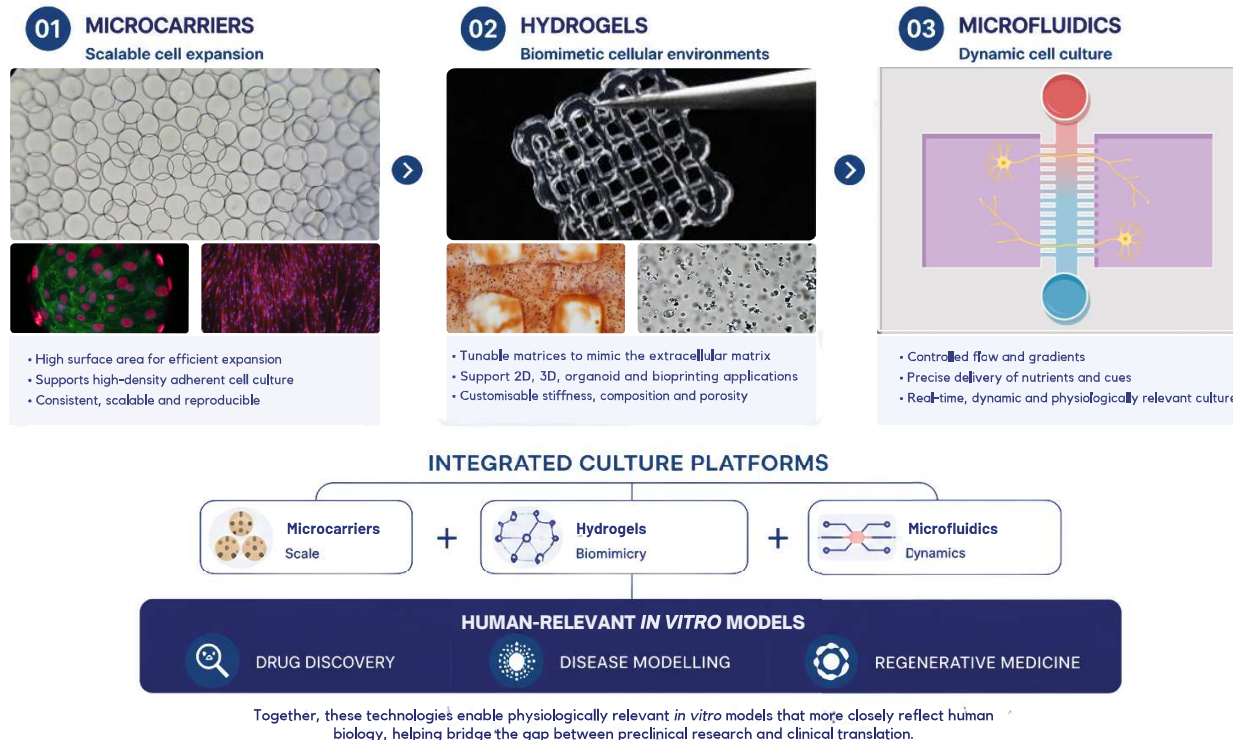


Figure 5. Conceptual framework illustrating the convergence of complementary cell culture technologies towards more physiologically relevant and translationally meaningful *in vitro* systems.

remarkably similar to that of the earliest cell culture pioneers: to understand how cells function within their native environment. As that gap continues to narrow, it raises an intriguing question: what will the next generation of human-relevant models look like?

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Peptide-to-Small-Molecule Scaffold Exploration with Amino Acid Mapping

Peptides and macrocycles can bind targets beyond the reach of many conventional smaller molecules. Converting that binding insight into a smaller scaffold with room for further development remains a substantial medicinal chemistry challenge. Conventional analogue design usually remains close to the structure that produced the original activity. That is useful for optimisation within a series, but less useful when the series itself has become the constraint.

drug2drugs AI-AAM® (Amino Acid Mapping) begins from a different premise. It maps amino-acid interaction features around an active reference ligand and uses those features to guide exploration of alternative molecular frameworks. The objective is to identify structurally differentiated molecules that may retain selected elements of the reference interaction profile.

