Adoptive cell transfer can lead to durable tumor response in patients with metastatic melanoma.

Adoptive Cell Transfer for Patients With Metastatic Melanoma: The Potential and Promise of Cancer Immunotherapy

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Background: Current FDA-approved therapeutic options for patients with metastatic melanoma include dacarbazine, interleukin 2, ipilimumab, vemurafenib, dabrafenib, and trametinib, but long-term tumor regression using available agents remains out of reach for most patients. Adoptive cell transfer (ACT) with autologous tumor-infiltrating lymphocytes (TILs) has shown encouraging results in clinical trials, with evidence of durable ongoing complete responses in patients with advanced melanoma. Emerging techniques to engineer T-cell receptors (TCRs) or chimeric antigen receptors (CARs) using lymphocytes from peripheral blood may offer new tactics in ACT.

Methods: We reviewed the literature to provide a synopsis on the development and clinical trial results of ACT, as well as the future outlook for using ACT in patients with metastatic melanoma.

Results: ACT with TILs as part of a lymphodepleting regimen has been shown in clinical trials to cause objective clinical responses in approximately 40% to 72% of patients with metastatic melanoma, with up to 40% of those patients experiencing complete responses lasting up to 7 years ongoing. Pilot trials using TCR-engineered cells against melanoma-associated antigens MART-1 and gp100 and the cancer-testis antigen NY-ESO-1 have shown clinical responses in patients with melanoma. CAR cells directed against melanoma have been tested only in preclinical models; however, CAR cells targeting other histologies such as lymphoma have elicited antitumor responses in patients.

Conclusions: An example of state-of-the-art personalized medicine, ACT is a potentially curative therapy for patients with metastatic melanoma. Ongoing trials aiming to simplify the regimens may allow a broader range of patients to be treated and enable ACT to be offered by academic cancer centers.

Introduction
Cancer immunotherapy can be separated into three broad categories: active immunization, nonspecific immune stimulation, and adoptive cell transfer (ACT). For patients with metastatic melanoma, active immunization with agents such as peptides or whole tumor cell vaccines, recombinant viruses encoding tumor-associated antigens, or dendritic cells has not been shown to produce consistent and clinically relevant rates of tumor regression, which generally have been
no more than 5%. In the adjuvant setting, even patients with melanoma who have a strong in vitro response to vaccinations with melanoma-associated antigens such as the gp100\textsuperscript{209–217} peptide (evidenced by the generation of high frequency of antigen-specific T cells) have experienced tumor recurrence.\textsuperscript{5} Non-specific immune stimulation with interleukin 2 (IL-2) and ipilimumab can lead to durable cancer regression, although the overall tumor response rates for each agent have been small (16% for high-dose IL-2\textsuperscript{6} and 11% for ipilimumab\textsuperscript{7}), with complete response (CR) rates of less than 10%.\textsuperscript{6,7} A pilot trial of 36 patients with melanoma treated with ipilimumab combined with high-dose IL-2 had overall response (OR) rates of 25%, with 17% achieving CRs lasting more than 8 years ongoing; however, this IL-2 plus ipilimumab combination has not been further tested to confirm these results. Anti-PD1 and anti-PD-L1 antibodies have been recently reported to have OR rates of up to 38%\textsuperscript{8,9} and 17%,\textsuperscript{10} respectively, in patients with melanoma, and OR rates of up to 40% when combined with ipilimumab,\textsuperscript{11} although the long-term durability of the responses is not yet known.

ACT entails the ex vivo identification (or production) of antitumor lymphocytes that are then expanded to large numbers and reinfused (“transferred”) back into the patient. ACT has theoretical and practical advantages over active immunization and nonspecific immune stimulation, including the ability to identify the exact population of T cells capable of in vitro tumor killing and select them for expansion. These cells can be activated ex vivo, free from the potentially suppressive tumor microenvironment that may prevent them from fully living up to their antitumor potential. Preparation of the host patient with lymphodepletion immediately before the transfer of the antitumor cells can eliminate potentially suppressive influences (such as regulatory T cells) to provide an optimal milieu for the cells to proliferate and become activated in vivo. When combined with a preparative lymphodepleting regimen pretransfer, ACT has consistently higher OR rates, from 40% to 72%, with long-term durable and potentially curative CR rates of up to 40%.\textsuperscript{12} This review briefly discusses the historical development of ACT, key clinical trials demonstrating its efficacy and potential, and ongoing and future developments that may lead to a wider use of ACT for patients with metastatic melanoma.

