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REVIEW

Efficacy of adoptive therapy with tumor-infiltrating lymphocytes and recombinant interleukin-2 in advanced cutaneous melanoma: a systematic review and meta-analysis

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Adoptive cell therapy (ACT) using autologous tumor-infiltrating lymphocytes (TIL) has been tested in advanced melanoma patients at various centers. We conducted a systematic review and meta-analysis to assess its efficacy on previously treated advanced metastatic cutaneous melanoma. The PubMed electronic database was searched from inception to 17 December 2018 to identify studies administering TIL-ACT and recombinant interleukin-2 (IL-2) following non-myeloablative chemotherapy in previously treated metastatic melanoma patients. Objective response rate (ORR) was the primary end point. Secondary end points were complete response rate (CRR), overall survival (OS), duration of response (DOR) and toxicity. Pooled estimates were derived from fixed or random effect models, depending on the amount of heterogeneity detected. Analysis was carried out separately for high dose (HD) and low dose (LD) IL-2. Sensitivity analyses were carried out. Among 1211 records screened, 13 studies (published 1988 — 2016) were eligible for meta-analysis. Among 410 heavily pretreated patients (some with brain metastasis), 332 received HD-IL-2 and 78 LD-IL-2. The pooled overall ORR estimate was 41% [95% confidence interval (CI) 35% to 48%], and the overall CRR was 12% (95% CI 7% to 16%). For the HD-IL-2 group, the ORR was 43% (95% CI 36% to 50%), while for the LD-IL-2 it was 35% (95% CI 25% to 45%). Corresponding pooled estimates for CRR were 14% (95% CI 7% to 20%) and 7% (95% CI 1% to 12%). The majority of HD-IL-2 complete responders (27/28) remained in remission during the extent of follow-up after CR (median 40 months). Sensitivity analyses yielded similar results. Higher number of infused cells was associated with a favorable response. The ORR for HD-IL-2 compared favorably with the nivolumab/ipilimumab combination following anti-PD-1 failure. TIL-ACT therapy, especially when combined with HD-IL-2, achieves durable clinical benefit and warrants further investigation. We discuss the current position of TIL-ACT in the therapy of advanced melanoma, particularly in the era of immune checkpoint blockade therapy, and review future opportunities for improvement of this approach.

Key words: adoptive cell therapy, tumor-infiltrating lymphocytes (TIL), advanced-melanoma, meta-analysis, immunotherapy

Introduction

Adoptive cell therapy (ACT) using tumor-infiltrating lymphocytes (TIL) is a personalized cancer treatment based on the infusion of autologous CD4+ and CD8+ T lymphocytes expanded from tumors in the presence of interleukin-2 (IL-2) [1] alone, or in combination with IL-7, IL-15, and/or IL-21 [2–4]. CD8+ T cells have historically been considered the primary T-cell mediators driving tumor rejection, although CD4+ T cells have also shown tumor killing potential [5, 6]. TILs are polyclonal populations enriched for lymphocytes recognizing tumor-specific antigens, including shared tumor-associated antigens as well as private tumor neoantigens. Clinical benefit from TIL-AC has been correlated with significantly higher tumor neoantigen load [7].

Development of TIL-ACT required multiple steps of optimization. Studies at the National Cancer Institute (NCI) initiated in 1980 demonstrated tumor regression in selected patients receiving adoptive transfer of lymphokine-activated killer cells in combination with recombinant IL-2 [8]. Rosenberg et al. developed subsequent methods for large-scale expansion of human TIL [9] and pioneered TIL-ACT clinical trials [10, 11]. Melanoma-reactive TILs were expanded from small surgically resected tumor specimens fragments [12], selecting for further expansion only cultures from fragments that yielded tumor-reactive T cells, based on interferon-gamma (IFN-γ) assays. Later studies used the 'young' TIL method, i.e. unselected, minimally cultured, bulk TILs derived from mixed fragments. 'Young' TIL simplified and shortened the TIL production process, enabling the accessibility of this approach to more centers worldwide [13]. Preconditioning with lymphodepletion [14] has been an important milestone in the development of TIL-ACT based on preclinical data [15–18]. Promising response rates were achieved when TILs were infused following non-myeloablating (NMA), lymphodepleting doses of fludarabine and cyclophosphamide [19], to promote production of T-cell homeostatic cytokines [20], which were reproduced by later studies [21–30].

As different centers undertook testing TIL-ACT, the length of TIL cultures, the number of TILs infused, and the dose of IL-2 administered immediately post-TIL infusion to support T-cell expansion *in vivo* have varied. Several important questions thus remain regarding the reproducibility of TIL therapy across centers and the best practices, including the duration of TIL culture, the number of TILs infused and the IL-2 dose. Furthermore, the parallel success of checkpoint blockade immunotherapy (CBI) has raised questions on what role, if any, TIL-ACT has in the current management of melanoma. Here we report the first systematic meta-analysis of all TIL studies administering NMA chemotherapy (or cyclophosphamide, before 2000) and adjuvant recombinant IL-2, which could help guide the development of TIL-ACT.

Methods

Analysis methods and inclusion criteria were specified in advance in a review protocol, but the study was not registered in any public domain. We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines as a basis for this report, since both randomized and non-randomized studies are included [31].

Eligibility criteria

Randomized and non-randomized studies, administering TILs with the addition of full NMA chemotherapy regimen (or cyclophosphamide before 2000) and IL-2 [low dose (LD): <720 000 IU/kg; high dose (HD): ≥720 000 IU/kg] without total body irradiation (TBI), were eligible for inclusion. Target population was advanced cutaneous-melanoma patients, refractory to several treatment lines, such as DTIC/temozolomide, bio-chemotherapy and high-dose IL-2. Tumor response according to standard oncologic assessment criteria had to be provided, while no limitation regarding metastases (including brain metastases) was imposed. Exclusion criteria included uveal/mucosal melanoma, genetically engineered T cells, TBI, intratumoral injections of TIL-ACT combined with kinase inhibitors (e.g. vemurafenib) and single-case reports.