A case study on peptide-to-small-molecule conversion using drug2drugs AI-AAM® is presented in this application note. In this published study on PD-L1, a 1,796 Da macrocyclic peptide was translated into a 482 Da small molecule with measurable activity, improved binding efficiency per molecular weight, and substantially greater membrane permeability; structure-based chemical modification then improved potency more than six-fold.

The Scaffold Limitation Problem in Lead Discovery

Analogue design can refine a known chemical series, but it rarely changes the central framework that defines the series' chemical, developability and intellectual-property constraints. This becomes a problem when the scaffold itself contributes to metabolic instability, poor solubility, excessive lipophilicity, difficult synthesis or overlaps with existing patents.

The challenge is greater for cyclic peptides and macrocycles. These ligands can bind difficult protein surfaces with high affinity, yet their size and polarity can restrict membrane permeability and complicate oral delivery. Conventional fingerprint and pharmacophore methods often favour structures that remain close to the starting ligand. Peptide-to-small-molecule conversion requires a search criterion that can retain selected binding-relevant features while allowing the framework to change.

drug2drugs AI-AAM®: *In Silico* Spatial Feature Mapping

drug2drugs AI-AAM® is a ligand-based computational service for scaffold exploration and molecule prioritisation from one active reference structure (Figure 1). The platform maps amino-acid interaction features around the reference ligand, then uses those features to guide generation or selection of alternative molecular frameworks. It delivers computationally prioritised molecules for synthesis and biological testing; experimental validation remains downstream.

AAM is the core descriptor. It records the spatial distribution of interaction features associated with each of the 20 natural amino-acid probe types around a ligand. The resulting four-dimensional map combines amino-acid identity with three-dimensional position. It provides a ligand-side representation of binding-relevant features, rather than a reconstruction of the complete protein binding site.

The atom-based generator designs molecules through sequential atom addition, deletion and bond formation while assessing the evolving structure against the reference AAM profile. Learned adjacent-atom patterns suppress chemically unreasonable local structures.

AAM similarity, diversity selection and project-defined computational filters then refine the generated pool into a focused molecule set.

AI-AAM proposes new bioactive candidates by scaffold hopping from an original bioactive compound

