



OPEN Characteristics of successful expansion of tumor-infiltrating lymphocytes from colorectal cancer liver metastasis

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Adoptive cell therapy (ACT) utilizing tumor-infiltrating lymphocytes (TILs) has emerged as a successful treatment modality for various malignancies. However, TILs cultured from colorectal cancer (CRC) liver metastasis remain underexplored. Fifteen CRC liver metastasis tissues underwent initial expansion (IE) of TILs and rapid expansion (REP). Histologic examination including the level of stromal TILs and Klintrup-Mäkinen score, were assessed by pathologists and deep learning-derived spatial analysis. We performed correlation analysis between expanded TILs and histopathologic factors. All cases exhibited successful IE, with a mean IE TIL count per fragment and total IE TIL per case of $2.59 \pm 2.79 \times 10^5$ cells and $167.79 \pm 126.97 \times 10^5$ cells, respectively. Five cases underwent REP, with a median fold change of 3,610 (range, 1,136–4,925). The median CD4+/CD8+ ratio in IE TILs and REP TILs were 3.66 and 0.68, respectively. A significant correlation was observed between the mean number of expanded TILs per fragment and KM score ($p=0.022$). Successful expansion of TILs from CRC liver metastasis was achieved. Assessment of KM score may serve as a predictive tool for the obtainable TILs before IE. These findings lay the groundwork for future studies to establish effective ACT in patients with metastatic CRC.

Keywords Colorectal cancer, Liver metastasis, Tumor-infiltrating lymphocytes, Adoptive cell therapy

Colorectal cancer (CRC) is one of the most common cancer worldwide. Substantial progress has been made over recent decades in the treatment of advanced CRC; however, the 5-year survival rate of patients with stage IV disease remains extremely low at 5.7%¹. Late-stage CRCs are prone to metastasis, and one of the most common sites of metastasis is the liver. Approximately 50% of patients with CRC develop liver metastasis as the disease progresses. The median survival for patients with liver metastasis is 6 to 9 months without proper treatment intervention². Liver resection currently stands as the gold-standard approach for achieving long-term survival and potential curative outcomes. Surgical resection yields 5-year survival rates as high as 71% for solitary CRC liver metastasis, with further improvement when coupled with chemotherapy or other adjuvant treatments³. However, up to 55–60% of patients experience recurrence, particularly within 2 years post-resection. Additionally, 60–70% of patients develop local, regional, or distant recurrence, and the outcomes of repeated curative resection remain comparable to the initial resection in terms of overall survival, while the complexity of the operation is heightened by adhesions resulting from repeated resections⁴.

There have been trials to use recently introduced checkpoint inhibitors and tropomyosin receptor kinase (TRK) inhibitors in patients with advanced CRC. However, most CRCs exhibit proficiency in mismatch repair, resulting in an immune-excluded microenvironment characterized by the absence or inactivity of CD8+ T lymphocytes and diminished expression of immune checkpoint proteins on tumor cells⁵. Although a small subset of patients may possess deficient mismatch repair and be eligible for immunotherapy, the benefits of immune checkpoint inhibitor without high levels of tumor mutation burden remain uncertain⁶. Recently, adoptive cell transfer (ACT) of expanded tumor-infiltrating T cells (TILs) has emerged as a promising treatment modality.

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Expanded TILs have already demonstrated efficacy in treating patients with advanced melanoma^{7,8}, cervical^{9,10}, breast¹¹, and ovarian cancers^{12,13}.

For successful implementation of TIL therapy, it is critical to obtain abundant expanded TILs. However, harvesting TILs from primary CRC is hindered by limitations from potential contamination by fecal bacterial colonies. Overcoming this challenge involves sampling from hepatic metastatic lesions. However, the liver maintains an immunosuppressive microenvironment, potentially impeding florid lymphocytic infiltration into the tumor¹⁴. The rate of successful expansion of TILs from CRC liver metastasis remains unexplored, and there is no predictive marker for successful TIL expansion in CRC liver metastasis. We therefore cultured TILs from metastatic CRC specimens and investigated the relationship between the histopathologic characteristics of the tumor and the number of expanded TILs.

