Synergy of an anti-HER2 ADC TAK-522 (XMT-1522) in combination with anti-PD1 mAb in a syngeneic breast cancer model expressing human HER2

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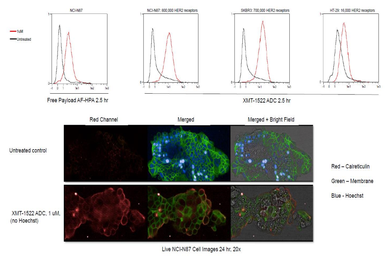
Abstract

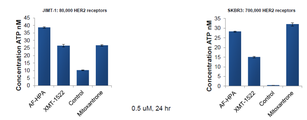
Antibody-drug conjugates (ADCs) are a highly potent class of drugs that specifically target cancer cells expressing a tumor associated antigen (TAA). The ADC TAK-522 (XMT-1522) consists of a novel human IgG1 anti-HER2 monoclonal antibody and a novel, auristatin-based cytotoxic payload (Auristatin F-hydroxypropylamide, AF-HPA). An average DAR of twelve AF-HPA molecules is achieved via a biodegradable polymer conjugation platform. We have characterized the ability of both the free payload AF-HPA and the ADC TAK-522 to induce immunogenic cell death (ICD) in vitro in multiple cell lines (NCI-N87, HT-29, SKBR3), as measured by cell surface expression of the ICD marker calreticulin (CRT) using microscopy and flow cytometry. CRT, usually contained in the lumen of the endoplasmic reticulum, translocated to the cell surface within a few hours after treatment with AF-HPA or TAK-522. Furthermore, we developed a novel syngeneic breast cancer (4T1) model expressing human HER2 at a relatively low antigen density. Treatment in this poorly immunogenic tumor model with TAK-522 but not Kadcyla showed significant inhibition of tumor growth *in vivo*. Importantly, a combination of anti-PD1 mAb and TAK-522 therapy substantially enhanced the anti-tumor efficacy synergistically, resulting in complete responses in some mice. The frequency of complete responders was further increased when the two drugs were sequentially, rather than concurrently, administered such that TAK-522 administration was followed by anti-PD1 mAb therapy. These results suggest an immunological mechanism involving induction of immunogenic cell death by TAK-522, which in turn may activate the adaptive immune system by releasing tumor specific antigens. TAK-522 is currently being tested in a phase-1b clinical trial in patients with advanced breast, lung and gastric cancer expressing HER2. Based on our data, TAK-522 represents a potential candidate for combination therapies with immune checkpoint modulators in patients with poorly immunogenic HER2 expressing tumors.

Immunological effects of TAK-522

TAK-522 induces Immunogenic Cell Death (ICD) hallmarks *in vitro*

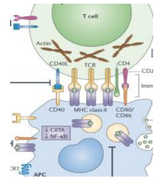


•The cell surface expression of calreticulin, CRT, usually contained in the lumen of the endoplasmic reticulum, translocated to the cell surface within a few hours after treatment with AF-HPA or TAK-522 alone



•ATP release was confirmed after treatment with AF-HPA or TAK-522

DC maturation/ activation induced by Aur F HPA Payload



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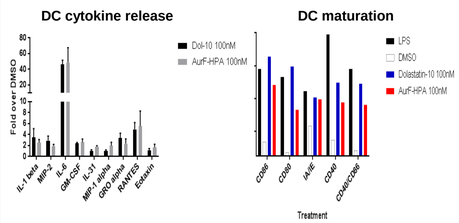
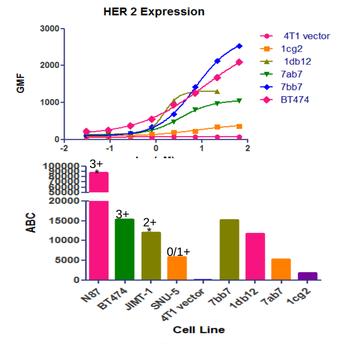


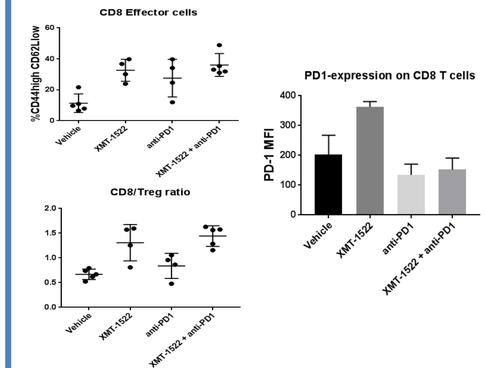
•Mouse bone marrow derived dendritic cells were treated with Dolastain-10 or AF-HPA for 20h. Bar graphs show (A) cytokine levels in supernatants and (B) DC activation/maturation markers measured by flow cytometry after respective treatments as labeled. LPS was used a control for DC maturation.

Generation of a mouse breast cancer model expressing human Her2



We lentivirally transduced 4T1 (a mouse triple negative breast cancer cell line) with human HER2. The transduced cells were selected and sub-cloned by limited dilution to generate four different human HER2 expressing clones, i.e. 7bb7, 1db12, 7ab7 and 1cg2. The expression level of human Her2 was tested in these clones and different human Her-2+ cancers (N87, BT474, JIMT-1 and SNU-5). We used the clone 4T1-7bb7, which expressed the highest level of human Her-2, to test the *in vivo* efficacy and immunological mechanisms of TAK-522 in a fully immune competent host.