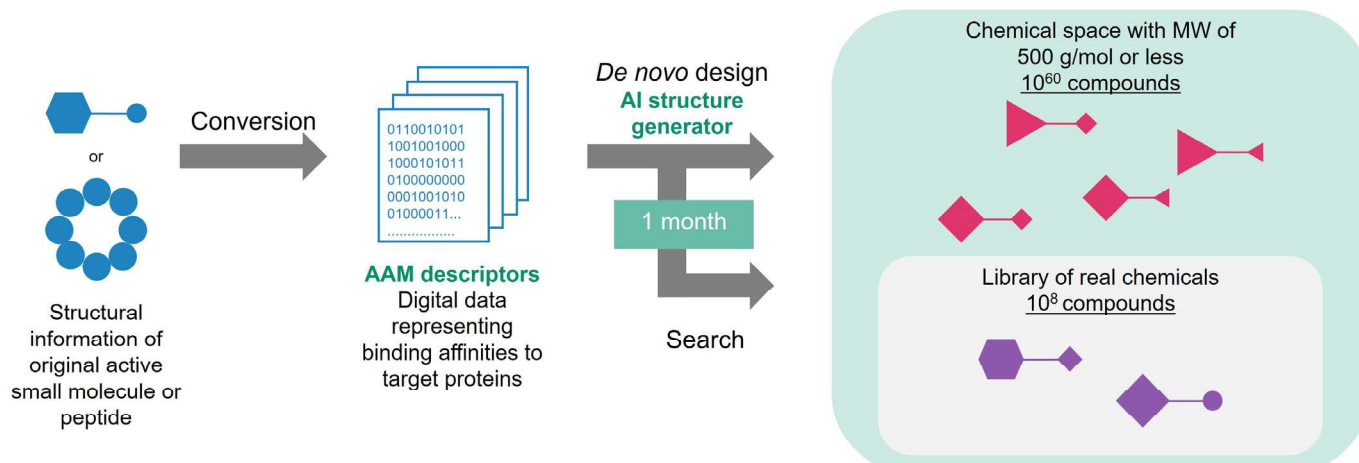


Figure 1. drug2drugs AI-AAM® converts an active reference compound into an AAM interaction profile, then uses that profile to support both library screening and atom-based de novo molecule design



AAM similarity can be evaluated during molecular generation because the comparison operates on ligand-side interaction maps rather than full protein calculations. A complete generation campaign can still require substantial resources: in the PD-L1 study, one run used multiple GPUs over approximately one month. The final output is typically a prioritised set of approximately 100 structures for further review and validation. During the computation phase, the generator also applies learned adjacent-atom patterns to suppress chemically unreasonable local structures, including implausible bond patterns and inappropriate connectivity. Computational indicators related to synthetic accessibility and stability can further refine the set of molecular structures. The workflow expands the search beyond close analogues of the reference scaffold (Figure 2).

AAM similarity compares amino-acid-specific spatial maps after relative alignment. Conventional fingerprint similarity rewards shared molecular connectivity; AAM similarity can rank molecules with unrelated cores when they present related interaction features in similar regions of space. The score is used to prioritise molecules within a defined system. It is not a direct measure of biological activity or a universal predictor of potency (Figure 3).

For larger ligands, the calculation can focus on selected regions rather than the complete molecule. This selected residue strategy is important for peptide-to-small-molecule

work because it directs the generator towards localised interaction motifs that may be represented within a lower-molecular-weight scaffold.

Peer-Reviewed Case Study: Macrocyclic Peptide to Small Molecule Conversion

To rigorously evaluate the platform, a proof-of-concept study was conducted and published in *Bioorganic and Medicinal Chemistry Letters* in 2026 (<https://doi.org/10.1016/j.bmcl.2026.130655>). The study investigated whether the AI-AAM platform could transform a high-molecular-weight macrocyclic peptide targeting the programmed death ligand-1 (PD-L1) interaction surface into an active, membrane-permeable small molecule.

The starting reference molecule was Peptide-71, a macrocyclic peptide inhibitor of the PD-L1 interaction possessing a molecular weight of 1796.12 g/mol and a measured inhibition constant K_i of 0.004 μM (4 nM, PDB ID: 5O45). The Binding Efficiency Index (BEI), defined as $-\log_{10}(K_i) / (\text{MW}/1000)$, was calculated at 4.7. To identify the critical interaction domain, FMO-based energy decomposition was applied to the PD-L1/Peptide-71 complex model. The total calculated FMO interaction-energy score for Peptide-71 was -90 kcal/mol.

Energy decomposition revealed that binding energy was unevenly distributed across the peptide backbone. Four spatially localised residues, namely 1Phe, 7NMePhe, 10Trp and 11Tyr, accounted for -61 kcal/mol, representing approximately

