Preclinical characterization of DF1001, a first-in-class dual NK and T cell engager targeting HER2-high and HER2-low tumors

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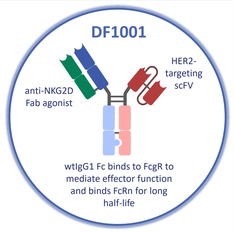
DF1001 harnesses multiple mechanisms to drive anti-tumor activity

DF1001 is a first-in-class TriNKET® immune engager, designed to stimulate both innate and adaptive immune effector cells, including NK, CD8⁺ T, NKT, and γδ T cells, through engagement of the activating receptors CD16a and/or NKG2D. DF1001 redirects immune cell effectors against tumor cells by anchoring to HER2, a clinically validated target that is widely expressed across breast, gastric, esophageal, lung, bladder, and other tumor indications.

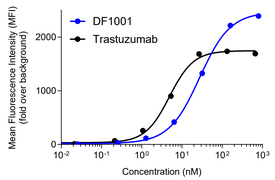


Images created with BioRender.com

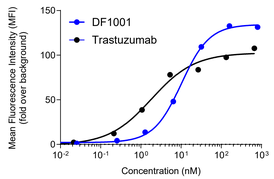
DF1001 is uniquely differentiated from HER2-targeted therapeutics due to its distinct immune-engaging mechanisms of action which are active even against HER2-low expressing cancers, where HER2 monoclonal antibodies such as trastuzumab are ineffective. In addition to DF1001's ability to target a range of HER2 expressing tumors, its favorable preclinical safety profile enables therapeutic combination with standard of care agents, including the Trop2-targeted ADC sacituzumab govitecan-hziy, which is approved for the treatment of metastatic breast cancer.

DF1001 binds to HER2 with high affinity inhibiting HER2-signaling leading to in vitro and in vivo anti-tumor activity

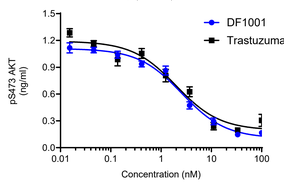
SKBR-3 (Breast) Tumor Cell Binding



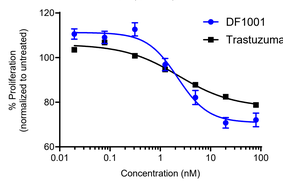
NCI-H661 (Lung) Tumor Cell Binding



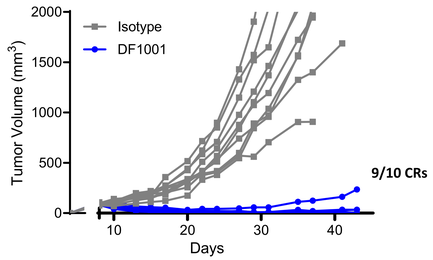
AKT Phosphorylation Inhibition
SKBR-3 (Breast) tumor cells



Tumor Cell Growth Inhibition
NCI-N87 (Gastric) tumor Cells

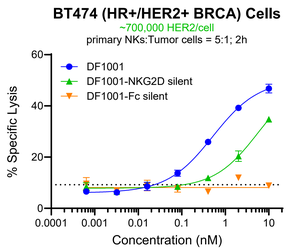


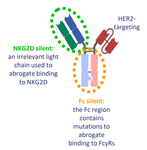
In Vivo Efficacy
OE19 (EAC) xenograph; nude mice
HER2-positive, erbb2 amp



Maximal cytotoxicity is achieved through engagement of both CD16a and NKG2D

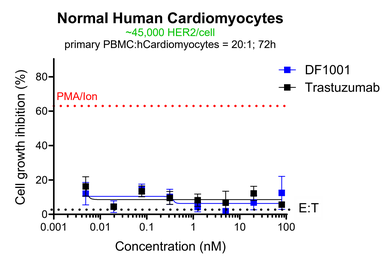
BT474 (HR+/HER2+ BRCA) Cells
~700,000 HER2/cell
primary NKs:Tumor cells = 5:1; 2h





