FairJourneyBio
THE ANTIBODY COMPANY

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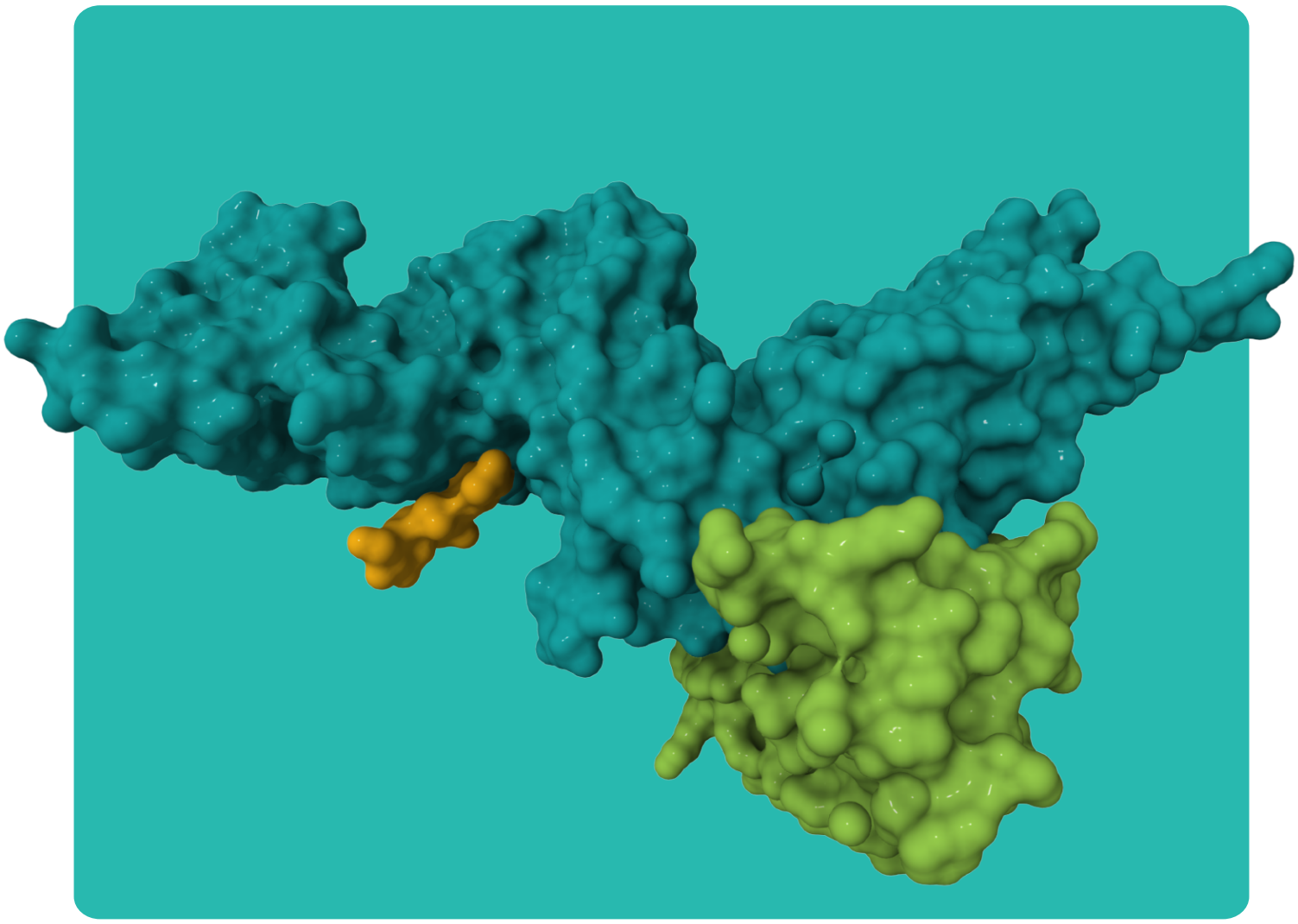
Dual-specificity Fab engineering for conditional cytokine activation

ANTIBODY DISCOVERY & ENGINEERING • CONDITIONAL CYTOKINE ACTIVATION

Reference: Kahn, J.D. et al. (2026). Conditional activation of IL-12 through a Fibronectin-EDB dependent switch gate. mAbs, 18(1). doi:10.1080/19420862.2026.2700812



