

# Structure-Based Drug Repurposing for MMP-13 Inhibition via the S1' Specificity Loop

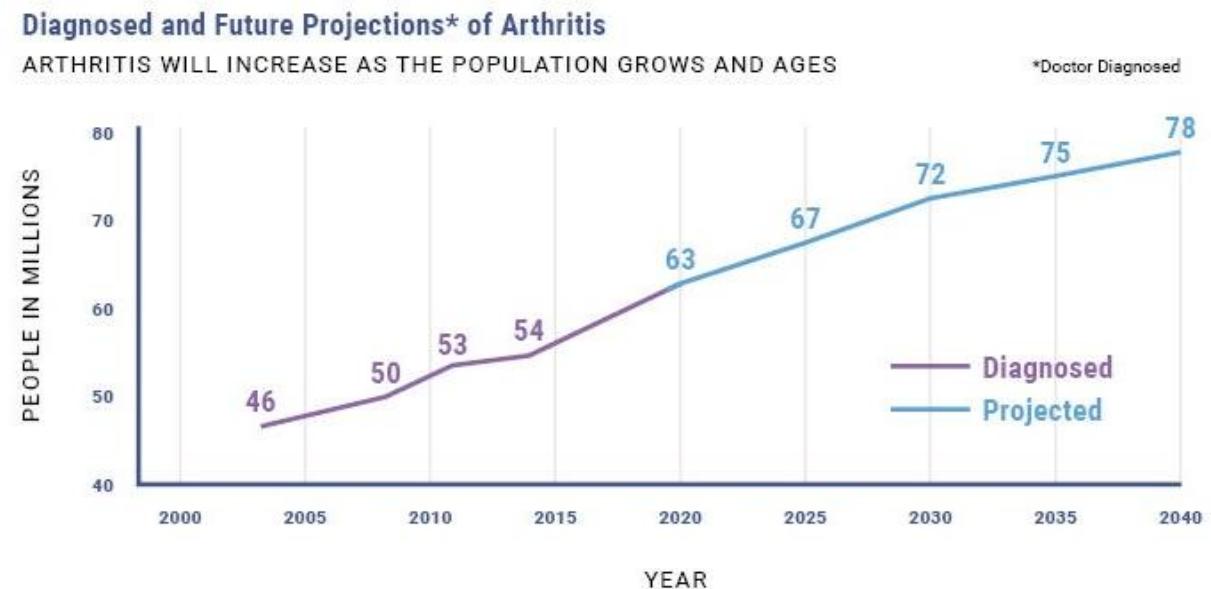
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RBIF – 110

# Motivation – The Gap in Osteoarthritis Treatment

- Osteoarthritis (OA) is a degenerative joint disease, characterized by the progressive breakdown of joint tissues, leading to pain, stiffness and instability (Branch, 2017).
- Chronic and episodic pain impacts mental health, mobility, and quality of life, contributing to depression, disability, and work limitations (OA Prevalence and Burden, n.d.).
- OA is the most common form of arthritis, affecting 32.5 million US adults, with cases projected to rise significantly (OA Prevalence and Burden, n.d.).
- Current treatments, such as NSAIDS and corticosteroids only manage symptoms and do not stop disease progression (Hu & Ecker, 2021)

**There are currently no disease-modifying drugs (DMDs) specifically approved for OA, highlighting the urgent need for new therapeutic strategies.**



Data from: National Health Interview Survey 2013–2015

Adapted from *OA Prevalence and Burden*, n.d.

# Target Information – Matrix Metalloproteinase-13 (MMP-13)

- MMP-13 is a collagenase that cleaves type II collagen, leading to cartilage destruction and OA progression (Hu & Ecker, 2021)
- Like other metalloproteinases, MMP-13 is zinc-dependent and requires a catalytic zinc ion (colored orange) for its enzymatic activity (Schnute et al., 2010)
- Structural calcium ions (colored blue) help maintain enzyme integrity and stability (Schnute et al., 2010)
- So, overexpression of MMP-13 in OA joints is strongly linked to disease progression by accelerating collagen breakdown
- Excessive MMP-13 activity contributes to joint inflammation by inducing pro-inflammatory cytokines (Hu & Ecker, 2021)

**Inhibiting MMP-13 could slow cartilage degradation and reduce inflammation, potentially offering long-term benefits beyond symptom relief.**