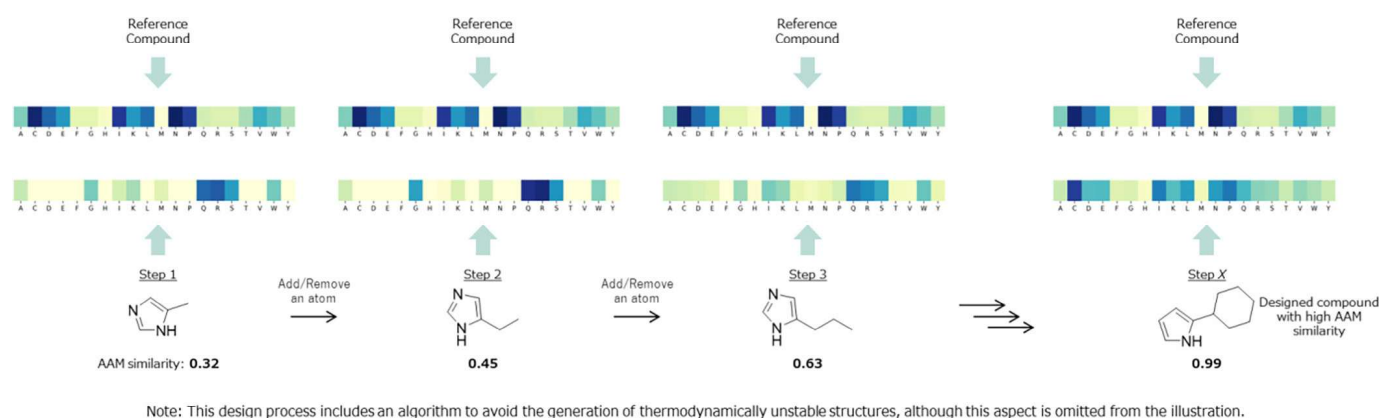


Figure 2. Atom-based generation modifies molecular structures iteratively while assessing similarity to the reference AAM profile

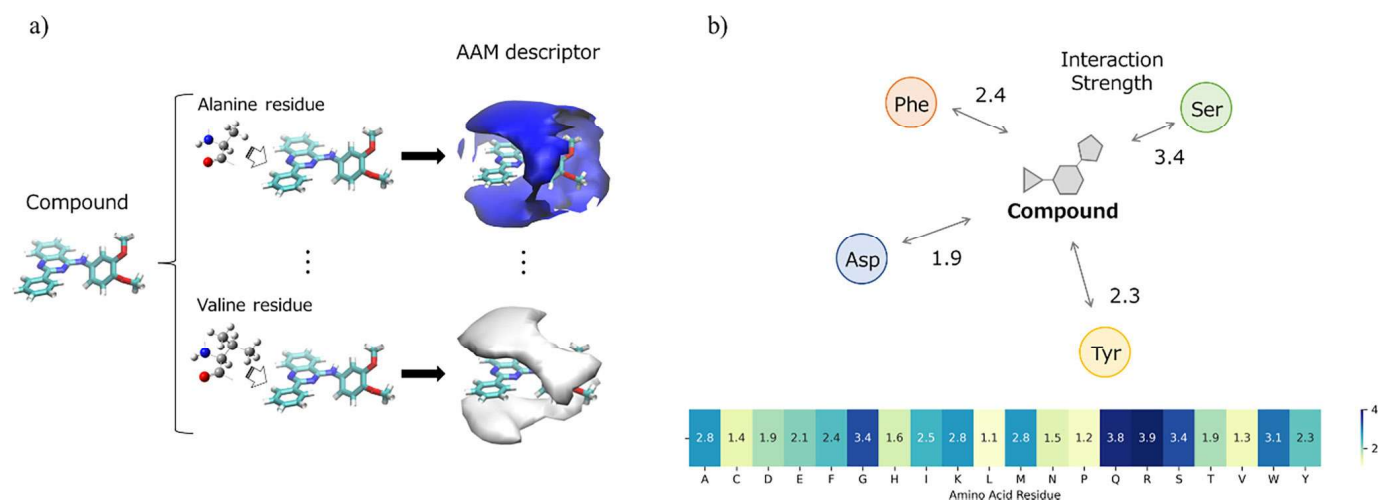


Figure 3. (a) The AAM descriptor maps the spatial interaction profile of each amino-acid residue type around a reference compound. Together, these amino-acid-specific 3D maps form a four-dimensional interaction descriptor. (b) A simplified heatmap view shows the relative interaction profile by amino-acid type

