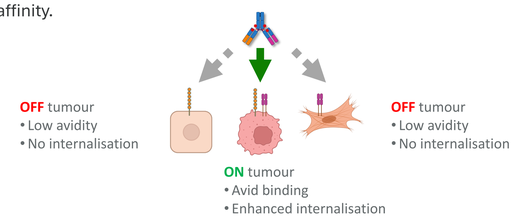
Hybrid Avidity-Gated EGFR x EphA2 Bispecific ADC Demonstrates Potent Anti-Tumour Efficacy while Sparing Keratinocytes

Daniel Holdbrook[1], PhD; Mark Edwards[1], DPhil; Roy Pettipher[1], PhD; Michael Hunter[1], PhD; Sophie Cullen[1]; Narinder Janghra[1]; I-Hsun Chiang[1], PhD; Chloe Shepherd[2]; Kazuyo Kaneko[2], PhD; Nick Holliday[2], PhD; Katherine Vousden[1], PhD
[1] Promatix Biosciences Ltd, [2] Excellerate Bioscience Ltd

Introduction

Targeted anti-EGFR therapy using TKIs or monoclonal antibodies is associated with on-target, dose-limiting skin toxicity which is a barrier to the development of EGFR ADCs.

Our approach exploits selective EGFR and EphA2 co-expression in colon cancer to enhance tumour selective targeting through the development of a bispecific antibody with low monovalent binding affinity.



TxPro mining reveals EGFR x EphA2 as a colon cancer selective target pair

TxPro is a genome-wide cancer proteomics resource built on the OGAP database

- >5,000 tissue samples profiled by high-sensitivity mass spectrometry and 15,192 proteins were quantified (6,807 membrane; 1,853 cell-surface).
- Matched normal vs cancer profiles enable identification of differentially expressed target pairs
- In silico mining over 5,000 EGFR bispecific partners for CRC selectivity revealed EGFR x EphA2
- EGFR and EphA2 known to associate and promote tumourigenesis[1]

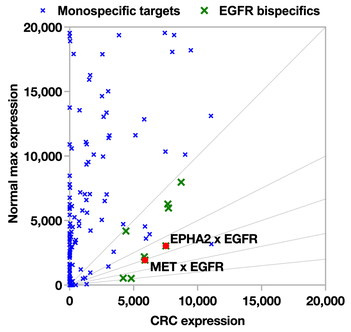


Figure 1a) Differential expression plot (Normal maximum expression signal vs CRC expression signal) for monospecific targets and EGFR bispecifics.

Expression values are predicted receptor copy numbers based on mean %mol values across data sets in TxPro.

EGFR x MET included as an AND-gate comparator.

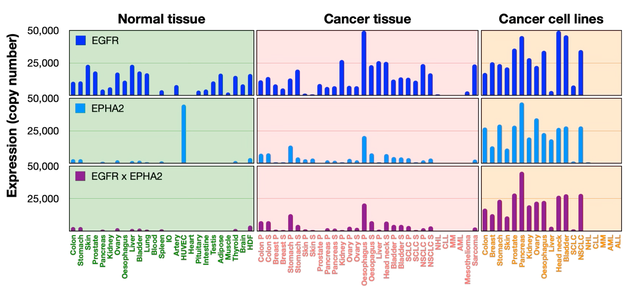
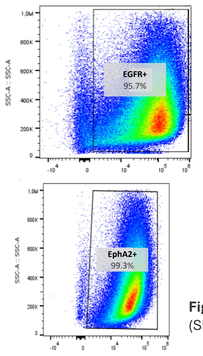


Figure 1b) TxPro Normal / Cancer tissue profile plot for EGFR, EphA2, and EGFR x EphA2

EGFR x EphA2 are co-expressed in CRC PDX

FACS analysis of eleven CRC PDX lines showed high level of EGFR and EphA2 co-expression at the single cell level (Figure 2a).