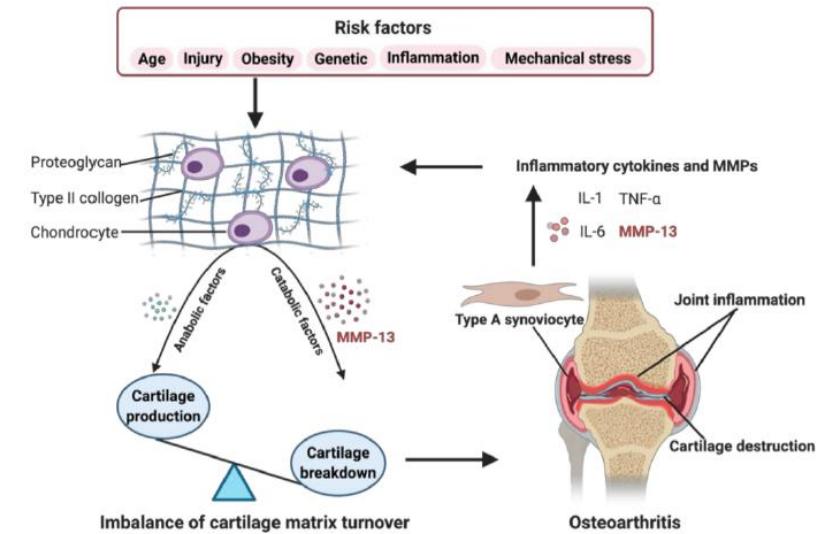
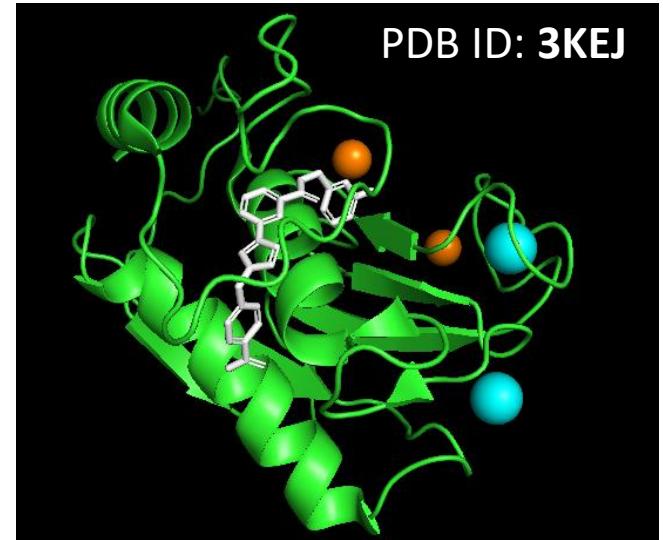
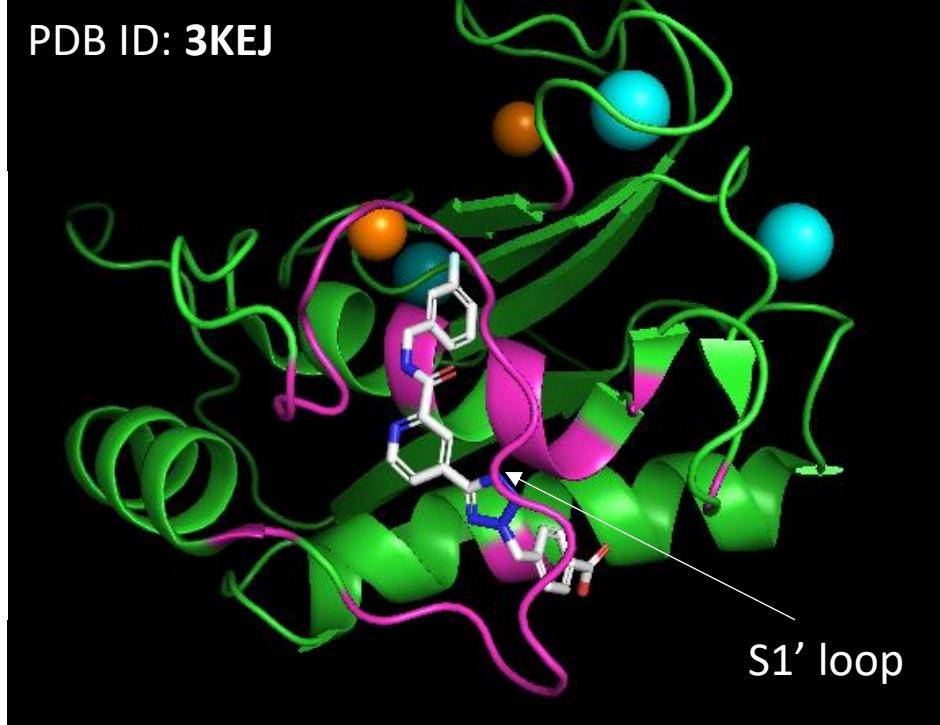
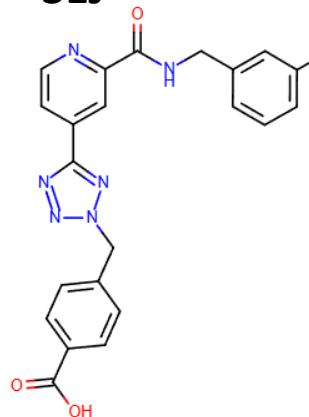


Figure 1 adapted from Hu & Ecker, 2021

# Target Information – Protein Structure & Reference Ligand

- Reference ligand will be PDB ID: **3EJ**
  - 4-[(5-{2-[(3-fluorobenzyl)carbamoyl]pyridin-4-yl}-2H-tetrazol-2-yl)methyl]benzoic acid
- Key molecular properties of 3EJ
  - Molecular Weight: ~432 Da
  - LogP: ~2.55
  - TPSA: ~123
- Targeted binding site will be S1' specificity loop (highlighted magenta)

2D Structure  
of PDB ID:  
**3EJ**



If PDB ID: 3EJ truly stops disease progression,  
then that means compounds similar to it should  
also stop disease progression with similar binding

Crystal Structure of Human MMP-13 complexed with  
a (pyridin-4-yl)-2H-tetrazole compound (colored  
white)