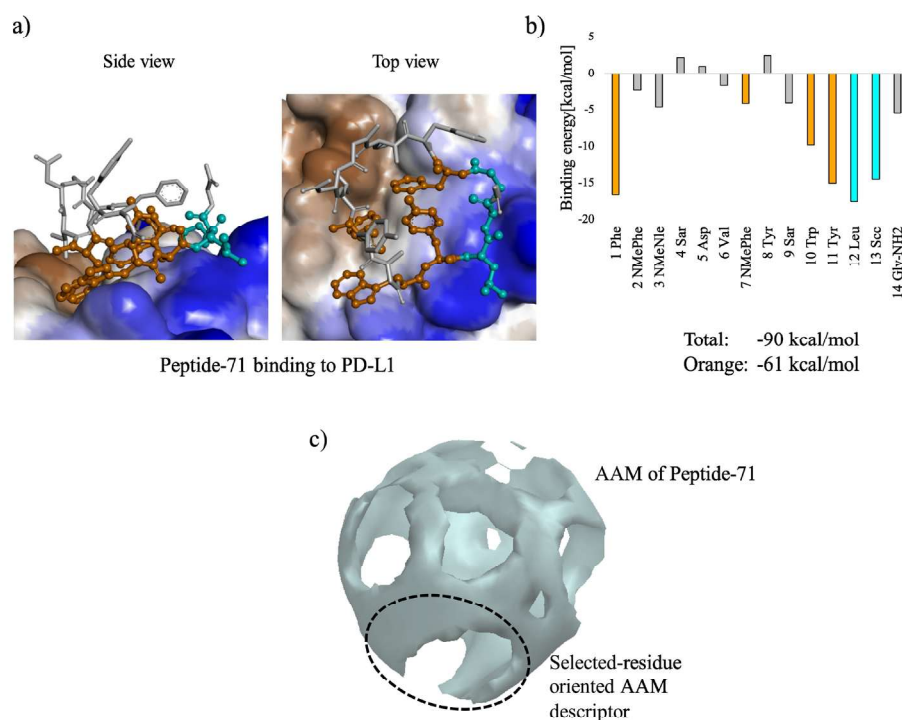


Figure 4. (a) Peptide-71 in the PD-L1 binding site. (b) FMO-derived residue interaction-energy contributions. (c) Selected-residue AAM descriptor derived from the localised peptide region chosen for small-molecule translation

68 per cent of the total interaction energy score. These four residues form a consolidated hydrophobic binding motif within a localised pocket on PD-L1. Other high-contribution residues, such as 12Leu and 13Sec, contacted exposed hydrophilic surface regions and were excluded from feature mapping to enable maximum molecular weight compression (Figure 4).

A selected-residue oriented AAM descriptor was generated specifically for the region encompassing 1Phe, 7NMePhe, 10Trp and 11Tyr. The atom-based generative engine then sampled potential small-molecule space, producing 78,765 unique molecules. The resulting AAM similarity scores had a median value of 0.54, with 95th and 99th percentile thresholds of 0.64 and 0.67, respectively.

FF-ATC-001 had the highest AAM similarity in the generation run, with a score of 0.73 and a molecular weight of 481.51 Da. It inhibited PD-L1 with a reported K_i of 39 μM , compared with 0.004 μM for Peptide-71. The approximately four-log reduction in potency illustrates the difficulty of compressing the peptide's interaction pattern into a 482 Da scaffold. FF-ATC-001 nevertheless retained measurable activity, achieved a BEI of 9.2 versus 4.7 for Peptide-71, and provided a structurally differentiated starting point for optimisation (Table 1).

Complex modelling of FF-ATC-001 aligned within the PD-L1 pocket yielded a calculated FMO interaction-energy score of -50 kcal/mol, closely matching the -61 kcal/mol baseline of the four targeted peptide residues. The compound also avoided the biphenyl-centred chemotype common among reported PD-L1 inhibitors, providing a distinct starting point for further optimisation. When benchmarked against the known small-molecule PD-L1 inhibitor BMS-202 (MW = 419.52 g/mol, BEI = 17.8), FF-ATC-001 (MW = 481.51 g/mol) represents a structurally distinct, non-biphenyl starting point derived directly from peptide feature translation. Crucially, in assay models measuring passive diffusion, FF-ATC-001

demonstrated an approximate 100-fold increase in cell membrane permeability compared to Peptide-71.