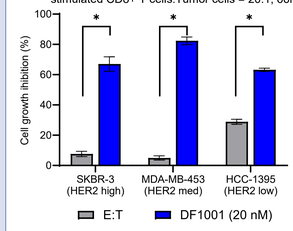
DF1001 spares healthy HER2-expressing cells

Normal Human Cardiomyocytes
~45,000 HER2/cell
primary PBMC:hCardiomyocytes = 20:1; 72h

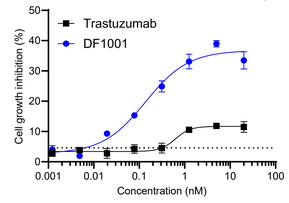


DF1001 triggers potent cytotoxic CD8+ T cell responses via NKG2D stimulation

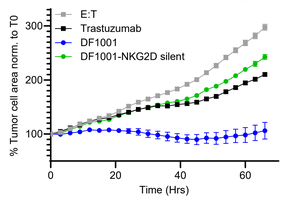
CD8⁺ T Cell-Mediated Tumor Killing
stimulated CD8+ T cells:Tumor cells = 20:1, 66h



CD8⁺ T Cell Killing: Dose Response
stimulated CD8+ T cells:SKBR-3 cells = 20:1, 24h



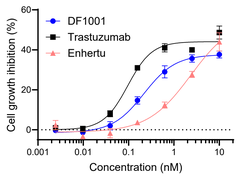
CD8⁺ T Cell-Mediated Killing: Timecourse
stimulated CD8+ T cells:SKBR-3 cells = 20:1; 20nM



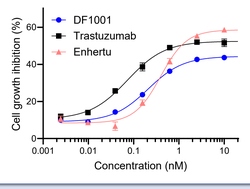
DF1001 demonstrates potent anti-tumor activity, outperforming clinical benchmarks in tumor cells expressing medium to low HER2 levels

HER2 High
IHC: 3+

SK-OV-3 (Ovarian) Cells
~870,000 HER2/cell
primary NKs:Tumor cells = 5:1; 72h

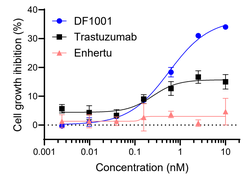


MDA-MB-453 (HER2+ BrCa) Cells
~90,000 HER2/cell
primary NKs:Tumor cells = 5:1; 72h

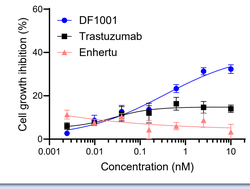


HER2 medium
IHC: 1-2+

MKN-45 (Gastric) Cells
~50,000 HER2/cell
primary NKs:Tumor cells = 5:1; 72h

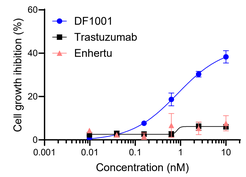


HT29 (CRC) Cells
~25,000 HER2/cell
primary NKs:Tumor cells = 5:1; 72h

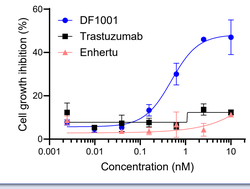


HER2 Low
IHC: "0"-1+

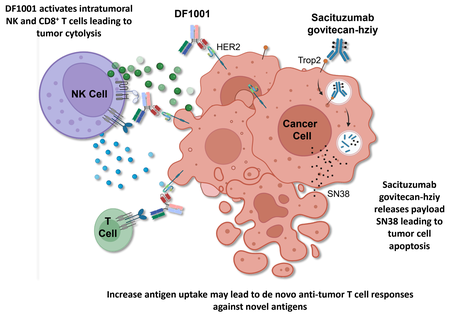
786-0 (RCC) Cells
~10,000 HER2/cell
primary NKs:Tumor cells = 5:1; 72h



