

Computational Design and Evaluation of ScFv-IFN- β Fusion Protein against HER1-positive Breast Cancer

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ABSTRACT

OBJECTIVE: To computationally design immunocytokines capable of targeting cancer cell antigens and modulating the immune response, representing a promising therapeutic approach for HER1-positive breast cancer

METHODOLOGY: This study was conducted at the National Center of Excellence, Molecular Biology, University of Punjab, Lahore, between 2021 and 2022. This is an in silico study in which a novel immunocytokine was engineered by linking an anti-EGFR/HER1 single-chain variable fragment to interferon- β . In this design, the ScFv moiety targets HER1-positive tumor cells, while the IFN- β domain retains its natural specificity for IFNAR1/IFNAR2 and is intended to provide localized immune-modulatory signaling at the tumor site. Sequence data for the ScFv and for IFN- β were concatenated to yield the ScFv-IFN- β design. Physicochemical metrics, including molecular weight, extinction coefficient, and instability index, were calculated using ProtParam and SOLpro. We predicted the 3D conformation using AlphaFold, followed by refinement with GalaxyRefine. We assessed structural quality using PROCHECK and RAMPAGE. Potential allergenic sites were mapped using AllPred, and the corresponding mRNA secondary structure was analyzed with RNAfold. Protein-receptor interactions were examined through docking on the HDock server.

RESULTS: The fused protein displayed acceptable physicochemical characteristics, retained a plausible and well-folded conformation, maintained a robust mRNA secondary structure, and demonstrated favorable docking poses for both HER1 and interferon receptors. Stability metrics from the dynamic simulations corroborated these findings.

CONCLUSION: The ScFv-IFN- β fusion protein emerges as a theoretically interesting design for targeted therapy against HER1-positive breast cancer, but the evidence at this stage is purely computational.

KEYWORDS: Breast cancer, Fusion protein, Human Epidermal Growth Factor Receptor 1 (HER1), Interferon Beta (IFN- β), immunocytokine, Single-chain Variable Fragment (ScFv).

INTRODUCTION

Breast cancer ranks as the second-highest cause of cancer-related mortality among women across the globe, right behind lung cancer, as noted by Afzal S et al.¹. Clinicians routinely grapple with the tumors' vast cellular diversity, or heterogeneity. Standard protocols -chemotherapy, radiotherapy, and surgery-tend to work best while the disease is still confined to localized sites; once it spreads, their impact often dwindles. During earlier stages, some patients discover that treatments are ineffective or cause more harm. Care decisions are usually based on the patient's genetic and epigenetic makeup². This reliance highlights a growing demand for therapies that move beyond traditional pathways. Researchers have begun exploring next-generation agents such as

immunotoxins, cytokine-chimeric fusions, and tumour-necrosis-factor-linked proteins, with experimental results appearing in various literature³. One of the promising methods involves immunocytokines, hybrid molecules that combine antibody-targeting precision with cytokine-driven immune boosting⁴. Sampson JF et al.⁵, demonstrated that immunocytokines can adapt to different tumor microenvironments. This adaptive behavior stems from the dual action built into each immunocytokine: one end binds a specific tumour antigen, while the other end sparks local immune activity. Early preclinical data summarized by Mobark N 2025⁶ show that this characteristic keeps leukocytes focused on the malignancy while sparing surrounding healthy tissue. Such tailor-made delivery could help oncologists customize therapy on a case-by-case basis rather than leaning on broad population averages.

Gout DY 2022⁷ points to a growing clinical interest in the promise of immunocytokines to inflict a customized punch on breast cancers. The HER1 receptor, embedded in the plasma membrane, is a tyrosine kinase whose activity is strongly correlated with the growth and development of certain breast tumours. In about 15 to 30 percent of cases,

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especially in triple-negative subtypes, overexpression of HER1 is followed by brisk tumor progression and shrinking therapeutic opportunities. EGF and TGF- β activate HER1, releasing PI3K/AKT and MAPK/ERK cascades that feed cell proliferation and motility.

Antibody constructs and small-molecule tyrosine kinase inhibitors now dot the clinic, demonstrating substantial benefit to HER1-positive patients. Yet, clinical experience reveals an unsettling truth: tumors often retool their signaling architecture and sidestep these targeted approaches.

