Engineering assembly and low pH hold stress of anti-CD3 scFv-based multispecific T cell engagers

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Background – Anti-CD3 scFv stabilization for multispecific TCEs

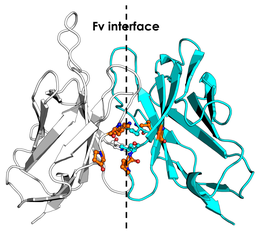
- T cell-engaging (TCE) multispecifics can utilize either HC-LC heterodimerization for cognate chain pairing[1], common LC, single domain[2], or scFv-based αCD3[3] arms to avoid light-chain mispairing.
- scFvs typically have higher aggregation propensity, especially at low pH, lower thermal melting transition (T_m) than Fab, and other manufacturing challenges.
- An engineered disulfide bond VH44–Vκ100 (Pastan DS)[4] has been shown to stabilize scFvs, but frequently compromises initial quality after primary purification.

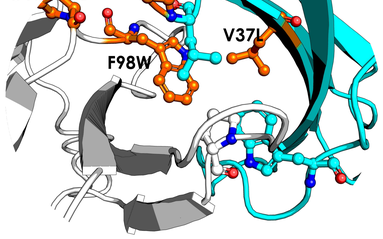
Strategy – Diversification and selection of αCD3 ADI-26909 with G93A mutation (medium affinity) scFv in Adimab's yeast platform yields ADI Sets 1 and 3

ADI Set 1: VL-F98W VH-L45P

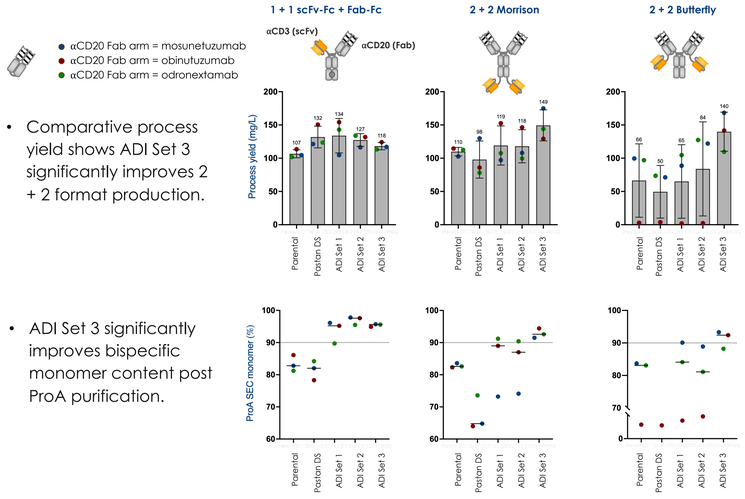
ADI Set 3: VL-F98W VL-G100P VH-V37L VH-R44P

- Boltz-2 model depiction of stabilizing mutations (orange) at the interface between VH (cyan) and Vκ (grey).





Results – Comparison of yield and quality across formats



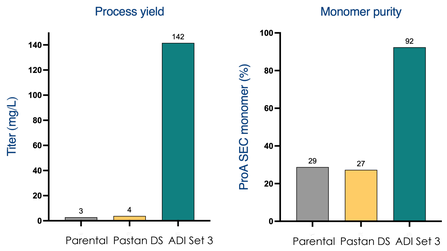
- Comparative process yield shows ADI Set 3 significantly improves 2 + 2 format production.
- ADI Set 3 significantly improves bispecific monomer content post ProA purification.

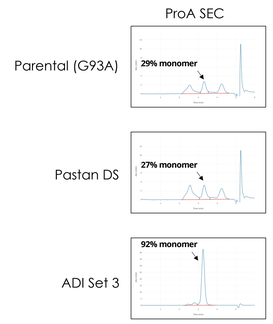
Results – ADI Set 3 rescues the obinutuzumab 2+2 Butterfly multispecific assembly

- Parental scFv and the Pastan DS scFv both fail to express in this format; ADI Set 3 rescues both yield and monomer purity.