Where protein structural information is available, AI-AAM-generated structures can provide starting points for subsequent structure-based optimisation. Because FF-ATC-001 was generated to reproduce the three-dimensional feature fields of Peptide-71, a complex model of FF-ATC-001 bound to PD-L1 was constructed by aligning the small molecule directly onto the spatial coordinates of the template peptide in PDB 5O45 without requiring docking calculations.

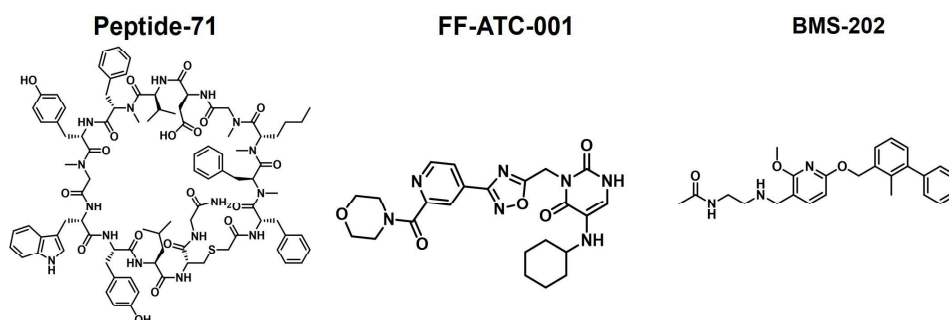
A model of FF-ATC-001 in the PD-L1 binding site identified Gln66 as an opportunity for an additional interaction. The researchers designed three analogues, FF-ATC-2a, -2b, and -2c, to engage this region. Their reported K_i values were 20, 11 and 5.7 μM , respectively (Figure 5).

FF-ATC-2c improved potency by more than six-fold relative to FF-ATC-001. FMO-derived interaction-energy scores for the analogues were approximately -60 to -61 kcal/mol, compared with -50 kcal/mol for FF-ATC-001. In this series, the experimental result was directionally consistent with the structure-based prioritisation hypothesis.

Drug2drugs AI-AAM®: Strategic Discovery Applications

For biopharma drug design teams and medicinal chemists, Fujifilm's drug2drugs AI-AAM® service supports programmes in which a known active compound provides a useful interaction reference but its scaffold limits further exploration. Typical uses include alternative scaffold generation from an active lead, backup-series exploration, peptide-to-small-molecule design and expansion of a narrow medicinal-chemistry series.

The service begins with an active reference structure and project-defined objectives. It generates or screens structurally



Molecular Format	Macrocyclic Peptide	Small Molecule	Small Molecule
K _i ([μM])	0.004	39	0.034
BEI ([pK _i /Mw×1000])	4.7	9.2	17.8
MDCK-II Permeability ([10 ⁻⁶ cm/s])	0.08±0.02	11.1±0.8	Not measured

Table 1. Scaffold compression reduced molecular weight and increased the reported binding-efficiency index and membrane permeability of FF-ATC-001 relative to Peptide-71 and BMS-202

differentiated molecules, ranks them using AAM similarity and complementary computational criteria, and delivers a focused set for customer-led synthesis and testing. Where a protein structure is available, it can support optional structure-based analysis, as demonstrated in the PD-L1 study.

Scope of the Method

Peptide-to-small-molecule exploration is most tractable when key interaction features are spatially localised and can be represented within a compact scaffold. Systems with widely dispersed epitopes, highly polar or solvent-exposed contacts, extensive backbone hydrogen-bonding networks or flexible binding conformations may be more difficult to translate.

A project begins with an active reference structure and defined objectives. Drug2drugs AI-AAM® calculates the AAM profile, generates or screens alternative structures, applies ranking and diversity criteria, and delivers a prioritised set of approximately 100 molecules for customer-led synthesis, docking or biological testing.

