

EXPERT INSIGHT

Current and future directions for tumor infiltrating lymphocyte therapy for the treatment of solid tumors

**Maria Fardis, Kelly DiTrapani, Cécile Chartier &
Friedrich Graf Finckenstein**

Cancer is the second leading cause of death in the USA. Over 90% of cancers involve solid tumors while 10% are hematological malignancies. Adoptive cell transfer (ACT) utilizing chimeric antigen receptor (CAR) T cells has recently been approved as treatment for a subset of hematologic cancers, changing the prospects of a small fraction of cancer patients. Patients with solid tumors though have not received significant benefit from CAR T therapy and many remain without therapeutic options after progressing on standard of care, including immune checkpoint inhibitors. First tested more than 30 years ago and optimized over the decades, ACT with tumor-infiltrating lymphocytes (TIL) was shown to be remarkably efficient for the treatment of metastatic melanoma, and is now re-emerging as a promising therapeutic option for heavily pre-treated patients with melanoma and other solid tumors. TIL therapy is a one-time treatment that involves the adoptive transfer of autologous T cells isolated from the tumor tissue and expanded *ex vivo* to a patient who has been lymphodepleted to remove their immunosuppressive tumor microenvironment which is supportive of a tumor in a cancer patient. The authors have established a streamlined GMP process for the production of TIL and demonstrated efficacy of the product in several highly unmet medical need patient populations, as evidenced by durable responses as assessed by RECIST 1.1. Two pivotal clinical studies in melanoma and cervical cancer indications are ongoing to support bringing this product to the market.

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Immunotherapy represents a potentially life-saving option in the treatment of patients with cancer. Because of the enhanced understanding over the past three decades of the adaptive immune system, as well as of immunologic signaling and immunosuppressive pathways in cancer, immunotherapy has emerged as a major focus of cancer research and a novel treatment option. However, currently approved immunotherapy drugs work through mechanisms that are not tumor specific and can lead to immune related organ toxicity that can limit benefit for patients [1]. In addition, various mechanisms can drive both primary and acquired resistance to currently available immunotherapeutics in a meaningful proportion of patients [2]. Despite novel advances geared at taking off the brakes on immune responses to tumors, treatment options remain limited for patients who cannot tolerate, do not initially respond, or develop resistance to currently approved immune checkpoint inhibitors.

As part of normal immune response, TIL migrate to the tumor site after circulating in blood and through recognition of chemokines produced by the tumor, penetrate the tumor stroma and engage in tumor cell killing. Cancer prevails in cases where the tumor microenvironment overpowers the immune response [3-6].

ACT utilizing autologous TIL is building on this tumor specific physiological immune response mechanism and has demonstrated the potential for durable complete responses in immunogenic tumors such as melanoma, in studies conducted at the National Cancer Institute (NCI) and other institutions globally [7]. Responses are demonstrated even in heavily pretreated patients irrespective of prior therapy, including checkpoint inhibitors [8-11]. The encouraging results of TIL therapy in melanoma have led to further exploration of ACT with TIL as a treatment option for multiple additional cancer indications [12,13].

The principle behind TIL therapy is to amplify and rejuvenate the cancer patient's immune system thereby enabling it to eliminate

tumor cells. To translate the approach in a commercially viable product, the authors initially focused on optimizing the manufacturing process. The original process from NCI required approximately 6 weeks for completion. A new manufacturing process for TIL was developed lasting only 22 days and called Generation 2 (Gen 2). The authors' Gen 2 manufacturing process is robust with well over 90% success rate in >300 patients treated to date. This product is investigated in two pivotal programs for melanoma and cervical cancers, with intent to commercialize the Gen 2 manufacturing product in the USA subsequent to submission of a BLA to the FDA.

TIL MANUFACTURING PROCESS

TIL manufacturing starts with the surgical resection of a tumor. The resected tumor is shipped to the central manufacturing facility where it is fragmented and placed in media. Upon placement of tumor fragments in the presence of IL-2, the TIL egress from the tumor while expanding in media. After completion of expansion, approximately 10^9 – 10^{11} cells are produced and harvested. The TIL cells are washed, placed in media in the infusion bags and cryopreserved (Figure 1).

