Highly cytotoxic, completely human constructs targeting HER2 and containing the immune-oncology payload granzyme B

Lawrence H. Cheung¹, Yunli Zhao², Khalid A. Mohamadali¹, Ana Alvarez-Cienfuegos¹, Walter N. Hittelman¹ and Michael G. Rosenblum¹
¹M.D. Anderson Cancer Center, Houston, TX; ²Shenyang Pharmaceutical University, Shenyang, China

Summary
- Our laboratory has generated a novel, construct composed of the anti-HER2 single-chain 4DS, GrB and human IgG heavy chain fragment.
- This is a bivalent, highly cytotoxic construct comparable in activity to Kadcyla.
- The construct is active against both log-phase and confluent cells.
- The GrB-4DS has a long serum half-life (~39 hrs) and is virtually non-toxic to mice.
- In vivo efficacy studies against human tumor xenografts are ongoing.
- This appears to be an excellent candidate for clinical development against HER2-expressing tumors.

Abstract
Recent immunotherapeutic approaches designed to augment T- and B-cell mediated killing of tumor cells has met with clinical success in recent years suggesting that immune-oncology (IO) approaches have tremendous potential for treatment in a broad spectrum of tumor types. After complex recognition of target cells by T and B cells, delivery of the serum protease granzyme B (GrB) to tumor cells comprises the cytotoxic insult resulting in a well-characterized, multimediated apoptotic cascade. We designed a recombinant fusion construct composed of a human anti-HER2 scFv fused to active GrB for recognition of tumor cells and delivery of GrB to tumor cells simulating T- and B-cell therapy. The GrB-4DS dimeric construct (MW 160 kDa) was generated and expressed in stably-transfected CHO-S cells at ~40 mg/L and purified to homogeneity. The enzymatic activity of the fusion construct was similar to commercially-available GrB and the affinity of the construct for purified Her2 extracellular domain (ECD) was 0.328 nM and was comparable to that of Herceptin (0.150 nM). The GrB-4DS construct was highly cytotoxic to HER2 positive cells such as SKBR3, MCF7 and MDA-MB-231 with IC50 values of 56, 99 and 27 nM respectively. Upon immunofluorescence, the fusion construct internalized rapidly into target (SKBR3 or SKOV3) cells within 1 hour of exposure rapidly delivering GrB to the cytoplasmic compartment in a manner similar to that of immune T- and B-cell targeting but without the action of the transmembrane pore-forming agent perforin. Against a large panel of various tumor types, GrB-4DS was highly cytotoxic to virtually all cells regardless of natural expression levels of the PI-9 inhibitor. Contemporaneous studies with Kadcyla demonstrate similar levels of in vitro activity against virtually all cell tested. Both GrB-4DS demonstrated activity against log-phase and confluent tumor cells. In keeping with its relatively high molecular weight (~160 kDa), the construct demonstrated an terminal-phase half-life of ~92 hours. In vivo efficacy studies were performed in several orthotopic xenograft models. Also ongoing are toxicology, histopathology and clinical chemistry studies. Research conducted, in part, by the Clayton Foundation for Research.

Mechanistic studies of GrB-4DS
GrB-Fc-4DS has similar cytotoxicity against both log-phase and confluent cells

Pharmacokinetic profile of GrB-Fc-4DS in BALB/c mice

Cytotoxicity of GrB-4DS is similar to Kadcyla in virtually all tested cell lines

DO NOT POST

Humanized Anti-HER2 scFv (4DS)

- 26 kDa humanized scFv antibody derived from the published Trastuzumab light and heavy chain variable domain sequence
- Linked together by a 218 flexible linker
- High affinity against the extracellular epitope p185 HER2/neu
- Internalized rapidly via receptor endocytosis

Granzyme B (GrB)

- 25 kDa Human serum protease
- Normally enters cells through perforin-generated pores
- Causes release of cytochrome c from mitochondria
- Initiates apoptosis by activating procaspases -3, -7 and -9
- Cleaves nuclear matrix proteins
- Can initiate apoptosis via caspase-independent mechanisms

GrB-Fc-4DS internalizes rapidly within 1 hour into both SKBR3 or SKOV3 cells

GrB-Fc-4DS administration at 100 mg/kg was well-tolerated in mice

Kadcyla was purified and administered at 35 mg/kg (400-µg GrB-4DS (or MAb) (Control)

Herceptin was diluted at 50 µg/ml

100% of mice were sacrificed 24th-hour post-therapy

Summary

- The construct is active against both log-phase and confluent cells.
- The GrB-4DS has a long serum half-life (~39 hrs) and is virtually non-toxic to mice.
- In vivo efficacy studies against human tumor xenografts are ongoing.
- This appears to be an excellent candidate for clinical development against HER2-expressing tumors.