PDX line	%EGFR^{+ve}	%EGFR EphA2^{+ve}	Overall DP%
CXF-269	94.9	99.3	94
GXA-MKN7	97.1	99.8	97
CXF-2029	98.2	82.3	81
CXF-2358	89.1	52.8	47
CXF-4086	97.3	45.6	44
CO-04-0108	43.8	75.0	33
CO-04-0280	17.3	30.0	5
CO-04-0311	51.0	83.0	42
CO-04-0314	22.3	92.0	21
CO-04-0722	80.1	88.0	70
CO-04-0727	96.6	95.0	92

Figure 2a) FACS analysis of tumour cells from PDX line (Shown is CXF-269; CRL) gated on EGFR and EphA2

IHC shows surface membrane co-localised EGFR and EphA2 staining within CRC PDX tissue (Figure 2b).

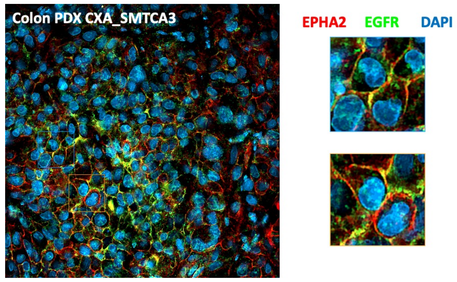


Figure 2b) IF co-staining analysis on CRC PDX line CXA-SMTCA3 (CRL)

Development of EGFR x EphA2 lead candidate PBS293-01A (MMAE, DAR=4)

PBS293-01A (Figure 3) is an EGFR x EphA2 full IgG1 bispecific ADC conjugated to MMAE via a cathepsin cleavable MC-VC-PAB linker (DAR 4). Affinity was tuned by targeted engineering of the variable regions to reduce monovalent binding and favour avidity-driven engagement.

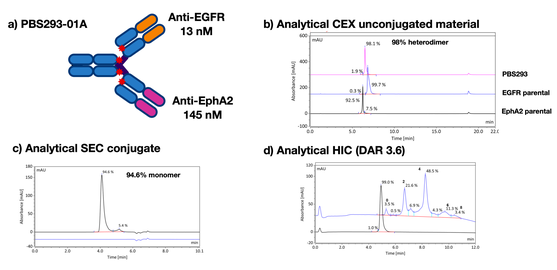


Figure 3) Schematic diagram of PBS293-01A and analytical characterization

PBS293 exhibits hybrid avidity in Cell Binding

Binding of PBS293 to CRC cell line HCT116 is approximately 10-fold higher when both EGFR and EphA2 are present (EC_{50} = 0.3 nM) assessed using KO lines (Figure 4a) or monovalent control constructs (Figure 4b).

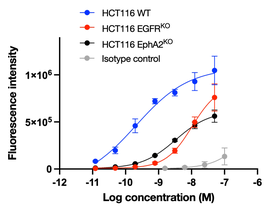


Figure 4a) PBS293 binding to HCT116 receptor knockout lines

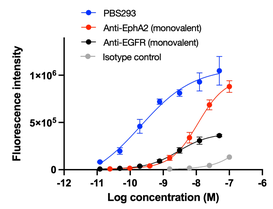
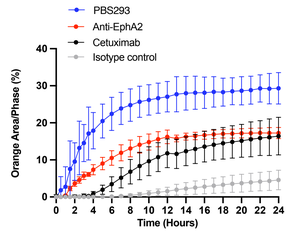


Figure 4b) PBS293 and monovalent control constructs binding to HCT116 w.t.

PBS293 internalises rapidly and exhibits potent cytotoxicity in HCT116 cells



PBS293 internalises significantly more than either cetuximab or anti-EphA2 bivalent controls (Figure 5a).

Figure 5a) Incucyte internalisation assay (HCT116 cells, 50 nM ligand)

Cytotoxicity data in w.t. and receptor knockout HCT116 cells indicate cytotoxicity is predominantly driven by EphA2 binding, consistent with this receptor's more rapid internalisation (Figure 5b).

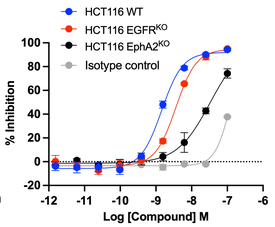


Figure 5b) Cytotoxicity of PBS293-01A in HCT116 knockout cell lines

PBS293-01A is more potent than cetuximab ADC in EGFR-low CRC cell lines

PBS293-01A is potent across a range of CRC cell lines, with IC_{50}s comparable or superior to parental monoclonal antibodies (Table 1).

