Mechanism of action of MCLA-128, a humanized bispecific IgG1 antibody targeting the HER2:HER3 heterodimer

Cecile Geuijen, Eric Rovers, Tristan Gallenne, David Maussang-Detaille, Arjen Kramer, Nellie Nieuwenhuizen, Katinka van Zoest, Roy Nijhuis, Therese Visser, Renate den Blanken-Smit, Abdul Basmeleh , Willem Bartelink, Vanessa Zondag-van der Zande, Carina Clements, Linda Kaldenberg, Pieter Fokko van Loo, Rob Roovers, Leo Price, Stefan Braam, Robert Doornbos, Setareh van Driel Shamsili, Lex Bakker, Ton Logtenberg, John de Kruif, Mark Throsby; **Merus BV, Utrecht, the Netherlands**

Background

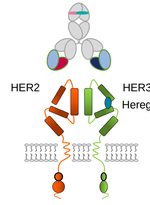
HER heterodimers and HER3 mediated resistance

- HER2 is amplified/overexpressed in ~20% of breast cancer patients
- Amplification/overexpression correlates with poor clinical outcome
- HER3 *buffering* of HER family member signalling is an important mechanism of adaptive resistance[1]
- HER3 or Heregulin (HRG) expression is a prognostic marker for shorter survival times (e.g. mCRC, mBC)[2]

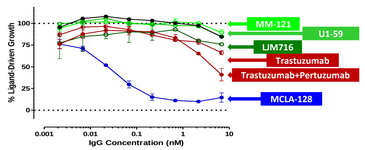
[1] Sergina, N.V., et al. (2007). Escape from HER-family tyrosine kinase inhibitor therapy by the kinase-inactive HER3. Nature *445*, *437-441*.
[2] Ocana, A., et al. (2012). HER3 overexpression and survival in solid tumors: a meta-analysis. J Natl Cancer Inst *105*, *266-273*.

MCLA-128 – HER2:HER3 bispecific antibody

- MCLA-128 combines common light chain Fab regions with CH3 electrostatic engineering in the constant region to drive asymmetric IgG1 formation
- MCLA-128 specifically targets the HER2:HER3 heterodimer and blocks HER3/HRG signaling
- MCLA-128 shows superior activity *in vitro* and *in vivo* compared to HER2 and HER3 monoclonal antibodies

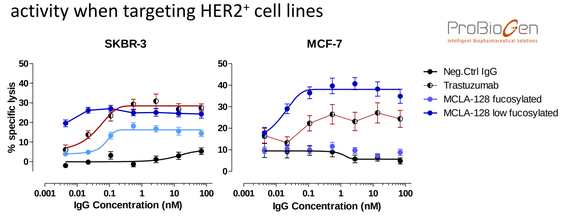


MCLA-128 – potently inhibits HRG mediated growth



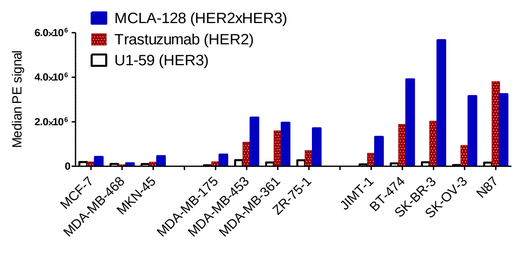
MCLA-128 has potent ADCC effector function

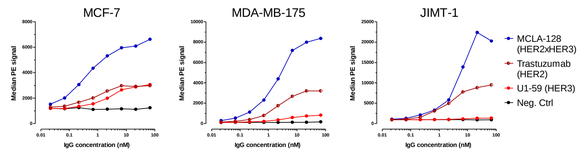
- MCLA-128 uses GlymaxX® technology to enhance ADCC activity
- MCLA-128, a low-fucosylated IgG1, has equivalent ADCC activity to Trastuzumab when targeting HER2^{+++} cell lines and superior ADCC activity when targeting HER2^{+} cell lines



MCLA-128 has superior binding than HER2 and HER3 mAbs

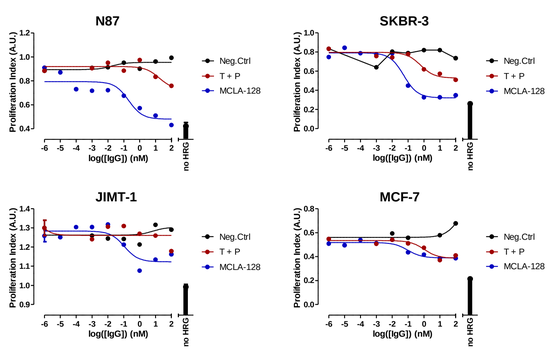
- MCLA-128 binding to a panel or cell lines was compared to HER2 and HER3 monoclonal antibodies using FACS
- MCLA-128 binds breast cancer cell lines expressing HER2 at different levels with greater avidity than HER2 & HER3 mAbs