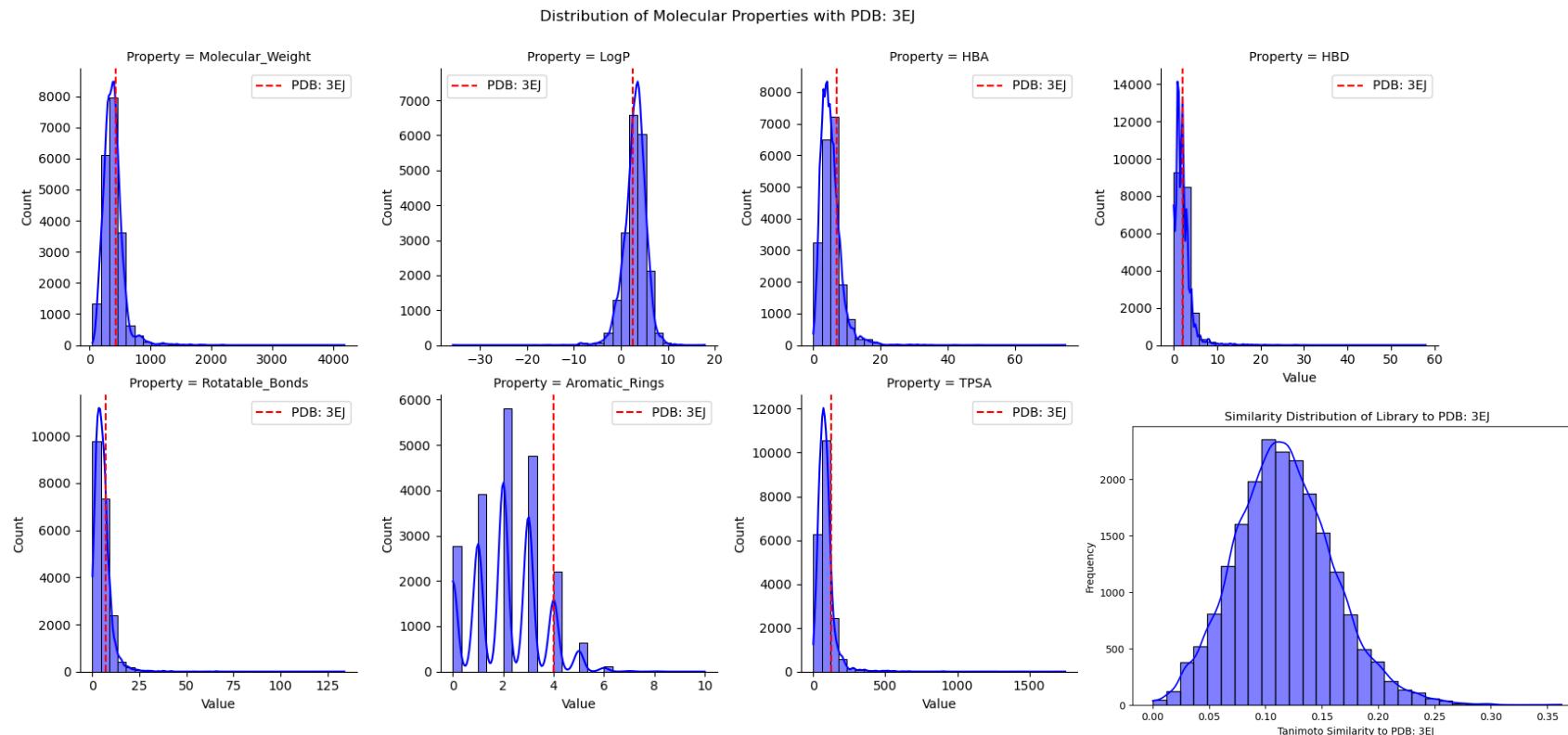
# Target Information – “Known” Inhibitors

Due to the limited number of known MMP-13 inhibitors that specifically target the S1' loop, the top S1' loop inhibitor candidates from the study conducted by Kalva et al., 2013, were selected as positive controls

Compound ID	Experimental Glide Score (XP)	2D Structure
ZINC 02535232	-9.58	
ZINC 08399795	-10.33	
ZINC 12419118	-10.43	
ZINC 00624580	-9.34	