FairJourneyBio

CASE STUDY

FACT SHEET | ANTIBODY DISCOVERY & ENGINEERING | CONDITIONAL CYTOKINE ACTIVATION

FairJourney Bio delivered antibody engineering for novel tumor-conditional IL-12 therapeutic approach that unlocks the potential of localized cytokine activation

>1,000-fold	0.33 nM	9-fold	927
AFFINITY GAIN, SWITCH1 VS THE H01 PARENT	IL-12 AFFINITY OF THE FINAL SWITCH ARM	FURTHER IL-12 GAIN FROM ONE HCDR2 MUTATION	IGG CLONES SCREENED BY SPR

BACKGROUND

Why partner with FairJourney Bio for dual-specificity Fab engineering

Cytokines are potent immune activators, but systemic toxicity has confined their clinical use to a narrow therapeutic window. IL-12 is a prominent example: early trials were halted by severe immune-related toxicity despite signs of anti-tumour activity, and later strategies – intra-tumoral dosing, half-life extension, protease-cleavable pro-drugs – have had limited clinical success. The approach described here keeps IL-12 inactive in systemic circulation and releases it locally only on binding Fibronectin Extra Domain B (FN-EDB), a matrix antigen abundant in the tumour extracellular matrix (up to 100 nM), largely absent from normal adult tissue, and retained rather than internalised. The sponsor, a venture-backed oncology company, designed the concept: a dual-binding Fab "switch" arm that binds IL-12 or FN-EDB competitively, paired with a targeting arm that drives tumour localization and avidity-driven unveiling. FJBio's Porto and Cambridge teams discovered and engineered that switch arm to a binding specification the sponsor fixed in advance.

STRATEGY

A phage discovery and engineering campaign run against a binding specification the sponsor fixed in advance by modelling

The sponsor's QSP modelling defined the ideal binding window before an antibody existed: high affinity for IL-12 (KD ~0.27 nM), low affinity for FN-EDB (~KD 160 nM) in the "switch" arm, and a targeting arm binding FN-EDB at 0.5 nM. These parameters drove a campaign combining naïve phage display, combinatorial library design, focused affinity maturation and kinetic screening – requiring the molecule to recognise IL-12 and FN-EDB competitively, engage a neutralising IL-12 epitope, minimise polyreactivity, and hold a precisely tuned affinity differential between the two targets. A panel of 17 FN-EDB binders was selected from FJBio's naïve phage display library using two antigen forms, counter-selected against EDB-lacking splice variants, and triaged on sequence diversity and dissociation kinetics against benchmark L19. Five oligoclonal libraries, co-designed with the sponsor and stratified by germline usage with HCDR1/HCDR2 diversified (theoretical diversity 5.2E+8–1.8E+9), underwent sequential selection alternating between IL-12 and FN-EDB, yielding 34 sequence-unique dual binders; competitive screening against ustekinumab identified H01 as the lead for further optimization. A two-stage library strategy then optimized simultaneous binding: LCDR1/LCDR2 (3E+9) and HCDR3/LCDR3 (2.8E+9) libraries were

separately selected at low stringency to remove deleterious variants, then recombined into a 1.7E+10 cfu library preserving the HCDR3–LCDR3 linkage. Stringent selection against both targets, followed by off-rate screening, identified Switch1, with >1,000-fold improved affinity to both antigens over the H01 parent. Further NGS and off-rate analysis, combined with machine-learning-guided design, generated Switch2, whose affinities matched QSP predictions and benchmark antibodies.

RESULTS

Switch2 achieved affinities comparable to two separate clinical benchmark antibodies from one Fab

Monovalent binding kinetics were determined by surface plasmon resonance (SPR) and fitted with a 1:1 Langmuir model. The parental FN-EDB binder A03 bound FN-EDB at 3,265 nM with no detectable IL-12 binding. The first dual binder, H01, bound IL-12 at 3,120 nM and FN-EDB at >10,000 nM. Switch1 improved >1,000-fold over H01 on both antigens, reaching 2.91 nM for IL-12 and 180 nM for FN-EDB. A single additional HCDR2 mutation gave Switch1 a further 9-fold gain in IL-12 affinity while retaining FN-EDB binding, yielding the final switch arm Switch2 at 0.33 nM and 250 nM respectively – in line with the sponsor's QSP-predicted window, and comparable to the single-target benchmarks ustekinumab (IL-12, 0.29 nM) and L19 (FN-EDB, 234 nM). Both Switch1 and Switch2 inhibited IL-12-induced IFN-γ release from NK-92 cells, with Switch2 showing an IC50 similar to ustekinumab.