TIL ADMINISTRATION TO PATIENT

Subsequent to TIL product manufacturing, the TIL which may recognize multiple patient-specific antigens expressed by the tumor, are now available in great numbers and with restored functionality. In preparation for the therapeutic TIL infusion, the patient receives non-myeloablative lymphodepletion (NMA-LD) with cyclophosphamide (60 mg/kg, IV x 2 doses) and fludarabine ($25 \text{ mg/m}^2 \times 5$ doses) to eliminate potentially suppressive immune cells which support the tumor and to maximize engraftment and potency of TIL therapy through homeostatic proliferation [14]. The patient is

then infused with their expanded therapeutic TIL (lifileucel [LN-144] or LN-145) and subsequently receives up to 6 doses of IL-2 (600,000 IU/kg) to promote activation, proliferation, and anti-tumor cytolytic activity of TIL (Figure 1).

The IL-2 is administered to allow for TIL to survive and expand *in vivo*. IL-2 administration is limited to up to 6 doses given over approximately three days, which compared to therapeutic IL-2 is significantly sub-therapeutic by dose and duration of administration which is limited to approximately 3 days.

Iovance's 22-day Gen 2 expansion protocol demonstrated significant improvement over classical methods of generating TIL which involve multiple *ex-vivo* incubation steps to yield a noncryopreserved, infusion product. The Gen 2 TIL manufacturing process abbreviates the *ex vivo* culture duration to 22 days, is suitable for centralized manufacturing and yields a cryopreserved TIL infusion product that brings convenience in scheduling, logistics, and delivery to clinical sites at commercial scale [15]. The release

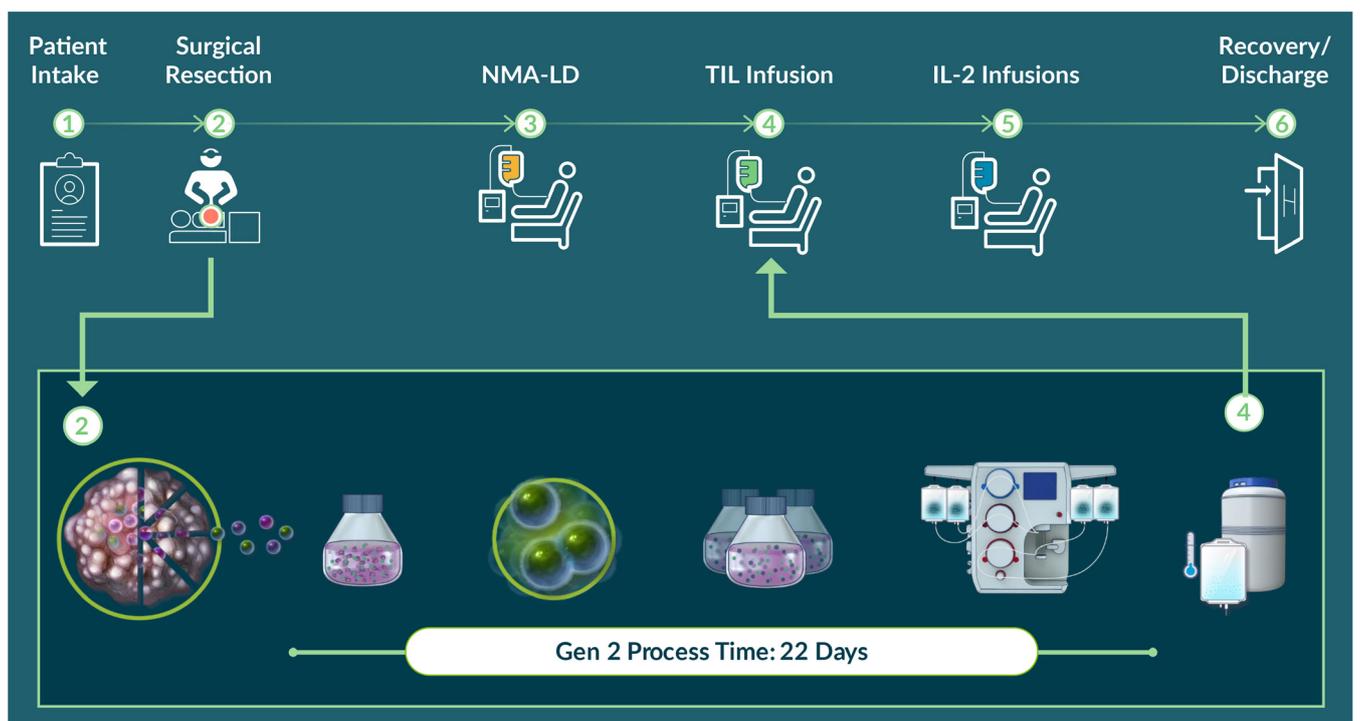
criteria have been well defined and observed for each patient. The Iovance TIL therapy administration process has been implemented globally in multiple institutions offering broad access for melanoma and cervical patient populations in multiple geographic locations.