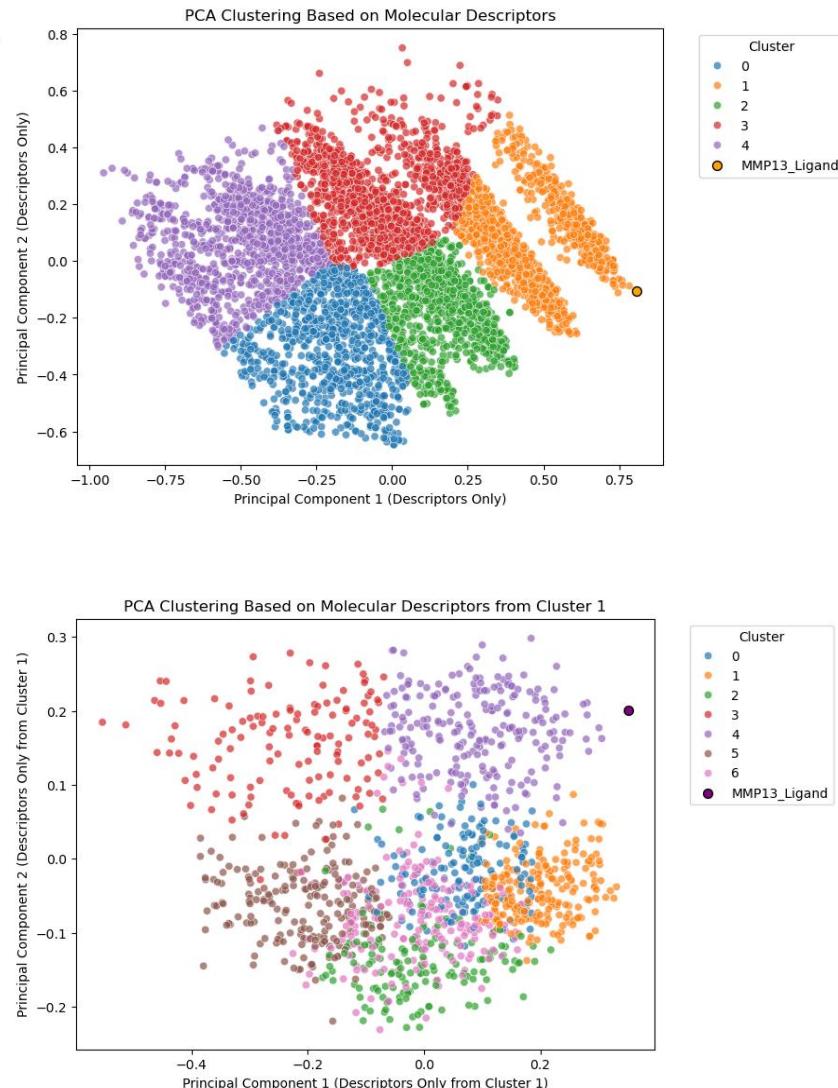
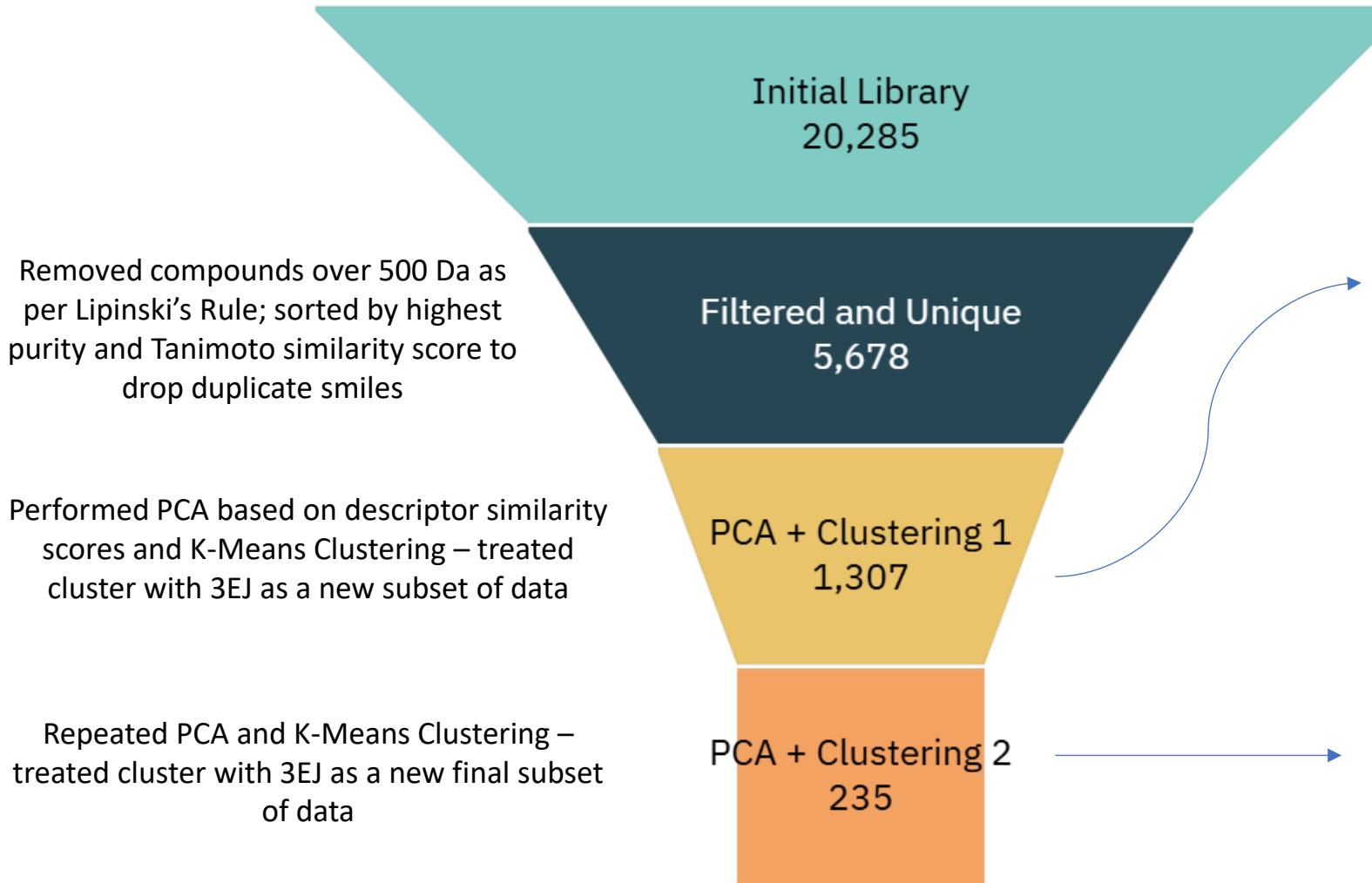
# Compound Selection – Initial Compound Library

- The initial library contained 20,285 compounds sourced from the **Broad Institute Drug Repurposing Hub**, comprising FDA-approved drugs, clinical trial candidates, and pre-clinical tool compounds

