FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Therapeutic Products
Office of Pharmacology/Toxicology
Division of Pharmacology/Toxicology 2
Pharmacology/Toxicology Branch

I concur with this review memo. S. Sanduja 02/06/24

BLA NUMBER:	STN #125773.000
DATE RECEIVED BY CBER:	March 27, 2023 Major amendment submitted on August 28, 2023 (CMC)
DATE REVIEW COMPLETED:	October 2, 2023
PRODUCT:	AMTAGVI (Lifileucel)
APPLICANT:	Iovance Biotherapeutics, Inc.
PROPOSED INDICATION:	Treatment of adult patients with unresectable or metastatic melanoma (MM) previously treated with a programmed cell death protein-1(PD-1) blocking antibody (Ab), and if proto-oncogene B-Raf (BRAF) V600 mutation positive, a BRAF inhibitor with or without a mitogen-activated extracellular signal-regulated kinase (MEK) inhibitor
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EXECUTIVE SUMMARY:

AMTAGVI (Lifileucel) is a cellular product comprised of autologous tumor-derived T lymphocytes intended for the treatment of adult patients with unresectable or metastatic melanoma (MM) who have not responded to a previous immune checkpoint inhibitor (ICI)

treatment alone or in combination with targeted therapy. AMTAGVI is administered as a single intravenous (IV) infusion of AMTAGVI, within a dose level range of (b) (4) viable cells/infusion, following a lymphodepletion (LD) regimen of cyclophosphamide (Cy), mesna and fludarabine. Recombinant human interleukin 2 (IL-2) is administered every eight to 12 hours for up to six IV infusions following AMTAGVI administration.

No nonclinical studies were conducted with AMTAGVI. Instead, the applicant cited published nonclinical data evaluating similar murine and human tumor-derived T lymphocytes to provide support for AMTAGVI. These studies demonstrate: 1) a positive correlation between the level of lymphocyte infiltration in primary MM of the skin and disease prognosis; 2) the ability of IL-2 to enhance *ex vivo* expansion of murine and human tumor-derived lymphocytes; 3) anti-tumor activity of tumor-derived lymphocytes, in combination with Cy and IL-2, in murine models of colon adenocarcinoma and sarcoma with lung and liver metastases.

No genotoxicity, carcinogenicity, and developmental and reproductive toxicity (DART) studies were conducted for AMTAGVI. These studies are not warranted based on the product characteristics, biological activity, and safety profile.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:

No nonclinical deficiencies are identified in this submission. There are no outstanding requests for additional nonclinical data to evaluate AMTAGVI. The nonclinical information in the BLA submission supports approval of the licensure application.

Formulation and Chemistry:

AMTAGVI is a cellular suspension containing live autologous T lymphocytes isolated from a patient's resected tumor. The cells are expanded *ex vivo* using the Generation 2 (GEN2) manufacturing process, which involves two sequential steps. The first step, the pre-Rapid Expansion Protocol, (b) (4) (b) (4) followed by the second step of rapid expansion (b) (4) (b) (4) containing IL-2 (b) (4) a murine anti-human CD3 monoclonal antibody (mAb; OKT3; (b) (4) AMTAGVI mainly consists of (b) (4) including $CD4^+$ T cells, $CD8^+$ T cells, (b) (4) The drug product is formulated with CryoStor CS10® (50%), Plasma-Lyte A (48%), human albumin (2% final concentration), and IL-2 (b) (4) IU/100 mL) for IV infusion. Each AMTAGVI administration contains (b) (4) (b) (4) viable cells in (b) (4) distributed across (b) (4) to four cryogenic bags.