Recombinant antibody engineering has recently pivoted toward a novel class of fusion proteins that combine the selective binding of an antibody fragment with the direct immune signaling of a cytokine. Versions of this concept are commonly grouped under the label immunocytokines. In the prototype molecule, IFN- and a recombinant single-chain variable fragment (ScFv)-built from the dispersed variable-heavy and variable-light domains-handle the cytotoxic and targeting tasks in tandem^{5,8-9}. By concentrating both effectors at the tumor interface, the chimeric design boosts local cell-killing activity while sparing healthy tissues from the widespread inflammatory surge typical of free cytokines¹⁰. Among the catalogue of possible immune signals, type I interferons- whether IFN- α or retain special prominence because they simultaneously provoke T-cell mobilization, activate dendritic and macrophage guardians, and curtail aberrant blood vessel sprouting^{3,4}. IFN- β further distinguishes itself by dampening the auto-growth circuits of neoplastic cells, inhibiting telomerase function, silencing c-myc transcription, and priming the TRAIL pathway that drives tumor-cell suicide⁹.

The origin of the immunocytokine concept stretches back to the late 1990s, but clinical momentum dissipated more than a decade later, long enough for skepticism to crystallize. Nonetheless, recent modeling studies hint that fusing a single-chain variable fragment with interferons can narrow the therapeutic window without flattening it entirely¹⁰. Greater design flexibility in the ScFv moiety lets researchers swap, truncate, or extend masking and targeting domains, so prototype molecules resemble less a rigid framework and more a modular kit¹¹. Toward that end, the current investigation fuses a HER1-directed ScFv domain to IFN- β . Hence, the antibody fragment localizes the construct to HER1-positive breast cancer cells, while IFN- β exerts its effects through its cognate interferon receptors (IFNAR1/IFNAR2) in the same microenvironment. In silico docking is therefore used to explore plausible engagement of the fusion with HER1 and interferon receptors, with the understanding that IFN- β itself does not bind HER1.

Despite the development of HER1-targeted therapies, the treatment of HER1-positive breast cancer remains difficult because of limited selectivity, off-target toxicity, and immune evasion. Immunocytokines, chimeric proteins of a tumor-targeting antibody

fragment and an immune-stimulating cytokine have the potential to resolve the issue by localizing therapeutic activity at the tumor with the activation of anti-tumor immunity. The targeting of an anti-HER1 single-chain variable fragment (ScFv) with interferon- β (IFN- β) may localize the therapy at the tumor site more effectively, giving double receptor-targeted and immunomodulatory activity, and minimize systemic adverse effects.

METHODOLOGY

Study Design

This in silico study describes the computational design and characterization of a novel immunocytokine (ScFv-IFN- β) generated by fusing an anti-EGFR/HER1 single-chain variable fragment (ScFv) with human interferon- β (IFN- β). The work was carried out at the Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan, during the period 2021–2022. All molecular modeling, sequence optimization, structure prediction, physicochemical analysis, docking simulations, and immunogenicity/allergenicity predictions were performed using publicly available bioinformatics tools and servers. This study did not include experimental wet-lab validation.

Sequence Analysis and In Silico Construct Design

The amino acid strings for Interferon- (IFN- β) and single-chain variable fragment (ScFv) were extracted from GenBank under accession numbers ABS89222.1 and AFF61391.1. In silico assembly of the ScFv domain involved concatenating the light-chain and heavy-chain variable regions with the flexible linker SSGGGSGGGSGGS. To connect the finished ScFv to the IFN- β compound, a second linker, GGGSGGGSGGGSGGS, was inserted; previous work shows such extensions improve folding and maintain structural integrity¹². A 6-His tag (HHHHHH) was appended to the N-terminus of the entire fusion for straightforward chromatographic purification.

Predicting Physicochemical Properties

A suite of standard physicochemical parameters was computed to gauge how the immunocytokine behaves in solution and interacts with cellular environments. These include estimated half-life, molecular weight, net charge, instability index, theoretical isoelectric point, aliphatic index, amino-acid profile, and the grand average of hydropathicity. For the calculations, ExPASy ProtParam provided a familiar workbench¹³. Solubility itself was modeled using SOLpro, a web-based platform that relies on a trained ensemble of machine-learning predictors. The system has empirical support from several wet-lab comparisons, making it a favoured choice when only sequence data are available¹⁴. Predictive solubility is reported in natural log units of milligrams per milliliter.