With the introduction of artificial intelligence (AI) into digital pathology, there has been pathologists' interest to establish a deep learning algorithm that can be used in diagnosis, pattern analysis, and spatial measurement of tumor components. This study conducted a computerized analysis using deep learning methods on hematoxylin and eosin (H&E) slides of representative tumors. The program classified tumor components into background, debris, normal liver, mucin, stroma, and tumor. Additionally, we employed the deep learning model to quantify TILs. We also analyzed the relationship between histopathologic characteristics of tumors, as derived from AI analysis, and the number of expanded TILs.

Materials and methods

Patients and tumor infiltrating lymphocyte cultures

An analysis involving 15 patients who underwent hepatectomy due to metastatic colorectal adenocarcinoma to the liver at Asan Medical Center, from April 2021 to June 2022 was conducted. Clinical information, including pathologic reports, sex, age, microsatellite instability (MSI) status, and history of chemotherapy after the tumor metastasis to the liver, was reviewed. This study was approved by the Institutional Review Board of Asan Medical Center (IRB No. 2020–0980). Informed written consent was obtained from all patients.

TIL culture

Cultures for T cell products underwent IE and rapid expansion (REP). In the initial expansion phase, metastatic tumor tissue was sectioned into fragments of 1–2 mm in diameter and plated into T75 flasks (12 fragments/flask) within 2 h of surgery. The TIL culture medium (AIM-V medium, Life Technologies, Carlsbad, CA, USA) was supplemented with 3% Stemulate (Sexton, Biotechnologies, WA, USA) and 1,000 IU/mL human recombinant interleukin-2 (Miltenyi Biotec, Bergisch Gladbach). Plates were incubated for 14 days at 37 °C in a 5% CO₂ incubator, with a medium addition period of 7, 10, and 12 days. Cultured TILs were cryopreserved until further analysis. In the REP stage, TILs were cultured in G-rex 100 M flasks with irradiated (80 Gy) allogeneic peripheral blood mononuclear cells (PBMCs) from one healthy donor in REP medium (AIM-V medium, Life Technologies) supplemented with 3% Stemulate. On the first day, 2,000 IU/mL of human recombinant interleukin-2 and 30 ng/mL of human anti-CD3 antibody (clone OKT3, Miltenyi Biotec) were administered, followed by 2,000 IU/mL of REP medium every 4, 6, 8, and 10 days.

Pathology data

The harvested hepatectomy specimen first underwent routine pathologic evaluation, and the H&E-stained slides were reviewed by two pathologists (JB and GC). The TIL status of each tumor was evaluated based on the stromal TIL (sTIL) and inflammatory cell infiltrate of the tumor invasive margin, using the Klintrup–Mäkinen (KM) assessment¹⁵. sTIL was defined as the guidelines of the International Immuno-Oncology Biomarkers Working Group¹⁶.

Histopathologic evaluation

The guidelines define sTIL as the area occupied by mononuclear inflammatory cells over a total stromal area. A comprehensive evaluation of the average sTIL in the entire tumor area is also required. The sTIL data were recorded in increments of 5%, with 1% representing the lowest score. Assessment of KM was scored from 0 to 3 (Fig. 1). A score of 0 indicated no increase in inflammatory cells, and 1 denoted a mild and patchy increase of inflammatory cells at the invasive margin, without destruction of invading cancer cell islets by the inflammatory cells. A score of 2 indicated inflammatory cells forming a band-like infiltrate at the invasive margin, with some destruction of cancer cell islets by inflammatory cells. Finally, a score of 3 was assigned to a very prominent inflammatory reaction, forming a cup-like zone at the invasive margin, with frequent destruction of cancer cell islets. The size of the metastatic tumor was also measured. The average sTIL and consensus value of the KM score of two pathologists was adopted. Additionally, the intratumoral proportion of tumor cells, stroma, mucin, and necrotic debris was independently assessed by two pathologists (JB and GC), and the average values were adopted.

