Optimization of lead antibody selection for XMT-1522, a novel, highly potent HER2-targeted antibody-drug conjugate (ADC)

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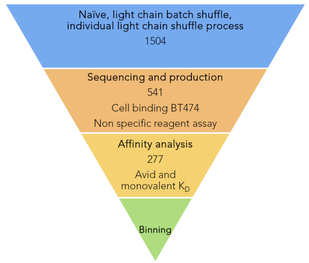
Summary

XMT-1522 is an anti-HER2 ADC that incorporates HT19, a novel, human anti-HER2 antibody optimized for cytotoxic payload delivery. Several parameters such as cell binding, internalization rate, cytotoxicity, antibody-dependent cell-mediated cytotoxicity (ADCC), downstream signaling, affinity, NHP cross-reactivity, normal human tissue cross-reactivity and in vivo efficacy were used to screen a wide range of antibodies to select a lead candidate optimized for use in ADC applications. In addition, HT19 was selected to be non-competitive for HER2 binding with existing therapies - trastuzumab or pertuzumab, to allow for potential combinability. In vivo efficacy as an ADC was not necessarily predictive of typical screening parameters such as in vitro cytotoxicity, internalization or binding affinity.

The lead antibodies were affinity matured, and despite increases in affinity, the corresponding ADCs showed significantly decreased ADC in vivo efficacy. This phenomenon was observed with all the antibody hits. HT19 showed antibody-dependent cell-mediated cytotoxicity activity. When administered as the unconjugated antibody in the NCI-N87 gastric cancer xenograft model it had biological activity at 20 mg/kg as well as at 3 mg/kg. Consistent with the hypothesis that a non-competitive ADC is combinable with current anti-HER2 regimens, the combination of XMT-1522 with trastuzumab and/or pertuzumab showed more rapid internalization, more complete HER2 degradation, and significantly greater anti-tumor activity in the NCI-N87 gastric cancer xenograft model relative to XMT-1522 alone or the combination of pertuzumab + trastuzumab.

Antibody Selection Process

8 naïve libraries and 6 LC batch libraries

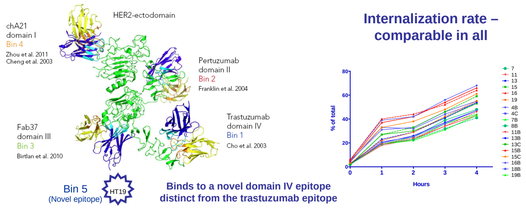


mAbs affinity

Clone Index	Fab KD human HER2-Fc (nM) monomeric	Fab KD rhesus HER2-Fc (nM) monomeric
HT1	P.F.	P.F.
HT2	+	+
HT3	+	+
HT4	+	+
HT5	P.F.	P.F.
HT6	++	++
HT7	++	++
HT8	++	++
HT9	+	W.B.
HT10	++	P.F.
HT11	++	N.B.
HT12	+	N.B.
HT13	+	+
HT14	+	N.B.
HT15	+	W.B.
HT16	++	+
HT17	+	W.B.
HT18	+	+
HT19	+	+

++ <10 nM
+ > 10 nM
P.F. poor fit
W.B weak binder
N.B. non binder

Binning by competition with known mAbs

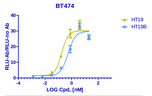


Internalization rate – comparable in all

ADC In vitro cytotoxicity – not necessarily predictive of in vivo efficacy

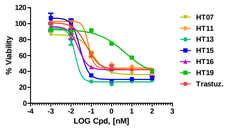
	HT1	HT2	HT3	HT4	HT5	HT6	HT7	HT8	HT9	HT10	HT11	HT12	HT13	HT14	HT15	HT16	HT17	HT18	HT19	Trastuzumab
EC50	1.30	1.12	1.27	1.24	0.80	0.09	0.12	1.24	0.76	0.86	0.08	0.95	0.02	0.02	0.04	0.02	0.08	0.84	0.32	0.06

HT19 Shows ADCC Activity



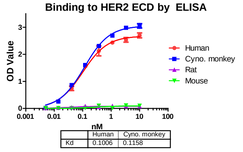
	HT19	HT19B (affinity matured)
EC_{50}	0.18	0.54