Information sources, search strategies and study selection

Studies were identified by searching the PubMed electronic database. The database search took place on 17 December 2018. No language or year restrictions were imposed.

A comprehensive three-step search strategy was used. First, eligibility assessment of identified records was carried out independently by three reviewers (OM, SML, VP) in two stages. The initial screening was carried out by reviewing title and abstract, excluding those studies that did not meet the inclusion criteria. Whenever a clear decision could not be made, the full text was reviewed. Secondly, the full text was reviewed for all studies. Disagreements were resolved through discussions, or with the help of an additional reviewer (ZT). Special attention was drawn to studies published more than once or with augmented cohorts. Double-counting was avoided by juxtaposing authors' names, treatment groups, sample size, outcome and recruitment period. Hence, if a patient cohort was described in more than one study, only data from the most recent publication was included in the analysis. In addition, if a study had sub-cohorts violating the inclusion criteria, these were excluded as well. Thirdly, the process involved consultation with specialists in the field of TIL-ACT therapy.

Data extraction process

Data extraction was carried out independently by two reviewers (VP, ZT) and was cross-checked by a third (DK), using a predefined standardized form. Information extracted from each study is provided in supplementary Table S1, available at *Annals of Oncology* online, and extraction process methodology in supplementary Table S2, available at *Annals of Oncology* online. Whenever data were available at patient level, these were also recorded. To ascertain the quality of the studies analyzed we used the Cochrane ROBINS-I tool [32], along with Egger's test and funnel plots [33].

Statistical analysis

The primary end point was objective response rate (ORR). Complete response rate (CRR), overall survival (OS), duration of response (DOR) and toxicity [by common terminology criteria for adverse events (CTCAE) version 4.0] were secondary end points. ORR with corresponding 95% exact binomial confidence interval (CI) is presented by study [34]. Pooled estimates across studies are derived either from fixed or random effects models (FEM, REM) [35], depending on the amount of heterogeneity detected [36, 37]. Heterogeneity was assessed via Cochran's Q test ($P < 10\%$) and the I^2 measure [38]. In cases where an FEM model is fitted, due to low amount of heterogeneity, an estimate based upon an REM model is also provided. Analysis was carried out separately for the two IL-2 level doses, since the dose level was expected to differentiate patients' response. Overall estimates are provided for illustrative purposes. Effect of number of cells infused and type of TIL administered (young/conventional) on outcome was also assessed.

Information on CRR is presented in an analogous way. For OS and DOR, Kaplan–Meier plots were produced, when data at patient level were available. Observed hazard differences between groups were assessed via the log-rank test (two-sided $\alpha = 5\%$). The number of patients experiencing specific severe adverse events (AEs) (grade ≥ 3) and the percentages over total number of TIL-ACT-treated patients were presented by study. For AEs of primary interest, probabilities of occurrence were summarized across studies and pooled estimates were derived. SAS v4 (SAS Institute, Cary, NC) and R. v3.4.2 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analysis.

Comparison with approved immunotherapy

Results on the primary and secondary efficacy end points obtained from the meta-analysis were compared with those of currently approved first- or second-line CBI in advanced melanoma.

Sensitivity analysis

Sensitivity analyses were carried out using three scenarios: (A) reduced cohort (excluding studies published before 2006 or with sample size <20); (B) extended cohort (analyzing all patients from all eligible studies—irrespective to whether they received TBI or NMA); and (C) re-classifying from LD to HD one study with IL-2 dose administered in a continuous decrescendo regimen [28].

Results

Studies included in the meta-analysis

We screened 1211 PubMed abstracts and deemed 67 eligible for full review, with 13 studies meeting all inclusion criteria (Table 1 and supplementary Table S3, available at *Annals of Oncology* online). Figure 1 outlines the selection process and reasons for exclusion. Study characteristics, treatment cohort and primary outcomes are summarized in Table 1. Information on prior treatments is provided in supplementary Table S4, available at *Annals of Oncology* online. From the 13 included studies, we excluded from our analysis subsets of patients who were not previously treated, or who received TBI or no NMA chemotherapy (or cyclophosphamide before 2000). All studies were considered at low risk of bias based on Cochrane ROBINS-I tool, and we detected no evidence of bias for ORR (supplementary Figure S1, available at *Annals of Oncology* online).

Cohorts

We gathered a total of 410 pretreated cutaneous melanoma patients (brain metastases included) who received TIL with IL-2 after NMA chemotherapy or cyclophosphamide for studies before 2000 (the standard of care at that time). TIL expansion failure is reported only on five studies (range 7%-33%; Table 1). Two studies were published in 1988; all others were after 2005 (Table 1 and supplementary Table S3, available at *Annals of Oncology* online). All patients had received several lines of prior treatment including immunotherapy, chemotherapy and/or radiotherapy, with the overwhelming majority not receiving CBI (almost 90%; supplementary Table S4, available at *Annals of Oncology* online). The LD-IL-2 cohort comprised 78 patients (5 studies), while the HD-IL-2 cohort comprised 332 patients (8 studies). 'Young' TIL were administered in 183 patients (4 studies) and conventional-cultured TIL in 227 patients (9 studies) (Table 1).