Tertiary Structure Prediction, Refinement, and Validation

AlphaFold (alphafold.ebi.ac.uk) delivered the initial three-dimensional model, a choice driven by its recent reputation for producing protein structures that rival

experimental data in terms of fidelity¹¹. To further refine the conformation, the top-scoring model was then run on the GalaxyRefine server that optimizes atomic contact with molecular dynamics-like procedures. Final stereochemical integrity was confirmed with the RAMPAGE as well as PROCHECK web servers, both of which highlight the torsion-angle distribution as well as the geometrical clashes within the chimeric construct^{15,16}.

Prediction of mRNA Stability

Detection of the secondary fold of an RNA chain can be indicative of biochemical functions hidden within ostensibly quiet sequences. The RNAfold web service (<http://rna.tbi.univie.ac.at/cgi-bin/NAWebSuite/RNAfold.cgi>), calculates such structures based on minimizing free energy and provides a quick snapshot of potential base-pairings for the transcript of the immunocytokine.

Molecular Docking

Molecular docking investigations typically depend on computational power to start with binding guesses, and the HDock web server-<http://hdock.phys.hust.edu.cn>-fills that gap by merging the template-free with the template-guided strategies. The server has itself been evaluated with various test sets and can therefore generate orientation predictions that still retain statistical significance. Without using the docking score as a direct surrogate for binding affinity, the results show how a ligand can fit into its receiving binding site even under default binding orientations, enabling quick, qualitative comparisons of docking poses across many complexes. As a result, rather than being quantitative estimates of binding free energy, the docking results are interpreted as relative pose preferences and interface patterns. The current exercise included six different combinations: the HER1 receptor with the solo ScFv; the receptor with the ScFv-IFN- β fused construct; the fusible module against either IFN R1 or IFN R2; and lastly, free IFN- β against both receptor chains. Each protein construct was represented in PDB format and submitted in one go, because treating them as a set simplified writing of the post-docking script and checking of the alignment.

Molecular-Dynamics Simulation

Docked protein structures were uploaded to the iMODs portal (<https://imods.iqfr.csic.es/>) for in-depth Molecular Dynamics Simulation. The web-based platform records how the torsional angles of the interface evolve overtime. Built-in Normal Mode Analysis quantifies each assembly's flexibility and stability while keeping processing costs manageable. From the same run, it extracts root-mean-square deviation profiles, eigenvalue distributions, covariance maps between residues, and local deformation patterns. Those metrics collectively flag regions of structural rigidity and slack, then summarize interatomic coherence. For this study, we retained the default parameters after submission to minimize the need for manual tuning.

RESULTS

Chimeric Protein Construction

Single-chain variable fragment and interferon-beta sequences were fused via a 15-residue linker to yield the ScFv- IFN- β construct. The antibody fragment contributes 242 amino acids, with a second linker of 15 residues uniting the light-chain and heavy-chain variable domains. For translational stability, we appended an N-terminal extension of methionine-glycine-serine-serine and a 6-histidine affinity tag. The interferon-beta moiety contains 167 amino acids, so the entire chimeric protein measures 434 residues. A schematic representation of the arrangement is provided in Supplementary Figure 1.

Physicochemical Properties and Solubility

ProtParam and the SOLpro provided a summary of the protein's baseline characteristics. ScFv-IFN- β weighed roughly 48,173 Da, a figure consistent with similar fusions. Because -3.777 appears in the GRAVY calculation, the construct is expected to prefer aqueous environments rather than membrane-like solvents. The aliphatic index of 73.38 suggests that a substantial fraction of its side chains is hydrophobic. A theoretical isoelectric point of 9.02 combined with a net charge of plus five indicates that the molecule will reside in the positive region of a standard pH gradient. A solubility score of 0.634 (probability threshold >0.5) indicates that the ScFv-IFN construct is predicted to be soluble, but this remains a probabilistic *in silico* estimate and not a guarantee of solubility across all experimental buffers.

3D Model Building, Refinement, and Validation

The AlphaFold web service produced four separate models of the fused protein, as well as individual ScFv, IFN- β , ScFv-IFN- β , and HER1 architectures; these representations are shown in **Figure 1** (A-D). Each construct was assigned a confidence score, and the single model accruing the highest c-score was retained for downstream evaluation.