MECHANISM OF ACTION

Mechanistically, the reinfused TIL circulate in the blood until they detect the tumor in the vicinity due to chemokines produced by the tumor. The TIL then depart the capillaries and migrate to the site of tumor (Figure 2). Upon arrival at the tumor, the TIL recognize tumor antigen peptides presented by MHC molecules on the surface of the tumor cells via their T cell receptors. Upon tumor antigen recognition, the TIL get activated and secrete perforin, a pore-forming protein. The newly formed pores allow for the delivery of granzyme, a pro-apoptotic protease which is also released by the activated TIL and causes lysis of the targeted cancer cell. The infused TIL

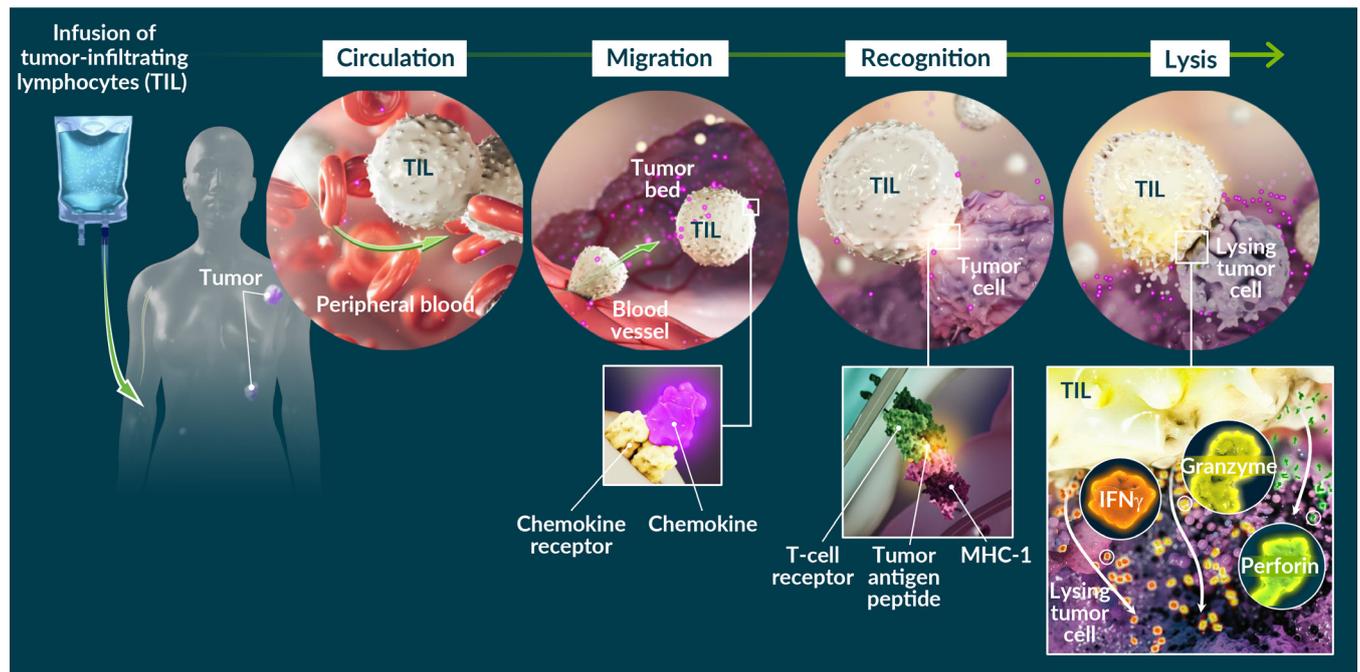
► **FIGURE 1**

Proprietary TIL therapy process.



► **FIGURE 2**

TIL mechanism of action.



thus mediate regression of tumors by direct cell kill but may also induce cytokine-mediated tumor cell killing [16,17].