Objective response rate

The overall ORR of the full cohort was 41% ($n = 170/410$ responses; CR 56; PR 114, 95% CI 35% to 48%; REM, $P = 0.049$; $I^2 = 43.10\%$, Figure 2). For the HD-IL-2 cohort, the pooled ORR estimate was 43% ($n = 141/332$ responses; CR 49; PR 92, 95% CI 36% to 50%; REM: Cochran's-Q $P = 0.075$; $I^2 = 45.67\%$; Table 1 and Figure 2). For the LD-IL-2 cohort the pooled ORR estimate was 35% ($n = 29/78$; CR 7; PR 22, 95% CI 25% to 45%; FEM $P = 0.15$; $I^2 = 41.28\%$; Table 1 and Figure 2). In all three sensitivity analysis scenarios considered, pooled ORR estimates for the HD-IL-2 cohort were comparable to the original meta-analysis cohort estimate of 43% (A: 42%, 95% CI 34% to 51%; B: 46%, 95% CI 38% to 54%; C: 42%, 95% CI 37% to 47%; supplementary Figures S2–S4 and Section S1, available at *Annals of Oncology* online). Inference was analogous for the LD-IL-2 cohort.

Complete response rate

A total of 56 CRs were observed. CRR estimate for HD-IL-2 cohort was 14% ($n = 49/332$, 95% CI 7% to 20%; REM, $P = 0.0024$, $I^2 = 68.42\%$) and for LD-IL-2 was 7% ($n = 7/78$, 95% CI 1% to 12%; FEM, $P = 0.52$; $I^2 = 0.0\%$) (Table 1 and Figure 3). Results from the sensitivity analysis were comparable to those of the primary analysis (supplementary Figures S5–S7 and Section S1, available at *Annals of Oncology* online).

Overall survival

Median OS of 17 months is reported for the HD-IL-2 cohort, based on the available individual patient data (IPD) from two studies (no difference in OS observed; supplementary Table S5 and Figure S8, available at *Annals of Oncology* online). Survival is

Table 1. Characteristics of studies included in the meta-analysis, along with information on primary outcome

IL-2 dose	Study year	Type of TILs administrated	Stage (AJCC/ WHO)	Age in years (median/ range)	Performance status	Pts with brain metastasis	Response determination criteria	*N* = 410 Included in the meta-analysis	CR	PR	*N* with TIL expansion failures	*N* with TIL expansion failures, refusals and failures to receive treatment
Low (<720 000 IU/kg)	Topalian-1988[a] [10]	Traditional	NA	41 (35–50)	NA	No	WHO	4[b]	0	1	–	–
	Rosenberg-1988[a] [11]	Traditional	NA	41.5 (21–59)	NA	No	WHO	20	1	10	30	37
	Ellebaek-2012[a] [23]	Traditional	M1a & c	53.5 (36–62)	0	Yes	RECIST 1.0	6	2	0	7	11
	Ullenhag-2012[a,c] [39]	Traditional	IV	55.5 (17–73)	0–3	Yes	RECIST 1.1	24	1	4	–	–
	Andersen-2016[a] [28]	Young	M1a-c	51.5 (25–68)	0–3	Yes	RECIST 1.0	24	3	7	–	25
High (≥720 000 IU/kg)	Dudley-2005 [44]	Traditional	IV	≥18	0–1	Yes	WHO	35	15	3	–	–
	Dudley-2010 [22]	Young	M1a-c	≥18	Good clinical performance	No	RECIST	33[d,e]	3	16	–	–
	Rosenberg-2011 [45]	Traditional	M1a-c	≥18	0–1	Yes	RECIST	43[d]	5	16	–	–
	Pilon-Thomas-2012[a] [40]	Traditional	M1b-c	49 (25–67)	0–1	Unknown	RECIST 1.1	13	2	3	14	−19
	Radvanyi-2012[a] [24]	Traditional	IIIc–IV	≥15	NA	Yes	irRC	31	2	11	–	–
	Besser-2013 [25]	Young	M1a-c	Mean age 54	0–1	Yes	RECIST 1.0	57	5	18	65	80
	Dudley-2013 [26]	Young	M1a-c	≥18	0–1	Yes	RECIST 1.0	69	5	14	86	101
	Goff-2016 [29]	Traditional	M1a-c	45	0–1	Unknown	RECIST 1.0	51[d,e]	12	11	–	–

[a]Information on number of cells infused available at patient level.

[b]Patients with renal, breast or colon cancer excluded.

[c]Related comment in 'Svane, I.M. Cancer Immunol Immunother (2012) 61: 747'.

[d]Patients treated with combination of NMA and TBI are excluded.

[e]There are patients without prior treatment that cannot be excluded from analysis, since they cannot be separated regarding response outcome from the other participants, $n = 8$ for Dudley-2010 and $n = 14$ for Goff-2016.

NA, not available.

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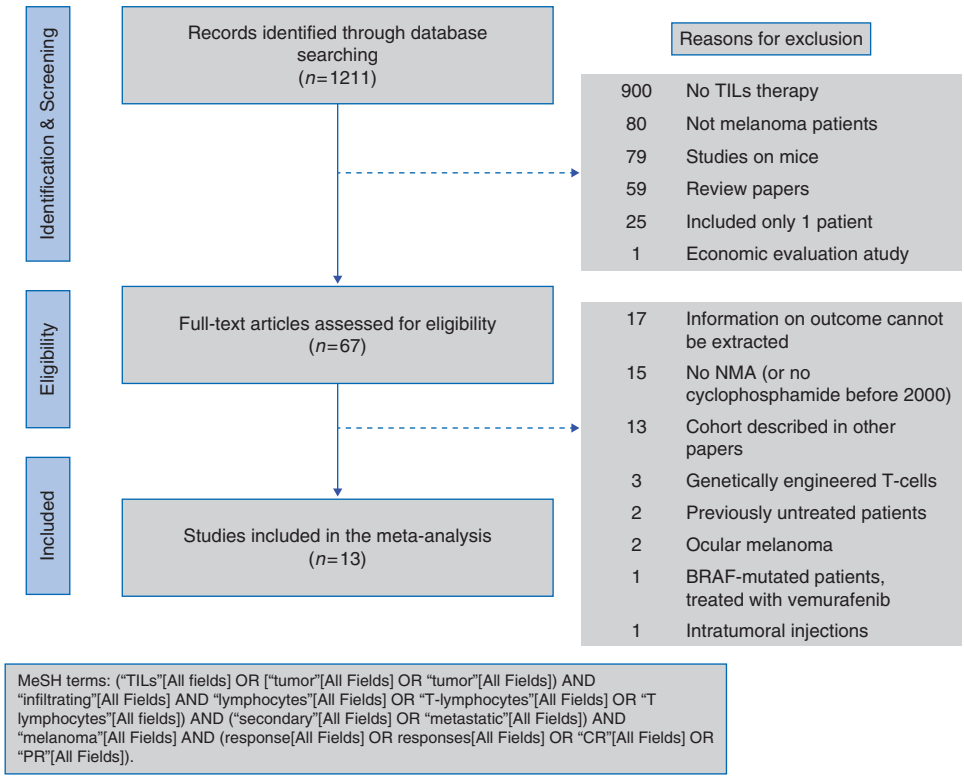