Cell line	EGFR sites / cell	EphA2 sites / cell	IC_{50} (nM) PBS293-01A	IC_{50} (nM) Cetuximab MMAE	IC_{50} (nM) Anti-EphA2 MMAE
HT1376	166,400	61,000	0.3	0.1	0.9
Lovo	23,600	91,600	0.3	0.2	0.6
LS123	52,500	110,000	0.5	0.2	0.1
HCT116	14,700	74,600	1.1	88.9	0.7
T84	18,900	28,600	1.3	4.3	3.1
HT29	17,900	29,400	2.1	16.0	1.1
SW1417	22,000	43,000	15.0	13.0	108.0
SW948	18,300	8,500	164.0	256.0	>300

Table 1) Cytotoxic activity of PBS293-01A and comparators across panel of tumour cell lines

PBS293-01A bispecific has superior efficacy than target-specific antibodies in HCT116 xenograft

PBS293-01A produced dose-dependent tumour growth inhibition in the HCT116 xenograft model (ED_{50} = 2.6 mg/kg; Figure 6a). Tumour growth suppression was significant at 3 and 10 mg/kg versus the conjugated isotype control (p<0.001). In a 45-day head-to-head study at 10 mg/kg i.v., PBS293-01A showed greater anti-tumour activity than cetuximab-MMAE or anti-EphA2-MMAE (p<0.001; Figure 6b).

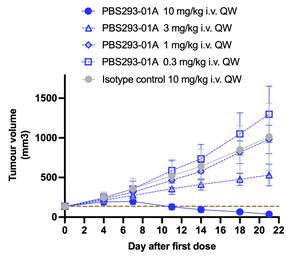


Figure 6a) Dose-response tumour growth inhibition with PBS293-01A in the HCT116 xenograft model

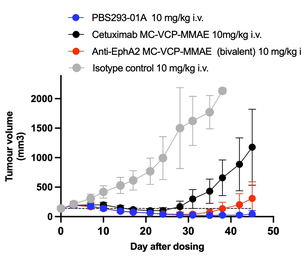


Figure 6b) Head-to-head tumour growth inhibition at 10 mg/kg i.v. in HCT116 CDX model

PBS293-01A spares keratinocytes but is more potent on HCT116 cells than cetuximab

Normal human epidermal keratinocyte (NHEK) cytotoxicity was used as a surrogate for EGFR-driven skin toxicity. PBS293-01A is 20-30-fold less toxic to keratinocytes than cetuximab (Figure 7a) but is ~ 100 x more potent on HCT116 cells (Figure 7b). Both graphs and values pooled from five experiments.

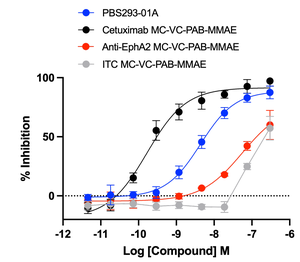


Figure 7a) Cytotoxicity of PBS293-01A and comparators on NHEK cells

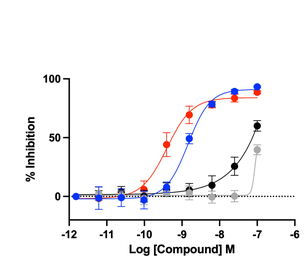


Figure 7b) Cytotoxicity of PBS293-01A and comparators on HCT116 cells

Conclusion

PBS293-01A is an EGFR x EphA2 bispecific MMAE ADC that uses avidity-gated dual binding to improve selectivity while maintaining strong anti-tumour activity.

- **Mechanism:** Avidity-gated co-engagement drives internalisation in dual-positive tumours
- ***In vitro*:** Strong HCT116 potency – outperforms cetuximab-MMAE and comparable to bivalent anti-EphA2-MMAE
- ***In vivo*:** Durable HCT116 xenograft tumour control vs cetuximab-MMAE and more persistent than bivalent anti-EphA2-MMAE
- **Safety:** Lower keratinocyte toxicity and only modest HUVEC killing

Contact

Michael Hunter, PhD
Promatix Biosciences Ltd
Email: mick.hunter@pro-matix.com
Website: www.pro-matix.com

References

1. Kim, J., Chang, I.-Y. & You, H. J. Interactions between EGFR and EphA2 promote tumorigenesis through the action of Ephexin1. Cell Death Dis 13, 528 (2022).

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