TAK-522 increases the tumor infiltration of CD8+ T cells



Tumors from mice treated with vehicle, TAK-522, and anti-PD1 alone or in combination as labeled were harvested for the analysis of different immune subsets using Flow Cytometry. A) TAK-522 treatment enhanced the frequency of effector CD8 T cells in the tumors, B) This correlated with a higher CD8/Treg ratio; a small but consistent decrease in Treg frequency was also observed (data not shown). C) Importantly, PD-1 expression on CD8 T cells was enhanced after TAK-522 treatment alone, which was reduced after combined therapy with TAK-522 and anti-PD1.

TAK-522 (XMT-1522)

Dolaflexin-based anti-HER2 ADC

Novel anti-HER2 Antibody:

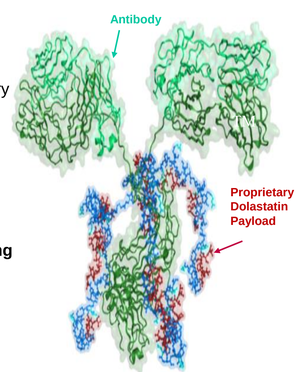
- Mersana proprietary
- Fully human IgG1 identified after screening Adimab yeast display library
- Optimized for internalization
- Binds to a **novel, distinct epitope** from trastuzumab or pertuzumab
  - Does not compete for binding

Novel Linker:

- Mersana Fleximer® polymer
- Allows for **much higher drug loading** (Average DAR ~15)
- Compatible with diverse payload classes

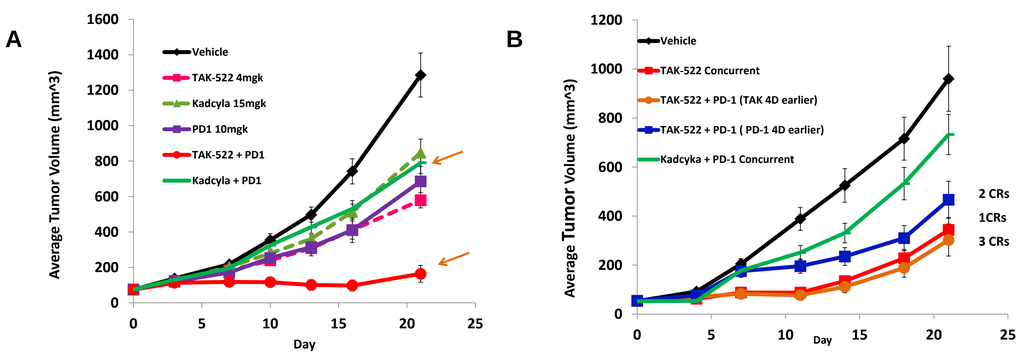
Proprietary payload:

- Dolastatin derivative with unique pharmacology



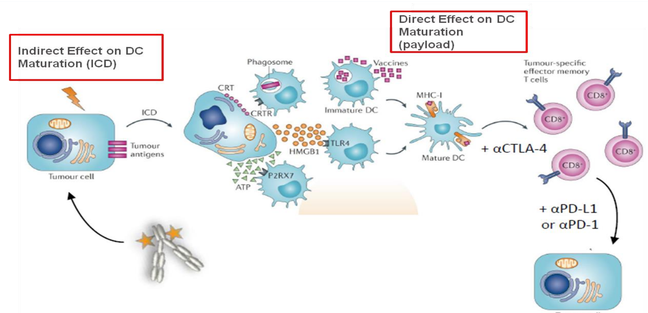
TAK-522 / XMT-1522

In Vivo efficacy Studies



Balb/c mice subcutaneously implanted with 4 x 10^4/mouse 4T1-7bb7 cells (n=10 mice/group) were treated with test compounds, TAK-522 DAR – 12.6, Kadcyla DAR – 4.3, anti-mouse PD1, and vehicle either alone or in different combinations, when tumors reached an average volume of 50 ± 80 mm^3. A) Treatment with TAK-522 or anti-PD1 as single agents showed significant inhibition of tumor growth *in vivo*. Importantly, a combination of anti-PD1 mAb and TAK-522, but not Kadcyla and anti-PD1 therapy, substantially and synergistically enhanced the anti-tumor efficacy, with complete response (CR) in one mouse. B) The frequency of complete responders was further increased when the two drugs were sequentially, rather than concurrently, administered such that TAK-522 administration was followed by anti-PD1 mAb therapy.

Summary and Model



➢TAK-522 induces immunogenic cell death (ICD) *in vitro* in multiple cell lines.

➢In a syngeneic breast cancer model expressing human HER2, TGI was observed with TAK-522 alone, which was enhanced in combination with anti-PD1 therapy, leading to complete responses in a few mice. Such activity was not observed with Kadcyla.

➢TAk-522 enhanced CD8+ T cell infiltration and PD-1 expression on CD8 T cells in the tumors.

➢The results are in support of a clinical trial withTAK-522/anti-PD1 combo in HER2 expressing cancers.