Figure 1. Flow chart of study selection.

presented separately by study, without IPD pooling for the LD-IL-2 studies, since the survival differed significantly among them with the more recent one with superior survival [28] ($P = 0.0002$; supplementary Figure S9, available at *Annals of Oncology* online). Of note, this study was re-classified as HD-IL-2 in the sensitivity analysis (scenario C). The 1-year OS rate in the HD-IL-2 group was 56.5% (95% CI 45.0% to 66.4%), consistently high across all HD-IL-2 studies (supplementary Figure S10, available at *Annals of Oncology* online).

Duration of response

DOR at patient level was available for 112 patients (HD: 100; LD: 12) derived from 7 studies (HD: 5, LD: 2; supplementary Table S6, available at *Annals of Oncology* online). Within the HD-IL-2 cohort, among the 100 responders (CR 28; PR: 72), 55.0% progressed and 45.0% sustained their response during follow-up, with median follow-up after response of 36 months. Median DOR for all responders in HD-IL-2 cohort was 21 months (Figure 4 and supplementary Figure S11, available at *Annals of Oncology* online). All complete responders but 1 (27/28) remained in remission during the extent of their follow-up, with median follow-up after response of 40 months. All 12 PRs of the LD-IL-2 cohort eventually progressed.

Effect of preparation and number of TIL infused

Pooled ORR estimates were comparable between 'young' (41%, 95% CI 28% to 53%) and conventional TIL cohorts (42%, 95% CI 36% to 49%; supplementary Figure S12 and Section S2, available at *Annals of Oncology* online). Corresponding results for CRR for conventional TIL was 14% (95% CI 7% to 22%) and 9% (95% CI 5% to 12%) for young TIL (supplementary Figure S13 and Section S2, available at *Annals of Oncology* online).

Information on the number of cells infused at patient level was available for 122 patients, including 11 CRs and 36 PRs. Significantly higher number of cells was infused in patients who eventually achieved response ($P < 0.001$; supplementary Figure S14, available at *Annals of Oncology* online). OS differed significantly by category of number of cells infused (high: $\geq 50\times 10^9$ cells received [median OS: not reached (NR)]; low: $<50\times 10^9$ cells received [median OS: 4.8 months]; N = 85; $P < 0.001$; supplementary Figure S15, available at *Annals of Oncology* online).

Comparison with approved immunotherapy

We compared TIL-ACT outcomes with cohorts of melanoma patients treated with standard-of-care CBI. For CBI-naïve patients, the pooled ORR estimate of 43% for the HD-IL-2 TIL-ACT cohort did not differ significantly from the estimate for nivolumab-alone (44%; 95% CI 39% to 50%, $P = 0.83$), while it was lower than the ORR estimate of 58% (95% CI 53% to 64%) for the nivolumab/ipilimumab combination ($P = 0.001$; supplementary Table S7, available at *Annals of Oncology* online). No significant difference was detected for CRR between the HD-IL-2 pooled estimate and either the estimate of the nivolumab ($P = 0.60$) or the nivolumab/ipilimumab arm ($P = 0.20$). The pooled ORR estimate of TIL-ACT (overall: $P = 0.043$; HD-IL-2: $P = 0.027$) compared favorably with the ipilimumab/nivolumab combination following anti-PD-1 failure [42] (ORR = 23%; 95% CI 9% to 44%; supplementary Table S8, available at *Annals of Oncology* online).