Abbreviations

Ab	Antibody
BRAF	Proto-oncogene B-Raf
IL-2	Recombinant human interleukin 2 or interleukin 2
ICI	Immune checkpoint inhibitor
IV	Intravenous
LAK	Lymphokine-activated killer cells
LD	Lymphodepletion
mAb	Monoclonal antibody
MM	Metastatic melanoma
PBL	Peripheral blood lymphocytes
TILs	Tumor infiltrating lymphocytes

Related File(s)

IND #16317: Iovance Biotherapeutics, Inc.; Autologous Tumor Infiltrating Lymphocytes Cultured with OKT3, Interleukin-2, and Allogeneic Mononuclear Feeder Cells for Advanced Melanoma

Table of Contents

INTRODUCTION

Melanoma of the skin is the fifth most common cancer in the United States (US) in 2023, with an estimated 97,610 new cases, and 7,990 deaths[1,2]. It originates from melanocytes, which produce

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin* 2023; 73:17-48
[2] American Cancer Society. Cancer Facts & Figures 2023. *Atlanta: American Cancer Society*; 2023

melanin in the skin, eyes, ears, and mucous membranes[3]. Cutaneous melanoma makes up over 90% of cases, while mucosal and uveal melanomas comprise less than 5% of cases. Melanoma is categorized into stages I (primary tumor at low-risk of recurrence), II (primary tumors at high-risk of recurrence), III (involving regional lymph nodes and in-transit or satellite metastasis), and IV (with distant metastases)[4,5,6]. Five-year melanoma-specific survival rates vary from 93% (stage IIIA) to 32% (Stage IIID). Despite the progress with immunotherapies and targeted therapies, development of relapsed/refractory disease after ICI treatment alone or in combination with targeted therapy remains an unmet medical need[4.]

AMTAGVI consists of autologous tumor-derived T lymphocytes isolated from a patient's resected tumor that are expanded *ex vivo*. Based on the referenced nonclinical studies, AMTAGVI, in combination with IL-2 and Cy, is hypothesized to have anti-tumor activity against autologous tumor cells, while sparing normal cells.

AMTAGVI is administered as a single IV infusion (within a dose level range of (b) (4) (b) (4) viable cells/infusion) after a LD regimen consisting of 60 mg/kg of Cy daily with mesna for two days followed by fludarabine at 25 mg/m^2 daily for five days. The LD regimen is intended to eliminate tumor-induced suppressor T cells and enhance *in vivo* growth and trafficking of administered cells[7,8]. Within three to 24 hours after AMTAGVI administration, patients receive up to six IV infusions of IL-2 (600,000 IU/kg/infusion) every eight to 12 hours to support cell survival, expansion, activation, and persistence *in vivo*[9,10].

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

No nonclinical pharmacology studies were performed for AMTAGVI. Instead, in Module 4 of the BLA the applicant submitted publications of nonclinical pharmacology studies assessing the

[3] Long GV, Swetter SM, Menzies AM, et al. Cutaneous melanoma. *Lancet* 2023; 402:485
[4] Kahlon N, Doddi S, Yousif R, et al. Melanoma treatments and mortality rate trends in the US, 1975 to 2019. *JAMA Network Open* 2022; 5(12):e2245269
[5] Tumor, node, metastasis (TNM) staging system and other prognostic factors in cutaneous melanoma - UpToDate; accessed September 26, 2023
[6] Gershenwald JE, Scolyer RA, Hess KR, et al. Melanoma Staging: Evidence-based changes in the American Joint Committee on Cancer Eighth Edition Cancer Staging Manual. *CA Cancer J Clin* 2017; 67(6): 472–492.
[7] North RJ. Cyclophosphamide-facilitated adoptive immunotherapy of an established tumor depends on elimination of tumor-induced suppressor T cells. *J Exp Med* 1982; 55:1063-1074
[8] Pockaj BA, Sherry RM, Wei JP, et al. Localization of ^{111}indium-labeled tumor infiltrating lymphocytes to tumor in patients receiving adoptive immunotherapy. *Cancer* 1994; 73:1731-1737
[9] Sarnaik AA, Hamid O, Khushalani NI, et al. Lifileucel, a tumor-infiltrating lymphocyte therapy, in metastatic melanoma. *J Clin Oncol* 2021; 39:2656-2666
[10] Chesney J, Lewis KD, Kluger H, et al. Efficacy and safety of lifileucel, a one-time autologous tumor-infiltrating lymphocyte (TIL) cell therapy, in patients with advanced melanoma after progression on immune checkpoint inhibitors and targeted therapies: pooled analysis of consecutive cohorts of the C-144-01 study. *J Immunother Cance*r 2022; 10:e005755

characteristics and biological activities of similar products, both alone and in combination with Cy and IL-2.