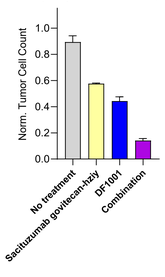
HCC1395 (TNBC) Cells
~2,000 HER2/cell
primary NKs:Tumor cells = 5:1; 72h



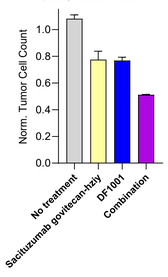
DF1001 and sacituzumab govitecan-hziy kill tumor cells via complementary mechanisms resulting in enhanced anti-tumor activity in combination



SK-OV-3 (Ovarian) Tumor Cells
HER2 high, TROP2 high
primary NKs:Tumor cells = 5:1; 96h

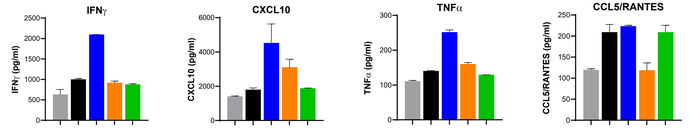


HCC1395 (TNBC) Tumor Cells
HER2 low, TROP2 low
primary NKs:Tumor cells = 5:1; 96h

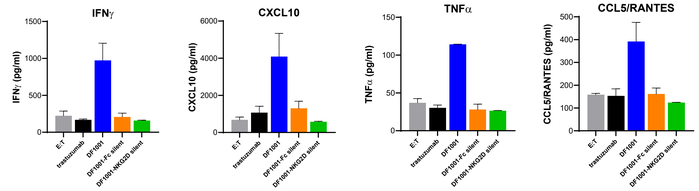


DF1001 promotes the release of pro-inflammatory factors upon tumor engagement, which can facilitate the recruitment of immune effector cells

SKBR-3 (Breast) Tumor Cells
HER2-positive; IHC 3+, erbb2 amp
~600,000 cell surface HER2 molecules
stimulated NK cells:Tumor cells = 1:1
15 nM test article; 24h

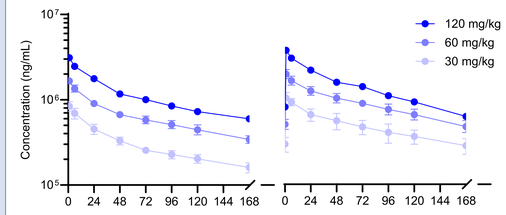


HCC-1395 (TNBC) Tumor Cells
HER2-negative; IHC"0"
~2,000 cell surface HER2 molecules
stimulated NK cells:Tumor cells = 1:1
60 nM test article; 24h



DF1001 was well tolerated in a repeat-dose GLP intravenous NHP study

DF1001 exhibits an antibody-like PK profile



DF1001 was well-tolerated up to the highest dose tested (120 mg/kg/week) in a 4-week GLP study in cynomolgus monkeys

- **DF1001 was well tolerated with no adverse toxicity**
  - No effects on body weight, ECG parameters, respiratory rate, blood pressure, ophthalmology findings
  - No elevation in systemic cytokines was observed
  - A higher incidence of non-adverse vascular/perivascular infiltrates was observed, which were reversible
- NOAEL identified in 4-week GLP study was top dose of 120 mg/kg/week

DF1001's potent anti-tumor activity and favorable preclinical safety profile make it a promising therapeutic for solid tumors

The DF1001 TriNKET® targets robust immune-mediated activities, which enhances cytolytic responses directed at both HER2-high and HER2-low tumors and elevates local release of inflammatory cytokines/chemokines without causing cytokine-release syndrome. DF1001's favorable preclinical safety profile and distinct immune-engaging MOA positions it to complement and synergize with SOC agents such as sacituzumab govitecan-hziy in the HER2-low solid tumor setting. DF1001 is currently under evaluation in patients with locally advanced or metastatic solid tumors in PhI/II trial (NCT04143711).