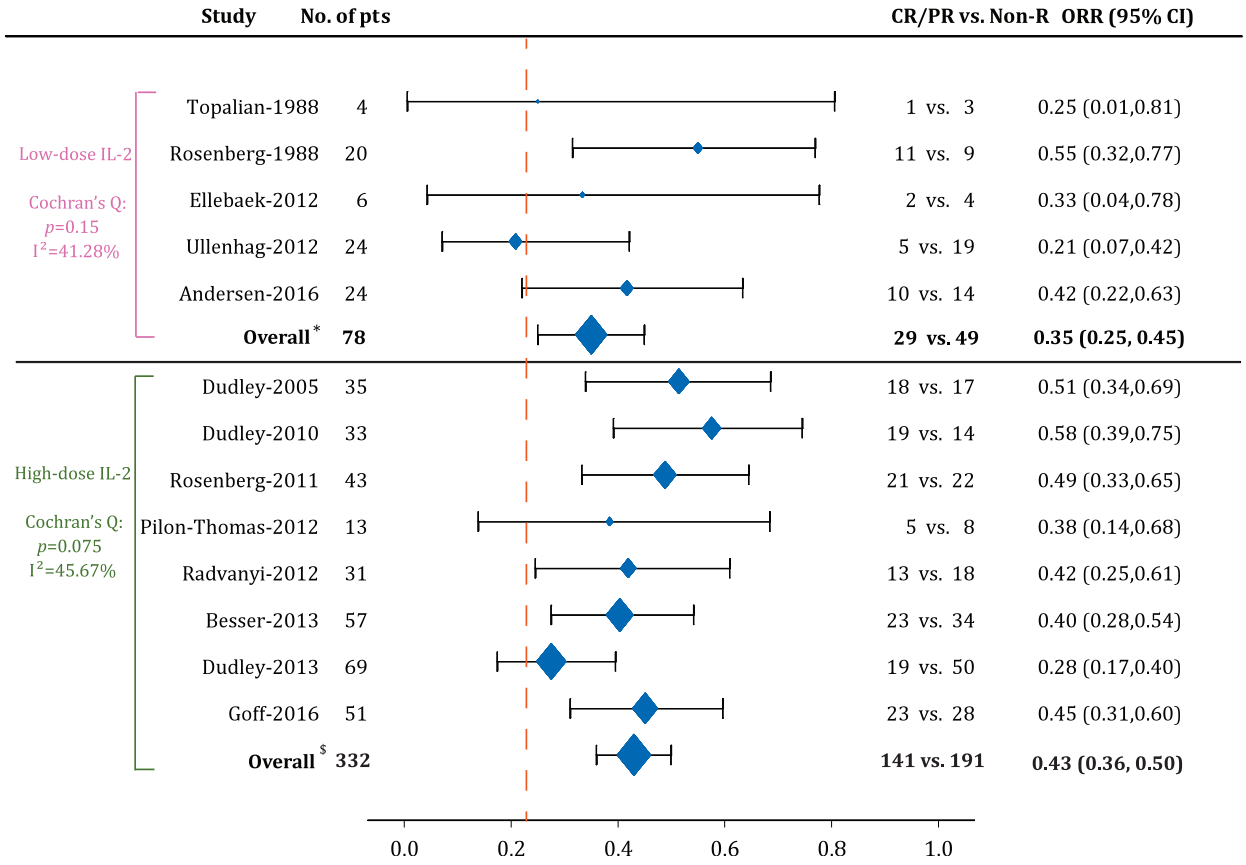

Figure 2. Forest plot for ORRs, by IL-2 dose level. Overall pooled ORR (irrespective of IL-2 dose level): 41%, with 95% CI 35% to 48% (random effects model; Cochran's Q $P = 0.049$; $I^2 = 43.10\%$). Reference line — — —: Zimmer et al. [42], ORR, 95% CI 23% [9% to 44%]. REM estimate for ORR for the LD-IL-2 group: 36%, 95% CI 22% to 50% (*estimate and 95% CI derived from a fixed effect model; $^{\$}$estimate and 95% CI derived from a random effects model; CR: complete response, PR: partial response, Non-R: non-response). Effect of IL-2 dose level $P = 0.32$ (random effects model; Cochran's Q $P = 0.050$; $I^2 = 44.15\%$).

Toxicity

Available information on toxicity is shown in supplementary Table S9, available at *Annals of Oncology* online. The most frequent AE reported was febrile neutropenia (supplementary Figure S16, available at *Annals of Oncology* online), attributable directly to NMA chemotherapy. Pooled estimates of the probability of febrile neutropenia were 35% (95% CI 9% to 62%) and 38% (95% CI 0% to 100%) for the HD and LD-IL-2 cohorts. Other AEs frequently reported were diarrhea, thrombocytopenia and vitiligo. The pooled estimates of the probability of experiencing the AE of 'infections and infestations' and 'respiratory, thoracic and mediastinal disorders' are in supplementary Figures S17 and S18, available at *Annals of Oncology* online. Finally, three cases of treatment-related mortalities were reported (supplementary Table S10, available at *Annals of Oncology* online). Comparison of young/conventional TIL on all secondary outcomes is shown in supplementary Section S2, available at *Annals of Oncology* online.

Discussion

Herein, we addressed some important questions for the clinical development of TIL-ACT in cutaneous melanoma through a meta-analysis of all available studies. Specifically, we sought to examine the efficacy of TIL-ACT across centers and relative to CBI, the influence of IL-2 dose levels, duration of TIL culture and TIL dose. One limitation of our study is that in most cases information was only available for patients with successful TIL expansion, while patients with non-successful TIL expansion have not been generally reported. Another limitation is the lack of IPD and the use of mostly aggregate data. However, IPD meta-analysis (IPD-MA) and aggregate data meta-analysis (AD-MA) have often reached similar conclusions [43], increasing confidence that our findings reveal important aspects of TIL-ACT.

Our analysis reveals reproducible tumor regression in advanced cutaneous melanoma with TIL-ACT regimens including NMA chemotherapy and systemic IL-2 across earlier studies [9, 11, 44] and all recent TIL-ACT trials (after 2006) across centers [22–26, 28, 29, 39, 40, 45]. The ORR ranged between 28% and 45%. For example, Besser et al. reported an ORR of 40% in 57 cutaneous melanoma patients treated with TIL-ACT, with 78% 3-year OS for responders, and a similar ORR even for patients who failed prior ipilimumab [25]. It is important to note that we did not include in our meta-analysis two recently published studies [30, 41], one due to possible overlap with previous studies analyzed, and the other due to differences in cohort characteristics (it included uveal or mucosal melanoma patients).