In vitro and in vivo Studies

Study Number	Publication Citation
1	Larsen TE and Grude TH. A retrospective histological study of 669 cases of primary cutaneous malignant melanoma in clinical stage I. *Acta Pathol Microbial Scand A.* 1978; 86A (6): 513-522
2	Rosenberg SA, Spiess P, LaFreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science* 1986; 233:1318-1321
3	Muul LM, Spiess PJ, Director EP, et al. Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma. *J Immunol* 1987; 138 (3): 989–995
4	Topalian SL, Muul LM, Solomon D, et al. Expansion of human tumor infiltrating lymphocytes for use in immunotherapy trials. *J Immunol Methods* 1987; 102:127-141
5	Schoof DD, Selleck CM, Massaro AF, et al. Activation of human tumor-infiltrating lymphocytes by monoclonal antibodies directed to the CD3 complex. *Cancer Res* 1990; 50(4):1138-1143

- ***Reviewer's Note*:**
  - This review memo summarizes the data from five key publications in the 'Overview of Pharmacology Studies' section below. Although the label specifies that the AMTAGVI is comprised of autologous tumor-derived T cells, this review memo utilizes tumor infiltrating lymphocytes (TILs) when referring to the cells cited in the publications since that is the nomenclature used in the publications.

Overview of Pharmacology Studies

Study #1:
Larsen TE and Grude TH. A retrospective histological study of 669 cases of primary cutaneous malignant melanoma in clinical stage I. *Acta Pathol Microbiol Scand A* 1978; 86A(6): 523-530

Objective: To evaluate correlation between TIL density, disease prognosis and tumor characteristics, including tumor cell type, pigmentation, cellular atypia, mitotic count, dermal invasion depth, tumor type and ulceration.

Methods: 669 primary MM (PMM) cases of the skin, clinical stage I, were evaluated. These cases include 86

This study included 256 men and 413 women, aged six to 93 years.

- MM cases were categorized according to Clark's classification (1967)[11].

- Lymphocyte infiltration in tumors was graded as + (slight), + + (intermediate), + + + (dense) or "not possible to judge."

- Tumor invasion depth followed Clark's level (1969)[12], consisting of five levels: I-epidermis (E); II-papillary dermis (PD); III-papillary-reticular interface (PRI); IV-reticular dermis (RD); and V-subcutis (S). If the lower part of the tumor was absent, the level was marked as "not possible to judge."

➢ ***Reviewer's Comment:***
- Since 2017 the American Joint Committee on Cancer categorizes MM cases based on mitotic rate, not Clark level, due to its greater predictive value[6]. It is not clear how this may impact interpretation of the data reported in this publication.

Results:
- Compared to slight (+) and intermediate (++) levels, dense lymphocyte infiltration (+++) in PMM was associated with a better prognosis and survival for up to ten years post-diagnosis.

- Among PMM subtypes with dense lymphocyte infiltration, NMM demonstrated a statistically significant worse prognosis.

- Dense lymphocyte infiltration was linked to superficial tumor invasion (levels E, PD and PRI) with higher survival rates for the PD invasion level, which declined as tumor invasion deepened from PRI to RD.

- Lymphocytic infiltration exhibited no correlation with patient demographics.

Conclusion:
Dense lymphocyte infiltration is positively correlated with improved prognosis in clinical stage I PMM of the skin, except for NMM.

