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

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Background
HER heterodimers and HER3 mediated resistance
- HER2 is amplified/overexpressed in ~20% of breast cancer patients
- Amplification/overexpression correlates with poor clinical outcome
- HER2 overexpressing of HER family member signaling is an important mechanism of adaptive resistance
- HER3 or heregulin (HRG) expression is a prognostic marker for shorter survival times (e.g. MBC, MB)
- HER2/HER3 heterodimer and blocks HER2/HER3 signaling
- HER3 monoclonal antibodies
- HER2 shows superior activity in vitro and in vivo compared to HER2 and HER3 monoclonal antibodies

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 HER2/HER3 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 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 has superior binding than HER2 and HER3 mAbs
- The effect of MCLA-128 on HER2 HER3 dimerization and HER2:PI3K kinase interaction was investigated in vitro 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 luminescent 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 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

Absence of MCLA-128 induced cardiotoxicity
- The potential toxicity of MCLA-128 on primary cardiomyocytes in the presence of Doxorubicin was assessed by measuring intracellular ATP levels
- In contrast to Trastuzumab (r- 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 (Promerge)
- 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/PI3K kinase pathway signalling
- MCLA-128 shows enhanced binding to HER2 expressing cell lines compared to Trastuzumab
- MCLA-128 exhibits potent ADCC activity regardless of HER2 and HER3 heterodimers
- A First-In-Human study with MCLA-128 is currently ongoing in patients with solid tumors

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 determined by proliferation inhibition and high content imaging assays in HER2 amplified cell lines
- Synergistic growth inhibition was observed in various agents including tyrosine kinase inhibitors and the PI3 kinase pathway

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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 PhosCan antibody arrays and Western blot experiments
- MCLA-128 inhibited HRG-induced HER3 and Akt phosphorylation more potently than Trastuzumab + Pertuzumab

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