Caninization of Adalimumab

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What is Caninization?

- Caninization describes the antibody engineering strategy in which the complementarity-determining regions (CDRs) of a donor antibody are grafted onto a canine antibody framework.
- The objective is to generate a therapeutic canine antibody by retaining the original antibody's antigen-binding properties, while replacing non-essential framework regions with canine sequences to improve compatibility and reduce immunogenicity in canine recipients.
- Caninization enables the rapid generation of new canine therapeutic candidates, removing time-consuming conventional antibody development processes, including immunization campaigns, hybridoma screening and antibody characterization.
- Adalimumab is a TNF-α inhibiting human-recombinant antibody, treating inflammatory autoimmune disorders such as Rheumatoid arthritis.
- Dog immunoglobulins exhibit a preference for the lambda isotype over the kappa isotype (approximately 91% of the light chain repertoire).
- Therapeutic kappa light chain antibodies may benefit from light-chain isotype switching to enhance structural resemblance to endogenous canine immunoglobulins and in turn reduce immunogenicity potential.

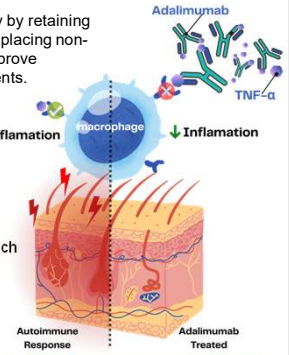


Fig 1. Adalimumab blocks the inflammatory cascade by binding to TNF-α and preventing TNF-α from binding to its receptors on macrophages to cause inflammation.

Protein Purification Results

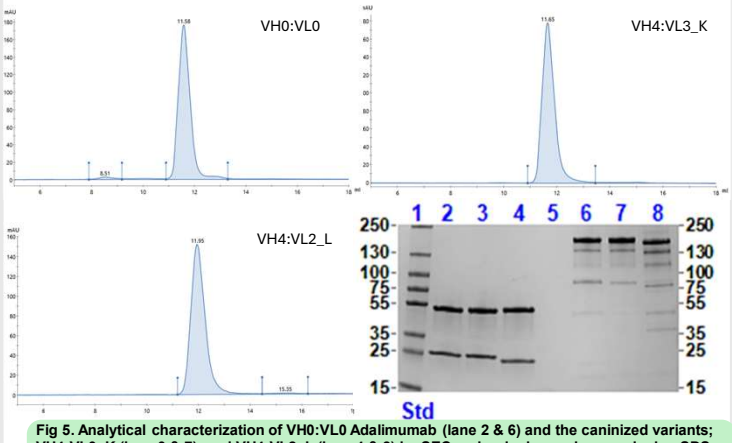


Fig 5. Analytical characterization of VH0:VL0 Adalimumab (lane 2 & 6) and the caninized variants; VH4:VL3_K (lane 3 & 7), and VH4:VL2_L (lane 4 & 8) by SEC and reducing and non-reducing SDS-PAGE. The SEC trace and SDS-gel results demonstrate that both variants were comparable to the parental antibody, with no observable changes in monodispersity (>95%) or purity. These caninized IgGs have an average molecular weight of 150kDa and a corresponding band can be seen.

Engineering

- Adalimumab's variable sequences were run through Fusion's in-silico library of canine frameworks. After selecting multiple donor sequences for CDR grafting, 5 heavy chains and 8 light chains (5 lambda and 3 kappa chains) were designed.

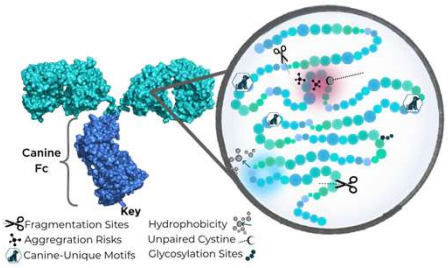


Fig 2. The sequence screening processes highlighted liabilities for removal and key structural residues to be maintained. The addition of an appropriate canine Fc was also essential in the design process.

- A matrix combining these sequences produced 40 different antibodies which were tested against the chimeric Adalimumab, the VH0:VL0 antibody.
- Predicted structural similarity of the designed antibodies' variable region showed that the caninized antibodies had RMSD value of less than 2Å against Adalimumab's structure. This highlights how well the structure of the caninized antibodies matches with Adalimumab's.