➢ ***Reviewer's Comment:***
- Based on the positive link between dense lymphocyte infiltration in the tumor and improved prognosis for clinical stage I PMM, this study supports the scientific rationale for using autologous TILs to target MM.

Study #2
Rosenberg SA, Spiess P, LaFreniere R. A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. *Science* 1986; 233:1318-1321

[11] Clark WH. A classification of malignant melanoma in man correlated with histogenesis and biologic behavior. In: *Montagna, W.* (Ed.): *Advances in biology of the skin: The pigmentary system. 1st ed. vol. VIII Pergamon Press, Oxford* 1967; pp. 621-647

[12] Clark WH, From L, Bernardino EA, et al. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. *Cancer Res* 1969; 29(3):705-727

Objective: To develop an *ex vivo* expansion method for murine TILs and evaluate their activity *in vitro* and in murine tumor models.

Methods:

- Murine Tumors: The authors employed murine MCA-105 sarcoma and MC-38 colon adenocarcinoma in syngeneic C57BL/6 mice. MCA-105 sarcoma was induced by intramuscular injection of 3-methylcholanthrene, while MC-38 murine colon adenocarcinoma was generated by subcutaneous injection of dimethylhydrazine.

- TIL Cultures: Harvested mouse tumors were enzymatically digested for one to two hours, and the resulting cell suspensions were filtered, washed, and cultured (2.5×10^5 cells/ml) in complete medium with 1000 U/ml IL-2[13]. Cells were cultured for several weeks until TILs were expanded to sufficient numbers needed for adoptive transfer.

- Adoptive Transfer of TILs: C57BL/6 mice with pulmonary or hepatic metastases from the MC-38 colon adenocarcinoma or MCA-105 sarcoma received an IV infusion of TILs alone, IL-2 alone, Cy alone, a combination of TILs + IL-2, a combination of Cy and IL-2, or a combination of TILs + IL-2 following Cy treatment. Animal survival was assessed after each treatment.

- *In vitro* and *in vivo* activities of syngeneic murine TILs and peripheral blood lymphocytes (PBL)-derived lymphokine activated killer (LAK) cells[14] were also compared.

Results:

- *Ex vivo* expansion with IL-2 significantly increased the TIL numbers, generating approximately 100-fold more TILs than the original cell number after 15 days of culture. Cytologic examination confirmed the absence of tumor cells in the expanded murine TILs.

- TILs derived from the MC-38 tumor specifically lysed MC-38 tumor cells *in vitro*, but not MCA-102 cells. LAK cells showed similar level of lysis between MCA-102 and MC-38 tumor cells but the cytolytic activity of LAK cells against MC-38 tumor cells was >10-fold lower compared to TILs.

- *In vivo* data:

    - In the MCA-105 sarcoma tumor model with 3-day pulmonary micrometastases, both TILs and LAK cells showed a dose-dependent anti-tumor response after an IV infusion, followed by intraperitoneal (IP) infusions of IL-2 (7,500 to 20,000 units three times daily).

        - TIL + IL-2 treatment was 50 to 100 times more effective in mice with metastatic MCA-105 sarcoma tumors than LAK cells + IL-2.

[13] Rosenberg SA, Grimm EA, McGrogan M, et al. Biological activity of recombinant human interleukin-2 produced in Escherichia coli. *Science* 1984; 223(4643):1412-4

[14] Rosenberg SA. Lymphokine-activated killer cells: A new approach to immunotherapy of cancer. *JNCI* 1985; 75 (4): 595-603

- Similar findings were observed in C57BL/6 mice with established pulmonary micrometastases from MC-38 colon adenocarcinoma, B16 melanoma, or the MCA-106 sarcoma[15].

- LAK administration was previously shown to be ineffective for large established pulmonary and hepatic metastases in mice[26]. Thus, the authors investigated the anti-tumor activity of TILs in murine tumor models with large established hepatic and pulmonary metastases. Data showed:

  - LAK cells (10^8 cells/IV infusion/mouse) or TILs (1.3×10^7 cells/IV infusion/mouse), in combination with IL-2 (25,000 units/infusion/mouse three times daily for five days), did not eliminate established 8-day liver metastases derived from MC-38 colon adenocarcinoma.

