Strategies to Improve Quality and Agility when Producing Monoclonal Antibody Biotherapeutics

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Introduction

The market for biotherapeutics remains buoyant with four monoclonal antibody-based (mAb) therapies among the top ten best selling drugs globally in 2022 (1). Humira (adalimumab) from AbbVie, a mAb used to treat a range of autoimmune diseases and Keytruda (pembrolizumab), a breast cancer therapy from Merck, were the biggest hitters at the number two and three slots (1). There is also a great appetite for developing more, too. According to ClinicalTrials.gov in October 2023, there were over 3400 therapeutics mAbs in development for a range of oncology indications alone (2).

However, developing mAbs is a time consuming business where failure rates are high. Attrition rates for mAbs have historically been poor, with 26% failing in Phase I development and 52% in Phase II, suggesting that most of the early-stage mAbs in the pipeline today remain at high risk of failure.

The quality of lead mAb candidates can have an impact on their developability, efficacy, manufacturability, formulation, and cost. If the quality of mAb candidates is poor, this can lead to the need for redevelopment which can slow down delivery of mAbs, which can also set back the development of novel combination therapies. The knock-on-effects of these delays could mean that some regions have limited or no access to life saving therapies, as well as being less responsive when needed to rapidly supply effective treatments for pandemic and other disease emergencies.

Ensuring that lead candidates exhibit drug-like characteristics across both efficacy and developability can ensure that lead candidates can be rapidly ready for use in First-in-human (FIH) trials. In this article, we describe how Just – Evotec Biologics' antibody selection and optimization programs, combined with its novel intensified continuous cell culture approach, can help overcome these challenges to ensure delivery of fit-for-purpose standard mAb candidates in around 12 months.

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Improving The Quality Of Lead Candidates
Ensuring that lead mAb candidates are high quality requires initial screening of suitable candidates for optimal properties. Two such critical properties are mAbs which have high affinity (strong binding) and specificity (selectivity for the intended target antigen). Such screening can be time-consuming, resource-intensive, and often requires a high level of manual and expensive skilled labor.

To overcome these bottle-necks, Just – Evotec Biologics uses its J.HAL® and J.MD™ in silico artificial intelligence (AI) and machine learning (ML) platforms to discover and optimize humanized mAb sequences that both represent natural repertoires and are fit for purpose before library construction and screening. The J.HAL® discovery methodology uses an Antibody-GAN (Generative Adversarial Network) (3) trained on a large, human-derived antibody sequence set (4) and to learn and produce features of a mature human antibody repertoire, including sequence characteristics and structure properties, while biasing the sequences toward developability, allowing for encoding a large range of combinatorial germline pairings to create diverse libraries (see Figure 1). This novel synthetic approach helps discover the best therapeutic candidate for both efficacy and developability. These GAN-generated sequences have been produced as Fab-on-phage libraries for therapeutic discovery and ML-intended data generation. Transfer learning methods as shown in Figure 1 may also be applied to shift Fv sequences toward desired properties.