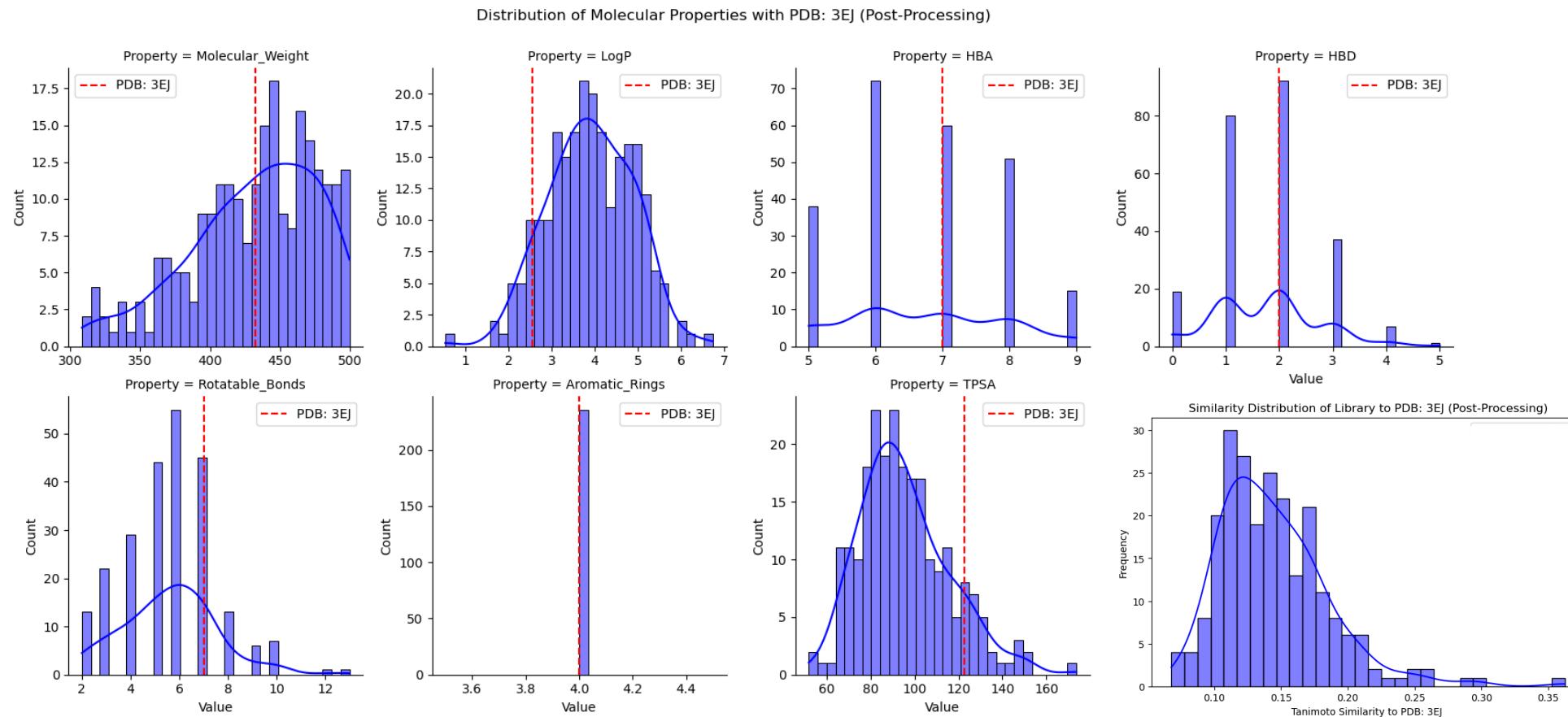


- The selected library exhibits a diverse range of molecular weights and properties, with a subset clustering around known drug-like space.
- Similarity to PDB: 3EJ varies, ensuring a balance of structural novelty and relevance
- Average Tanimoto Similarity is around 10% with no compounds exceeding 35%