CellTiter-Glo® Cell Viability Assay



Lead Antibody is cross-reactive to NHP

Binding to HER2 ECD by ELISA

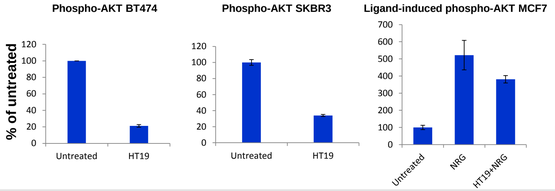


Kd	Human	Cyno. monkey
	0.1006	0.1158

HT19 Binding Kinetics
Human and Cynomolgus monomeric HER2-ECD; FortéBio

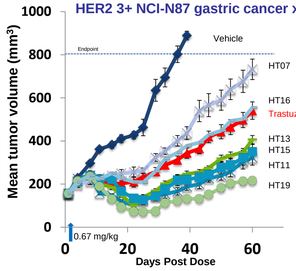
Cross-reactivity	KD (nM)	k_{on}(1/Ms)	k_{dis}(1/s)	n
Human	18.0	4.60E+05	7.54E-03	3
Cynomolgus	15.5	3.14E+05	4.56E-03	3

Lead Antibody inhibits the HER2 signaling pathways



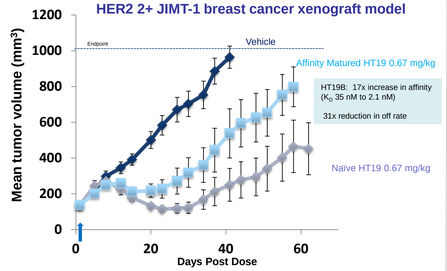
In Vivo Lead Selection, Dolaflexin ADCs

HER2 3+ NCI-N87 gastric cancer xenograft model



In vivo performance: possible correlation with high off rate

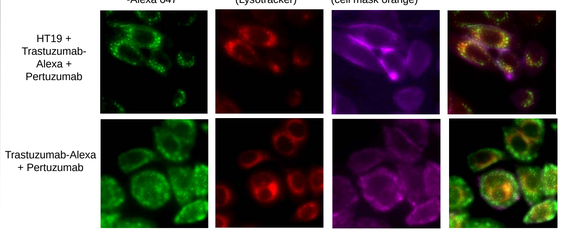
HER2 2+ JIMT-1 breast cancer xenograft model



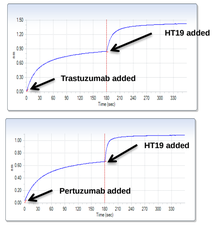
Affinity maturation resulted in in vivo activity drop

Lead antibody HT19 is combinable with trastuzumab and pertuzumab

Enhanced internalization

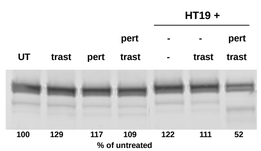


HT19 binds to a different HER2 epitope than either trastuzumab or pertuzumab



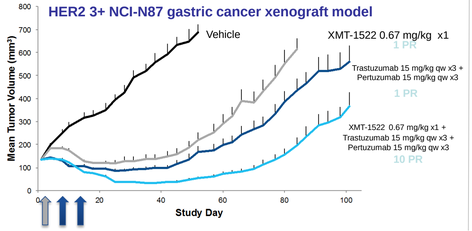
HT19 in combination causes degradation of HER2

SKBR3 cell line, 24 hr



HT19 ADC in combination with existing therapies improves in vivo efficacy

HER2 3+ NCI-N87 gastric cancer xenograft model



Discussion and Conclusions

- In vivo data is crucial to select lead mAb for ADC
- In vitro cytotoxicity is not necessarily predictive of in vivo efficacy
- Affinity maturation did not translate into increases in ADC in vivo efficacy for the ADCs. All ADCs of affinity matured leads were inferior to parental versions
- High off rate could be a factor for high in vivo efficacy
- Lead antibody HT19 shows ADCC activity
- Lead antibody HT19 inhibits the HER2 signaling pathways
- Lead antibody HT19 binds to a different epitope of HER2 than either trastuzumab or pertuzumab and therefore is combinable with trastuzumab and pertuzumab
- The combination of XMT-1522 with trastuzumab and/or pertuzumab showed more rapid internalization, more complete HER2 degradation, and significantly great anti-tumor activity in the NCI-N87 gastric cancer xenograft model relative to XMT-1522 alone or the combination of pertuzumab + trastuzumab

Acknowledgements

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