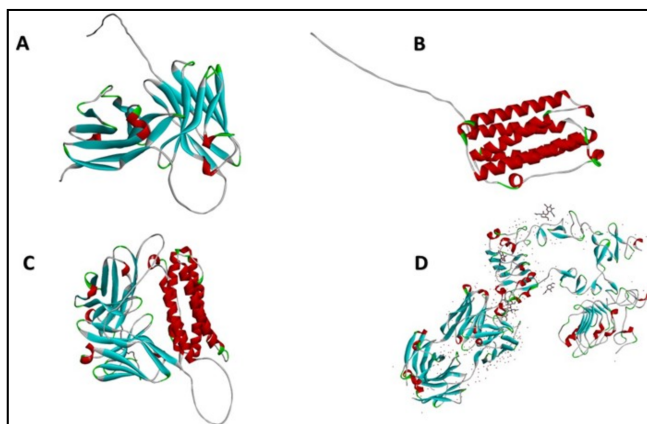


Figure 1: AlphaFold's 3D structural predictions of single proteins

(A) The ScFv structure illustrates an immunoglobulin fold with well-defined VL and VH domains united by a

flexible linker, supporting its antibody-like binding function.

(B) IFN- β has a helical cytokine structure required for receptor binding and for signal transduction downstream.

The ScFv-IFN- β fusion protein illustrates spatial separation of the antibody and cytokine domains by the inter-domain linker, which prevents steric hindrance and allows the domains of function independently.

The structural architecture of the HER1 receptor is depicted, symbolizing its docking surface. The predicted structures were used as a reference point for the assessment of protein-protein interactions and stability by docking and dynamics calculations.

The AlphaFold predictions provided per-residue pLDDT confidence scores, with most residues in the structured domains falling in the high-confidence range (pLDDT > 70), indicating generally reliable backbone placement in these regions. In contrast, flexible loops and linker segments showed lower confidence values, consistent with increased structural uncertainty. To further improve the structure, the initial 3D coordinate set was submitted to GalaxyRefine, which proposed five distinct refinements. Of those five candidates, the model that showed the greatest accumulation of favored conformations in Ramachandran space was ultimately selected, as illustrated in **Figure 2**. Model quality assessment was conducted via PROCHECK and RAMPAGE, enabling a systematic comparison between the pre-refinement and post-refinement data.

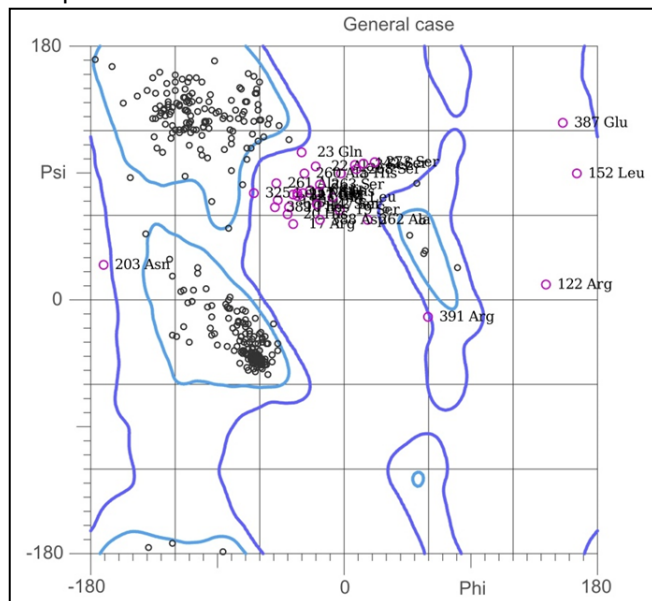


Figure 2: Ramachandran plot of refined 3D model of ScFv-IFN- β fusion protein

Allergenicity predictions

Allergenicity predictions were generated using the AlgPred web portal, yielding a score of -0.74675496.

Because scores below the default threshold of -0.4 indicate a probable non-allergen, this *in silico* method predicts the chimeric protein to be non-allergenic. However, a negative AlgPred score only suggests low allergenicity risk and does not constitute definitive proof of safety. Final confirmation of non-allergenic status requires experimental testing, such as *in vitro* IgE-binding assays or appropriate clinical studies.

mRNA folding stability

The strongly negative ΔG value indicates a thermodynamically stable predicted mRNA secondary structure, but it does not guarantee efficient expression; actual expression levels would need to be assessed experimentally.