# Compound Selection – Filtering Process

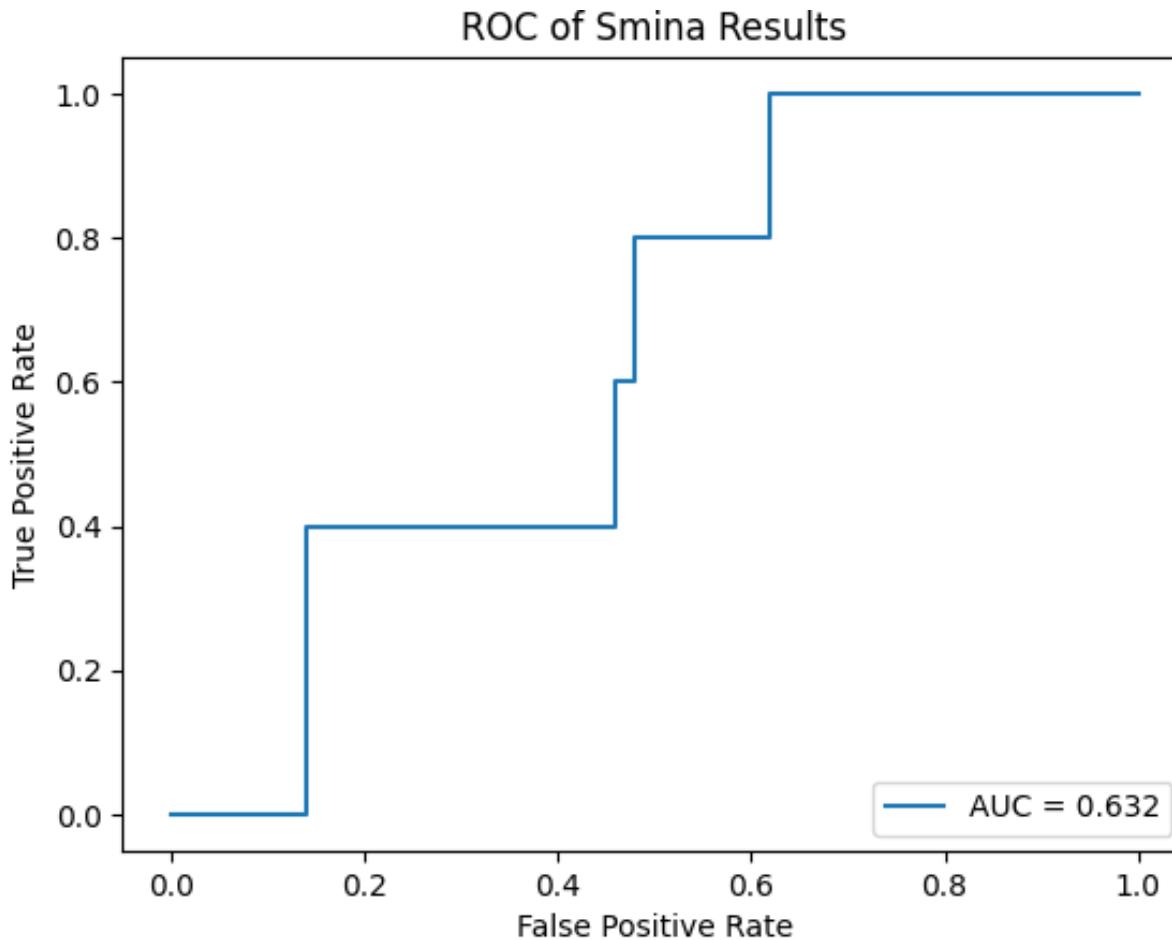


# Compound Selection – Final Selection of 235 Compounds



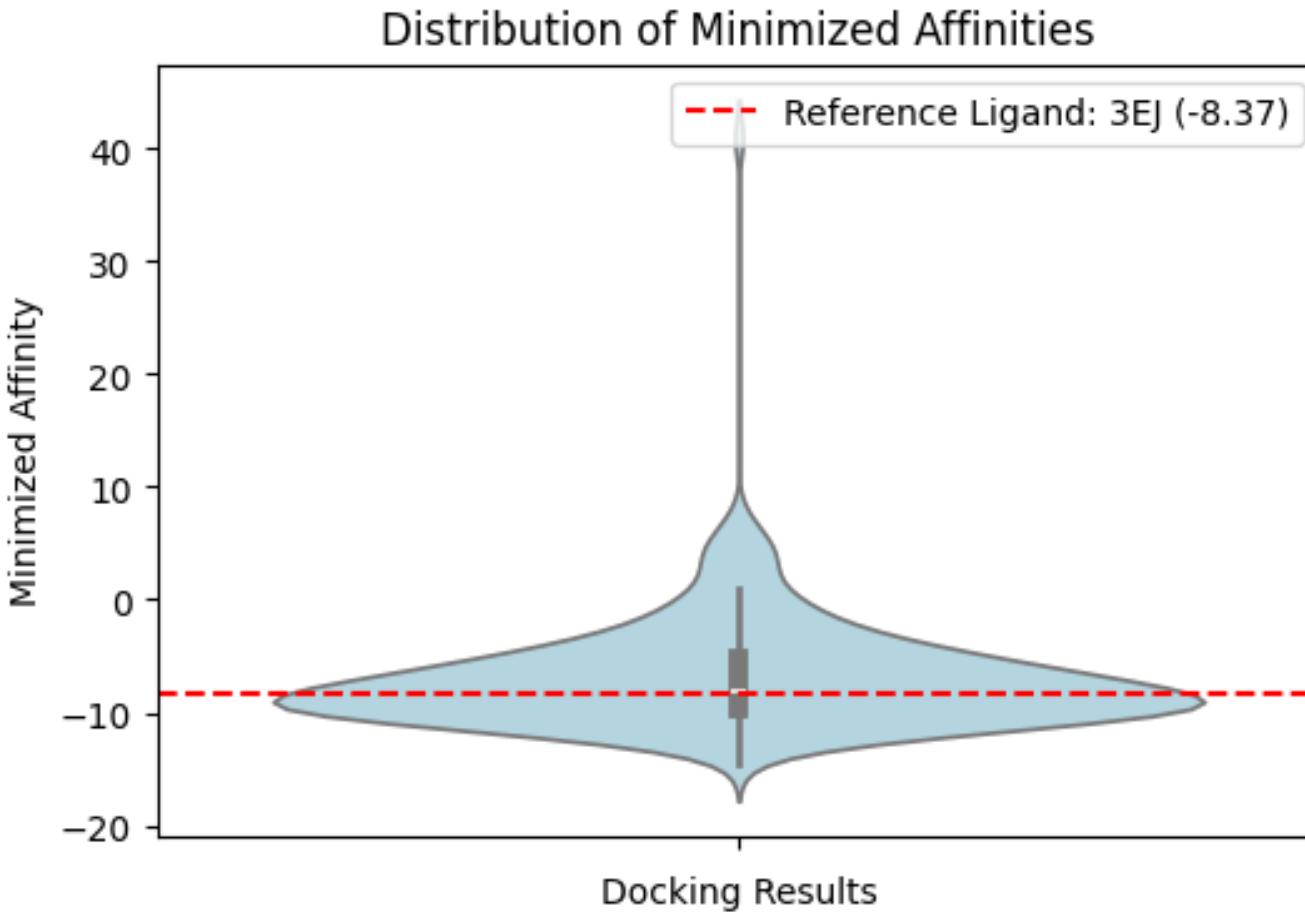
Final compound selection was based on key molecular properties relative to the reference ligand (PDB: 3EJ) from PCA and K-Means Clustering. The following distributions compare our library to the reference, ensuring structural relevance. The mean similarity of the final selection is around the same as the initial library ~10%.