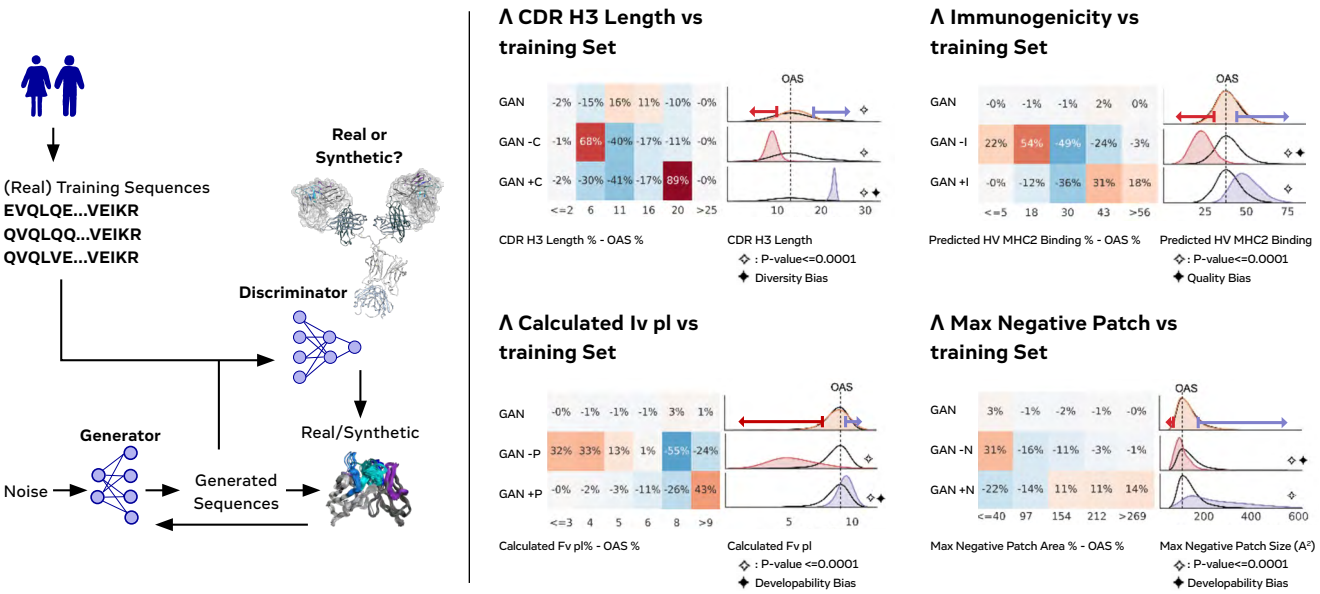

Figure 1: *Overview of Just – Evotec Biologics J.HAL® platform*

Case Study 1: Designing mAbs for Efficacy.
To demonstrate that using the J.HAL® platform can discover active mAbs, we performed dose dependent activity screens for agonist activity, a key indicator of a mAbs binding ability and efficacy. We used mAbs from a library designed using the J.HAL® platform (designated seq 201 – seq 215) and compared them with a positive control mAb (CP-870, a fully human, CD40-specific agonist) and an isotype control mAb. Antibody binding was determined using cell-associated CD40 and flow cytometry whereas activity was determined with a cell-based reporter assay. The results (Figure 2) show that mAb 207 exhibits a 4.8ug/mL half maximal effective concentration (EC50) whereas the positive control, CP-870 has a 19ng/mL EC50. This data indicates that using our J.HAL® platform, we can discover mAbs which potentially have desirable binding and functional properties and have the potential for further improvement with affinity maturation.

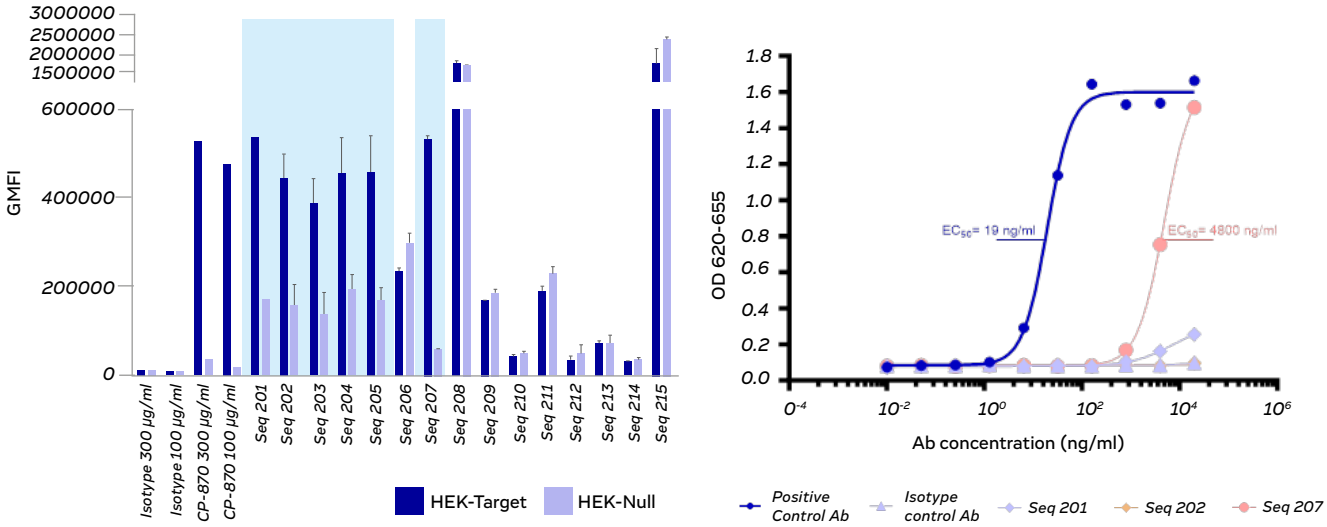

Figure 2: *In vitro assay data demonstrating binding and functional activity of mAbs designed using the J.HAL® platform, compared to a positive control mAb (CP-870) and an isotype control mAb.*

