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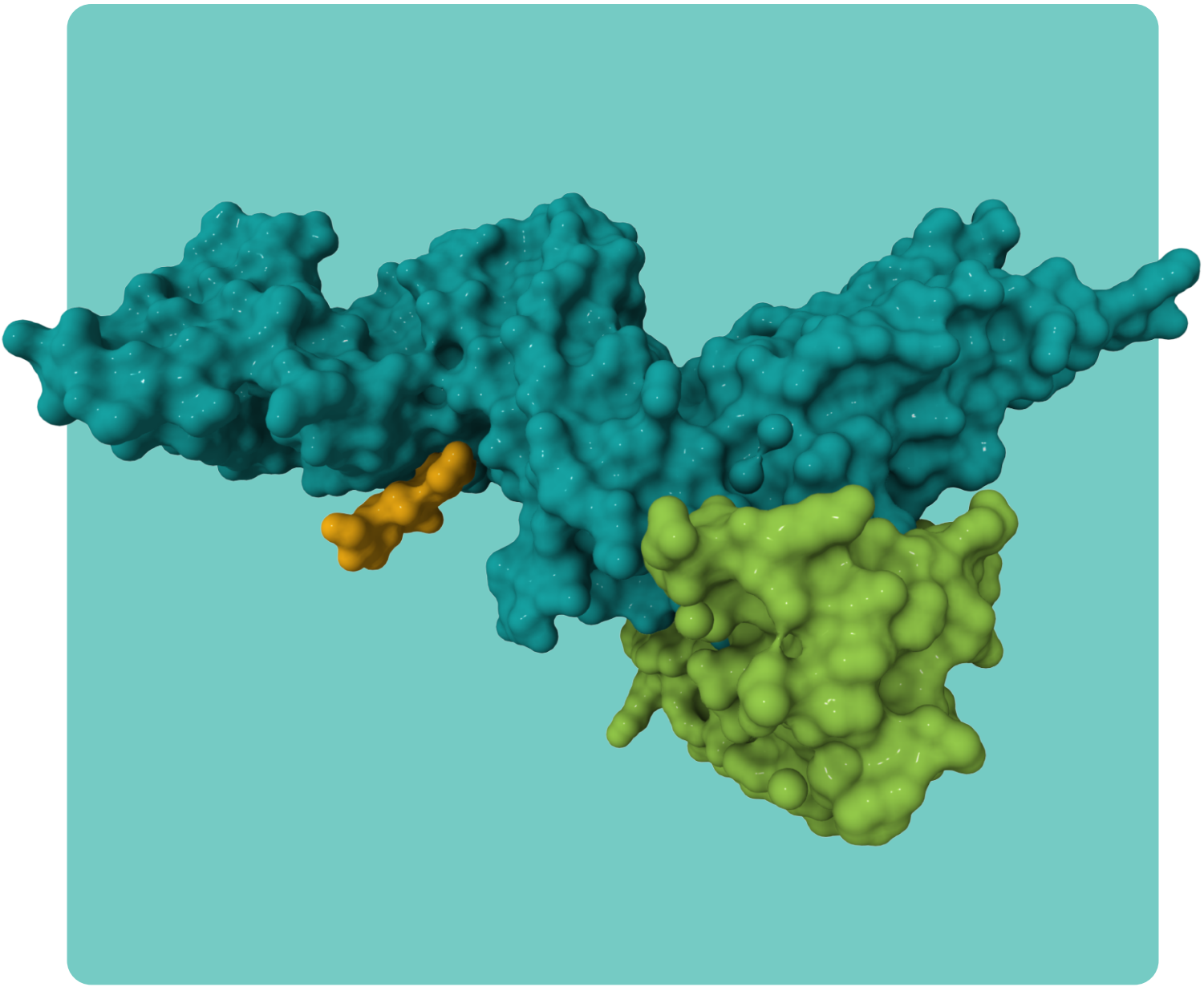
Fact Sheet

Conditional Cytokine Activation Dual-specificity Fab engineering

ANTIBODY DISCOVERY & ENGINEERING • CONDITIONAL CYTOKINE ACTIVATION



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FACT SHEET

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

WHY PARTNER WITH FJBIO FOR DUAL-SPECIFICITY FAB ENGINEERING

Cytokines are potent activators of the immune system, but their clinical application has been limited by systemic toxicity that restricts dosing to a narrow therapeutic window. Interleukin-12 is a prominent example: early trials were terminated because of severe immune-related toxicities despite signs of anti-tumour activity, and subsequent strategies (intra-tumoral administration, half-life extension, protease-cleavable pro-drugs) have seen limited clinical success so far. The approach described here keeps IL-12 masked in systemic circulation and makes it available only on binding Fibronectin Extra Domain B (FN-EDB), a matrix antigen abundantly expressed in the tumour extracellular matrix at levels reaching 100 nM while largely absent from normal adult tissues and retained rather than internalised. The whole concept rests on one component: a dual-binding Fab, or switch arm, that binds tethered IL-12 or FN-EDB in a competitive manner, paired with a targeting arm that promotes avidity-driven unveiling in the tumour. FairJourney Bio's teams in Porto and Cambridge carried out the discovery and engineering of that switch arm.