**Historical Milestones in the Development of ACT**

As early as 1922, it was suggested that the presence of a lymphocytic infiltration within resected tumor specimens was associated with longer postoperative survival compared to tumors that lacked lymphocytic infiltration.\textsuperscript{13} In 1954, Billingham et al\textsuperscript{14} reported that allograft immunity could be transferred by using regional draining lymph node cells and termed it “adoptively acquired immunity, in which a normal subject becomes immune as a result of the transference, not of preformed antibody, but of immunologically activated tissue,” demonstrating the role of the cellular arm of the immune system in tissue rejection. Limited by the ability to grow T lymphocytes in vitro, the evaluation and manipulation of this “adoptively acquired immunity” in the next several decades utilized cells obtained fresh from immunized animals.

In 1976, Morgan et al\textsuperscript{15} reported that nontransformed, bone marrow–derived T cells could be cultured in vitro using conditioned medium from stimulated T cells. In 1980, Smith et al\textsuperscript{16,17} identified IL-2 as the soluble “T-cell growth factor” responsible for the initiation and proliferation of T lymphocytes. The development of recombinant IL-2 in 1984 led to the mass manufacture of IL-2 for use in humans.\textsuperscript{18} A report in 1985 showing that IL-2 (given along with lymphokine-activated natural killer [LAK] cells which are nonspecific non-T and non-B lymphocytes) could cause tumor regression in humans was the first to show the feasibility and potential efficacy of manipulating the immune system in human cancer therapy.\textsuperscript{19} A follow-up randomized trial showed that the tumor response was due to IL-2, not due to the nonspecific LAK cells.\textsuperscript{20} The search for a more specific cause of immune-mediated tumor rejection led to the identification of tumor-infiltrating lymphocytes (TILs) which are capable of killing established tumors in murine models at much greater efficacy than LAK cells.\textsuperscript{21} Unlike LAK cells, TILs are classical T cells that become activated only by recognizing, with its T-cell receptor, a specific peptide presented by a human leukocyte antigen (HLA) complex on an antigen-presenting cell or tumor cell. The discovery that TILs grown from human melanoma tumors can lyse fresh autologous tumor cells but not autologous normal cells\textsuperscript{22} led to a phase I clinical trial published in 1988 involving 12 patients with advanced cancer of varying histologies\textsuperscript{23} who had metastases that could be resected to grow TILs while still having residual evaluable tumors. A single dose of cyclophosphamide was used as a preparative regimen (mainly as an “immunomodulatory agent to inhibit suppressor cell function”), and patients were infused with varying numbers of TILs (10\textsuperscript{8} to 10\textsuperscript{11} cells) and varying doses of IL-2. Predictable toxicities attributable to IL-2 were seen, but none were directly attributable to TILs. Tumor response was seen in 1 patient with melanoma and 1 with renal cell carcinoma. These results showing the potential of treating patients with TILs led to further trials that assessed the exact preparative lymphodepleting regimens, the role of IL-2, and the nature of the infused lymphocytes needed to attain the full power of ACT.
Tumor-Infiltrating Lymphocytes
Generating TILs involves resecting a tumor deposit (generally > 1 cm, preferably ≥ 2 cm in diameter) and establishing multiple individual microcultures grown in vitro from either single-cell suspensions or 1 to 2 mm³ tumor fragments in media containing IL-2 (Figure). Appropriately expanded TIL cultures should reach several million cells (combined) in 2 to 3 weeks and have the capacity to kill autologous tumor cells present within the individual cultures. These T cells then undergo a rapid expansion protocol (REP) using the T-cell–stimulating antibody muromonab-CD3, resulting in billions of cells for patient infusion. In a retrospective study evaluating surgical resections for TILs in 402 patients from 2002 to 2007 at the Surgery Branch of the National Cancer Institute, TILs were successfully generated in 677 (86%) of the 787 specimens from all tumor sites, although tumors from the gastrointestinal tract had a decreased rate of TIL growth (70%; \(P = .008\)).

