Nano-BiTEs: Bispecific T cell engagers based on nanobodies

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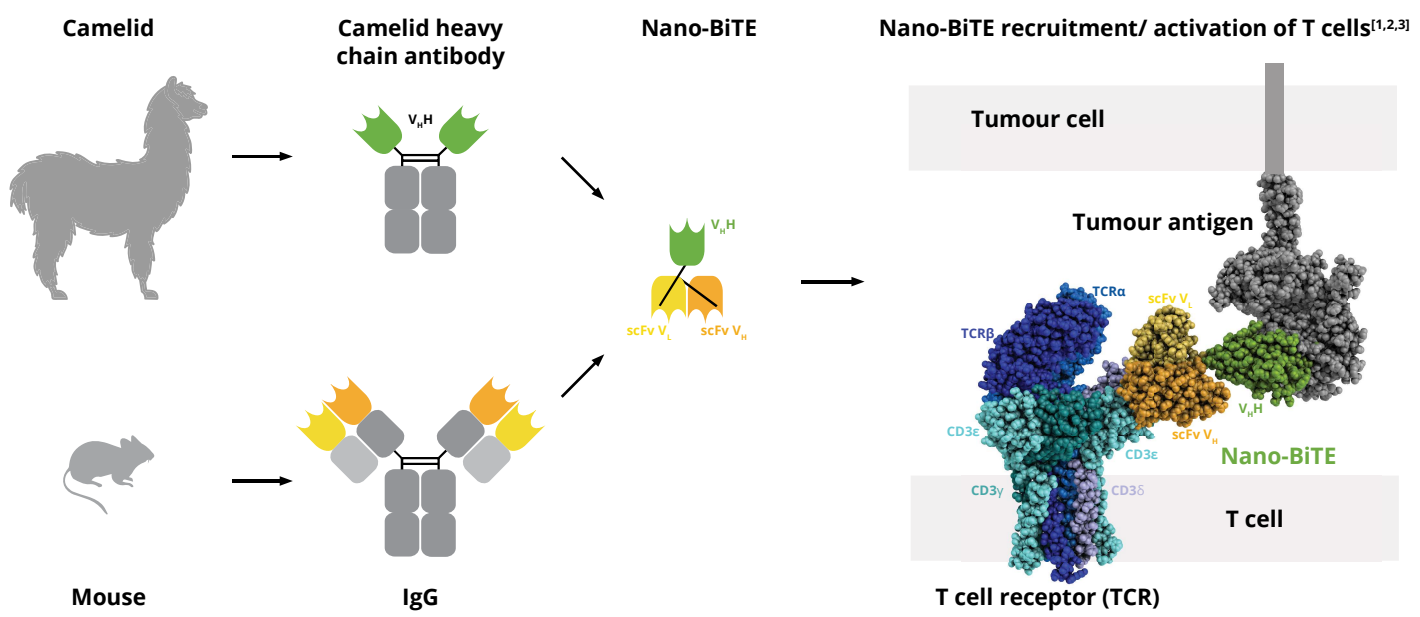
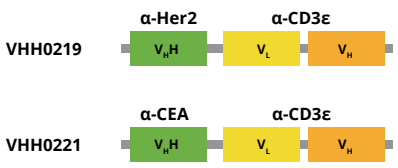
Based on our long-standing experience in the field of nanobodies (V_HH), we have developed Nano-BiTEs. These bispecific T cell engagers are fusions of a nanobody against the tumor antigens Her2 or CEA and an scFv against the T cell receptor (TCR) subunit CD3ε.

Nano-BiTEs are designed as reagents for the efficient activation of T cells in the context of tumour cells.