Molecular docking

Protein-Protein docking was carried out on six protein-protein complexes, and the individual arrangements were grouped accordingly. For each configuration, scoring metrics such as the absolute docking score, the confidence score, and the ligand root-mean-square deviation (measured in angstroms) were recorded; a summary appears in Table 1. As shown in panels A and B of Figure 4, the designed ScFv-Interferon- β fusion protein generated one of the favorable docking poses toward the epidermal growth factor receptor 1 (HER1). However, given the high ligand RMSD values (45–86 Å), these docking models are interpreted as qualitative representations of potential binding interfaces rather than evidence of strong, well-defined binding affinity. In contrast, IFN- β alone showed significantly lower RMSD values (~1 Å) with IFN receptors, indicating more stable and reproducible binding orientations. Residues Q65, S147, A151, D236, Y238, G251, K254, A262, G277, N368, and H371 formed multiple hydrogen-bond contacts with HER1 residues in the top-ranked HER1-ScFv-IFN- β pose, suggesting a plausible interaction interface. However, because of the high ligand RMSD values (45–86 Å), these models are interpreted only as qualitative representations of potential contact regions and not as evidence of strong or well-defined binding affinity.

Molecular dynamics simulation

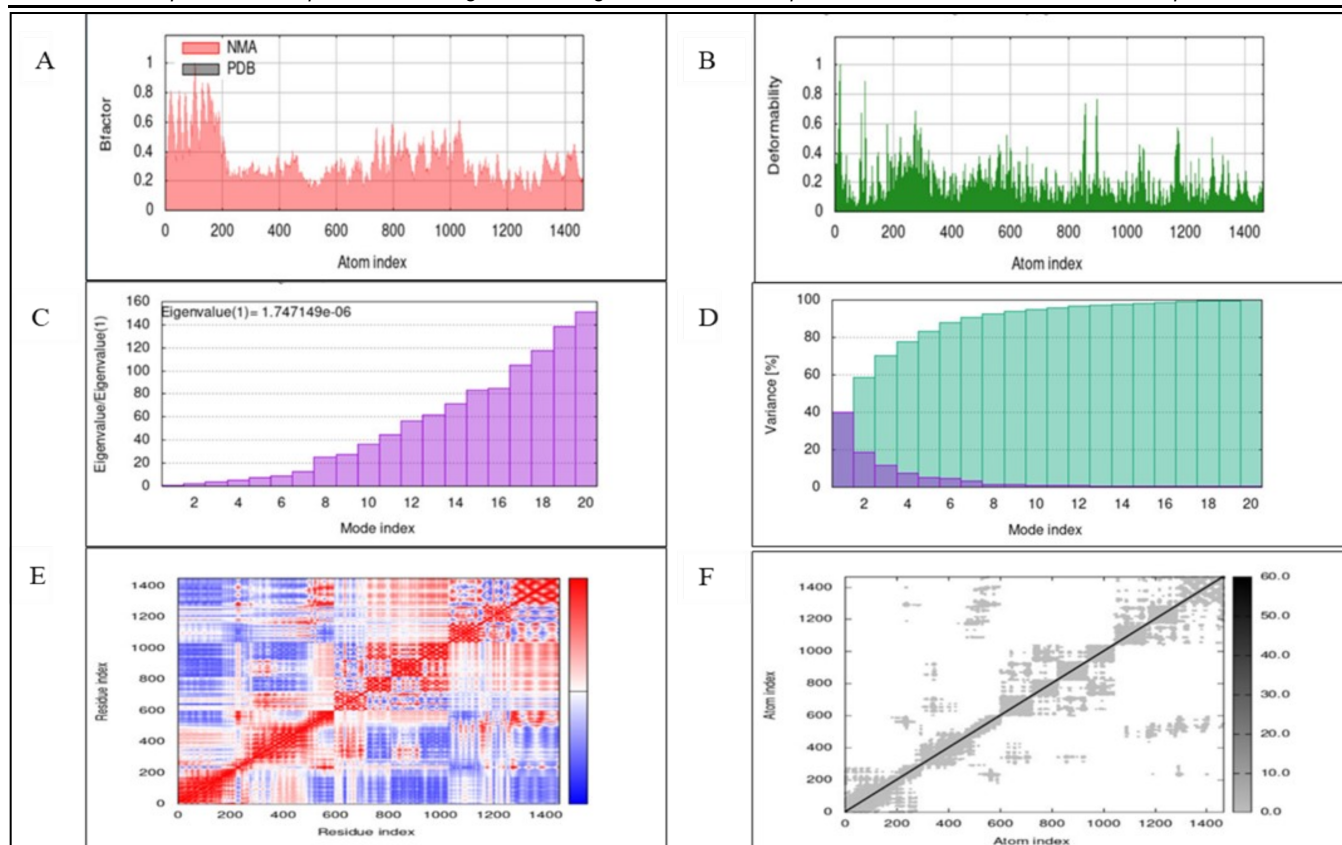
The simulation model displayed a steady increase in flexibility across the amino acid sequence, underscored by an eigenvalue of 1.747149×10^{-6} , a numeric indicator of low mechanical stiffness. Heat-map correlations, coupled with the observed low root-mean-square deviation, confirmed that many side chains interacted strongly with one another (shown in **Figure 5**). **Figure 6** assembles key results from iMODS molecular-dynamics analysis: panel A plots the temperature-factor distribution, panel B tracks local deformability, and panels C through F present eigenvalue dispersion, variance color-coding, covariance mapping, and a network elasticity scheme, respectively.