Unmasking of IL-12 was dependent on FN-EDB in both biochemical and cell-based assays

Conditional IL-12 availability was analysed by ELISA with a biotinylated anti-IL-12 Fab, against an Always ON control (IL-12 tethered to L19, which cannot bind IL-12 and so cannot mask it) and an Always OFF control (IL-12 tethered to ustekinumab). Captured via their Fc domains to mimic systemic circulation, both Switch-IL-12 candidates showed minimal IL-12 availability, similar to Always OFF – confirming the intended masked conformation. On FN-EDB-coated plates, Switch1 showed robust IL-12 availability similar to Always ON, confirming localized conditional activation; Switch2 showed more modest availability, apparent only at high concentrations. Switch2's higher IL-12 affinity raises its release threshold, further limiting systemic-activation risk (the plated FN-EDB here sits ~100-fold below concentrations reported in human tumours). In a functional co-culture assay, Switch1 induced dose-dependent IFN-γ release from IL-12 receptor-expressing NK-92 cells only in the presence of FN-EDB, with no detectable release in its absence, normalized to the Always ON control.

Table 1 · Switch arm binding affinities (KD, monovalent SPR, 1:1 Langmuir fit)

QSP specification	~0.27 nM IL-12; 160 nM FN-EDB
A03, parental FN-EDB binder	No IL-12 binding; 3,265 nM FN-EDB
H01, first dual binder	3,120 nM IL-12; >10,000 nM FN-EDB
Switch1, after affinity maturation	2.91 nM IL-12; 180 nM FN-EDB
Switch2, final switch arm	0.33 nM IL-12; 250 nM FN-EDB
Benchmark antibodies	Ustekinumab 0.29 nM IL-12; L19 234 nM FN-EDB

DISCUSSION

A format- rather than a target-specific outcome and what the data does and does not show

The switch arm is a core component: the "Switch" mechanism can in principle improve the therapeutic window of IL-12 or other cargoes by keeping them masked in systemic circulation and releasing them locally only in the tumour extracellular matrix upon FN-EDB binding. Achieving this required two unrelated specificities arranged competitively on a single dual-binding Fab and tuned to a window defined in advance by modelling. Whilst this is an attractive and innovative approach with potential for therapeutic application of tumor-targeted IL-12, several caveats apply: the improved therapeutic window is a QSP model prediction, not a measured in vivo outcome. Human IL-12 does not cross-react with the murine IL-12 receptor and no directly translatable murine model was available. FN-EDB concentrations achievable in vitro were ~100-fold lower than those reported in human tumours, and in vitro models cannot fully capture FN-EDB's three-dimensional intratumoral presentation – likely underestimating FN-EDB-dependent IL-12 release. Switch1 showed better unmasking than Switch2 in both the ELISA and the IFN-γ release assay, likely because of its weaker masking. To further validate conditional tumor-localized IL-12 activity, patient-derived tumour fragments or ex vivo tumoroid models that preserve native matrix architecture are proposed as a route.

BOTTOM LINE

A dual-binding Fab was engineered to bind two unrelated antigens competitively, at affinities separated by design – matching two clinical benchmark antibodies in one, one of them deliberately at low affinity. Unmasking of IL-12 was shown to be FN-EDB-dependent in both a biochemical and a cell-based assay, with the predicted therapeutic window matching QSP modelling rather than in vivo measurement. The same switch-arm format can in principle gate other cargoes, given a suitable tissue-restricted antigen.

CONTRIBUTION

The sponsor designed the conditional-activation format, performed QSP modelling to set the binding specification, contributed to library design, built the machine-learning models guiding variant design, and advised on functional assay set-up. **FairJourney Bio built and selected the naïve phage libraries used, co-designed and built the engineering libraries, ran the discovery and affinity maturation campaigns, reformatted and expressed the IgG panels, and generated the SPR, biochemical and cell-based datasets, across its Porto and Cambridge sites. They also generated the machine-learning datasets utilised in this study.**

WHAT THIS MEANS FOR PARTNERS

A sponsor arrived with a binding window derived from modelling: sub-nanomolar on one antigen, mid-nanomolar on a second unrelated antigen, competitive rather than additive, on a single Fab surface. FJBio delivered a molecule precisely conform that window. If you need a discovery & engineering partner who can hit the specification you set, or need a partner to develop a similar design, contact us.

Click here to talk to our team →

Let's talk about your programme

Bring your target, your timeline and your tough questions to a scoping conversation.

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