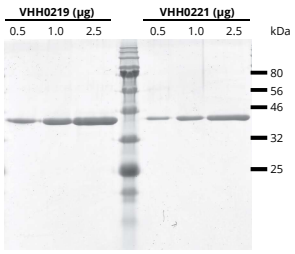


Nano-BiTE format and affinity

Nano-BiTE constructs

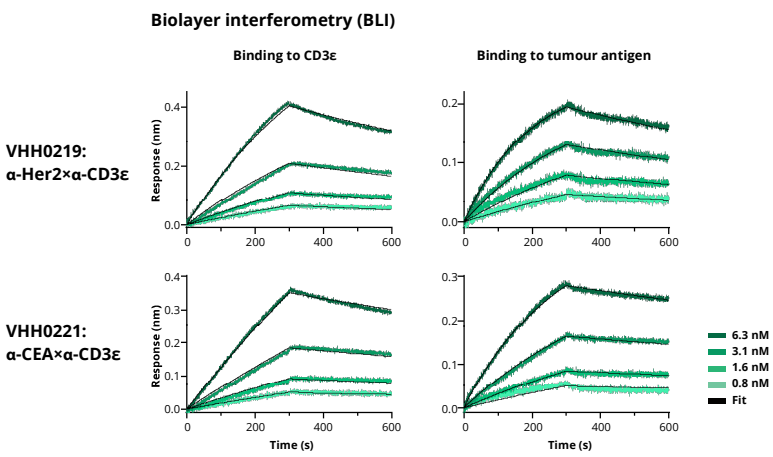


Format of Nano-BiTEs:
- Fusion of V_HH and scFv
- scFv anti-human CD3ε



Production:
- Mammalian expression
- Affinity chromatography
- Endotoxin-free

High affinity of the Nano-BiTEs

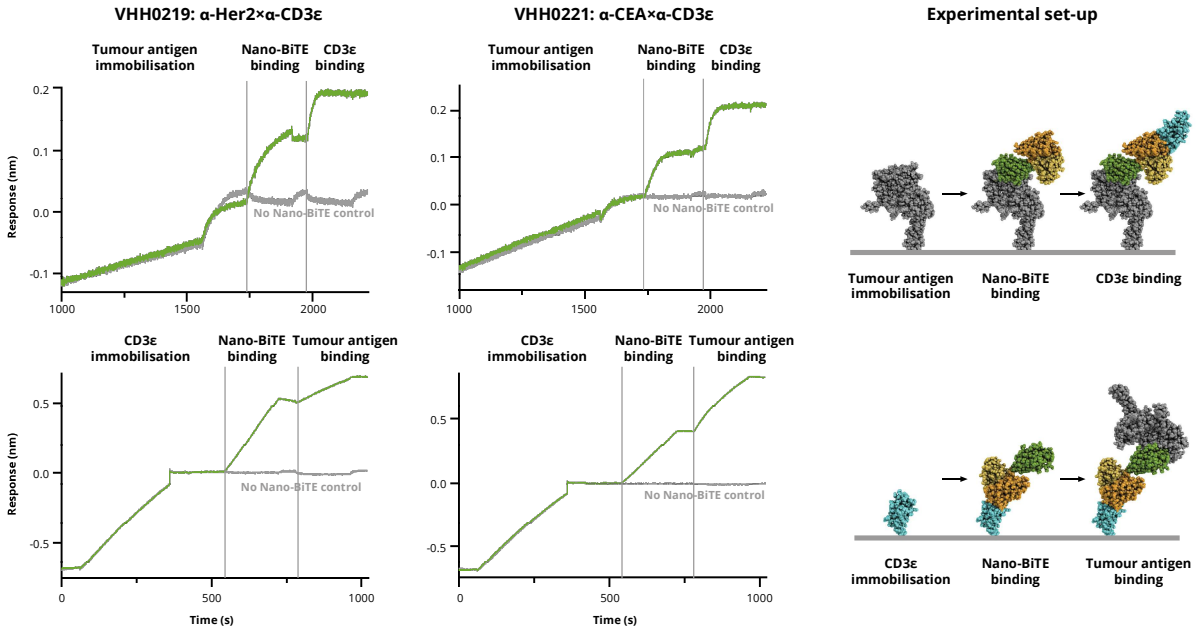


	Ligand	K_D (nM)	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})
VHH0219 α-Her2×α-CD3	CD3ε	3	$2.5 \cdot 10^5$	$7.8 \cdot 10^{-4}$
	Her2	1	$7.2 \cdot 10^5$	$7.3 \cdot 10^{-4}$
VHH0221 α-CEA×α-CD3	CD3ε	12	$4.2 \cdot 10^5$	$5.0 \cdot 10^{-4}$
	CEA	0.8	$5.3 \cdot 10^5$	$4.1 \cdot 10^{-4}$

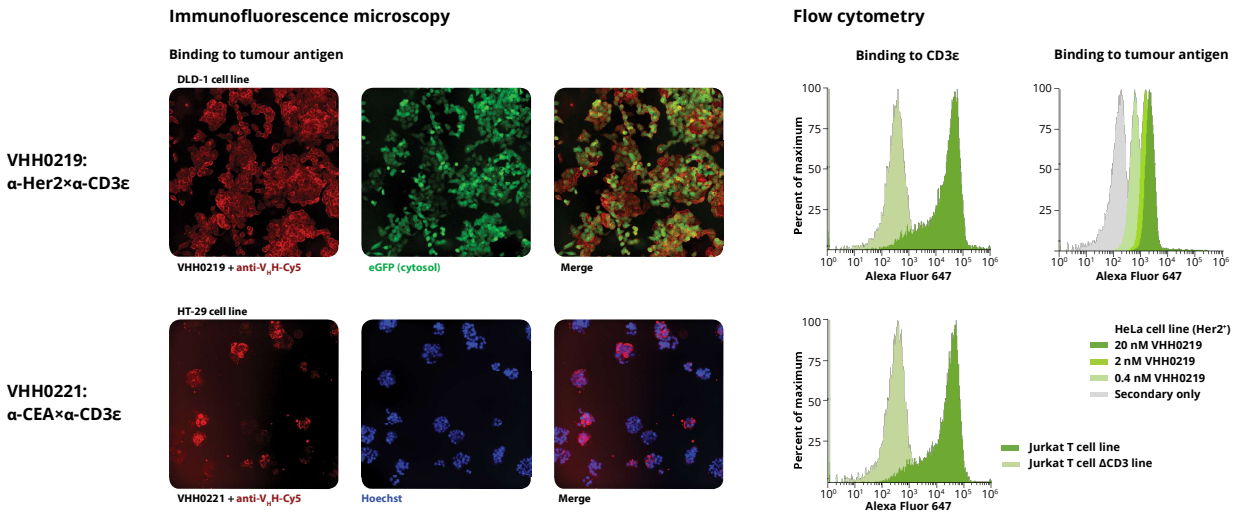
→ **High affinity of Nano-BiTEs will allow targeted tumour and T cell binding.**

Antigen-binding of Nano-BiTEs

Concomitant binding to CD3ε and tumour antigen *in vitro* (BLI)



Binding to CD3ε and tumour antigens on cells



→ **Nano-BiTEs recognise their antigens CD3ε and Her2 or CEA *in situ* on cells.**

Conclusion

Our Nano-BiTEs are a novel tool format to activate T cells specifically by recruiting them to tumour cells. Nano-BiTEs can be used in immuno-oncology as standards or to achieve a defined level of T cell activation to quantify the effect of novel drug candidates.

Acknowledgements:
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References:

[1] Zheng, L. et al. 2019: Structural basis of assembly of the human T cell receptor–CD3 complex. Nature 573, pp. 546-552. PDB 6JXR. [2] Garboczi, D. N. et al. 1996: Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature 384, pp. 134-141. PDB 1AO7. [3] Kjer-Nielsen, L. et al. 2004: Crystal structure of the human T cell receptor CD3εγ heterodimer complexed to the therapeutic mAb OKT3. PNAS 101, pp. 7675-7680. PDB 1SY6.