Table I: Molecular docking of protein complexes

No.	Docked complexes	Docking score	Confidence score	Ligand RMSD (Å)
1	HER1 receptor vs ScFv	-259	0.89	45.89
2	HER1 receptor vs ScFv-IFN- β	-285.3	0.94	53.23
3	ScFv-IFN- β vs IFNR1	-255.9	0.89	86.07
4	ScFv-IFN- β vs IFNR2	-255.5	0.89	64.31
5	IFN- β vs IFNR1	-390.7	0.99	1.03
6	IFN- β vs IFNR2	-456	0.99	0.6

Abbreviations.

HER1; Human Epidermal Receptor 1, ScFv; Single Chain Fragment Variable, IFN- β ; Interferon beta, IFNR; Interferon Receptor

**Figure 5: The results of a molecular dynamics simulation study of the ScFv-IFN- β and HER1 receptor docked complex**

Results of molecular dynamics simulation of the ScFv-IFN- β and HER1 receptor docked complex in iMODs. (A) B-factor plot indicates thermal motion, with low values indicating stable regions of binding.

(B) Deformability analysis reveals few peaks above residues, indicating minimal flexibility and a rigid, stable interaction.

(C) An eigenvalue of 1.747149e-06 reflects low stiffness, i.e., the complex can dissipate dynamic interactions without sacrificing structural integrity. The variance plot shows correlation, which is typical for a stable docked state. (E) The covariance matrix confirms high residue correlation of residues that are interacting with one another, indicating coordinated motion on interaction. (F) Elastic network model (shaded areas = stiffness) reveals extremely few high-

stiffness areas, again demonstrating the structural rigidity of the complex.

DISCUSSION

The global incidence of breast cancer continues to rise, a trend most observers blame partly on the lack of highly selective treatment protocols that are also gentle on normal tissues^{17,18}. Researchers remain troubled by the disease's unrelenting high mortality and morbidity, facts that continue to pressure the laboratory to refine existing therapies and create new ones that truly enhance survival prospects. They still rely on surgery, radiotherapy, and chemotherapy today, but each of them carries old familiar baggage-recurrence, cruel side effects, and in some settings, infuriatingly brief prolongations of life^{19,20}. Targeted

immunotherapy has begun to carve out a different narrative, and early reports suggest it often outperforms older strategies when the tumour microenvironment allows²¹. One subcategory attracting attention is the immunocytokine, a chimeric molecule that can home in on multiple tumor-associated antigens such as carbonic anhydrase IX, EGFR, EpCAM, FAP, and splice variants of fibronectin and tenascin²². This poly-specificity may be especially advantageous for breast-cancer patients whose tumors exhibit marked amplification of the HER1 receptor.

The present study engineered an immunocytokine by pairing a HER1-directed single-chain variable fragment with an interferon- β coding sequence to amplify the oncolytic response. Comparable constructs have outperformed standard systemic therapies in numerous preclinical models and are now under scrutiny in first-in-human trials²³.

Single-chain fragments have repeatedly proven stable and fold quickly into functional conformations, even after overnight incubation at elevated temperatures¹⁰. Their modest molecular weight promotes rapid intratumoral distribution while limiting unintentional engagement of Fc receptors on macrophages, dendritic cells, and both T- and B-cell populations.