# Smina Docking – Method Validation



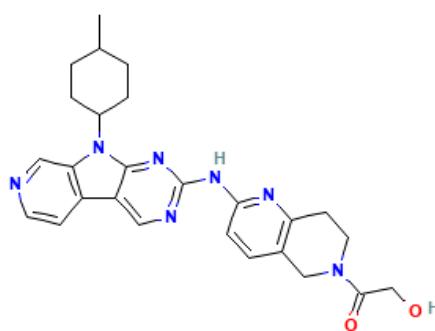
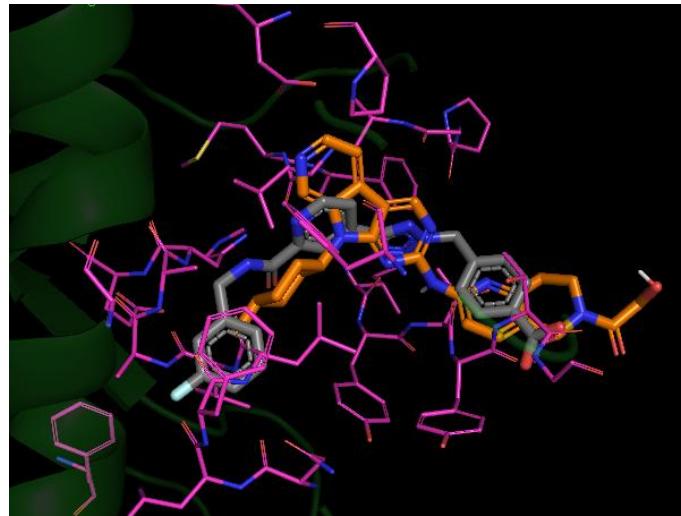
- Smina method validation library consisted of 55 compounds
  - **5 positive controls** (4 from Kalva et al., 2013; 1 native ligand [3EJ])
  - **50 negative controls** sourced from ChEMBL database (explore all activities)
    - Subset of compounds with known bioactivity for MMP-13 (filtered for pChEMBL score < 5, and took a random sample of 50)
- The resulting **AUC (Area Under the Curve) = 0.632**, which indicates that the docking model has moderate discriminatory ability between active and inactive compounds.
  - This lower than ideal value could be due to the nature of the positive controls used. Still, this value being above 0.5 means that it is better than random guessing.

# Smina Docking – Summary of Results



- Most candidate compounds exhibited comparable or better docking scores than the reference ligand, suggesting strong binding potential to MMP-13.
- Candidate compounds were prioritized mainly based on minimizedAffinity score.
- Compound consideration criteria included:
  - Equal or better (more negative) minimizedAffinity score compared to the reference ligand
  - Non-lethal / has dire side-effects
  - Docking image showed that it was reasonably situated within the pocket

# Smina Docking – Top 4 Candidates

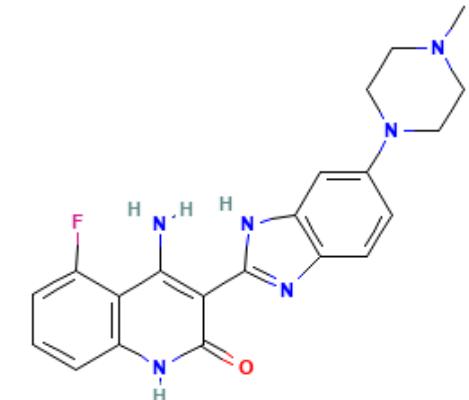
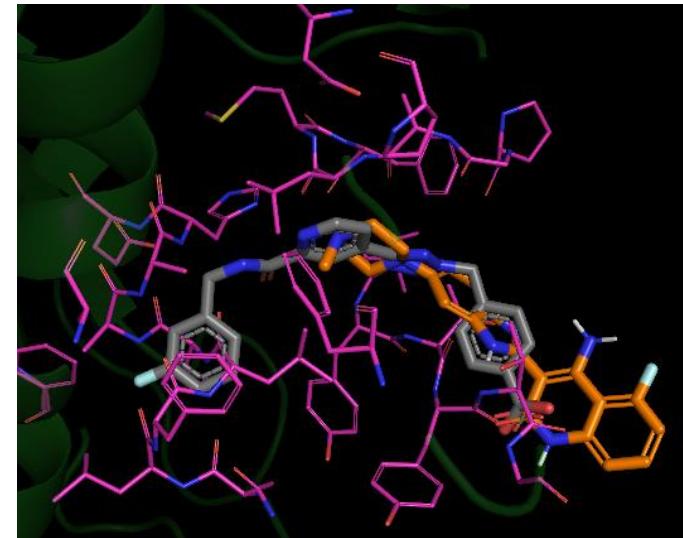


**AMG-925 | BRD-K97452254-001-01-3**

Mainly a dual inhibitor targeting FLT3 and CDK4, kinases involved in cell proliferation and survival, making it a candidate for treating certain leukemias. Targeting MMP-13 could be challenging, despite low affinity score (PubChem, n.d.).

**MinimizedAffinity:** -12.85738

**Last Known Clinical Phase:** Phase 1



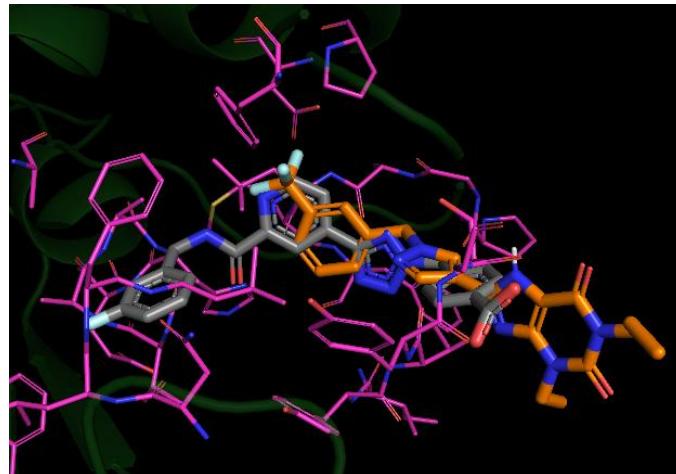
**Dovitinib | BRD-K85402309-406-01-8**

Orally active small molecule that exhibits potent inhibitory activity against multiple RTKs involved in tumor growth and angiogenesis. Some TKIs have been shown to inhibit MMPs indirectly by modulating tumor microenvironment pathways, though inhibiting multiple kinases can lead to serious side effects (PubChem, n.d.).