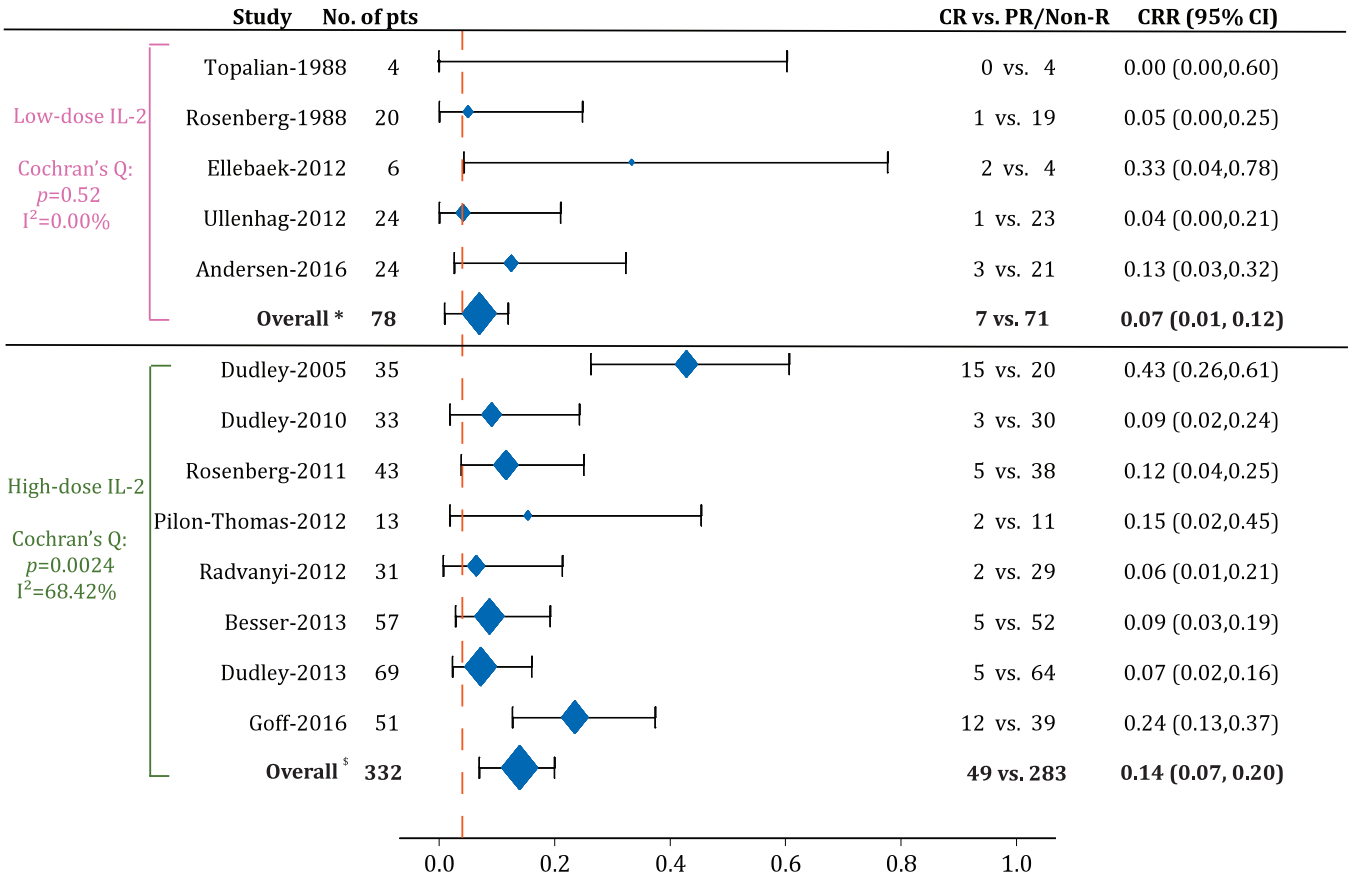

Figure 3. Forest plot for CRRs, by IL-2 dose level Overall pooled CRR (irrespective of IL-2 dose level: 12%, 95% CI 7% to 16%; REM; Cochran's Q $P = 0.0070$; $I^2 = 56.01\%$). Reference line — — —: Zimmer et al. [42] ORR, 95% CI 4% [0% to 20%]. REM estimate for CRR for the LD-IL-2 group: 7%, 95% CI 1% to 12% (*estimate and 95% CI derived from a fixed effect model; $estimate and 95% CI derived from a random effects model; CR: complete response; PR: partial response; Non-R: non-response). Effect of IL-2 dose level $P = 0.32$ (random effects model; Cochran's Q $P = 0.008$; $I^2 = 56.68\%$).

The former compiled the NCI experience on 144 patients in trials from 2001 to 2016 and reported an ORR of 45% (49% of patients without brain metastases) [41], marginally higher than ours. The highest response rates reported to date with TIL-ACT were in a landmark study from the NCI group comparing preparative lymphodepleting regimens using NMA chemotherapy alone, or combined with 2 or 12 Gy TBI in heavily pretreated patients, with 49%, 52%, and 72% ORR and high durable CRRs [45]. Since then, a subsequent randomized study showed no benefit of added TBI and increased toxicities [29]. TBI has not been further pursued, and it is therefore not included in the current meta-analysis.

The 'young' TIL approach allows faster delivery of TIL therapy to eligible patients [22] and yielded in our analysis similar ORR and OS as conventional TIL, while the CRR, which correlates with long-term benefit (although arithmetically lower with 'young' TIL) was also found not significantly different ($P = 0.37$; FEM model, Cochran's Q $P = 0.0056$, I^2=58.37%). In theory, conventional TILs may be more enriched in tumor-specific T-cell clones, due to their selection process. However, longer duration of TIL cultures may have also biased the cohort for slowly progressing patients, potentially explaining the clinical differences reported. In addition, we found a beneficial effect of the TIL dose, with ≥50 billion TIL being associated with better response in 122 patients with available data. This was similar for OS ($n = 85$). This is consistent with mouse data highlighting the importance of the T-cell dose in adoptive T-cell therapy [46–48]. A minimum number of tumor-specific T cells must be infused in order for the T cells to be able to overcome barriers at the tumor microenvironment that prevent full engraftment and function of T cells [47]. In the clinic, the frequency of tumor-specific T cells within the TIL product may vary among patients. A higher dose of TIL might mean a higher absolute number of tumor-specific T cells and/or reflect a higher frequency and/or proliferation capacity of tumor-specific TILs from the original fragments. The relative contribution of CD8+ versus CD4+ T-cell subsets remains incompletely defined; however, some studies have correlated ORR with the absolute number of infused CD8+ cells [25].