Interestingly, one-domain scaffolds such as nanobodies or fibronectin domains achieve their tertiary structure with minimal post-translational refinement, a striking contrast to the complex glycosylation and multi-sheet folding of conventional IgGs¹⁰. Interferon- β is not resting on its laurels; when a cell senses a virus or simply overheats, it releases this cytokine as an early warning flag²⁴. Receptor docking studies consistently show that it binds more strongly than its partner, a feature reproduced by tighter immunological grip²⁵. Clinical optimism is moderated, however, as in the bloodstream the protein has a half-life of under 90 minutes and can become hypersensitive to normal tissue, side effects that dampen its anticancer activity²⁵. Recent technological developments have extended that short half-life to approximately 15 hours in adult immunocytokines, at least giving physicians a better opportunity to schedule effective doses^{24,25}. Attachment of the cytokine to one-chain antibody variable fragment from an HER1-targeting antibody generates a guided missile: the payload targets, adheres, and only then releases its immune storm²⁶. Once inside a tumor, Interferon holds the cellular clock still at S phase and quietly silences the c-myc switchboard, preventing runaway growth²⁷. By waving the Fas and TRAIL flags, it sets off the caspase-8 execution pathway, effectively marking diseased cells for demolition²⁸. Even in trace amounts, the cytokine throttles new blood vessel construction, starving tumors of nutrients. In vitro research suggests that the cytotoxic impact of IFN- β -coupled immunocytokines is primarily attributable to interferon activity itself, rather than to any antibody component²⁹. Parallel in vivo

experiments show that an ErbB2-targeting formulation of IFN- β can completely stall the growth of humanized -mouse gastric-cancer xenografts, an outcome linked to mobilization of the host immune system³⁰.

A different set of experiments led by Chan Gyu Lee and colleagues noted favorable therapeutic responses when IFN- β was paired with trastuzumab, based on trials performed across several cancer-cell lines²⁵. In an independent project, Asma Vafadar and coworkers engineered a recombinant single-chain variable fragment (ScFv) fused to the Cj-CdtB toxin. They confirmed that the ScFv remains a stable polypeptide with strong binding to the HER2 receptor³¹. Parnian Navabi and associates pursued a separate immunocytokine strategy, linking the same ScFv to an IL-2R α module and demonstrating robust HER2 engagement as well as successful expression in the periplasmic space of *E. coli* BL21 (DE3)³². Our research confirms these results of demonstrating an analogous, favorable interaction between ScFv-IFN- β and the HER1 receptor.

Molecular docking generated a favorable docking score for the ScFv-IFN- β chimeric protein complexed with the HER1 extracellular domain (score = -285.3; confidence score = 0.94). However, this score cannot be interpreted as a quantitative binding affinity measurement. The high ligand RMSD value (53.23 Å) indicates substantial pose variability and limits confidence in the predicted interface. For comparison, IFN- β alone produced even more favorable scores with IFN receptors (-390.7 and -456) but achieved significantly lower RMSD values (~1 Å), reflecting more stable and reproducible docking poses. Evidence supporting that prediction is enhanced by a docking consensus measured at better than 0.94 over replicate runs.

The fusion protein also generated docking poses with interferon receptors IFNR1 and IFNR2; however, these complexes exhibited even higher RMSD values (64–86 Å), making them unsuitable for structural interpretation. The computational data thus support the feasibility of the fusion engaging multiple receptors, but do not constitute evidence of superior dual targeting compared to the individual components. Quantitative analysis of the HER1 pose revealed an unexpected set of hydrogen bonds linking receptor side chains to fragment residues; such hooks tend to confer higher specificity and kinetic stability in cellular contexts. If the in-silico image holds up against cellular assays, then immunocytokine ought to differentially label HER1-overexpressing breast carcinoma because it alone spares most normal epithelia. Binding affinity is only half the story; within the same molecule, the yeast-derived ScFv component provides antigen selectivity, while the interferon payload delivers anti-proliferative and immune-modulating activity to the same target. Personalized medicine applications are immediate, especially for patients no longer benefiting from conventional monoclonal antibodies or small-molecule TKIs. Although the

