Antibody-Coupled T cell Receptor (ACTR) engineered autologous T cells in combination with trastuzumab for the treatment of HER2-positive malignancies

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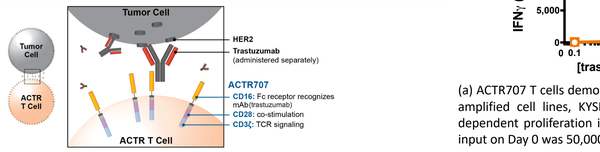
Introduction

The Antibody-Coupled T cell Receptor (ACTR) platform is an autologous engineered T cell therapy developed to combine with tumor-targeting antibodies to exert potent anti-tumor immune responses and tumor cell killing. The ACTR construct is composed of the extracellular domain of the high-affinity V158 variant of CD16 fused to CD3ζ signaling and T cell co-stimulatory domains. ACTR-expressing T cells are universal in that they can be paired with a therapeutic antibody to target specific antigens on tumor cells. The ACTR707 construct, which contains the CD28 costimulation domain, is being tested in combination with rituximab in a dose-escalation study in subjects with CD20+ B cell lymphoma (NCT03189836, Motz et al., AACR-NCI-EORTC 2017, Poster #B105).

While autologous T cell therapies, such as chimeric antigen receptor (CAR) T cells, have demonstrated clinical activity in hematological cancers, the therapeutic potential of this approach has yet to be established in solid tumors. Challenges associated with targeting solid tumors with CAR-T cells include tumor antigen heterogeneity and antigen expression on normal tissues. HER2 is a well-established therapeutic target that is over-expressed in a number of cancers. HER2 is also expressed at low levels on normal epithelial cells, creating a risk for on-target/off-tumor toxicities of HER2-targeted CAR-T cells. We have previously shown that ACTR707 + trastuzumab and HER2-targeting CAR-T cells have comparable in vivo anti-tumor activity against HER2-amplified xenograft tumors (O'Callaghan et al., AACR-NCI-EORTC 2017, Poster #A163). Here we demonstrate that trastuzumab-mediated activity of ACTR707 T cells is antigen density-dependent, with robust activity observed against HER2-amplified tumor cell lines, and modest activity against tumor cells that lack genetic amplification of HER2. In contrast, HER2 CAR-T cells have equivalent potency against HER2-high and HER2-low tumor cells. Furthermore, antibody-mediated activity of ACTR707 T cells was antigen-specific and titrated with antibody concentration, thereby allowing for control of ACTR activity by modulation of trastuzumab concentration. On primary human cells that express low levels of HER2, ACTR707 + trastuzumab had negligible activity in comparison to HER2 CAR-T cells, suggesting that ACTR + trastuzumab may exhibit an improved clinical therapeutic index. Together, these data demonstrate the specificity of the ACTR T cell therapeutic approach to target HER2-amplified tumors, and support clinical testing in combination with trastuzumab..

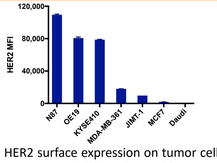
The Phase 1 study, ATTCK-34-01 (NCT03680560), is a multicenter, single-arm, open-label dose escalation study evaluating ACTR in combination with trastuzumab in subjects with advanced HER2-positive malignancies. The primary study objectives are to assess the safety and tolerability of the combination, and to define dose recommendations for further study. The ATTCK-34-01 study is expected to initiate by the end of 2018.

The Antibody-Coupled T cell Receptor (ACTR) platform



Schematic of the ACTR platform. The high-affinity variant of the Ig Fc receptor CD16 (V158) engages the Fc domain of tumor-targeting human IgG1 antibodies and triggers T cell activation and tumor cell cytotoxicity. The ACTR platform has demonstrated activity with multiple tumor-targeting antibodies. ACTR T cells combined with rituximab or an afucosylated BCMA-targeting antibody are currently in clinical trials (NCT02776813, NCT03189836, and NCT03266692).

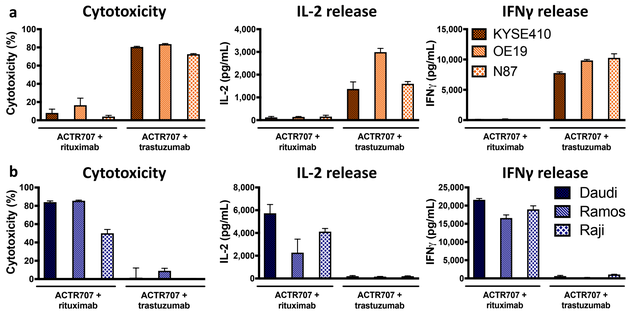
HER2 protein expression across tumor cell lines