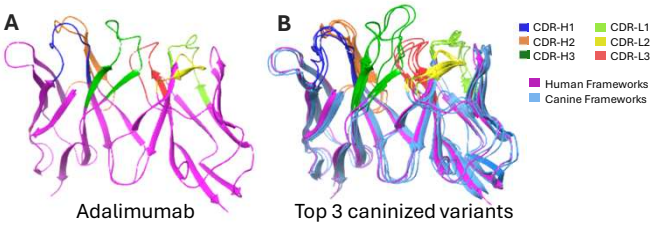
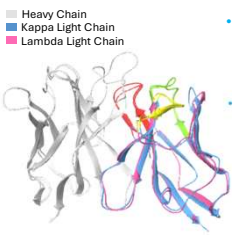


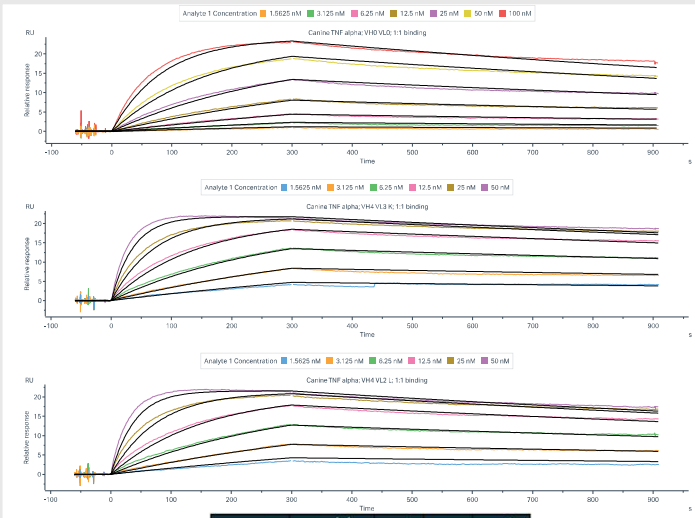
Fig 3. Ribbon models of (A) VH0:VL0 Adalimumab and (B) our top 3 caninized antibodies. SPR identified the top 3 antibodies as VH4:VL3_K, VH4:VL1_K and VH4:VL2_L.



- To reduce the risk of immunogenicity, we conducted light chain type-switching while caninizing Adalimumab's kappa light chain.
- These antibodies remained stable and functional and 4 out of our 10 top antibodies had a lambda light chain.

Fig 4. Ribbon structural model of our best caninized lambda antibody variant, VH4:VL2_L, aligned against Adalimumab and its kappa light chain.

SPR Results



Antibody ID	k_a ($M^{-1}s^{-1}$)	kd (s^{-1})	KD (nM)	Chi²
(1) VH0:VL0	1.15E+05	2.77E-04	2.41	0.67
(28) VH4 VL3 K	5.42E+05	3.50E-04	0.65	0.28
(30) VH4 VL2 L	5.38E+05	4.61E-04	0.86	0.39

Fig 6. Affinity characterization of the interaction between caninized anti-TNF-alpha IgG's and Recombinant Canine TNF-alpha protein (Sino Biological Cat #70003-DNAE). Antibody variants were captured to a Protein A sensor chip and tested with varying concentrations of TNF-alpha antigen. The data obtained from the binding response was fitted to a 1:1 binding model, yielding the rate constants (Ka & Kd) and equilibrium dissociation constant (KD) above. Performed on a Biacore 1k+. 19 antibodies were analyzed in total with the KD values lying within the range of 0.65 nM – 7.34 nM.

Conclusions

- 19 anti-TNF-alpha antibodies were successfully generated through the caninization of Adalimumab and retained high affinity to recombinant canine TNF-α.
- SPR analysis confirms the antibody with the greatest affinity for recombinant TNF-alpha is **VH4:VL3_K** with a KD of **0.65 nM**, significantly greater than the chimeric Adalimumab antibody **VH0:VL0** at **2.41nM**.
- The light chain isotype-switch from a kappa in the parental Adalimumab to a lambda has been successful. Our 3rd ranked antibody **VH4:VL2_L**, possess a lambda light chain and binds to recombinant Canine TNF-alpha with a KD of **0.86nM**.
- Fusion Antibodies' approach to caninization highlights how kappa and lambda light chains can be used interchangeably whilst retaining a high affinity for the target antigen.

Acknowledgements

Thank you to the Future Institute of Medicine for providing us access to the Biacore 1k+ SPR instrument. Structural Models were produced with Schrodinger. Illustrative figures were created using Canva.com

References

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