Conclusion

The example shown in this Application Note demonstrate how Drug2drugs AI-AAM® can support scaffold exploration for a peptide-to-small-molecule challenge. In the case study, selected peptide interactions were translated into a smaller active scaffold, then improved through structure-based optimisation. We are continuing to develop our technology for the conversion of peptides into small molecules using Drug2drugs AI-AAM® and are identifying metrics to guide this process. These findings will be reported in due course.

Drug2drugs AI-AAM® gives discovery teams a route to examine alternative chemical frameworks from a known active compound before committing to a conventional analogue campaign. The output is a prioritised set of structurally differentiated molecules for synthesis, testing and further structure-activity relationship (SAR) study.

Discuss a known active small molecule, peptide, macrocycle, natural product or constrained lead series with Fujifilm.

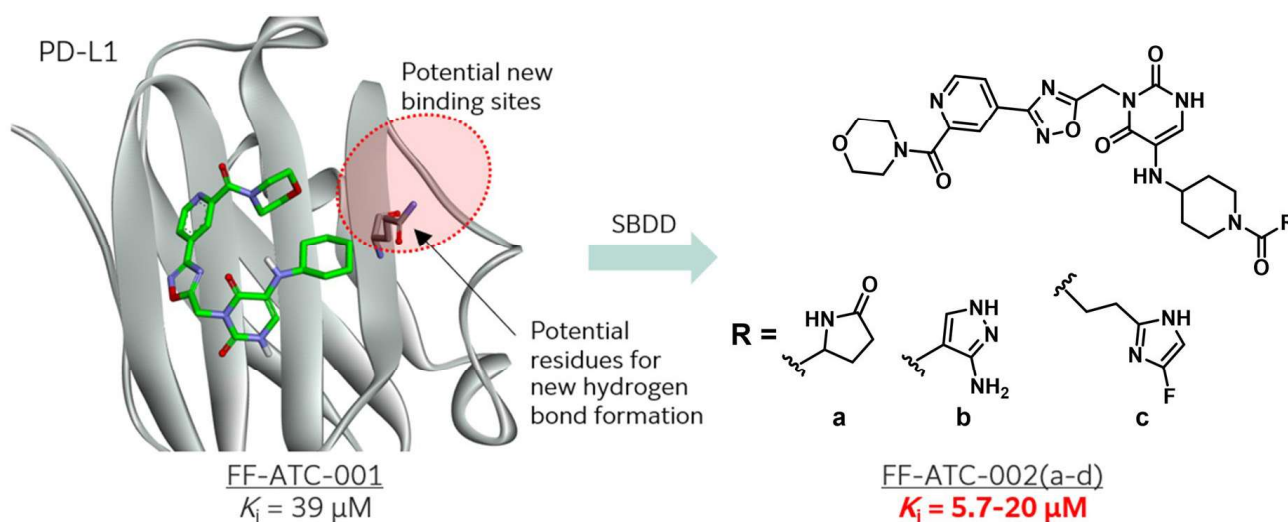
