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Tumor-infiltrating lymphocyte immunotherapy comes of age: a journey of development in the Surgery Branch, NCI

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ABSTRACT

The early development of tumor-infiltrating lymphocytes into an effective clinical strategy was fundamentally the work of hundreds of scientists and clinicians within the Surgery Branch of the National Cancer Institute under the leadership of Steven Rosenberg. That journey brought new insights into the tumor–immune cell interface and ultimately helped create a new first-in-class therapeutic for patients with metastatic cancer.

Nearly 40 years ago, a milky white infusion bag was carried from a cramped research lab to the bedside of a patient with metastatic cancer at the Clinical Center of the National Institutes of Health. Weeks earlier, one of the patient's tumors had been resected and cultured in conditions that would allow the tumor's infiltrating lymphocytes (TIL) to emerge and proliferate. That first-in-human infusion and hundreds that followed contributed to the stepwise development of TIL therapy in the labs of the Surgery Branch of the National Cancer Institute. After demonstrating meaningful clinical responses, the concept of TIL spread to laboratories across the world, into commercial development, and now into hospitals as standard-of-care for patients with metastatic melanoma (figure 1).

In hindsight, the concept seemed intuitively simple: if T cells exist that can recognize and eliminate cancer, they have likely migrated from the peripheral blood into the tumor. Testing that hypothesis was, and continues to be, complex.

DEVELOPING TIL AS A TREATMENT FOR METASTATIC MELANOMA

The Rosenberg lab was already deeply committed to investigating immune strategies to eliminate cancer and had demonstrated clinical success with the use of interleukin-2 (IL-2) that ultimately led to the first US Food and Drug Administration approval for a cancer immunotherapy.[1 2] At that time, lymphokine-activated killer (LAK) cells were also administered, harvested from the peripheral blood during post-IL-2 lymphocytosis. Preclinical lab efforts turned to the study of lymphocytes grown from tumor suspensions and, unlike LAK, TILs were demonstrated to be effective at eliminating large, vascularized tumors.[3]

Despite early negative results in the first-in-human effort of TIL,[4] the team persevered in patients with metastatic melanoma and established proof-of-principle in a cohort of twenty patients. Treatment responses to the earliest regimen (single dose of cyclophosphamide, TIL, and IL-2) were seen in patients whose tumors were naïve to immunotherapy and those in which IL-2 alone failed to control disease.[5] In follow-up studies, an additional 86 patients were treated with a TIL-based regimen (with and without cyclophosphamide and varying doses of interleukin-2) that resulted in an objective response rate of 34%. TILs were capable of mediating regression in metastatic deposits in skin, viscera, and bone.[6] While there were a few durable complete responses, the median duration of partial responses was only 4 months, and more work was necessary to create consistent benefit for patients.

The next decade was spent further refining techniques and elucidating underlying principles in the nascent field of cellular immunotherapy, often testing small changes sequentially in the same subject. One such patient was reported in detail, illustrating not only the ability of TIL to eliminate tumor previously uncontrolled by an infusion of peripheral blood clones but also the reliance of TIL on expression of human leukocyte antigen (HLA).[7] The generation of TIL evolved from culturing single-cell suspensions of mechanical and/or enzymatic digests