Cell line	Tissue	*HER2* status
N87	gastric	amplified
OE19	esophagus	amplified
KYSE410	esophagus	amplified
MDA-MB-361	breast	amplified
JIMT-1	breast	amplified
MCF7	breast	non-amplified
Daudi	Burkitt's lymphoma	negative

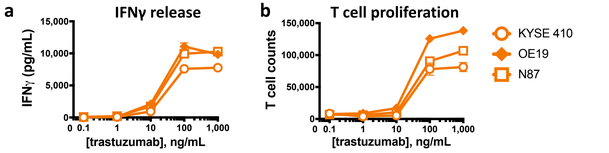
HER2 surface expression on tumor cell lines was determined by flow cytometry after staining with an anti-human HER2 antibody. Mean fluorescence intensity (MFI) of stained cells is represented on the bar graph. *HER2* gene copy number was described in the Cancer Cell Line Encyclopedia (CCLE).

Antibody-coupled ACTR707 T cell activity on HER2-amplified tumor cell lines is antigen- and antibody-specific



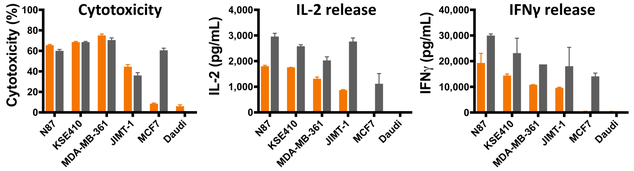
(a) ACTR707 T cells demonstrate functional activity on HER2-amplified tumor cells (KYES410, OE19 and N87) in the presence of trastuzumab (1 μg/mL), but not in the presence of the CD20-targeting antibody, rituximab (1 μg/mL). (b) On CD20-positive, HER2-negative lymphoma cell lines (Daudi, Ramos and Raji), ACTR707 has activity in the presence of rituximab but not trastuzumab.

Trastuzumab-mediated ACTR707 T cell activity on HER2-amplified tumor cell lines titrates with antibody concentration



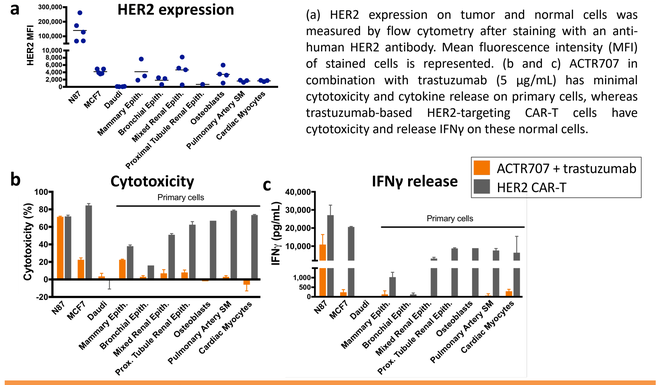
(a) ACTR707 T cells demonstrate trastuzumab concentration-dependent IFNγ release in the presence of the HER2-amplified cell lines, KYSE410, OE19 and N87. (b) ACTR707 T cells demonstrate trastuzumab concentration-dependent proliferation in the presence of the HER2-amplified cell lines, KYSE410, OE19 and N87. Total T cell input on Day 0 was 50,000 cells. T cells were quantified on Day 7 by flow cytometry after staining for CD3.

Activity of ACTR707 + trastuzumab, but not HER2-targeting CAR-T, is restricted to HER2-amplified cell lines



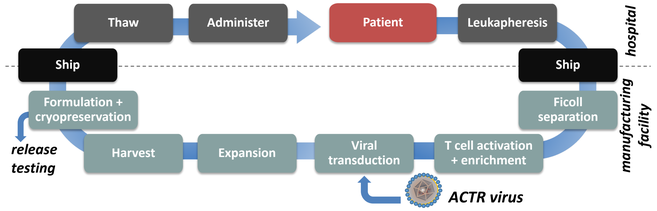
ACTR707 in combination with trastuzumab (1 μg/mL) mediates cytotoxicity and produces cytokines on tumor cells with moderate to high HER2 expression. Trastuzumab-based HER2-targeting CAR-T cells are cytotoxic and release cytokines in the presence of HER2-amplified and non-amplified target cell lines.

Differential in vitro activity of ACTR707 + trastuzumab compared to HER2 CAR-T on primary cells



(a) HER2 expression on tumor and normal cells was measured by flow cytometry after staining with an anti-human HER2 antibody. Mean fluorescence intensity (MFI) of stained cells is represented. (b and c) ACTR707 in combination with trastuzumab (5 μg/mL) has minimal cytotoxicity and cytokine release on primary cells, whereas trastuzumab-based HER2-targeting CAR-T cells have cytotoxicity and release IFNγ on these normal cells.