The first phase II TIL-based ACT trial for patients with metastatic melanoma was reported in 1988. The trial involved 20 patients treated with up to \(2 \times 10^{11}\) TILs and high-dose IL-2 (720,000 IU/kg) given every 8 hours as tolerated as previously described. Patients also received a single infusion of 25 mg/kg of cyclophosphamide 36 hours before they were given TILs. Eleven patients experienced objective tumor regression occurring in multiple metastatic sites (from subcutaneous tissue to liver and lung). A follow-up of this trial involving additional patients for a total of 86 revealed an OR rate of 34%, which was similar among those who had previously received (but did not respond to) IL-2 and those who were IL–2-naive, suggesting that the tumor responses were due to the combined regimen involving TILs and not necessarily due to the IL-2 component only.

Concurrent studies in murine models demonstrated that more aggressive lymphodepletion prior to cell transfer resulted in higher response rates suggesting...
that beyond the immunosuppressive effects of regulatory T cells, endogenous immune cells may compete with the adaptively transferred cells for homeostatic cytokines.\textsuperscript{30,31} Lymphodepletion was also associated with increasing levels of homeostatic cytokines IL-7 and IL-15, which may improve the expansion and activation of the transferred cells.\textsuperscript{32} Thus, pilot trials were conducted to explore the extent of lymphodepletion needed to increase the efficacy of TIL ACT.\textsuperscript{13,32} A nonmyeloablative, lymphodepleting chemotherapy regimen used for allogeneic peripheral blood stem cell transplant that consisted of 2 days of cyclophosphamide (60 mg/kg per day) followed by 5 days of fludarabine (25 mg/m\textsuperscript{2} per day) was used.\textsuperscript{33} The day after receiving the last dose of fludarabine, patients received TIL cell infusion followed by high-dose IL-2 as tolerated (Table 1).\textsuperscript{13} In two additional sequential trials, in addition to the lymphodepleting regimen described above, patients also received either a total of 2 Gy or 12 Gy total body irradiation (TBI) to further deplete endogenous lymphocytes prior to TIL cell infusion.\textsuperscript{13} The day following TIL infusion, patients receiving TBI also received at least $2 \times 10^6$/kg of autologous CD34+ hematopoietic stem cells harvested from a granulocyte colony-stimulating factor–mobilized apheresis performed at least 1 week prior to starting cyclophosphamide. With a median follow-up time of 90 months (43 patients with no TBI), 58 months (25 patients with 2 Gy TBI), and 41 months (25 patients with 12 Gy TBI; Table 2), objective tumor responses were seen in 56% of the 93 patients enrolled.\textsuperscript{13} Responses were seen in all affected organs, including the lungs, liver, and brain, and could affect large bulky tumor burden.\textsuperscript{13,35,36} Complete tumor disappearance was seen in 12% of patients without TBI, in 20% of those treated with 2 Gy, and in 40% of those treated with 12 Gy. Nineteen of the 20 patients with CR remain free of disease with some response durations lasting up to 82 months ongoing (82+, 81+, 79+, 78+, 64+, 68+, 64+, 60+, 57+, 54+, 48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, and 19 months).\textsuperscript{13}

Although the data suggest that the patients who received the most aggressive lymphodepleting regimen with the addition of 12 Gy TBI experienced higher OR rates and potentially curative CR rates, these three trials were sequential and not randomized; thus, comparisons between the trials are meant to be hypothesis-generating only. The role of TBI