Case Study 2: Designing mAbs for Manufacturability. To demonstrate that using the J.MD™ platform can help design mAbs for manufacturability, we used this *in silico* platform to predict a range of properties from sequences and calculated structures, such as CDR lengths, sequence diversity, potential post-translational modifications, potential machine-learned stability violations, surface properties, machine-learned immunogenicity, isoelectric point, and germline background, to rank order 283 mAbs from convalescing human patients. The four (designated mAbs 1–4) top-ranked mAbs were deemed suitable for development and were chosen for *in vitro* stability screening. Our results (Figure 3) demonstrate that mAbs 1 and 2 had optimum stability profiles and were both productive mAbs with consistently good titers of 4g/L/day (results not shown). While mAb 3 had a SINS issue (self-interaction), and this was of minor concern, we opted to drop this candidate. mAb 4 showed thermal stability issues and a hydrophobic column interaction, eliminating it from consideration. Again, our data indicates that using our J.MD™ platform we can select for mAbs which are manufacturable and can be easily formulated, thus improving quality and accelerating development timelines.

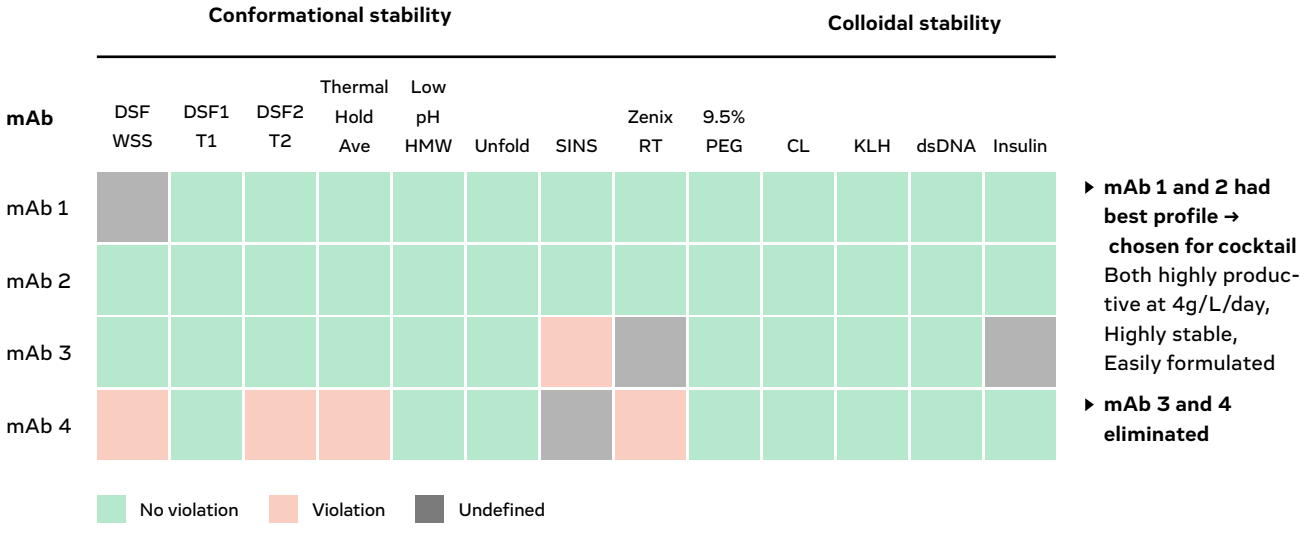

Figure 3: *In vitro assay data ranked for stability of mAbs designed using the J.MD platform.*

Case Study 3: Designing mAbs for Manufacturability and Formulation.

