Comparability and Compatibility Studies of TBI-2001: a Novel CAR T Cell Product with a JAK/STAT Signaling Domain.

Nguyen, L[1], Dojo Soeandy, C[1], Fisher, K[1], Fyrsta, M[1], Le, M[1], Lo, C[1], Murakami, K[1], Nie J[1], Scheid E[1], Saso, K[1], Ohashi, P[1,2], Nakanishi, R[3], Nomura, K[3], Tanaka, M[3], Butler, M[1,2,4], Hirano, N[1,2];
[1]Tumor Immunotherapy Program, Princess Margaret Cancer Centre, University Health Network, Toronto, ON, CANADA, [2]Department of Immunology, University of Toronto, Toronto, ON, CANADA, [3] Takara Bio, Inc., Shiga, JAPAN, [4]Department of Medicine, University of Toronto, Toronto, ON, CANADA.

BACKGROUND

A phase I/Ib clinical trial has been developed for a first-in-human Chimeric Antigen Receptor (CAR) T cell product called "TBI-2001".

TBI-2001 recognizes CD19 and expresses a signaling domain that leads to the activation of JAK/STAT pathways. The CAR construct has shown enhanced antitumor activity in preclinical studies (Kagoya et al., Nat Med. 2018).

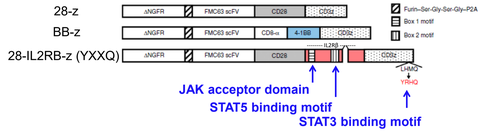
The trial is a single-centre study at the Princess Margaret Cancer Centre (PMCC) in Toronto.

Manufacturing of TBI-2001 is performed at the point of care. The manufacturing process was developed by Takara Bio Inc; technology transfer was performed from Takara to the PMCC. Subsequent preclinical studies included comparability and compatibility studies which were performed by Takara and the PMCC.

Kagoya Y, et al., Nature Medicine 2018, 24(3), 352-359

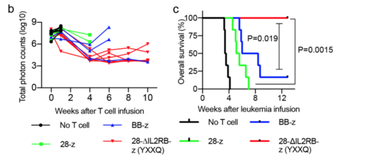
A novel chimeric antigen receptor containing a JAK–STAT signaling domain mediates superior antitumor effects

Yuki Kagoya[1], Shinya Tanaka[1,2], Tingxi Guo[1,3], Mark Anczurowski[1,3], Chung-Hsi Wang[1,3], Kayoko Saso[1], Marcus O Butler[1,3,4], Mark D Minden[5] & Naoto Hirano[1,3]

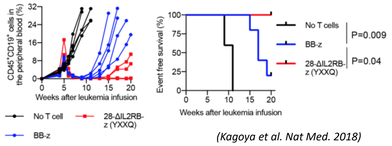


Structure of the novel CAR in TBI-2001. *The CAR is composed of a CD19-specifc scFv antigen recognition domain, a CD28 transmembrane domain, and intracellular domains derived from CD28, IL-2 receptor beta (IL-2Rß), and CD3ζ, with a STAT-3 binding YXXQ motif.*

NALM-6-Luc (high tumor burden) in NSG mice



Primary B-ALL in NSG mice



(Kagoya et al. Nat Med. 2018)

JAK-STAT CD19 CAR-T cells have superior antitumor effects in vivo. *T cells expressing previous-generation or novel CARs were adoptively transferred into mice bearing NALM-6 tumor cells (top graphs) or primary B-ALL cells (bottom graphs). Tumor burden and overall or event free survival were assessed.*

PREPARATION FOR CLINICAL TRIAL WITH POINT-OF-CARE MANUFACTURING

Takara Bio

Process Development: Manufacturing and quality testing. Manufacture of retrovirus particles.	Validation runs (3x)	Preclinical studies using products from validation runs: pharmacology, biodistribution, stability, etc

Princess Margaret Cancer Centre

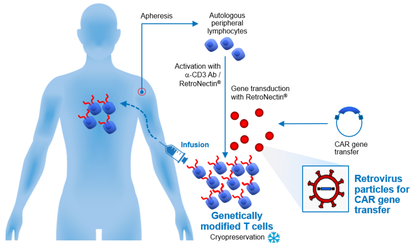
Technology transfer to PMCC	Validation runs (3x) (1 run split-source*)

Princess Margaret Cancer Centre

Preclinical studies: stability, comparability, compatibility	Submission of Clinical Trial Application	Single centre clinical trial with point-of-care manufacturing

The manufacturing procedure and quality testing procedures were developed by Takara Bio. (Japan). Retrovirus particles for the transduction of the CAR gene was manufactured by Takara Bio. Three validation batch runs were performed at Takara, and a technology transfer was performed to establish manufacturing at the Princess Margaret Cancer Centre (PMCC) (Canada). Three validation batch runs were performed at the PMCC; one of which was performed using starting material from a split-source apheresis collection that was also used by Takara. Preclinical studies were performed using validation batches from both sites, generating data for submission of the Clinical Trial Application to Health Canada for a single-centre study at the PMCC. *July 2023 FDA Guidance Document *Manufacturing Changes and Comparability for Human Cellular and Gene Therapy Products*.