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\hline
\textbf{ACT Without TBI} & & & & & & & & & & & \\
\hline
Chemotherapy & Cy & Cy & Flu & Flu & Flu & Flu & Flu & & & & \\
\hline
Cells & & & & & & & & & & & \\
\hline
Cytokines\textsuperscript{a} & & & & & & & & & IL-2 & IL-2 & IL-2 & IL-2 \\
\hline
\textbf{ACT With 2 Gy TBI} & & & & & & & & & & & \\
\hline
Chemotherapy & Cy + Flu & Cy + Flu & Flu & Flu & Flu & & & & & & \\
\hline
Radiation & & & & & & & TBI & & & & \\
\hline
Cells & & & & & & & Cells & & & & \\
\hline
Cytokines & & & & & & & IL-2 & IL-2 & IL-2 & IL-2 & \\
\hline
Stem cells & & & & & & & CD34+ & & & & \\
\hline
\textbf{ACT With 12 Gy TBI} & & & & & & & & & & & \\
\hline
Chemotherapy & Cy + Flu & Cy + Flu & Flu & Flu & Flu & & & & & & \\
\hline
Radiation & & & & & & & TBI & TBI & TBI & & \\
\hline
Cells & & & & & & & Cells & & & & \\
\hline
Cytokines & & & & & & & IL-2 & IL-2 & IL-2 & IL-2 & \\
\hline
Stem cells & & & & & & & CD34+ & & & & \\
\hline
\end{tabular}
\caption{Protocols for ACT Trials at the Surgery Branch of the National Cancer Institute}
\end{table}

\textsuperscript{a} For protocols in which cytokines such as IL-2 are given.

ACT = adoptive cell transfer, Cy = cyclophosphamide (60 mg/kg per day), Flu = fludarabine (25 mg/m\textsuperscript{2} daily), IL-2 = interleukin 2 (760,000 IU/kg per dose, given every 8 hours as tolerated up to maximum of 12 doses), TBI = total body irradiation.

lymphodepletion in affecting the efficacy and toxicity of TIL ACT is currently under investigation: an ongoing clinical trial is randomizing patients with metastatic melanoma to receive preparative lymphodepletion with cyclophosphamide and fludarabine or with cyclophosphamide, fludarabine, and 12 Gy TBI (NCT01319565). In the trials listed in Table 2, the TILs used were “selected” TILs. In other words, the individual start-up microcultures were separately tested for antitumor recognition by co-culture assays against either autologous tumor or melanoma cell lines, and only microcultures showing expected antitumor reactivity were selected to undergo REP expansion leading to clinical use. This step of selecting for reactivity required additional time and prevented some patients from undergoing therapy if their TIL cultures did not pass the test (approximately one-third of patients did not have adequate in vitro TIL reactivity25) or if rapid disease progression causing a significant decline in performance status occurred during the growth of the TILs (4 to 6 weeks). Furthermore, data emerging from both mouse models and human clinical trials have indicated that TILs grown for a shorter time in culture have characteristics (eg, longer telomeres37 and CD27+38,39) associated with higher proliferative potential and higher rates of tumor regression. Techniques to grow “unselected” TILs without screening for tumor reactivity were developed and shortened the time TILs spent in culture to 10 to 18 days before undergoing REP expansion.40 When comparing “selected” and “unselected” TILs, the efficacy rate was similar by in vitro testing40 and was subsequently tested in a pilot trial involving 33 patients who experienced similar tumor response rates (58%) as those seen in prior “selected” TIL trials.41

This and other simplifications in techniques led other centers to adapt methods to perform TIL trials. Besser et al22,23 at Sheba Medical Center (Israel) treated 32 patients with metastatic melanoma using (unselected) TIL and IL-2 with the same lymphodepleting cyclophosphamide and fludarabine regimen. Fifteen (48%) of 31 evaluable patients experienced ORs, with 4 (13%) achieving CRs. Pilon-Thomas et al44 at Moffitt Cancer Center treated 13 patients with melanoma using selected TIL and IL-2 with the same lymphodepleting regimen. Five patients achieved ORs (38% response among those treated) with 2 achieving CRs. Ullenlag et al45 from Uppsala University (Sweden) grew TILs from core biopsies rather than surgical excisions and used low-dose subcutaneous IL-2. They treated 24 patients with metastatic melanoma, with 5 (21%) achieving ORs and 1 achieving a CR. Other groups currently investigating TIL therapy in patients with melanoma include the MD Anderson Cancer Center (NCT00338377)46 and Copenhagen University Hospital (Denmark)47 (NCT00937625), which reported 2 patients achieving CRs out of 6 treated with TILs and low-dose subcutaneous IL-2.