  - Mice treated with Cy (100 mg/kg via IV infusion) six hours prior to TILs + IL-2 administration survived longer (over 100 days) than mice receiving a combination of Cy (100 mg/kg) with LAK + IL-2 (up to 30 days).

- In mice with established 14-day MC-38 pulmonary metastases, administration of high-dose Cy (100 mg/kg) in combination with TILs (2×10^7 TILs/mouse) + IL-2 resulted in the longest survival (75% survival on Day 100) compared with the administration of high-dose Cy and IL-2 (10% survival on Day 100), or low-dose Cy (20 mg/kg) with TILs + IL-2 (0% survival on Day 100).

Conclusion:
In a murine metastatic tumor-bearing model, administration of TILs in combination with high dose Cy and IL-2 resulted in the greatest increase in survival. Therefore, this study supports the rationale for the administration of autologous TILs in combination with Cy and IL-2.

Study #3
Muul LM, Spiess PJ, Director EP, et al. Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma. *J Immunol* 1987; 138 (3): 989–995

Objective: To develop a method for *ex vivo* expansion of human TILs from freshly resected MM nodules and evaluating their ability to target and eliminate autologous tumor cells *in vitro*.

Methods: Please refer to the "MATERIALS AND METHODS" section in this publication for specific experimental details.

- Tumor Cell Suspension and TIL Expansion Steps: Freshly resected and minced tumor nodules from six advanced MM patients were enzymatically digested for 16 hours. Isolated

[15]Spiess PJ, Yang JC, and Rosenberg SA. *In vivo* antitumor activity of tumor-infiltrating lymphocytes expanded in recombinant interleukin-2. *JNCI* 1987; 79:1067-1075

viable cells were first cultured for 7-13 days in 24-well plates with complete medium containing 1000 U/ml IL-2, followed by subculturing in 750-ml tissue culture flasks every 4-8 days, spanning 6-24 passages. Culture duration ranged between 27-181 days between different tumor samples.

- TIL Phenotypic Analysis: Mature TIL cultures were subjected to FACS analysis using various Abs.

- Chromium (Cr)-Release Assay for TIL Cytotoxicity: The effector cells were TILs and LAK cells from each patient. ^{51}Cr-labeled target cells included autologous or allogeneic melanoma tumor cells, sarcoma cells, an NK cell-sensitive K562 cell line, and normal human cells. ^{51}Cr-labeled target cells without effector cells were included as a control. % lysis of target cells was calculated after co-culturing the target cells with effector cells.

Results:

- TIL Expansion:
    - TIL expansion varied among six MM patients, ranging from 2.4-fold to 466-fold by week three of expansion and 3-fold to 95,652-fold at culture termination. The fold expansion was not correlative of initial percentages of mononuclear cells isolated from the resected tumors.
    - According to the authors, TIL cultures were free from residual tumor cells as confirmed by cytologic examination.
- TIL Phenotypic Analysis:
    - The expanded TILs were primarily CD3+ T cells, with most being CD4+ T cells in five of the six patients.
    - The number of CD3+ T cells increased rapidly, constituting more than 90 % of the cell population by week 4 in culture. CD8+ T cells comprised approximately 50% of the cell population by week 2 but declined over time. By week 3 the percentages of CD8+ and CD4+ cells were similar, with a rapid increase in CD4+ T cell numbers from week 2 to week 6.
- TIL Cytotoxicity:
    - TILs cultured from all MM patients displayed cytotoxic activity against autologous melanoma tumor cells but not against autologous or allogeneic normal human cells.
    - TILs from some patients also exhibited lysis of allogeneic melanoma cells, sarcoma cells and NK-sensitive K562 cells. The lysis of K562 cells suggests the presence of NK cells in patients' TILs.