TIL have clear advantage in treatment of solid tumors due to multiple differentiating factors:

1. Tumor recognition: TIL therapy is autologous, targeted, and enriched for tumor-specific T cells because the TIL were isolated from the site of tumor, where they have previously experienced the tumor-specific antigens [18];
2. Personalized: in solid tumors a single common target neoantigen has not been identified to date. In absence of such a target, TIL therapy relies on the recognition of patient specific tumor peptide antigens by the correct T cells;
3. Polyclonal: the mutational load is high in solid tumors when compared to hematologic malignancies. The polyclonality of TIL that can recognize an array of different tumor antigens best addresses this high mutational diversity. This is a significant strength of TIL as a

therapeutic option, and possibly is why TIL is able to generate clinical response in diseases with high mutational load such as melanoma [19];

4. Neoantigen-specific: the spectrum of neoantigens that need targeting to drive an antitumor response is unknown and highly specific to each patient. Per design, the TIL process ensures the inclusion of neoantigen-specific T cell clones without prior knowledge of the number or identity of those neoantigens;
5. Clinical efficacy: ultimately, clinical data from clinical trials in melanoma and cervical cancer clearly indicate the effectiveness of the polyclonal T cell.

CLINICAL TRIALS

A total of four company sponsored studies in locally advanced, recurrent or metastatic cancers including melanoma, cervical cancer, head and neck, and non-small cell lung cancer are currently being conducted (Table 1).

▶ TABLE 3

C-144-01 Cohort 2 treatment-emergent adverse events occurring in ≥20% of patients.

Preferred term, n (%)	Cohort 2 (N=66)		
	Any grade	Grade 3/4	Grade 5
Number of patients reporting at least one treatment-emergent adverse event	66 (100)	64 (97)	2 (3)†
Thrombocytopenia	59 (89.4)	54 (81.8)	0
Chills	53 (80.3)	4 (6.1)	0
Anemia	45 (68.2)	37 (56.1)	0
Pyrexia	39 (59.1)	11 (16.7)	0
Neutropenia	37 (56.1)	26 (39.4)	0
Febrile neutropenia	36 (54.5)	36 (54.5)	0
Hypophosphatemia	30 (45.5)	23 (34.8)	0
Leukopenia	28 (42.4)	23 (34.8)	0
Fatigue	26 (39.4)	1 (1.5)	0
Hypotension	24 (36.4)	7 (10.6)	0
Lymphopenia	23 (34.8)	21 (31.8)	0
Tachycardia	23 (34.8)	1 (1.5)	0

†One death was due to intra-abdominal hemorrhage considered possibly related to TIL and one was due to acute respiratory failure assessed as not related to TIL per investigator assessment. Patients with multiple events for a given preferred term are counted only once using the maximum grade under each preferred term. Treatment-emergent adverse events refer to all AEs starting on or after the first dose date of TIL up to 30 days.

Treatment options are particularly limited for these patients given that 40–65% of all metastatic melanoma patients are primary refractory to initial immune checkpoint inhibitor therapy [23].

An important consideration is the relative safety associated with TIL therapy. This one-time autologous treatment involves a product individually derived for each patient, it is not selected for the recognition of shared antigens that would be expressed in normal tissues, and is specific to the tumor neoantigens, reducing the risk for autoimmune toxicity. In addition, the TIL mechanism of action does not rely on engineered receptors

but maintains some physiologic control and avoids hyperactivation that may be responsible for complications from CAR-T cell therapy such as cytokine release syndrome or neurotoxicity. TIL therefore offers a differentiated safety profile compared to CAR-T products or immune checkpoint inhibitors and confirms the differentiation discussed above.

C-144-01 is the first study to demonstrate the scalability and reproducibility of a centrally manufactured frozen TIL product. Cohort 4 of the study (N=75) is the pivotal cohort in support of registration of lifileucel in post- anti-PD-1 melanoma patients. Enrollment in Cohort 4 completed in Jan 2020, in approximately 8 months, well in advance of the expected enrollment target possibly indicating the unmet need in this patient population.

C-145-04 metastatic or persistent cervical carcinoma

Cervical cancer is a leading cause of cancer-related death in women with over 12,000 new

▶ TABLE 4

C-145-04 efficacy outcomes.

Response	Patients, N = 27 n(%)
Objective response rate	12 (44.4)
Complete response	3 (11.1)
Partial response	9 (33.3)
Stable disease	11 (40.7)
Progressive disease	4 (14.8)
Non-evaluable	0
Disease control rate	23 (85.2)
Median duration of response	Not reached
Min, max (range)	2.6+, 9.2+

▶ TABLE 5
C-145-04 treatment emergent adverse events.