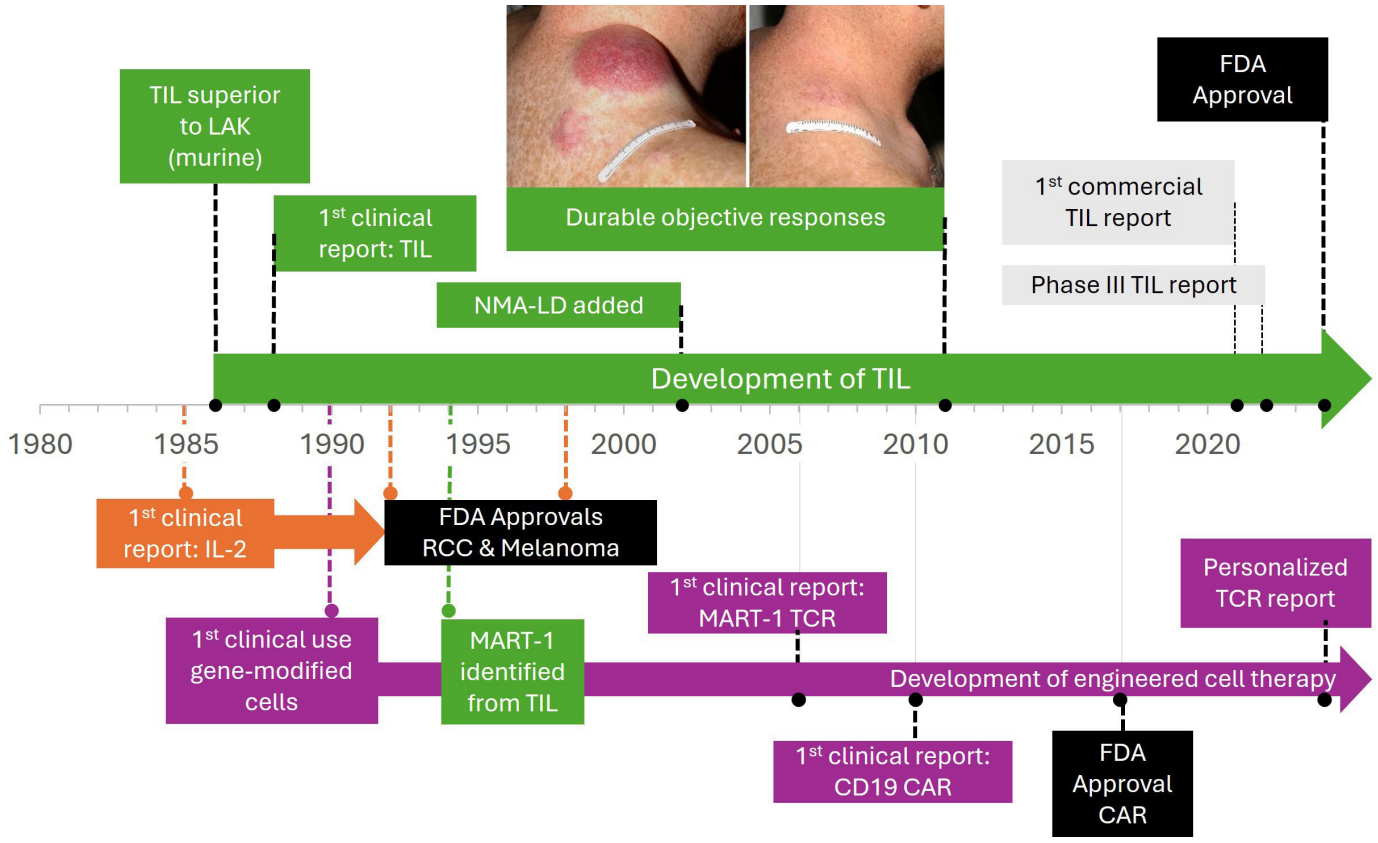

Figure 1 Timeline of TIL development. Interleukin-2 and TIL were developed concurrently in the Surgery Branch, and TIL served as a platform for the development of engineered cell therapy. After the demonstration of clinical objective responses, TIL development continued commercially and in global academic laboratories (gray boxes). Gene engineering was first used in TIL, and the techniques were developed to allow insertion of clinically active T-cell receptors (TCR) or chimeric antigen receptors (CAR). Middle top: Reduction of subcutaneous tumor nodule twelve days after infusion of TIL. FDA, Food and Drug Administration; LAK, lymphokine-activated killer; MART-1, melanoma antigen recognized by T lymphocytes; NMA-LD: non-myeloablative, lymphodepleting chemotherapy; RCC, renal cell carcinoma; TIL, tumor-infiltrating lymphocytes.

to sharply dissected small fragments of fresh tumor. Cultures were chosen for treatment on a variety of factors including proliferation, composition, and tumor recognition. Production timelines became more predictable with the development of a rapid expansion protocol in flasks with gas-permeable membranes that enabled consistent cell doses on the order of 10^{10} TIL. While other cytokines were explored in the Surgery Branch, the treatment culture conditions have remained reliant on high-dose interleukin-2.

The largest clinical change was in the composition of the chemotherapy preparative regimen. Adopting a non-myeloablative regimen similar to that used in bone marrow transplantation, high-dose cyclophosphamide and fludarabine were delivered safely to patients resulting in a transient induction of the desired lymphopenia. In this immunosuppressed state, patients also tolerated more doses of IL-2, reducing the side effect profile of the combination protocol.[8] The tripartite regimen of lymphodepleting, nonmyeloablative chemotherapy, cells, and high-dose intravenous IL-2 became a therapy for metastatic melanoma around which further innovation was studied, yielding a 49% objective response rate.[9]

Clinical trials at the Surgery Branch initially suggested that a deeper lymphodepletion with the addition of total body irradiation could improve the regimen, but a randomized trial showed no difference in complete response rates (24% in each arm).[10] Studies were underway to evaluate a potential reduction in lymphodepletion when the approval of checkpoint blockade inhibitors shifted the landscape of therapy for patients with metastatic melanoma.