Conclusion:
This study provides POC for manufacturing human TILs capable of inducing cytotoxic activity against autologous tumor cells, but not autologous normal cells.

- ***Reviewer's Comments:***
  - The expansion process for AMTAGV1 includes additional modifications to the method described in this publication. These modifications are intended to enhance the proliferation, differentiation, and antitumor activities of TILs.

  - This publication lacks sufficient detail regarding the sensitivity, specificity, and reproducibility of the method used to distinguish tumor cells from mononuclear cells in TIL cultures. Therefore, this reviewer cannot comment on the author's conclusion of absence of tumor cells in the expanded product.

Study #4
Topalian SL, Muul LM, Solomon D, et al. Expansion of human tumor infiltrating lymphocytes for use in immunotherapy trials. *J Immunol Methods* 1987; 102(1):127-41

Objective: To develop a scalable expansion method for TILs derived from various solid tumor types for use in cancer immunotherapy clinical trials.

- ***Reviewer's Note*:**
  - The manufacturing processes in Studies #3 and #4 are similar. Study #4 extended the methodology to expand TILs from various solid tumor types and confirmed the production of larger TIL quantities required for clinical trials.

Methods:
- Tumor Cell Suspension and TIL Expansion Steps: The authors obtained solid tumor samples from 24 patients, including six melanomas, ten sarcomas, and eight adenocarcinomas. Eight patients with metastatic disease (4 melanomas, 2 colon adenocarcinomas, 1 breast carcinoma, 1 one renal cell carcinoma) also underwent tumor resection to generate the TIL quantities required for administration in subjects. Enzymatically digested tumor cell suspensions from each solid tumor were either cryopreserved for long-term storage or used for *ex vivo* TIL expansion. Tumor cell suspensions were diluted in culture medium (2.5-5.0 x 10^5 viable cells/ml) containing 20% LAK cell culture supernatant and 1000 U/ml IL-2. TIL cultures were distributed into various types of culture vessels for expansion, maintaining viable cell concentrations at 2.5 x 10^5 cells/ml at each passage.

- TIL Phenotypic Analysis: The number of mononuclear cells in tumor-derived cell suspensions at Day 0 were determined through trypan blue dye visualization or cytologic analysis using hematoxylin and eosin staining. Mature TIL cultures were subjected to FACS analysis using various Abs.

- Cr-Release Assay for TIL Cytotoxicity: ^{51}Cr-labeled target cells included fresh, non-cultured tumor cells, normal human cells, K562 cells, and Daudi (an NK-resistant lymphoma cell line) cells. Cytotoxicity was determined after co-culturing effector (TIL or LAK cells) and target

cells at various effector: target ratios (40:1, 10:1 and 2.5:1) for 4-6 hours. ^{51}Cr-labeled target cells without effector cells were included as a control.

Results:

- TIL Expansion on Experimental Scale: TILs from 24/25 human tumor samples were able to expand. Expansion of TILs from frozen (n=13) and fresh tumor (n=12) preparations showed similar characteristics, except for one TIL culture failure from a cryopreserved colon adenocarcinoma.

  - Lymphocytes ranged from 3% to 74% in tumor cell suspensions and expanded within a range of 2.9-fold to 9.1 x 10^8-fold during a culture period of 14 to 100 days.

  - Culture growth was marked by gradual tumor cell disappearance and lymphocyte outgrowth, occurring between nine to 28 days after culture initiation.

  - Based on the cell surface phenotyping of 16 expanded TIL cultures (five melanomas, four sarcomas, and seven adenocarcinomas), the majority of lymphocytes were CD3+ (83%), CD8+ (58.9%) and CD4+ T cells (25.2%). A significant number of cells expressed the IL-2 receptor (29.4%) and HLA-DR (85.2%), while some cells (13-18%) expressed NK cell markers.