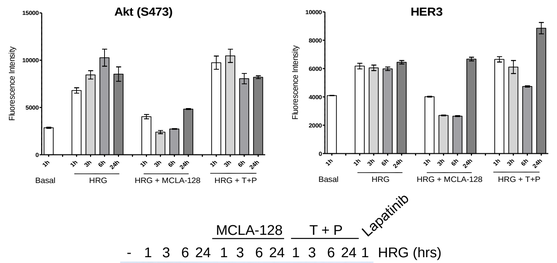
MCLA-128 inhibits cell cycle progression

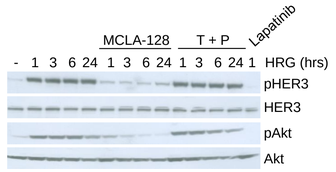
- The effect of MCLA-128 on cell cycle progression was measured in different cell lines incubated with heregulin at high concentration
- MCLA-128 inhibits cell cycle progression with a higher potency than the combination of HER2 mAbs Trastuzumab + Pertuzumab



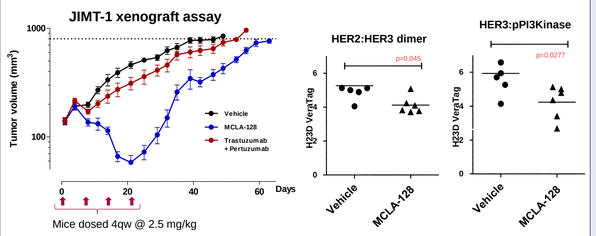
MCLA-128 inhibits HER3 and Akt phosphorylation and HER2:HER3 dimerization *in vitro* and *in vivo*

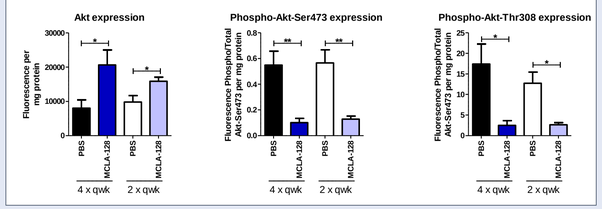
- Phosphorylation of HER receptors and downstream signaling pathways were analyzed in HRG stimulated N87 cells using PathScan antibody arrays and Western blot experiments
- MCLA-128 inhibited HRG-induced HER3 and Akt phosphorylation more potently than Trastuzumab + Pertuzumab





- The effect of MCLA-128 on HER2:HER3 dimerization and HER3:PI3Kinase interaction was investigated *in vivo* in the Trastuzumab-resistant JIMT-1 cell line using VeraTag™ assay
- The effect of MCLA-128 on Akt phosphorylation *in vivo* was investigated using the luminex beads assay
- Growth inhibition by MCLA-128 was correlated with a reduced HER2:HER3 dimerization and a profound inhibition of the PI3K pathway

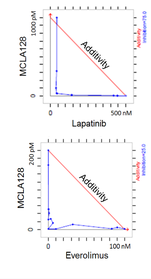


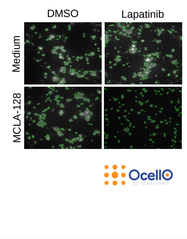


MCLA-128 synergizes with small molecule inhibitors

- Activity of MCLA-128 in combination with Tyrosine kinase inhibitors, small molecules targeting the MAPK and PI3 kinase/Akt pathways in the presence of HRG at high concentration was determined by proliferation inhibition and high content imaging assays in HER2 amplified cell lines
- Synergistic growth inhibition was observed with various agents including tyrosine kinase inhibitors and inhibitors of the PI3 kinase pathway

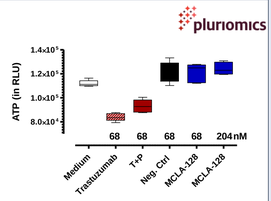
Synergistic drug	Target
Lapatinib	EGFR/HER2
Apatinib	EGFR/HER2
Neratinib	EGFR/HER2
BYL719	PI3 Kinase
MK-2206	Akt
Everolimus	mTOR
Saracatinib	Src/Abl
Vorinostat	HDAC
Paclitaxel	Tubulin





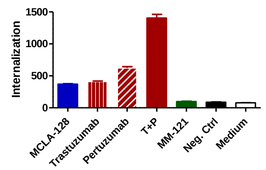
Absence of MCLA-128 induced cardiotoxicity

- The potential toxicity of MCLA-128 on primary cardiomyocytes in the presence of Doxorubicin was analysed by measuring intracellular ATP levels
- In contrast to Trastuzumab (+/- pertuzumab), MCLA-128 did not show any sign of cardiotoxicity *in vitro*



MCLA-128 shows low-level of internalization

- The ability of MCLA-128 to induce receptor internalization was investigated in SKBR-3 cells using pH-sensitive dye labelling (Promega)
- MCLA-128 showed a similar internalization as Trastuzumab and lower internalization than the combination Trastuzumab + Pertuzumab



Conclusions

- MCLA-128 blocks heregulin mediated resistance
- The unique simultaneous targeting of MCLA-128 to HER2 and HER3 leads to potent inhibition of AKT/PI3 kinase pathway signalling
- MCLA-128 shows enhanced binding to HER2 expressing cell lines compared to Trastuzumab
- MCLA-128 exhibits potent ADCC activity regardless of FcγRIIIa affinity
- A First-In-Human study with MCLA-128 is currently ongoing in patients with solid tumors