**Genetically Modified T-Cell Receptors**

Several important limitations of TIL therapy exist: (1) the need to perform an invasive procedure to obtain tumor tissue to grow TILs, (2) tumor sites that are not easily accessible can increase risks of postoperative morbidity; some tumor sites (eg, lung hilum, head of the pancreas) exclude the option for TIL resection given the potential high morbidity of surgery, and (3) the inability to grow TILs in a small but real number of patients (~ 10% to 15% of patients with melanoma25). The ability to modify genes of any lymphocyte to induce expression of the desired T-cell receptor (TCR) can help bypass these limitations. As an alternative to TILs, genes encoding TCRs that recognize cancer antigens can be introduced into a patient’s peripheral blood lymphocytes (obtained from an apheresis or blood draw) using retroviral or lentiviral vectors.48,49 High-avidity TCRs can be identified either by isolating highly reactive T-cell clones from patients (usually after extensive in vitro sensitization of peripheral blood lymphocytes with the target tumor antigen) or by immunizing transgenic mice (with human HLA) using the target human antigen. The TCR α and β chains from the reactive T-cell clones can then be isolated and cloned into a gene expression viral vector subsequently used to transduce any lymphocyte into becoming antigen specific similar to the parental clone.

The first ACT clinical trial using genetically engineered TCRs involved 15 patients with metastatic melanoma treated with anti–MART-1 TCR-engineered lymphocytes and high-dose IL-2 (after receiving a cyclophosphamide and fludarabine lymphodepleting

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**Table 2.** — Objective Tumor Regressions by RECIST in Selected, Sequential TIL Trials Using Differing Lymphodepleting Regimens

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. of Patients</th>
<th>Partial Response, n (%)</th>
<th>Complete Response, n (%)</th>
<th>Overall Response, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No TBI</td>
<td>43</td>
<td>16 (37)</td>
<td>5 (12)</td>
<td>21 (49)</td>
</tr>
<tr>
<td>2 Gy TBI</td>
<td>25</td>
<td>8 (32)</td>
<td>5 (20)</td>
<td>13 (52)</td>
</tr>
<tr>
<td>12 Gy TBI</td>
<td>25</td>
<td>8 (32)</td>
<td>10 (40)</td>
<td>18 (72)</td>
</tr>
<tr>
<td>Total</td>
<td>93</td>
<td>32 (34)</td>
<td>20 (22)</td>
<td>52 (56)</td>
</tr>
</tbody>
</table>


- Patient response to treatment was assessed utilizing Response Evaluation Criteria in Solid Tumors (RECIST) guidelines.30
- Specifics of the regimens are detailed in the text and in Table 1.
- TBI = total body irradiation, TIL = tumor-infiltrating lymphocyte.
Chimeric Antigen Receptors

Although the use of TCR-transduced T cells eliminates the need for tumor excision for TIL ACT, the specific nature of the TCR, which is HLA-restricted, limits its applicability in some patients. For example, the NY-ESO-1 TCR used in the study by Robbins et al\textsuperscript{53} can recognize and interact only with HLA-A*0201; lymphocytes transduced with that NY-ESO-1 TCR would recognize and interact only with HLA-A*0201; lymphocytes transduced with that NY-ESO-1 TCR would recognize and interact only with HLA-A*0201 and synovial cell sarcoma expressing NY-ESO-1, tumor response was seen in 4 out of 6 patients with synovial cell sarcoma and in 5 out of 11 patients with melanoma.\textsuperscript{53} No TCR-specific toxicity was seen. This study was the first to show that TCR-transduced lymphocytes could cause objective cancer regression in a nonmelanoma tumor, suggesting the possibility of expanding ACT to nonmelanoma cancers traditionally thought to be less immunogenic.
tient with CLL treated with a different anti-CD19 CAR after undergoing lymphodepletion with pentostatin and cyclophosphamide; no cytokine was administered after the cell infusion in this study. Because IL-2 was not needed for these CAR cells to cause tumor regression, the anti-CD19 CAR trial at the Surgery Branch of the National Cancer Institute no longer includes IL-2 (NCT00924326).