2.8 → 141.6
titer mg/L (50× over parental)

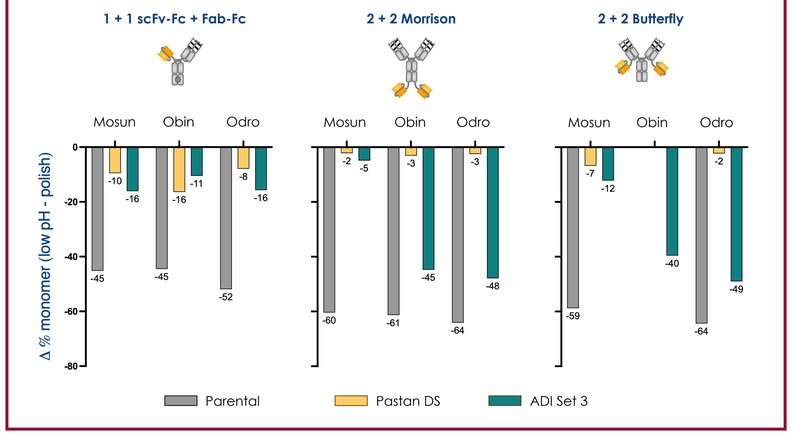
29% → 92%
ProA SEC monomer purity





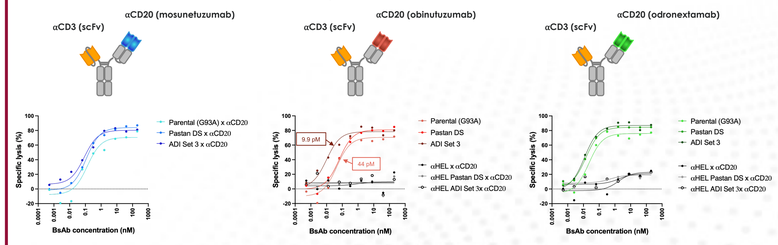
Results – Format dependency — Set 3 rescues 1+1; partial in 2+2 (arm-dependent)

- Low pH stress assay consists of a 1 hour hold at pH 3.5
- Set 3 rescues low pH stability 1+1; in 2+2 the residual Δ depends on the CD20 arm identity.



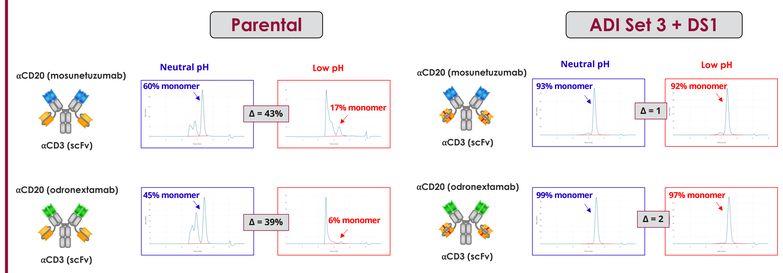
Results – TDCC across three CD20 arms — Set 3 improves potency

- T-cell-dependent cellular cytotoxicity dose-response across obinutuzumab, mosunetuzumab, and odronextamab CD20 arms.
- Obinutuzumab arm EC50: 44 → 9.9 pM



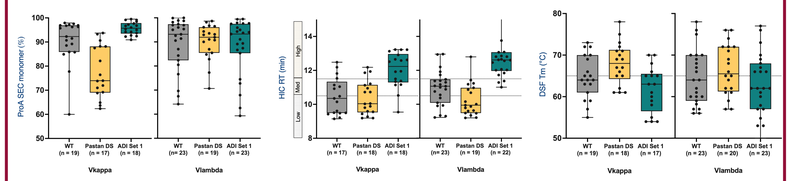
Results – Set 3 + novel disulfide (DS1) rescues 2 + 2 low pH stability

- Combination of ADI Set 3 and a novel disulfide (DS1) stabilizes the 2 + 2 butterfly format for concentration and exchange into neutral phosphate buffer and low pH stress.



Results – L45P + F98W (Set 1) broadly transferable across Vκ

- Yeast scFv-Fc panel with a diversity of human Vκ and Vλ germlines, ProA SEC monomer across biophysical attributes.
- Vκ median ProA SEC monomer: 92.3 → 95.7% with ADI Set 1 , Vλ unchanged.
- ADI Set 1 slightly increases HIC RT and reduces thermal stability.



Conclusions – ADI Set 3 outperforms the Pastan DS across the developability stack

- Set 3 surpasses Pastan DS on monomer %, titer, and low-pH in 1+1.
- Rescues assembly of the obinutuzumab 2+2 Butterfly that fails with parental and Pastan DS.
- Higher functional potency suggest more productive binding to CD3 on T cells
- Broadly transferable across Vκ germlines in yeast platform.

Path forward – Extending mutation sets across lineage, germline, and format space

- Identify Vκ and Vλ residues that improve HIC RT and DSF Tm.
- Explore stabilization of Pastan DS for full low-pH rescue of difficult 2+2 formats (DS1 resides within CDRs).

References

1. Barlow *et al.*, **mABs** 2025 Dec; 17(1).
2. Khalifé *et al.*, **Proc. AACR Ann. Meeting** 2025 Apr 25-30.
3. Liu *et al.*, **mABs** 2023 Jan-Dec; 15(1).
4. Jung and Pastan, **Proteins**1994 May 19(1).