Preferred term, n (%)	Cohort 2 (N=27)		
	Any grade	Grade 3/4	Grade 5
Number of patients reporting at least one treatment-emergent AE[†]	27 (100)	26 (96.3)	0
Chills	21 (77.8)	0	0
Anemia	15 (55.6)	15 (55.6)	0
Diarrhea	14 (51.9)	2 (7.4)	0
Pyrexia	14 (51.9)	1 (3.7)	0
Thrombocytopenia	14 (51.9)	12 (44.4)	0
Neutropenia	11 (40.7)	8 (29.6)	0
Vomiting	11 (40.7)	1 (3.7)	0
Hypotension	10 (37.0)	4 (14.8)	0
Dyspnea	9 (33.3)	1 (3.7)	0
Febrile neutropenia	9 (33.3)	8 (29.6)	0
Hypoxia	9 (33.3)	3 (11.1)	0
Leukopenia	9 (33.3)	6 (22.2)	0
Hypomagnesemia	8 (29.6)	0	0
Sinus tachycardia	8 (29.6)	0	0

[†]Treatment-emergent adverse events refer to all AEs starting on or after the first dose date of TIL up to 30 days. Patients with multiple events for a given preferred term are counted only once using the maximum grade under each preferred term. Safety terms which describe the same medical condition were combined.

cases and 4,000 deaths in the USA alone [24]. Most patients are young and survival rates are poor. Objective response rates (ORR) for second-line therapies in the metastatic setting, are between 4 and 14% for chemotherapy and recently approved immunotherapy, pembrolizumab [25].

C-145-04 (NCT03108495) is a multi-cohort, Phase 2 clinical trial, enrolling patients with recurrent, metastatic or persistent cervical carcinoma which have exhausted the therapeutic options with surgery and/or (chemo) radiation, as well as palliative chemotherapy administered in the metastatic setting. The clinical trial is designed to determine if this investigational TIL therapy (LN-145) is safe and effective for the treatment of recurrent, metastatic or persistent cervical carcinoma. Initial data from N=27 patients demonstrated an ORR of 44.4% with a median DOR of not reached at a median study follow up of 7.4 months (Table 4). Adverse events in the cervical study were consistent with what was noted in the melanoma program (Table 5) [13].

Ongoing & future research

We are at early stages of understanding capability of TIL therapy and exploration. Understanding of the indications in which TIL therapy can be effective is at early stages and ongoing. Furthermore, genetic modifications, selection of tumor-exposed TIL as well as various operational efficiencies, such as further shortening the TIL manufacturing process and use of core biopsies are all opportunities that are being pursued by Iovance.

Work continues on optimizing TIL manufacturing and potency. Iovance has recently demonstrated the ability to utilize the Gen 2 manufacturing method reliably to expand TIL from core biopsies in multiple tumor types, yielding comparable final therapeutic products [26]. The company continues to seek further improvements by creating new generations of TIL, including exploration of abrogating PD-1 within the TIL product to reduce PD-L1-dependent TIL inactivation, and via intrinsic silencing of PD-1 in our TIL products.

TRANSLATION INSIGHT & OPPORTUNITIES

Relapsed, refractory and metastatic cancers represent high unmet medical need. Despite recent advances in immunotherapies in addressing multiple solid tumor indications, very few options are available to treat patients who progress on immune checkpoint inhibitors or never respond to such treatments.

TIL generated using Iovance's 22-day Gen 2 expansion process have demonstrated anti-tumor efficacy including durable responses in

heavily pretreated metastatic melanoma and cervical carcinoma patients irrespective of prior therapy. This work is the first demonstration of the ability to produce therapeutic TIL in a rapid, centralized fashion with capability to serve multiple global treatment centers. Iovance intends to submit for regulatory approval, based on these data demonstrated in metastatic melanoma and cervical carcinoma, Development of newer generation of polyclonal TIL will continue in order to develop more potent and novel products with differentiated properties.

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AFFILIATIONS

Maria Fardis
Iovance Biotherapeutics

Kelly DiTrapani
Iovance Biotherapeutics

Cécile Chartier
Iovance Biotherapeutics

Friedrich Graf Finckenstein
Iovance Biotherapeutics

AUTHORSHIP & CONFLICT OF INTEREST

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