The binding mode of the bispecific anti-HER2xHER3 antibody MCLA-128 is responsible for its potent inhibition of HRG-driven tumorigenesis

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Introduction

- 20-25% of breast cancer patients overexpress HER2 and receive anti-HER2 targeted therapies.
- Resistance to treatment can occur through activation of the HER2:HER3 signaling pathway by the HER3 ligand heregulin (HRG).
- The anti-HER2xHER3 bispecific antibody MCLA-128 was developed to inhibit HRG-induced HER2:HER3 signaling.
- MCLA-128 showed to be more potent than other anti-HER2 and anti-HER3 antibodies to inhibit proliferation of HER2-amplified cells at high concentrations of HRG. (Soucy et al. 2015 ASCO)

Specific inhibition of dimerization

- Ligand-induced dimerization of ErbB family members (EGFR, HER2, HER3 and HER4) was evaluated in β-galactosidase complementation assays.
- MCLA-128 specifically inhibited HER2:HER3 dimerization. Pertuzumab inhibited all HER2-containing dimer pairs with EGFR, HER3 or HER4.

Dose-dependent inhibition of JIMT-1 tumors

- JIMT-1 cells are isolated from a HER2-amplified breast cancer patient who was resistant to Trastuzumab treatment.
- JIMT-1 xenografts (CB17, SCID mice) were treated with different doses of MCLA-128 weekly and tumor volume as well as IgG titers were evaluated 24 days post treatment initiation.
- MCLA-128 treatment efficacy correlated with IgG serum levels.

Increased in vivo tumor-targeting of bispecific antibody

- Anti-HER2 and anti-HER2xHER3 antibodies with the same epitope-targeting at MCLA-128 were radio-labelled with 111In for PET-CT imaging to evaluate tumor-targeting activity and biodistribution in mice bearing JIMT-1 xenografts.
- Bispecific anti-HER2xHER3 antibody better targeted JIMT-1 tumors in comparison to the anti-HER3 monospecific antibody and presented a higher percentage of injected dose per gram tissue in the tumors.

Inhibition of tumor growth in HRG-rich environment

- ST1360B patient-derived xenograft (PDX) tumor cells were engrafted into the brain of mice by stereotactic injection and tumor growth was monitored by T2-weighted magnetic resonance imaging (MRI).
- ST1360B have been generated from HER2-amplified breast cancer patient and express high levels of HER2 and HER3 protein.
- Mice treated with MCLA-128 (25 mg/kg, twice per week) survived during the 3-week treatment period (blue shaded), while control animals all succumbed.

Inhibition of NRG1 gene fusion-mediated signaling

- NRG1 gene fusions activate HER2:HER3 signaling.
- The DOCA-NRG1 and CLU-NRG1 gene fusions are expressed in the MDA-MB-175 cell line (breast) and in the OV-10-0050 PDX (ovarian), respectively.
- In vitro, MCLA-128 treatment inhibits MDA-MB-175 cell proliferation.
- In vivo, MCLA-128 treatment (25 mg/kg weekly until day 28) reduced tumor growth and eliminated tumors in 6/8 animals.

Crystallography studies

- Fab:ECD structures were solved at 3 Å (Rfree/Rwork of 0.23/0.27) and 3.4 Å (Rfree/Rwork of 0.21/0.25) for HER2 and HER3, respectively.
- Small Angle X-ray Scattering (SAXS) model was performed in solution with HER2 and HER3 ECDs in complex with the full IgG of MCLA128.
- Anti-HER2 Fab (blue, green) binds to C-terminal part of HER2 (sand) domain I.
- Anti-HER3 Fab (orange, green) binds HER3 (yellow) domain III, nearby the putative HRG binding site.
- MCLA-128 binds HER2 and HER3 ECD in solution with a 140 Å distance between HER2 and HER3 epitopes.

Epitope mapping

- Shotgun mutagenesis analysis identified: T514, R316 and R318 as critical residues for binding of Fab to HER2 ECD;
- P172 and G179 as secondary critical residues for binding of Fab to HER2 ECD;
- R426 as critical residue for binding of Fab to HER3 ECD.

MCLA-128 Fab (blue, green) interacts with HER2 (sand) via the three CDR regions of the heavy chain (interaction surface of 683 Å2).

MCLA-128 Fab interacts with HER3 (yellow) via both heavy and light chain (interaction surface of 238 and 188 Å2, respectively).

Conclusion

- MCLA-128 specifically inhibits the HER2:HER3 dimer and targets JIMT-1 tumors in vivo to a greater extent than anti-HER3 antibody.
- The growth of HRG-driven tumor models are efficiently inhibited by MCLA-128.
- MCLA-128 docks to HER2 domain I (exposed N-terminal domain) and blocks HER3 domain III (putative HRG binding domain).