**MinimizedAffinity:** -11.93030

**Last Known Clinical Phase:** Phase 3

# Smina Docking – Top 4 Candidates

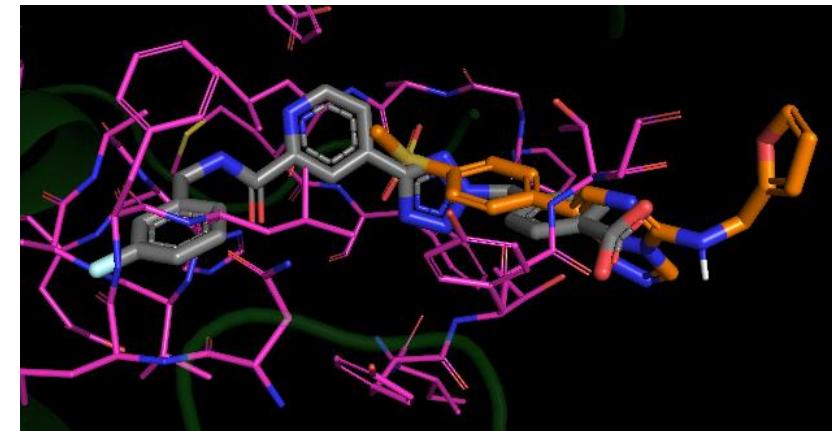
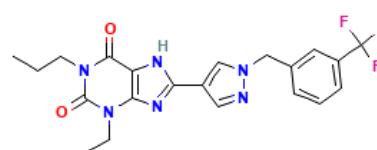


**GS-6201 | BRD-K68453341-001-01-1**

Orally available A2B-adenosine receptor antagonist; studied for the potential treatment of asthma and other conditions related to inflammation and fibrosis. Given that MMP-13 plays a role in inflammation, anti-inflammatory small molecule like GS-6201 could offer dual benefits. GS-6201 was not developed as an MMP inhibitor - binding affinity and inhibition mechanism needs more validation (PubChem, n.d.).

**MinimizedAffinity:** -11.55530

**Last Known Clinical Phase:** Phase 1

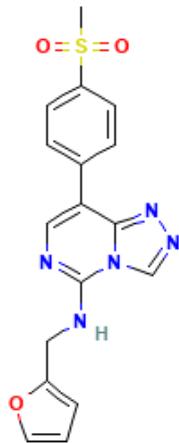


**EED226 | BRD-K19002914-001-01-3**

Allosteric inhibitor of the polycomb repressive complex 2 (PRC2), impacting gene silencing mechanisms. Its ability to bind allosteric sites suggests potential to modulate MMP-13 activity. PRC2 inhibition differs significantly from metalloproteinase inhibition (PubChem, n.d.).

**MinimizedAffinity:** -11.43799

**Last Known Clinical Phase:** Preclinical



# Budgeting and Experimental Considerations

## Total of 4 candidate compounds will be tested

In vitro screening these compounds will tentatively consist of the following tests:

- HPLC to determine purity
- Fluorescence Polarization assay for binding confirmation
- Fluorogenic Substrate Cleavage Assay for IC<sub>50</sub> determination
- Solubility testing to ensure compound dissolution in assay buffer
- Selectivity panel to confirm specificity to MMP-13
- X-ray Crystallography to confirm pose is in active state
- Basic ADMET

## Other cost considerations:

- Laboratory equipment and reagents
- Staffing and facility
- Data analysis / software licenses
- Preclinical animal studies

Compound	Unit Quantity	Price (USD)	Vendor
AMG-925	2 x 10 mg	252.00	MedChemExpress
Dovitinib	1 x 25 mg	168.00	MedChemExpress
GS-6201	1 x 25 mg	334.00	MedChemExpress
EED226	1 x 25 mg	173.00	MedChemExpress
MMP-13 Protein	1 x 10 ug	480.00	R&D Systems
	Total Price	1,407.00	

## In vitro timeline

- Compound procurement and preparation: 2 weeks
- Initial screening (HPLC, binding assays, solubility tests): 4 weeks
- Selectivity and crystallography studies: 6 weeks
- Basic ADMET profiling: 4 weeks

# Future Outlook and Challenges

## Stage 1

### Preclinical Studies (3–6 months)

- **In Vitro Testing** – MMP-13 enzymatic inhibition assays, fluorescence polarization binding assays, and IC<sub>50</sub> determination.
- **Solubility & Stability Testing** – Ensure compounds remain stable in physiological conditions.
- **X-ray Crystallography** – Validate ligand binding poses in the active site.

## Stage 2

### Animal Validation (6–12 months)

- **Pharmacokinetics & Toxicity** – Evaluate absorption, metabolism, clearance, and potential toxicity in rodent models.
- **Efficacy Studies** – Assess reduction in cartilage degradation and inflammatory markers in OA-induced animal models.
- **Joint Function Assessment** – Measure improvements in mobility and pain response in treated animals.

## Stage 3

### Clinical Trials (≥1 year)

- **Phase I** – Safety & tolerability testing in healthy volunteers, focusing on adverse effects and MMP-13 selectivity.
- **Phase II** – Efficacy trials in OA patients, measuring pain reduction, inflammation markers, and cartilage preservation.
- **Phase III** – Large-scale trials to confirm efficacy and compare with existing OA treatments for regulatory approval.

## Hurdles

- **Regulatory Challenges** – Repurposed drugs require new efficacy trials for OA. Early engagement with FDA/EMA can clarify approval pathways.
- **Patient Variability** – OA is heterogeneous. Biomarker stratification may help identify the most responsive patient groups.
- **Compensatory Mechanisms** – Blocking MMP-13 may activate alternative cartilage degradation pathways. Transcriptomics and proteomics studies can help mitigate this risk.

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