The optimal dose of IL-2 remains an open question, since a randomized phase II study (METILDA, NCT01995344) was not completed. In the NCI series, tolerance to IL-2 has been highly variable among patients, with the highest ORR obtained with three to five doses of HD-IL-2 [29]. When combining all available information in the present meta-analysis, the dose of IL-2 had a positive impact in all efficacy measures, reaching significance for DOR ($P < 0.001$), albeit with a small sample size. Impressively, over 90% of patients who achieved CR with HD-IL-2 remained in remission with a median follow-up of 40 months. Also, the

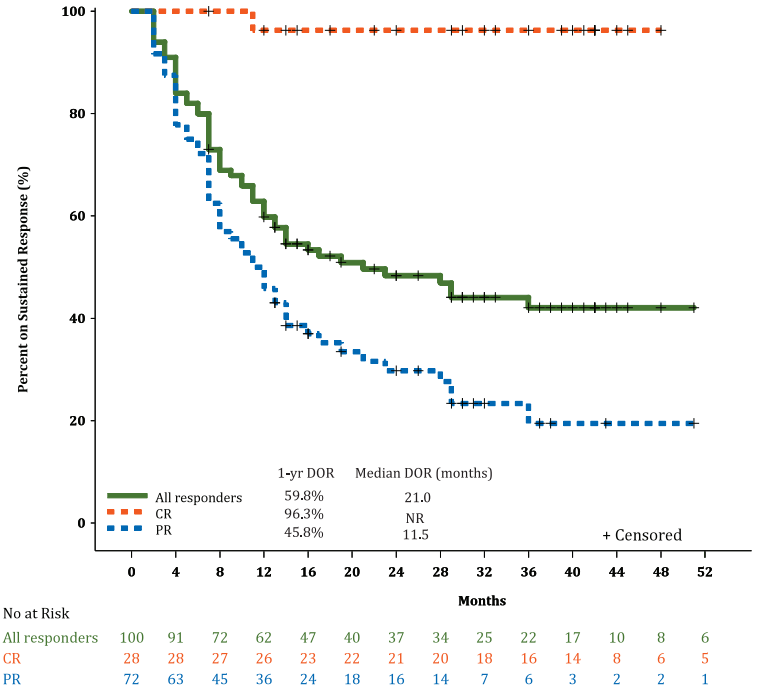

Figure 4. Kaplan–Meier plot for duration of response by type of response, HD-IL-2 studies only (complete versus partial). Data for KM plot derived from Dudley et al. [22, 26, 44], Rosenberg et al. [45], and Goff et al. [29] studies. KM plot lines for complete and partial responders are presented only for illustrative purposes (CR: complete response; PR: partial response).

observed median OS was 17 months, with a 56.5% 1-year OS rate; these data are limited to only 27% of the HD-IL-2 cohort patients (88 out of 332 patients; 2 studies). Consistent with this assertion, a recent report of a phase II study of 12 patients with metastatic cutaneous melanoma treated with a modified TIL-ACT protocol utilizing subcutaneous LD-IL-2 (125 000 IU/kg/day over 12 days) showed an ORR of 22% (2/9), with no CRs and no durable PRs [49].

The current meta-analysis examined studies spanning a wide time period, during which new treatments became available. The above limitation notwithstanding, results in more recent studies (years after 2006) were consistent with the meta-analysis findings, indicating that TIL-ACT is still important in the era of CBI and targeted therapies for advanced cutaneous melanoma. The constraint, however, with respect to current practices, is that in this meta-analysis we could not assess directly the efficacy of TIL-ACT following CBI treatment, since almost 90% of the included patients did not get prior CBI, the now-standard frontline therapy for advanced melanoma [50]. Since many patients with advanced cutaneous melanoma do not benefit from or have acquired resistance on CBI [51, 52], they could be subsequently offered TIL-ACT. In an indirect comparison, the ORR of 33% reported for TIL-ACT as second-line therapy following PD-1 blockade failure [30] was not significantly different from the ORR of 21% reported with the nivolumab/ipilimumab combination in the same population (uveal/mucosal cases included) [42]. Although the TIL-ACT ORR of 33% for this cohort was based on only 9 patients (3 PRs) [30], an ORR of 38% to TIL-ACT (2 CRs) was recently reported in a multi-center study involving 55 patients who failed prior CBI [53]. Similarly, an ORR of 32% was reported following failure of PD-1 blockade by the Copenhagen group [54].

From the emerging data it appear that the ORRs, and especially the CRRs, as well as the DOR to TIL-ACT administered following CBI failure are lower than previously reported in CBI-naïve patients. This should come as no surprise. Primary resistance to CBI may be mediated by a variety of mechanisms including paucity of immunogenic tumor antigens [55], impaired tumor antigen presentation, including loss of beta-2-microglobulin (β2M) [56, 57], somatic mutations leading to loss of IFN signaling and T-cell exclusion [51], or compensatory upregulation of alternate coinhibitory receptors [58–61]. Similar mechanisms may account for failure of TIL-ACT [62, 63], suggesting that tumors which have already escaped CBI may escape subsequent TIL-ACT. Frontline PD-1 blockade likely extracts the best responders from the overall patient population, leaving behind patients with tumors characterized by a variety of resistance mechanisms, only some of which may be overcome by TIL-ACT. A similar 'pruning' phenomenon is probably responsible for the difference in ORR to nivolumab/ipilimumab combination between frontline (all comers, 58%) [64] and second-line following PD-1 failure (21%) [42].