current findings remain proof-of-concept, they justify rapid progression into complementary in vitro and rodent xenograft studies before any clinical heuristic. The research examined a suite of characteristics for the recombinant ScFv-IFN- β fusion protein: physicochemical stability, solubility at physiological salts, structural integrity, potential allergenicity, mRNA longevity in vitro, and binding affinity to target receptors. We retrieved the polypeptide sequences of IFN- β and the antibody-derived ScFv from the NCBI GeneBank database under accession numbers ABS89222.1 and AFF61391.1, respectively. A flexible SSGGGGSSGGGSSGGS linker stitched together the variable light and heavy chain domains, and a second GGGGSSGGGSSGGS extension fused the ScFv to the cytokine to create the ScFv- IFN- β construct. Previously published reports indicate that both the total length and specific residue arrangement of linker segments can dramatically influence the proper folding and thermal stability of ScFv scaffolds^{31,33}. The predominance of small hydrophobic residues, primarily glycine and serine, confers an intrinsically flexible profile to the connector peptide without enforcing rigid secondary structures. For practical chromatography applications, we appended a Gs4Hs3 tag immediately upstream of the coding sequence to streamline purification and isolate the target fusion in near-native form.

The physicochemical properties of ScFv-IFN- β were assessed using ProtParam and SOLpro. ProtParam measures a variety of physicochemical parameters, including molecular weight, isoelectric point, extinction coefficient, instability index, and grand average of hydropathicity. SOLpro provides information about protein solubility based on the amino acid sequence. Recent studies show the importance of designing a stable 3D structure and its effect on the molecular functions of chimeric proteins³⁴. AlphaFold was used to predict the 3D structure of the ScFv-IFN- β protein, and GalaxyRefine was used to refine the structure. The structural integrity of the model was assessed using PROCHECK and RAMPAGE. AlgPred was used to predict allergenicity, and RNAfold was used to analyze ScFv-IFN β mRNA stability. HDock was used for docking and to calculate the binding affinity of ScFv-IFN β with HER1. iMOD was used for protein-protein interaction and molecular dynamics simulations.

Immunocytokines have the potential to treat different cancer types. However, limited studies have been conducted on immunocytokines. Antigen expression and mutation status of cancer cells may challenge their effectiveness. This study aimed to in silico study and design an effective immunocytokine with favourable binding affinity for HER1. However, further research is needed to validate the effectiveness of immunocytokines, particularly ScFv-IFN β against HER1-positive breast cancer, which could lead to the development of novel cancer therapeutics.

CONCLUSION

This research provides exploratory in silico modelling of the ScFv-IFN- β fusion protein against HER1-overexpressing breast cancer; however, several important caveats must be emphasized. First, the high RMSD values for fusion protein-receptor complexes (45–86 Å) indicate unreliable docking convergence and prevent confident structural interpretation of binding interfaces. Second, computational scores alone cannot rank binding strength, as evidenced by the more favorable HDock scores for IFN- β alone than for the fusion. Third, the work is purely theoretical, with biological efficacy, immunogenicity, and pharmacokinetics completely unproven in vitro or in vivo. In vitro and in vivo tests will need to confirm binding affinity, expression stability, and therapeutic effect. Second, variability in cancer heterogeneity and HER1 expression or mutation status might influence responsiveness; therefore, more detailed patient data analysis or personalized strategies may be required. Although accurate computational software such as HDock, AlphaFold and RNAfold were utilized, cross-validation against other software or more up-to-date models such as RoseTTAFold or ChimeraX would increase prediction certainty. Subsequent research should include expression and purification of the construct in *E. coli* or mammalian cell systems, assessment of bioactivity in HER1-positive breast cancer cell lines, and in vivo efficacy in xenograft models. Further investigation into potential immune-related adverse events or off-target effects is necessary.

Recommendations

Further in vitro and in vivo work is required to confirm the therapeutic activity, stability, and safety of the ScFv-IFN- β chimera. Furthermore, comparative analysis with existing therapeutic strategies should be conducted to evaluate the potential of the ScFv-IFN- β chimera.

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Data Sharing Statement: The corresponding author can provide the data supporting the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Khan MI: Conceived, designed and drafted the study, conducted all experiments

Hassan M: Contributed to the experiments and assisted in drafting the manuscript

Afandi MH: Contributed to manuscript drafting and critical revision

Sohail A: Assisted in performing experiments

Ahmad N: Critically reviewed and revised the manuscript.

Idrees M: Supervised, contributed to study design, and critically revised the manuscript

Bhatti R: Contributed to reviewing and revising the manuscript

Khan MA: Supervised, contributed to design and finalized the manuscript

All authors read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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