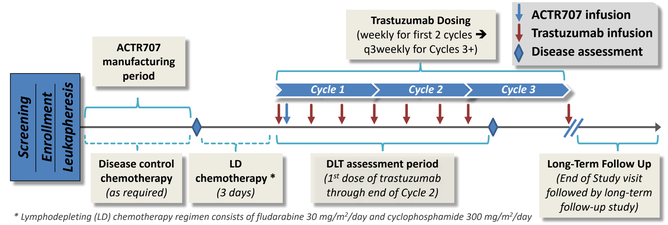
ACTR T cell manufacturing overview



ATTCK-34-01: Study design

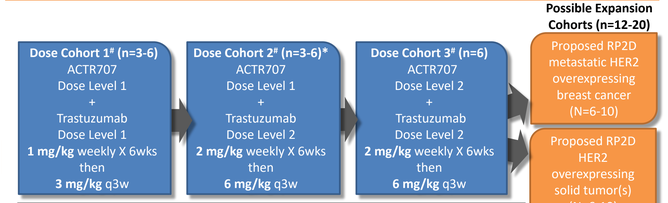
Design	▪ The study has two phases: a dose escalation phase to determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D), and an expansion phase to evaluate the proposed RP2D of ACTR707 in combination with trastuzumab ▪ Adaptive design using Bayesian logistic regression model (BLRM) for 2 agents, guided by the escalation with overdose control (EWOC) principle to determine the MTD and the RP2D of ACTR T cell product when used in combination with trastuzumab ▪ Multiple dose levels of ACTR707 and trastuzumab given in combination will be explored based on review of evolving safety and efficacy data originating from this trial
Primary Objective	▪ To assess the safety and tolerability of the combination regimen, and to define dose recommendations for further study
Secondary Objectives	▪ Anti-tumor activity ▪ ACTR707 T cell expansion and persistence ▪ Trastuzumab PK ▪ Inflammatory markers, cytokines, and chemokines after ACTR T cell administration
Key subject eligibility criteria	▪ Histologically confirmed, HER2-positive, advanced solid tumor malignancy (including breast cancer, gastric or gastroesophageal junction adenocarcinoma, or other locally advanced, recurrent or metastatic malignancy) ▪ Disease progression during or immediately following the immediate prior therapy, or within 6 months of completing adjuvant therapy for subjects with breast cancer

ATTCK-34-01: Study treatment regimen schema



* Lymphodepleting (LD) chemotherapy regimen consists of fludarabine 30 mg/m^2/day and cyclophosphamide 300 mg/m^2/day

ATTCK-34-01: Study design schema



#Additional de-escalations, dose escalations or intermediate dose levels allowed based on DEC review
*Concurrent dose escalation of ACTR707 at cleared trastuzumab starting dose allowed in a separate cohort

Sponsor decision: histologically defined safety expansion cohorts with DSMC review

Key
DEC: Dose Escalation Committee; DSMC: Data Safety Monitoring Committee; RP2D: Recommended Phase 2 Dose

ATTCK-34-01: Safety assessments and monitoring

- **Risk Minimization Tools**
  - Patient Alert Card
  - Event management guide
- **Focused Patient Monitoring**
  - Outpatient visits post ACTR infusion
  - Patient travel limitations from treatment center
  - At home temperature monitoring
  - At home neurological assessments
- Adverse events of special interest (AESIs) of pulmonary toxicity and cardiotoxicity defined to expedite data collection
- Specific exclusion criteria defined to account for patients with history of cardiac or lung disease
- Cardiac function testing q6weeks for all subjects
- Trastuzumab safety-related dose modifications incorporated (as per the USPI)

Conclusions and next steps

Here we demonstrated that ACTR707 T cell activity in the presence of trastuzumab is restricted to tumor cell lines with moderate to high levels of HER2 expression. ACTR707 T cell activity is antigen- and trastuzumab-specific, and titrates with antibody concentration. In vivo, ACTR707 + trastuzumab has comparable anti-tumor activity to trastuzumab-based HER2 CAR-T against HER2-amplified xenograft tumors. In contrast, ACTR707 + trastuzumab has differential activity on primary cells compared to HER2 CAR-T, supporting a hypothesis for an improved therapeutic index with ACTR707 + trastuzumab.

HER2-expressing solid tumors are associated with significant unmet need. HER2-directed therapies in metastatic breast and gastric cancer are generally not curative and relapsed/refractory patients have a poor prognosis. In the planned Phase 1 clinical trial, ATTCK-34-01 (**NCT03680560**), the safety and anti-tumor activity of ACTR707 + trastuzumab in patients with HER2-positive advanced malignancies will be evaluated. This study is expected to initiate by the end of 2018.