

Novel EGFR antibodies overcome acquired resistance

Escape-variant binding enables effective T-cell redirection beyond conventional EGFR blockade

MEDICAL NEED: EGFR is a validated therapeutic target in colorectal, head-and-neck and lung cancers, but EGFR-directed antibodies are limited by intrinsic and acquired resistance. A clinically important escape route is missense mutations in extracellular domain III (ECD III), targeted by cetuximab (Cet), panitumumab (Pan) and necitumumab (Nec). Variants such as S492R and G465R can impair antibody binding while preserving receptor signaling. Downstream alterations such as activating KRAS mutations can also confer resistance to EGFR blockade. Antibodies that retain recognition of escape variants and can be deployed in signaling-independent formats could therefore extend EGFR targeting beyond conventional receptor blockade.¹

From antibody escape to a new EGFR binding solution

In a collaboration between the University of Stuttgart, TUBS, and YUMAB, Kühl and colleagues developed novel human EGFR antibodies.² The parental antibody MKU011-A7 (A7) was identified by phage display and showed partial EGFR-blocking activity. Affinity maturation by VL shuffling yielded YU250-A02 (A02) and YU250-H10 (H10). H10 improved EGFR affinity from 32.0 nM for A7 to 4.17 nM, approaching cetuximab (1.69 nM). A02 and H10 recognized human, cynomolgus and mouse EGFR with comparable ELISA binding, supporting broader preclinical evaluation.

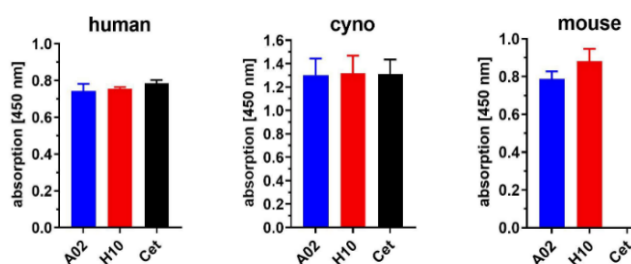


Fig. 1 ELISA binding of A02, H10 and Cet to human, cynomolgus and mouse EGFR.²

On EGFR-positive cancer cells with different surface densities, both affinity-matured antibodies inhibited receptor phosphorylation, downstream ERK activation and proliferation similarly to cetuximab.²

A different core epitope preserves binding to escape variants

Parental A7 and V_L-shuffled H10 both bind ECD III in a region overlapping those of approved EGFR antibodies, but mutational mapping revealed a distinct core interaction pattern. H10 retained binding to all tested clinically relevant ECD escape variants except the rare Q435P substitution, including S442R, S464L, G465R, K489E and S492R. Cetuximab, panitumumab and necitumumab lost or reduced binding to different subsets of these variants.

Antibody	Q435P	V441D	V441G	S442R	S464L	G465R	K489E	S492R
H10	×	✓	✓	✓	✓	✓	✓	✓
Cetuximab	×	×	✓	~	×	×	~	×
Necitumumab	~	×	~	×	✓	~	~	✓
Panitumumab	✓	~	~	×	×	×	~	✓

Tab. 1 Escape-variant coverage of H10 compared with approved EGFR antibodies (adapted from Kühl et al., Fig. 2c).²

✓ binding retained; ~ binding reduced; × binding abolished/no detectable binding. The simplified categories reflect the qualitative interpretation by the authors.

Consistent with this profile, A02 and H10 blocked EGF-induced EGFR and ERK phosphorylation in cells expressing G465R or S492R, whereas cetuximab failed to inhibit either mutant receptor.²

Turning resistance-robust binding into T-cell redirection

The authors next converted parental A7 and affinity-matured H10 into bispecific EGFR × CD3 T-cell engagers (TCEs) using 1+1 and 2+1 elg-based architectures^{3,4}. Both formats bound EGFR-positive tumor cells and CD3-positive T-cells and induced target-dependent cytotoxicity. Across several cell lines, the 2+1 format was generally more potent than the corresponding 1+1 molecule, particularly at low or intermediate EGFR density, consistent with avidity provided by the second EGFR-binding arm. The effect was most pronounced for the lower-affinity parental A7 binder. The pronounced format effect of A7 highlights how EGFR affinity and valency may be tuned to balance potency and selectivity in future bispecific concepts.

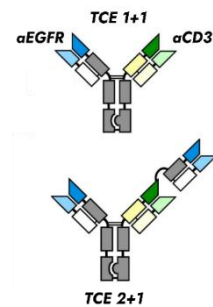


Fig. 2 Composition of 1 + 1 and 2 + 1 TCE.

Escape mutations remain targetable by H10 TCEs

H10-based TCEs efficiently killed cells expressing wild-type EGFR as well as G465R and S492R. Cetuximab-based TCEs remained active on wild-type EGFR but were inactive against both escape variants. H10 TCEs additionally killed KRAS-mutant HCT-116 and CT-26 cells, illustrating that T-cell-mediated cytotoxicity is independent of intact EGFR signaling.²

In vivo proof-of-concept – and the remaining challenge

In a syngeneic CT-26 model expressing human wild-type EGFR, murine CD3-reactive surrogate H10 TCEs suppressed tumor growth. The 2+1 surrogate significantly inhibited tumor growth at 30 µg and 3 µg after two treatments, although tumor regression was not observed and anti-drug antibody responses limited longer-term evaluation. A key translational challenge is EGFR expression in normal tissues. The study therefore supports optimization of format, valency and safety mechanisms to widen the therapeutic window of EGFR-directed T-cell engagement.²

Verified Feature	MKU11-A07 (Parental Antibody)	YU250-H10 (Affinity-Matured Variant 2)
Targeting of Domain III (EGF-binding domain)	✓	✓
EGF-Blocking (Inhibits Signaling / Cell Proliferation)	✓	✓
High-Affinity Binding	32 nM	4 nM
Human / Cyno / Mouse Species X-Reactivity	na	✓
Binding to a Unique Epitope	✓	✓
Resistant to Clinically Relevant EGFR-Escape Mutations	✓	✓
Internalizing	na	✓
Validated in-vivo as Bispecific T-Cell Engager	na	✓

Tab. 2 Feature summary of parental versus affinity matured antibody

CONCLUSION

Kühl and colleagues combine two complementary strategies to address acquired EGFR resistance: an antibody epitope that remains accessible across major ECD escape mutations and a T-cell-engaging mechanism independent of intact EGFR signaling. The data provides a rationale for further development in EGFR-positive tumors with primary or acquired resistance, while making safety engineering the next critical step toward clinical translation.

REFERENCES

1. Ríos-Hoyo et al. Unveiling Acquired Resistance to Anti-EGFR Therapies in Colorectal Cancer: A Long and Winding Road. *Frontiers in Pharmacology* 15, 1398419, (2024).
2. Kühl, L. et al. Novel bispecific T-cell engagers overcoming acquired EGFR resistance. *mAbs* 18, 2674236 (2026).
3. Kühl, L. et al. elg-based bispecific T-cell engagers targeting EGFR: format matters. *mAbs* 15, 2183540 (2023).
4. Kühl, L. et al. The elg Technology to Generate Ig-like Bispecific Antibodies. *mAbs* 14, 2063043 (2022).