OVERVIEW OF MANUFACTURING



Autologous peripheral lymphocytes are isolated from an apheresis product. Lymphocytes are activated with RetroNectin® and anti-CD3 mAb (OKT3). Prior to the transduction procedure, retrovirus particles are pre-loaded in RetroNectin®-coated bags at 4°C (Katsuyuki Dodo, et al., PLoS ONE 9(1): e86275). The activated lymphocytes are then seeded in the pre-loaded bags. After the transduction procedure, the lymphocytes are expanded, followed by cell harvest, final formulation and cryopreservation.

COMPARABILITY STUDY

Purpose:
The scope of the Comparability Study was to assess data from TBI-2001 full-scale validation batches manufactured at the PMCC against data from test batches manufactured at Takara. The purpose of this assessment was to confirm that the controlled process used for the manufacture of TBI-2001 minimizes variability such that data from pre-clinical studies and stability studies generated using Takara's batches are relevant to the PMCC's Clinical Trial Application (CTA).

Approach:

1. Comparability Assessment Based on Batch Characteristics

   Batch manufacturing data and quality testing data were evaluated as follows:
   - Release tests with defined acceptance criteria, e.g. identity, purity, potency, safety.
   - Additional characterization tests with defined acceptance criteria.
   - In-process control tests assessed qualitatively for comparability.

2. Comparability Assessment Based on Stress Testing

   Degradation of the final product under stress conditions was evaluated as follows:

   - Final Product was subjected to stress conditions.
   - Product degradation was assessed over time.
   - Results were assessed against predefined numerical acceptance criteria.
   - The overall rate of degradation was also assessed qualitatively.

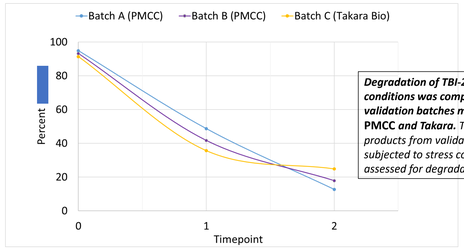
Results:

1. Comparability Assessment Based on Batch Characteristics

- All product specification test results from the batches manufactured at the PMCC batches met the target ranges as established by the Takara batches.
- All results from the additional characterization tests also met the target ranges.

2. Comparability Assessment Based on Stress Testing

- Stress induced degradation of TBI-2001 validation batches manufactured at the PMCC and at Takara met acceptance criteria for comparability.



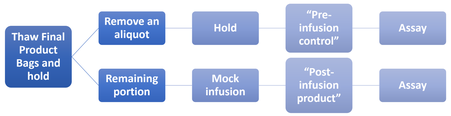
Degradation of TBI-2001 under stress conditions was comparable for validation batches manufactured at the PMCC and Takara. *TBI-2001 final products from validation batches were subjected to stress conditions and assessed for degradation over time.*

Conclusion:
Validation batches manufactured at the PMCC were comparable to the validation batches manufactured at Takara.

COMPATABILITY STUDY

Purpose:
The purpose of the Compatibility Study was to assess the suitability of administration materials for the infusion of TBI-2001.

Approach:



- Two final product concentrations were tested in duplicate.
- Examples of administration materials: non-filtered administration sets, peripherally inserted central catheter (PICC), tubing, port connectors and adaptors.
- Maximum allowable hold-time prior to infusion was tested.
- Readouts: pre-infusion and post-infusion samples assayed for four relevant release tests.
- Acceptance criteria for compatibility: post-infusion cells meet final product specifications for batch release.
- Assessment of cell recovery (yield) was also performed.
- Qualitative comparison of results from pre- and post-infusion samples was performed.

Results:
- All acceptance criteria were met.
- Cell recovery (yield) was high.
- Assay results from pre- versus post-infusion samples showed nominal changes of < 2%.

Conclusion:
The data from this Compatibility Study supported the use of the administration materials for TBI-2001.

CLINICAL TRIAL: OPEN TO ACCRUAL

ClinicalTrials.gov ID	NCT05963217
Sponsor	Princess Margaret Cancer Centre, University Health Network
Title	Phase I/Ib Study of TBI-2001 for Patients with Relapsed or Refractory CD19+ B-cell Lymphoma, Chronic Lymphocytic Leukemia (CLL), Small Lymphocytic Lymphoma (SLL)
Study site	Princess Margaret Cancer Centre, Canada (single centre study)
TBI-2001 manufacture site	Princess Margaret Cancer Centre, Canada
Investigators	PI: Marcus Butler. Co-Is: Christine Chen, John Kuruvilla, Linh Nguyen
Lymphodepleting chemotherapy	IV cyclophosphamide (500 mg/m^2 once daily) and IV fludarabine (30 mg/m^2 once daily) to be given concurrently for 3 days starting from -10 to -5 days before cell infusion.
TBI-2001 Dose	**Phase I:** Cohort: — Dose (CAR-positive cells): Cohort 1 — 3 x 10^5 CAR-positive cells/kg Cohort 2 — 1 x 10^6 CAR-positive cells/kg Cohort 3 — 3 x 10^6 CAR-positive cells/kg (maximum of 2 x 10^8 CAR-positive cells/patient) **Phase Ib:** RP2D
Frequency of Administration	Single dose
Planned sample size	19 – 28