High levels of protein aggregation at low pH can put a hold on the development of a therapeutic mAb as it can cause manufacturing and formulation issues. To demonstrate that using the J.MD™ platform can help design mAbs for improved manufacturability and formulation, we used this in silico platform to redesign the sequences of two mAbs (designated mAbs 1 and 2) to reduce their potential for protein aggregation (5). Our results (Figure 4) show that with both mAbs 1 and 2 their ability to aggregate at low pH was significantly reduced, demonstrating that using our J.MD™ platform we can design mAbs which have a higher probability of success in manufacturing and formulation, providing better quality and agility with mAb development.

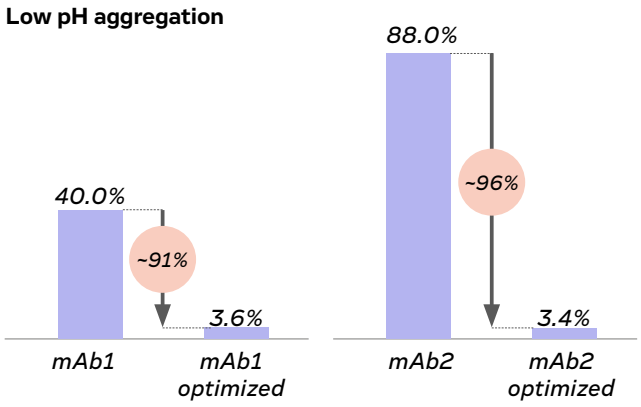

Figure 4: *Low pH aggregation potential of two mAbs designed with and without sequence optimization using the J.MD™ platform.*

Case Study 4: Screening and Optimizing mAb Titer for Increased Productivity.

Optimization of therapeutic mAbs can greatly improve production titers (6) which could reduce cost per dose. This is because having low titer at the development stages of mAb production can mean that when the process is scaled-up, manufacturing costs can become prohibitively high. To demonstrate that using the J.MD™ platform can help design mAbs for higher titer, we used this in silico platform to redesign the sequences of a low titer mAb to produce a panel of 16 mAb variants (designated mAbs MS-1783-MS-1799).

Screening mAbs for titer traditionally requires the use of time consuming cell culture, which if performed manually using shake flasks or benchtop bioreactors can take many weeks and be costly in terms of media and labor use. At Just – Evotec Biologics, we have automated and miniaturized our cell culture screening and have used 24-deep well plates with high throughput screening technology as a rapid, lower cost method of determining the titers of these mAb variants. Our results (Figure 6) demonstrate that all the mAb variants produced a 1.5 to nearly 2.5-fold increase in titer compared to the parent mAb, indicating that by using our J.MD™ platform we can design mAbs which produce a higher titer in cell culture. This higher productivity increases the probability of successful development and manufacturing, as well as better quality and faster delivery of mAbs.