Deep learning-derived spatial analysis

Two automated models were developed: histologic component classifier and TIL quantifier. For the histologic classifier, the ResNet50 architecture and ImageNet pre-trained CNN were adopted to classify histologic image tiles into six classes: background, debris, liver, mucin, stroma, and tumor. From the in-house image dataset and the part of NCT-CRC-HE-100 K dataset, a total of 76,923 image tiles of 224 × 224 pixels were extracted. These image tiles were subsequently randomly partitioned into a training set (65%) and a testing set (35%). We applied augmentation methods including rotation, color jittering, and color normalization. The training process involved computing the loss through cross-entropy and optimization using the Adam optimizer, with

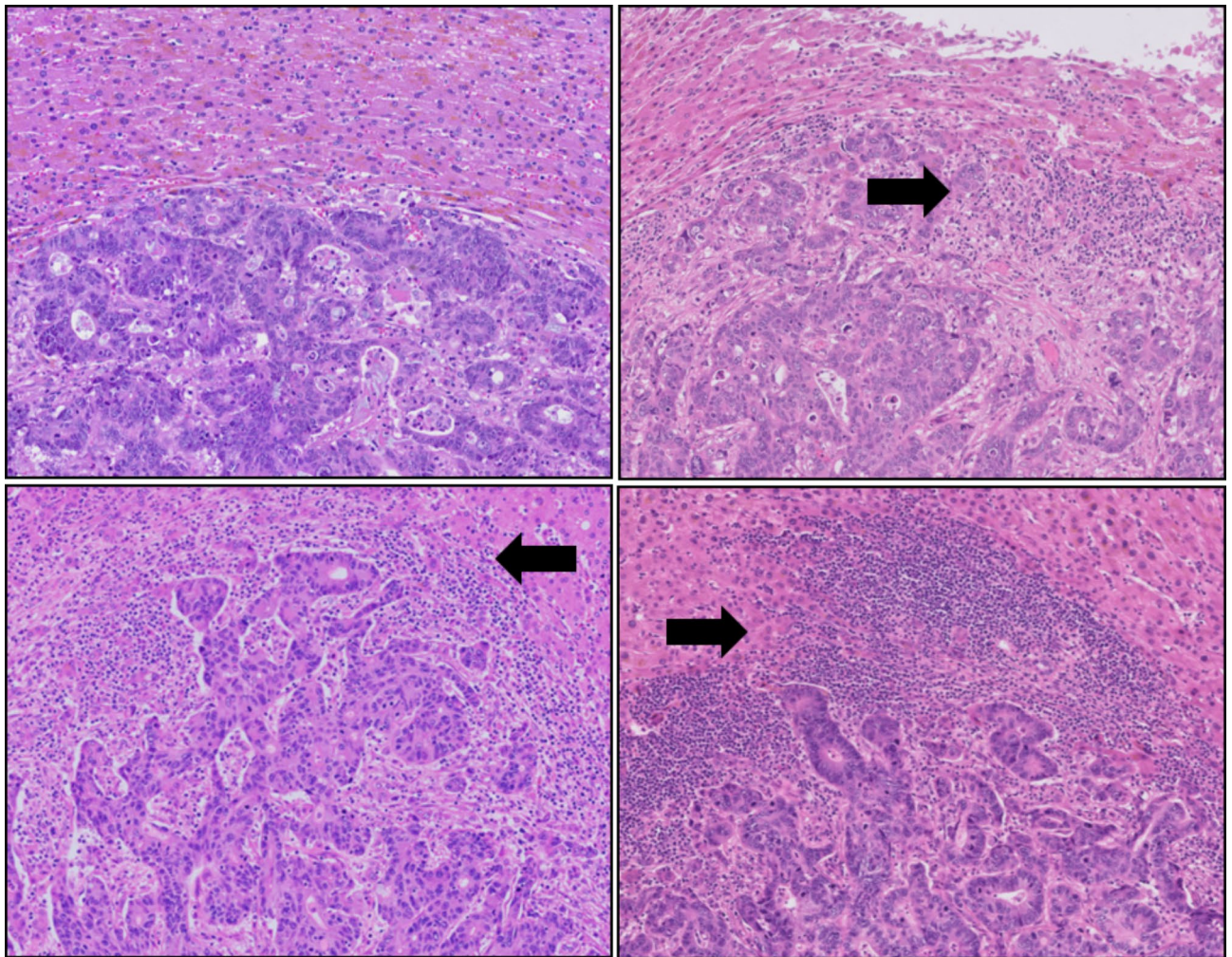


Fig. 1. Representative images of evaluation of Klintrup–Mäkinen (KM) assessment. KM score was scored from 0 to 3 (0, upper left; 1, upper right; 2, lower left; 3, lower right). The black arrows indicate immune cells located at the invasive front. H&E stain, 40× magnification.

specific parameter settings: a batch size, optimizer learning rate, and epochs were configured at 256, 3e-04, and 15, respectively. The performance of the model was evaluated based on average accuracy and F1-Score, both exhibiting values within the range of 0.99 to 1.

For TIL quantification, we developed DeepLabV3+ architecture-based semantic segmentation model with ResNet50 backbone. The image dataset was built using representative slides from 15 breast cancer samples. Each H&E-stained slide was de-stained and subsequently re-stained using a combination of immune cell markers (CD3, CD20, and CD79) and scanned, producing two whole slide images for each slide. The corresponding coordinates of immune cell marker-stained areas in the digitized H&E slides were used as ground truth. The model was trained using a learning rate of 0.001, with a batch size of 16, over a course of 50 epochs. Using these two models, we could count the average number of TIL per image tile of each tumor component.