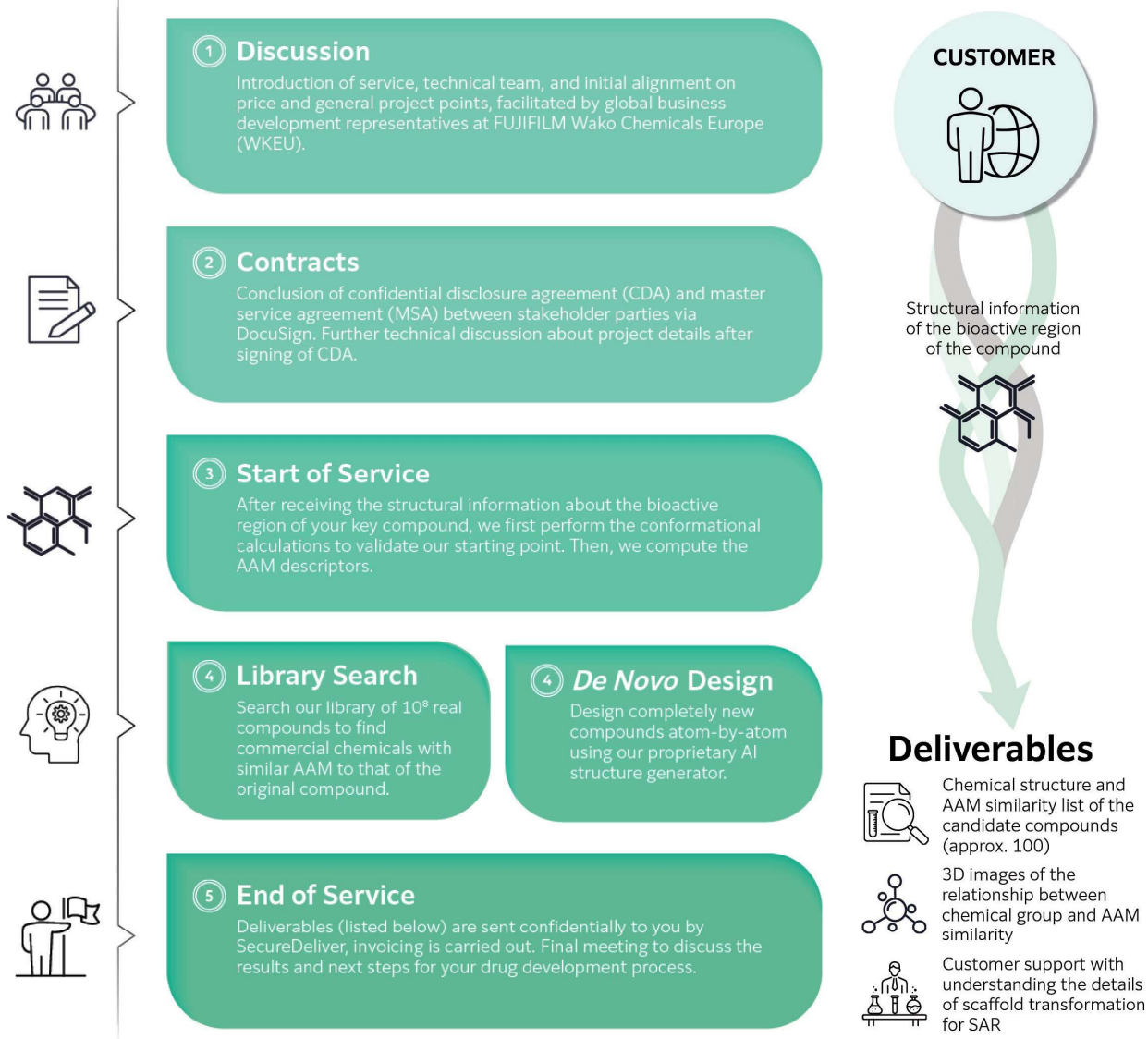


Figure 5. The model of FF-ATC-001 identified Gln66 as an opportunity for an additional PD-L1 interaction



Service Flow & Deliverables



Drug2drugs AI-AAM® can assess whether its AAM interaction profile is suitable for scaffold exploration and define a new scaffold-generation strategy around the objectives and constraints of the programme.

transformation of PD-L1 inhibitory peptides into small molecules using amino acid mapping descriptor and activity improvement through structure-based drug design. *Bioorganic and Medicinal Chemistry Letters*, 137, 130655.

Consider drug2drugs AI-AAM® if your programme needs to:

- Explore structurally differentiated backup series
- Translate selected peptide or macrocycle interactions into smaller scaffolds
- Expand beyond a narrow analogue series
- Investigate alternative chemical matter around an active reference compound
- Generate a prioritised set of molecules for synthesis and biological testing

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