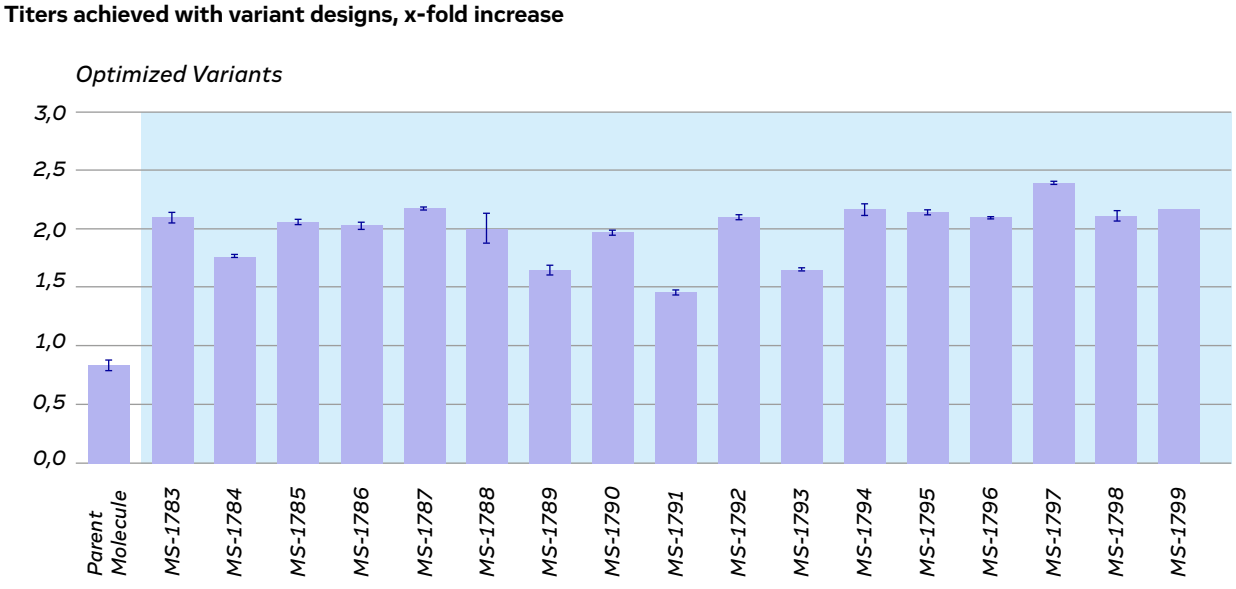

Figure 5: *Titers achieved from a panel of sequence optimized variant mAbs compared to the parental mAb molecule.*

Case Study 5: Reducing Modifications to the Protein Structure of mAbs.

Generating mAbs in cell culture can be problematic if the mAb is in culture for a long period. For example, a mAb can experience degradation caused by peptides expressed and secreted in the cell culture medium (7). This can cause modifications to the mAb' s protein structure which could potentially change glycosylation or charge for example and as a result affect a mAbs efficacy, manufacturability and safety profile.

At Just – Evotec Biologics we use an intensified perfusion cell culture process to produce mAbs (see Figure 7) which helps streamline processes, reduce human errors, and improves culture consistency. Cells also maintain high viability as fresh media is frequently being added, and waste products are being removed all the time. Additionally, the mAb product is being harvested for purification from the bioreactor as it is expressed, which means it is in contact for less time with cells, cell culture components and impurities that could cause product degradation.

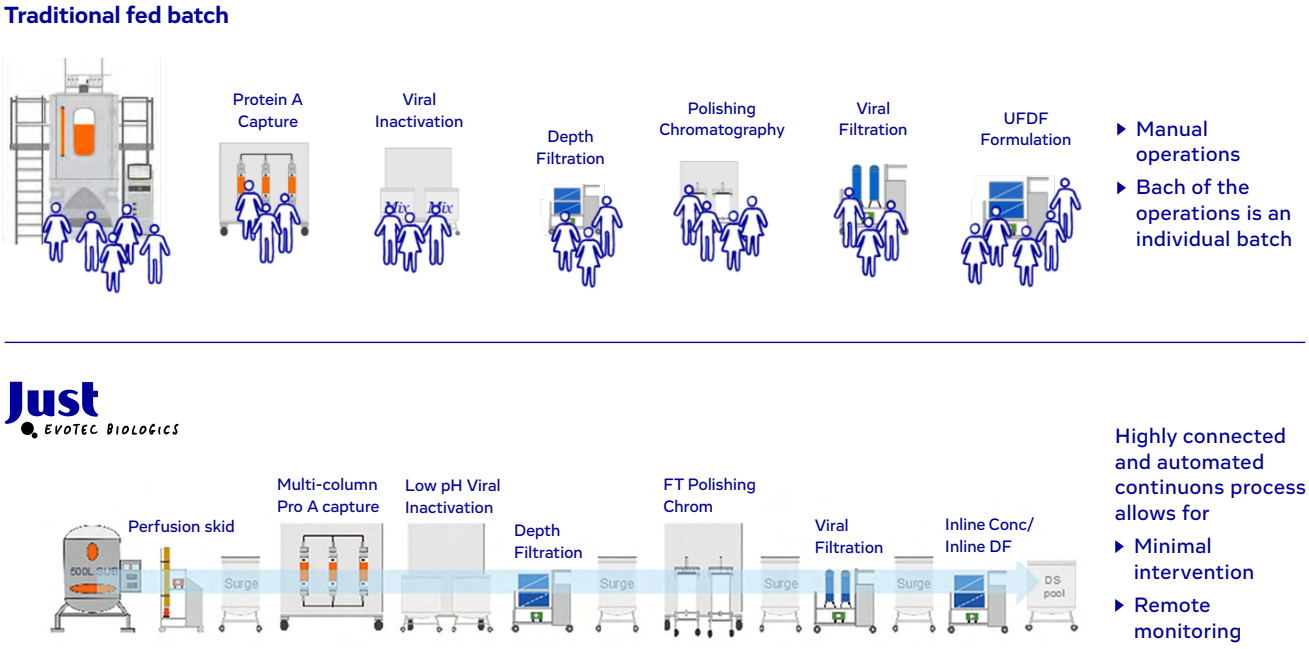

Figure 6: *Traditional fed-batch process (top) compared to Just – Evotec Biologics continuous process for mAb production (bottom).*