Although toxicities such as fever, tumor lysis syndrome, transient hypotension, and transient renal and hepatic insufficiency were reported in some CAR studies, many adverse events could be attributable to the administration of IL-2, and the patients recovered from these toxicities. However, unexpected deaths have also been reported in 1 patient treated with anti-CD19 CAR cells and 1 patient treated with anti-HER2 CAR cells. Soon after cell infusion, both patients developed profound hypotension, respiratory distress, and multiorgan failure associated with markedly elevated levels of inflammatory and homeostatic cytokines. Although severe sepsis could cause this clinical scenario given that these patients were neutropenic around the time of cell infusion, this lethal “cytokine storm” could have been due to the overwhelming activation and proliferation of the transferred CAR cells upon target recognition; such events had not been observed in other ACT trials using TILs. Modification of the CD19 trial to decrease the number of CAR cells transferred has allowed additional patients to be safely treated.

**Discussion**

Metastatic melanoma remains a formidable disease with 1-year survival rates for patients with M1a, M1b, and M1c being only 62%, 53%, and 33%, respectively. In 2011, ipilimumab and vemurafenib were approved by the US Food and Drug Administration for use in patients with metastatic melanoma, adding to the previously limited therapeutic armamentarium that consisted of IL-2 (approved in 1998) and dacarbazine (approved in 1975). Dabrafenib and trametinib were approved in May 2013 for patients with mutated BRAF. Anti-PD1 and anti-PD-L1 antibodies may be added to the list in the near future. The most beneficial sequence of therapy for patients is not yet determined, and we and others are evaluating whether these agents may provide synergism with ACT. Clinical trials are ongoing to evaluate TILs with ipilimumab (NCT01701674), TILs with vemurafenib (NCT01585415, NCT01659151), and TILs with dendritic cell immunization (NCT00338377).

Compared with immunomodulatory drugs such as IL-2 or ipilimumab alone, ACT can theoretically overcome the immunosuppressive tumor microenvironment that prevents naturally existing antitumor lymphocytes from proliferating, becoming activated, and killing tumor cells. ACT may be able to sidestep this limitation because activation and expansion of the antitumor lymphocytes occur ex vivo. Furthermore, the manipulation of the host patient precell transfer with lymphodepletion may create an environment conducive for further cell expansion and activation and may also prevent immunosuppressive regulatory T cells from interfering with the full capacity of the transferred cells. Consistent results of multiple clinical trials confirm the potential power of ACT to induce durable clinical responses. Although the ACT regimens with the longest follow-up (and thus having stronger evidence of durable tumor regression) use TILs, TCR- and CAR–engineered cells have elicited notable antitumor responses in clinical trials and may provide options to patients who do not qualify for TIL therapy. Given the simplicity of obtaining lymphocytes for transduction by just blood draws, T-cell engineering technology may in the future lead to “off-the-shelf” reagents that are personalized to an individual’s HLA and tumor antigen status.

The ACT schema we have used most often, which consists of preconditioning with cyclophosphamide and fludarabine precell transfer and high-dose IL-2 postcell transfer, is rigorous. Patients need to be medically fit to safely tolerate the regimen. Because a significant contributor to the adverse events seen in these protocols is the high-dose IL-2 component, ACT trials with low-dose or no IL-2 are being evaluated. A TIL trial currently enrolling patients with melanoma is using the preparative cyclophosphamide and fludarabine regimen but does not administer IL-2 or any cytokine following cell transfer; preliminary evaluations have shown some clinical responses, although whether the results are similar to TIL trials using high-dose IL-2 remains to be seen (NCT01468818). Based on encouraging results from murine models, another trial is using TILs transduced to express IL-12, an immunostimulatory cytokine, upon antigenic stimulation of the native T-cell receptor (NCT01236573). IL-2 is not given in this trial, and preliminary evaluations have shown some objective responses in patients with metastatic melanoma, including complete responses. Beyond decreasing the incidence of toxicities attributable to high-dose IL-2 and expanding the patient population eligible to receive ACT, these trials may ultimately help simplify the ACT regimens and allow more medical centers to offer these potentially curative therapies.

**Conclusions**

The cure of patients with solid tumors such as metastatic melanoma requires the induction of durable complete tumor responses. Adoptive cell transfer has the potential to provide lifelong antitumor immunity and the promise of a cure for some patients. Active research is ongoing to bring this to fruition for all patients.
2443-2454.

CD8+ T cells in patients with melanoma.

occur despite the induction of very high levels of self/tumor antigen-specific

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