  - 9/24 TIL cultures effectively lysed fresh autologous tumor targets, including melanoma-derived TILs (4/6), sarcoma-derived TILs (3/10) and colon adenocarcinoma-derived TILs (2/8). TIL cultures exhibited significant lysis of K562 and/or Daudi target cells, suggesting the cytotoxicity is mediated by both NK and T lymphocytes. TILs lysed the autologous tumor cells to a greater degree than LAK cells, while LAK cells showed a greater degree of cytotoxicity against allogeneic tumor cells compared to TILs.

- TIL Expansion on a Large-Scale: TIL cultures were derived from 8 fresh tumor samples (4 melanomas, 2 colon carcinomas, 1 breast carcinoma, and 1 renal cell carcinoma). 5/8 TIL cultures successfully generated $\geq 10^{10}$ TILs within a 3-6 week culture period, including 2 melanoma-derived TIL cultures.

  - According to the authors, all 5 of these TIL cultures were free from residual tumor cells as confirmed by cytologic examination.

  - Time-dependent phenotypic changes were observed in TIL cultures derived from different tumors.

    - 4/8 cultures with lower initial T cell percentages (64%-71%) transitioned to > 90% CD3+ T cells over the course of culture with IL-2, with a decline in B cell population. The other 4 cultures were predominantly composed of T cells.

    - The CD4+/CD8+ T cells ratios evolved over time. Initially, 6/8 cultures had a higher number of CD4+ T cells than CD8+ T cells, but in mature TIL cultures 5/8 TIL cultures contained more CD8+ T cells than CD4+ T cells.

- In the early culture period (Day 10), all five TIL preparations effectively lysed the autologous tumor cells, with a decline in cytolytic activity over time.

- Optimal Expansion Conditions: Expanded TILs exhibited the most rapid growth in 24-well plates and gas-permeable culture bags, while growth was poor in roller bottles, possibly due to rotation and limited gas exchange.

Conclusion:
This study demonstrates a method for large-scale TIL expansion that generated over 10^{10} functional TILs from 5/8 tumor samples, including melanoma, sarcoma, and adenocarcinoma, for clinical applications.

- ***Reviewer's Comment:***
  - The GEN2 manufacturing process for AMTAGV1 is different from the TILs expansion method in this publication. Therefore, this reviewer cannot comment on whether AMTAGV1 exhibits comparable cytotoxicity against autologous tumor cells as TILs in this publication.

Study #5
Schoof DD, Selleck CM, Massaro AF, et al. Activation of human tumor-infiltrating lymphocytes by monoclonal antibodies directed to the CD3 complex. *Cancer Res* 1990; 50(4):1138-1143

Objective: To assess the impact of solid-phase anti-CD3 activation on the phenotype and function of human TILs *in vitro*.

Methods:
- Tumor Cell Suspension and TIL Expansion Steps: TILs from enzymatically digested human tumor cell suspensions were cultured with a high concentration of IL-2 (1,000 U/ml) for the required duration to allow sufficient TIL expansion.

- Solid Phase Anti-CD3 Activation of TILs: A surface-coating of anti-CD3 mAb (1.0 μg/ml) was applied to the culture flasks or microtiter plates. After incubation, the surface was washed. Lymphocytes were then added to the culture dish without exogenous IL-2 for a two-day activation period. Subsequently, cells were recovered, washed, and re-plated in culture medium containing 50 U/ml of IL-2.

- TIL Phenotypic Analysis and Cytotoxicity: Flow cytometry analysis was performed for the lymphocyte surface antigens (CD3, CD4 and CD8) on TILs to define their phenotypes. The cytotoxicity of TILs against autologous and heterologous tumor cells (K562 and Daudi cells) was assessed using ^{51}Cr-release assay after co-culturing effector cells (TIL or LAK cells) with target cells for 4 hours.

Results:
- TIL Expansion: Maximum TIL proliferation required IL-2 concentrations of 200-1000 U/ml during the first several weeks of the culture.

- Exposure to 1000 U/ml IL-2 beyond 10 or 11weeks halted TIL proliferation.