To demonstrate that using an intensified perfusion cell culture can reduce post-translational modifications, we compared the post-translation modification profile of a mAb drug substance we produced in our continuous intensified process with the same mAb produced in a fed-batch process (8) using a multi-attribute method (MAM) based on mass spectrometric peptide mapping. Our results (Figure 8) showed improved quality profiles with lower levels of degradation including, oxidation, deamidation and glycation compared to the mAb produced in fed-batch cell culture, indicating that product quality is significantly improved using this approach.

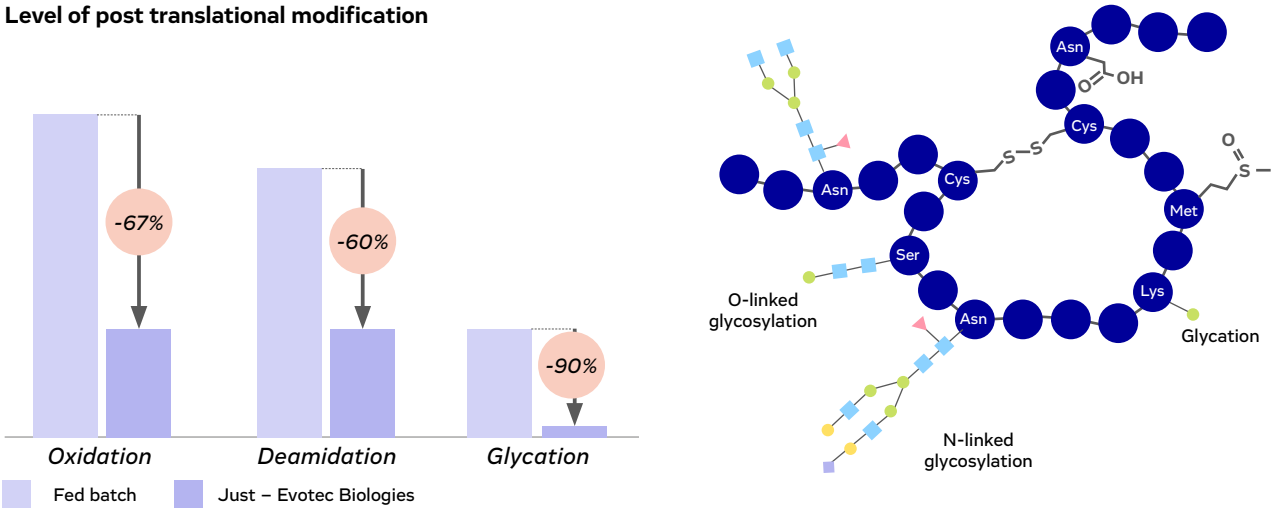

Figure 7: *Post-translational modifications of a mAb produced using Just-Evotec Biologics continuous process compared a mAb generated in fed-batch culture.*

Conclusions

With high attrition rates of mAbs in early phase clinical trials, it is becoming increasingly challenging for biopharmaceutical companies to rapidly deliver high quality therapeutic mAbs using conventional antibody screening and fed-batch bioprocessing methods. This is why new Quality by Design (QbD) approaches such as using in silico AI and ML platforms to discover and optimize mAb sequences, high-throughput screening, and continuous intensified manufacturing processes such as those used at Just – Evotec Biologics are critical for enabling a paradigm shift in reducing attrition rates. As detailed in this article, optimizing mAb design, using automated, miniaturized screening, and minimizing time in culture can deliver high-quality mAbs for FIH trials in rapid response times of around 12 months. In the future using this approach could expand access to life changing treatments, as well as support a rapid response to global health emergencies.

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