Fluorescence-activated cell sorting

The population of cultured TILs was estimated using fluorescence-activated cell sorting (FACS). To analyze the molecules expressed on cell surfaces, cells were stained with specific antibodies, including CD3-APC- cy7 (Biolegend, San Diego, CA, USA), CD4- FITC (BD Biosciences, Franklin Lakes, NJ, USA), CD8-Perpc CY5.5 (BioLegend), CD45RA-PE (BioLegend), CCR7-PE-cy7 (BioLegend), CD45-BV510 (BioLegend), and CD95-APC (BioLegend), for 30 min at 4 °C in the dark. After washing with FACS buffer, the cells were stained with 4',6-diamidino-2-phenylindole solution to distinguish dead cells. The cells were then washed and resuspended with FACS buffer. Flow cytometry was performed using the FACS Canto II device (BD Biosciences, San Diego, CA, USA). Data were analyzed using FlowJo software (Tree Star, Ashland, OR, USA). CD4+ and CD8+ T cells are subclassified as effector memory T cells (Tem, CD45RA-CCR7-), effector T cells (Teff, CD45RA+ CCR7-), central memory T cells (Tcm, CD45RA-CCR7+), and naïve T cells (Tnaïve, CD45RA+ CCR7+).

Statistical analysis of clinical and pathology data

All statistical analyses were performed using SPSS 20.0 statistical software (SPSS Inc, Chicago, IL, USA). Pearson’s correlation test, simple linear regression, and Mann–Whitney U tests were employed as appropriate. A *P*-value of <0.05 was considered statistically significant.

Results

Clinicopathologic evaluation

A total of 15 patients were included in our study, comprising 5 females and 10 males. The median age of the patients was 66 years (range 53–79). Among the patients, only one (Case 14) had unstable microsatellite instability (MSI) status, while 14 were stable. Four patients received chemotherapy before the surgical procedure. The median tumor size was 3.7 cm (range 2.1–11). On follow-up in October 2023, 7 of the 15 patients showed no signs of recurrence of metastasis. However, metastasis to other organs or recurrence at the stump of the colectomy site was observed in 8 patients (Table 1).