- TILs initially unresponsive to a prolonged exposure to 1000 U/ml of IL-2 continued proliferation after two days of solid phase anti-CD3 activation, followed by culturing with reduced IL-2 concentration (50 U/ml) for four days. These TILs also expressed higher levels of IL-2 receptor alpha and secreted higher concentrations of IL-2.

- TIL Phenotypic Analysis: After 8 weeks of anti-CD3 activation, TIL cultures were predominantly CD3+, CD8+ and TCR+ and lacked CD4+ and CD16 + cell populations.

- TIL Cytotoxicity:

  - Compared to TILs expanded in 1000 U/ml of IL-2 alone, TILs activated with anti-CD3 + 50 U/ml of IL-2 demonstrated higher cytotoxicity against autologous tumor cells but not against Daudi, K562, or histologically matched heterologous tumor cells.

  - Anti-CD3 activation did not improve cytotoxic activity of CD4+ TIL cultures . suggesting a difference in susceptibility to anti-CD3-induced cytotoxicity between CD4+ and CD8+ populations.

Conclusion:
Human TILs can be activated through the CD3 complex expressed on their surface.

- ***Reviewer's Comment:***
  - The TIL expansion method in this study differs in culture duration and anti-CD3 stimulation compared to the GEN2 manufacturing process for AMTAGV1.

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies were conducted for AMTAGV1. Given the characteristics and biological activity of AMTAGVI, this omission is acceptable.

PHARMACOKINETIC STUDIES (Cell Distribution)

No nonclinical studies were conducted to evaluate distribution of AMTAGVI. Based on the available information on the *in vivo* distribution of intravenously administered T cell products, this omission is acceptable.

TOXICOLOGY STUDIES

No toxicology studies were conducted for AMTAGVI. Given the characteristics and biological activity of AMTAGVI, this omission is acceptable.
Referenced publications (Studies #3 and #4 summarize above) demonstrate autologous tumor cell killing by culture expanded TILs from patients. These studies also showed that culture

expanded human TILs were free from tumor cells. Furthermore, during the clinical development, AMTAGVI lot release specifications included measures to control residual tumor cell levels in the final drug product.

Development and Reproductive Toxicology (DART) Studies:

No DART studies were conducted for AMTAGVI. Given the characteristics and biological activity of AMTAGVI, this omission is acceptable.

Genotoxicity Studies:

No genotoxicity studies were conducted for AMTAGVI. Given the characteristics and biological activity of AMTAGVI, this omission is acceptable.

Carcinogenicity/Tumorigenicity Studies:

No tumorigenicity studies were conducted for AMTAGVI. This is acceptable since Studies #3 and #4 provided evidence that the expanded human TIL cultures in the studies were free of tumor cells. AMTAGVI specifications also include measures to control residual tumor cell levels in the final drug product. Furthermore, the available clinical data for autologous TILs, including AMTAGVI therapy, do not indicate any tumorigenicity issues[9,10].

Other Safety/Toxicology Studies:

No safety/toxicology studies were conducted for the additional concomitant medications mentioned in the proposed product label, including: 1) a LD regimen of Cy, mesna and fludarabine; and 2) IL-2. The applicant states that each of these drug products is FDA-approved. For detailed information, please consult the package insert for each product.

APPLICANT'S PROPOSED LABEL

Sections 8.1-8.3 and Section 13 of the proposed label are acceptable.

CONCLUSION OF NONCLINICAL STUDIES

The nonclinical data for autologous tumor-derived lymphocytes support approval of the license application for AMTAGVI.

KEY WORDS/TERMS

AMTAGVI; Lifileucel; autologous tumor-derived T lymphocytes; autologous tumor-infiltrating lymphocytes; unresectable or metastatic melanoma; *ex vivo* expansion; murine tumor models, lymphodepletion regimen; IL-2; pharmacology; phenotypes; cytotoxicity; prognosis; toxicology.