CURRENT STATE OF TIL THERAPY

Ultimately, the Surgery Branch experience demonstrated the durable nature of the TIL response. Among the 48 patients with complete tumor regression, 46 never needed another treatment for their melanoma and had a 96% ten-year melanoma-specific survival.[11]

In contemporary studies, the response rate to TIL immunotherapy was lower in patients after checkpoint blockade inhibition but still led to the commercial approval of lifileucel in the USA and point-of-care hospital manufacturing in Denmark and the Netherlands for patients with disease refractory to checkpoint inhibition.[11–13]

While a TIL strategy is likely not appropriate for every patient, given the possibility of severe, transient side effects, the durable complete response rate and subsequent freedom from treatment could make TIL an attractive first-line therapy. A randomized study is underway to evaluate TIL+pembrolizumab versus pembrolizumab for those patients.

TIL therapy has expanded beyond cutaneous melanoma. Small trials in lung cancer, cervical cancer, ocular melanoma, breast cancer, and gastrointestinal malignancies have been reported, but the strategy remains in development.

At the Surgery Branch, the delivery of bulk TIL grown as developed for melanoma did not yield clinical responses in patients with gastrointestinal or breast cancer. However, rapidly expanding cultures chosen by identification of neoantigen reactivity have mediated clinical responses in an ongoing clinical trial.[14–16]

TIL AS A PLATFORM FOR RESEARCH DISCOVERY

Beyond the use as an autologous cancer therapy, the scientific development of TIL led to important advances in cell and gene therapy. Of historical note, TILs were the vehicle for the first foreign genes to be inserted into humans.[17] In the early development of TIL, one of the many concepts for improving the clinical efficacy of the cells was the insertion of functional genes. While the Surgery Branch did eventually test the insertion of functional genes (encoding tumor necrosis factor, IL-2, and IL-12), the original highly scrutinized experience used retroviral techniques to insert neomycin resistance genes to allow for molecular tracking of transferred lymphocytes.[17] The Branch's expertise with gene insertion eventually led to the first application of chimeric antigen receptors targeting CD19 for B-cell malignancies.[18]

The discovery of melanoma-related antigens came from early studies of TIL, independently identifying MART-1 (melanoma antigen recognized by T lymphocytes)[19] while others identified the same antigen (Melan-A) in peripheral blood. A T-cell receptor (TCR) recognizing MART-1 in the context of HLA-A*02:01 was the first to be inserted for successful engineered TCR cell therapy.[20] However, the observation of autoimmune toxicities with TIL and TCRs targeting melanocyte antigens highlighted the limitations of on-target/off-tumor tissue destruction with implications for all engineered cell therapy.

As research into the human genome matured, the Branch's biorepository allowed for retrospective studies of the TIL–tumor interactions of clinical responders. The identification of neoantigen reactivity was key to understanding the highly personalized nature of each patient's response.[21] It also provided a blueprint to developing potentially better treatments from tumors with lower tumor mutational burden.[22] Analysis of melanoma TIL also highlighted the complex role of PD-1 as a marker of tumor-specific reactivity but also transient functional impairment.[23–25] High-dimensional analyses of infusion products have also helped define desirable phenotypic states, identify reactive clonotypes for engineered therapy, and may yield selectable surface markers to improve TIL therapy.[26 27]

THE FUTURE OF TIL

The broader availability of TIL in the USA and parts of Europe will build familiarity with the concept and the clinical expertize needed to treat patients with cell products for metastatic solid cancers. The research impetus now is to improve the therapy to increase the likelihood of response and broaden the indications to improve the survival of patients with metastatic cancer.

Potential barriers to response may exist within the patient, the tumor, or the infusion product. Recognizing that some patient factors may not be modifiable, the next generation of TIL is likely to focus on curating or creating more favorable TIL. The Surgery Branch, academic laboratories, and commercial entities have been exploring ways to improve the TIL repertoire: broadening recognition through in vitro stimulation; preventing exhaustion or promoting "stemness" through alternative culture conditions; or enhancing function through gene modification, among others.

The durable nature of complete response to TIL provides optimism that its use can be extended to larger numbers of patients with a variety of cancer types.

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