The choice of nivolumab/ipilimumab versus TIL-ACT in the population failing PD-1 blockade could become an important question in advanced cutaneous melanoma. If an ORR of around 35% is confirmed in this population, this would be theoretically non-inferior or even superior to nivolumab/ipilimumab (ORR 21%) [42]. Independently of efficacy, the expected rate of serious AEs is much higher for the nivolumab/ipilimumab combination compared with TIL-ACT, with treatment-related AEs of grade 3/4 occurring in 59% of the patients in the nivolumab/ipilimumab group [64], and up to 40% of patients discontinuing the combination due to toxicity. For nivolumab/ipilimumab, treatment complications are mainly related to autoimmune toxicity, which

may be protracted and lead to important morbidity, in part related to required immunosuppressive therapy. On the other hand, TIL-ACT is a one-off therapy, and toxicity of TIL-ACT is short-lived, well managed by expert teams, related mostly to NMA chemotherapy and to a lesser extent to HD-IL-2. We found that toxicity was tolerable across TIL-ACT studies. Furthermore, although the costs of TIL-ACT may presently be elevated due to product manufacturing costs and hospitalization, the real costs of managing the toxicity of anti-CTLA-4/PD-1 combination should be also taken into consideration. This analysis will greatly benefit from completion of comparative studies, with one randomized presently under way (NCT02278887). Such studies should consider not only clinical efficacy but also toxicity, quality of life, and pharmacoeconomic end points. Previous economic analysis, e.g. demonstrated a more favorable role for TIL therapy than ipilimumab alone [65].

While TIL-ACT presently offers an acceptable response rate even after CBI, this strategy still presents important opportunities for enhancement [66, 67]. Gene engineering of TIL with cytokines could obviate the need for long-term support with exogenous cytokines. For example, ACT with human T cells overexpressing IL-2 could be used to prolong the *in vivo* survival of transferred cells, which has yielded promising results with respect to persistence of transduced TIL [68, 69]. Other cytokines such as IL-15 or IL-12 could be interesting [70, 71], although TIL engineered with IL-12 were not well tolerated [72]. A PEGylated form of IL-2 enabling the slow release of IL-2 molecules with a biased affinity towards the βγ-chains of the IL-2 receptor [73], was recently shown to be very well tolerated, resulting also in 10-fold rise in tumor CD8+ T and NK cells with minimal modifications in T-regulatory cells [74]. Such approach could facilitate longer-term use of IL-2 post-ACT. Other cytokines (IL-7, IL-15, IL-18) are emerging as key supporters of T-cell expansion and function *in vivo* [75, 76] and could find important applications in TIL-ACT.

T-cell-intrinsic characteristics such as durability, longevity, and functionality play significant roles in determining immunotherapy effectiveness [77]. Importantly, the cellular energetic pathways used by T cells control each of these qualities [78], and there is proof that T-cell metabolism can be modulated to improve melanoma TIL phenotype and function [79, 80]. Success in T-cell cancer therapy requires appropriate TIL activation and competition for nutrients in an immunosuppressive environment [79]. For example, PD-1 signaling induces T-cell inhibition in part through repression of the PI3K pathway [81, 82]. Similarly, increased potassium concentrations in the tumor microenvironment due to tumor necrosis drive TIL to an unreactive 'lymphoplegic' state, characterized by high autophagy and limited terminal differentiation [83, 84]. Future studies examining T-cell metabolic demands *in vitro* and *in vivo* may have a significant impact on TIL-ACT. Specifically, reduced metabolic activity in T cells during *in vitro* development and priming induces the development of T cells with enhanced self-renewal and persistence [79]. Supplementing amino acids (e.g. arginine) [85], decreasing the activity of fatty acid and cholesterol biosynthesis pathways [86], or altering T-cell mitochondrial dynamics [87] during *in vitro* culturing may also lead to enhanced cell persistence and *in vivo* antitumor activity. Finally, T-cell costimulation is a key factor enabling serial target killing and tumor control, as revealed by the use of T cells transduced with second-generation chimeric antigen receptors [88]. Costimulatory signals such as 4-1BB (CD137) or OX40 (CD134) [89] could be also exploited during TIL expansion [90–93].

Additional opportunities emerge from enriching the TIL product. Immune recognition of mutated tumor neoantigens, which can be highly immunogenic, may be partly responsible for tumor rejection upon both CBI [94–98] and TIL-ACT [99]. Current technological developments have enabled the identification of patient-specific tumor-associated antigens, and successful attempts have been reported with TIL-ACT using T cells enriched for recognition of tumor neoantigens [5, 100], although tumor evolution can lead to loss of neoantigen expression and immune escape [101]. Finally, preclinical research from several groups has shown that the antitumor activity of TIL *in vivo* could be enhanced by prior genetic modification endowing novel properties to the cells. For example, transduction with chemokine receptors such CXCR2 can facilitate their entry into the tumor site[102, 103] while incorporating a dominant negative form of the transforming growth factor beta receptor 2 (TGF-βRII) or Fas can protect TIL against local immune suppression in the TME [104, 105]. A novel method developed for the efficient manufacturing of large numbers of GMP-grade gene-modified TIL is presently being tested in clinical trials [106]. Pre-TIL strategies designed to enhance recruitment and expansion of T-cells *in vivo* before harvest using oncolytic viruses [107–109] or TLR9 agonists (CpG) could also be considered [110–112].

Conclusions

TIL-ACT therapy is an effective treatment of patients with advanced cutaneous melanoma, as confirmed by the present meta-analysis, but is currently developed in only a few specialized centers worldwide. The positive evidence accumulated worldwide and reanalyzed herein should incentivize institutional investments towards TIL manufacturing infrastructure, adapting surgical practices, and training teams to manage expected treatment-related toxicities. Developing and providing this personalized treatment in more centers would make an effective treatment available to a substantial number of advanced melanoma patients.

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