STRATEGY

QSP modelling defined the binding parameters required to achieve a positive therapeutic window, predicting that a switch arm with high-affinity IL-12 binding (KD ~0.27 nM) and weaker FN-EDB binding (KD ~160 nM), combined with a high-affinity FN-EDB targeting arm (KD ~0.5 nM), could reduce systemic IL-12 receptor occupancy while maintaining tumour-localised activity. These modelled parameters were translated into a systematic antibody engineering campaign combining naïve phage display, combinatorial library design, focused affinity maturation and kinetic screening. The engineering strategy was designed to achieve more than dual specificity, requiring the molecule to recognise IL-12 and FN-EDB competitively, engage a neutralising IL-12 epitope, minimise unwanted polyreactivity, and maintain a precisely tuned affinity differential between the two targets. A panel of 17 FN-EDB binders was selected from a naïve phage display library against two forms of the antigen and over splice variants lacking EDB, with clone triage also weighing sequence diversity and dissociation kinetics against the clinical benchmark L19. Five oligoclonal libraries were built by germline usage with HCDR1 and HCDR2 diversified (theoretical diversities 5.2E+8 to 1.8E+9); sequential selections alternating between IL-12 and FN-EDB yielded 34 sequence-unique dual binders, and competitive screening against ustekinumab identified H01. Two-stage affinity maturation followed, separate LCDR1/LCDR2 (3E+9) and HCDR3/LCDR3 (2.8E+9) libraries, low-stringency library focusing because the full combinatorial diversity of 5.8E+18 could not be explored outright, then recombination by inverse PCR into a final library of 1.7E+10 cfu preserving HCDR3–LCDR3 linkage. Off-rate screening identified Switch1. Machine learning models using ESM2 embeddings, fine-tuned on single-antigen SPR off-rates and phage

next-generation sequencing, then designed 48 variants across four backbones, informed by off-rate and sequence data for 927 IgG-reformatted clones screened by high-throughput SPR.

RESULTS

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

Monovalent binding kinetics were determined by surface plasmon resonance 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 more than 10,000 nM. Switch1 exhibited more than 1,000-fold improved binding affinity relative to the H01 parent on both target antigens, reaching 2.91 nM for IL-12 and 180 nM for FN-EDB. A variant of Switch1 carrying a single additional mutation in HCDR2 achieved 9-fold higher affinity to IL-12 while retaining comparable binding to FN-EDB, giving the final switch arm Switch2 at 0.33 nM and 250 nM respectively. These affinities were in line with those predicted to confer a positive therapeutic window by QSP modelling, and comparable to the single-binding benchmark antibodies ustekinumab, a clinically approved IL-12 neutralising antibody, at 0.29 nM, and L19, a clinically validated anti-FN-EDB antibody, at 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

IL-12 availability was probed by ELISA with a biotinylated anti-IL-12 Fab, against an Always ON control (IL-12 tethered to L19, which cannot bind and therefore cannot mask IL-12) 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 the Always OFF control – the intended masked conformation. On plates coated with FN-EDB, Switch1 showed robust IL-12 availability similar to the Always ON control. The candidate containing the Switch2 switch arm showed more modest availability, apparent only at high molecule concentrations; the authors attribute this to the higher release threshold created by its tighter IL-12 affinity in an assay where plated FN-EDB sits approximately 100-fold below the concentration 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, and release was not detectable in its absence when normalised to the L19-IL-12 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 component rather than a molecule tied to one cargo: the mechanism is described as having the potential to improve the therapeutic window of IL-12 or other cargo molecules by keeping the cargo masked in systemic circulation while enabling localised availability in the tumour extracellular matrix on FN-EDB binding. The engineering requirement is the one addressed here — two unrelated specificities arranged competitively on a single dual-binding Fab and tuned to a window defined in advance by modelling. Several limits should be read alongside the results. 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, so no directly translatable murine model was available. FN-EDB concentrations achievable in vitro were approximately 100-fold lower than those reported in human tumours, and in vitro models cannot fully capture the three-dimensional intratumoral presentation of FN-EDB, which is expected to lead to an underestimation of 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. Patient-derived tumour fragments or ex vivo tumoroid models that preserve native matrix architecture are proposed as a route to test IL-12 activity further.

BOTTOM LINE

A dual-binding Fab was engineered to bind two unrelated antigens competitively, at affinities separated by design, matching two clinical benchmark antibodies at the same time — 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 derived from QSP modelling rather than in vivo measurement. The same switch-arm format can in principle gate other cargoes, given a suitable tissue-restricted antigen.

From dual-specificity switches to multi-specifics.
Explore FairJourney Bio's antibody engineering capabilities for complex formats

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