In the pathologic evaluation, the mean percentage of sTILs in 15 patients was 7.7% (range, 10–30). Five (33.3%) cases were KM grade 1, 8 cases (53.3%) were KM grade 2, and 2 cases (13.3%) were KM grade 3. On AI model evaluation, the average number of TIL per stroma tile (A-TIL) was 2.84 (range, 0.77–7.82). Further analysis showed the mean percentage distribution of background, debris, liver, mucin, stroma, and tumor as 0.59, 25.45, 15.43, 10.11, 13.91, and 34.52, respectively (Fig. 2a, and Table 2). The average value of stromal TILs evaluated by the two pathologists (P-TIL) demonstrated a significant correlation with A-TIL (Pearson *R* = 0.774, *p* = 0.001). This correlation was comparable to the level of agreement observed between sTILs evaluated by the two pathologists (Pearson *R* = 0.711, *p* = 0.003) (Fig. 2b).

Expansion of TILs from metastatic colorectal carcinoma

All cases demonstrated successful TIL expansion. The mean number of IE TILs per fragment and total IE TILs per case were 2.59 ± 2.79e5 cells (range, 0.16–8.75e5) and 167.79 ± 126.97e5 cells (range, 15.3–420e5), respectively (Table 1). Among all patients, five underwent REP step, with a median fold change of 3,610 (range, 1,136–4,925).

Cell populations of expanded TILs by FACS

The phenotype of expanded TILs was assessed using FACS, with a focus on effector memory T, effector T, central memory T, and naïve T cells in 11 IE TILs and 3 REP TILs, all of which were derived from MSS tumors (Fig. 3a). In IE TILs, the median proportion of CD4+ and CD8+ T cells was 72.4% and 19.8%, respectively, while in REP TILs, these proportions were 30.4% and 44.7%, respectively. The median CD4+/CD8+ ratio was 3.66 in IE TILs and 0.68 in REP TILs (Fig. 3b). The median proportions of IE and REP CD8 effector, effector memory, central memory, and naïve T cells were 21.2% and 1.15%, 78.8% and 98.5%, 1.12% and 0.22%, and 0.064% and 0.13%, respectively (Fig. 3c, left). Additionally, the median proportions of IE and post-REP CD4 effector, effector memory, central memory, and naïve T cells were 3.01% and 0.28%, 95.7% and 98.2%, 2.25% and 0.33%, and 0.53% and 0.03%, respectively (Fig. 3c, right).

Case	Clinical data				Pathologic data				TIL culture		
	Age	Sex	MSI	CTx	Tumor size (cm)	P-TIL (%)	A-TIL	KM score	No. of fragment	Post-IE TILs/fragment (e5)	Total IE TILs (e5)
1	73	M	MSS	No	2.1	7.5	5.62	3	48	8.75	420
2	69	M	MSS	No	2.6	7.5	3.74	1	48	4.06	195
3	66	M	MSS	No	5.5	15	2.10	2	96	1.13	108
4	79	M	MSS	No	2.7	3	1.32	1	96	0.16	15.3
5	53	F	MSS	No	3.7	10	4.03	2	96	0.93	88.8
6	61	M	MSS	Yes	11	10	4.39	1	168	0.36	61.2
7	51	M	MSS	Yes	2.3	15	3.77	2	120	3.69	443
8	61	M	MSS	Yes	6.2	5	1.61	1	168	0.50	84.7
9	62	M	MSS	No	3.8	5	3.42	2	120	1.98	237.6
10	70	F	MSS	No	3.8	5	1.06	2	24	8.29	198.96
11	63	M	MSS	No	3.6	3	1.10	2	24	0.95	22.8
12	73	F	MSS	No	3.5	3	0.92	1	360	0.28	100.7
13	57	F	MSS	Yes	4	7.5	1.01	2	72	2.73	196.56
14	66	M	MSI-H	No	4.3	1	0.77	2	192	0.73	140.16
15	71	F	MSS	No	3	30	7.82	3	48	4.25	204

Table 1. Clinicopathologic characteristics of 15 cases colorectal cancer liver metastasis. M, male; F, female; MSI, microsatellite instability; MSS, microsatellite stable, MSI-H, microsatellite instability-high; CTx, chemotherapy; P-TIL, pathologist derived tumor infiltrating lymphocyte; A-TIL, artificial intelligence model derived tumor infiltrating lymphocyte; KM, Klintrup-Mäkinen; IE, initial expansion.

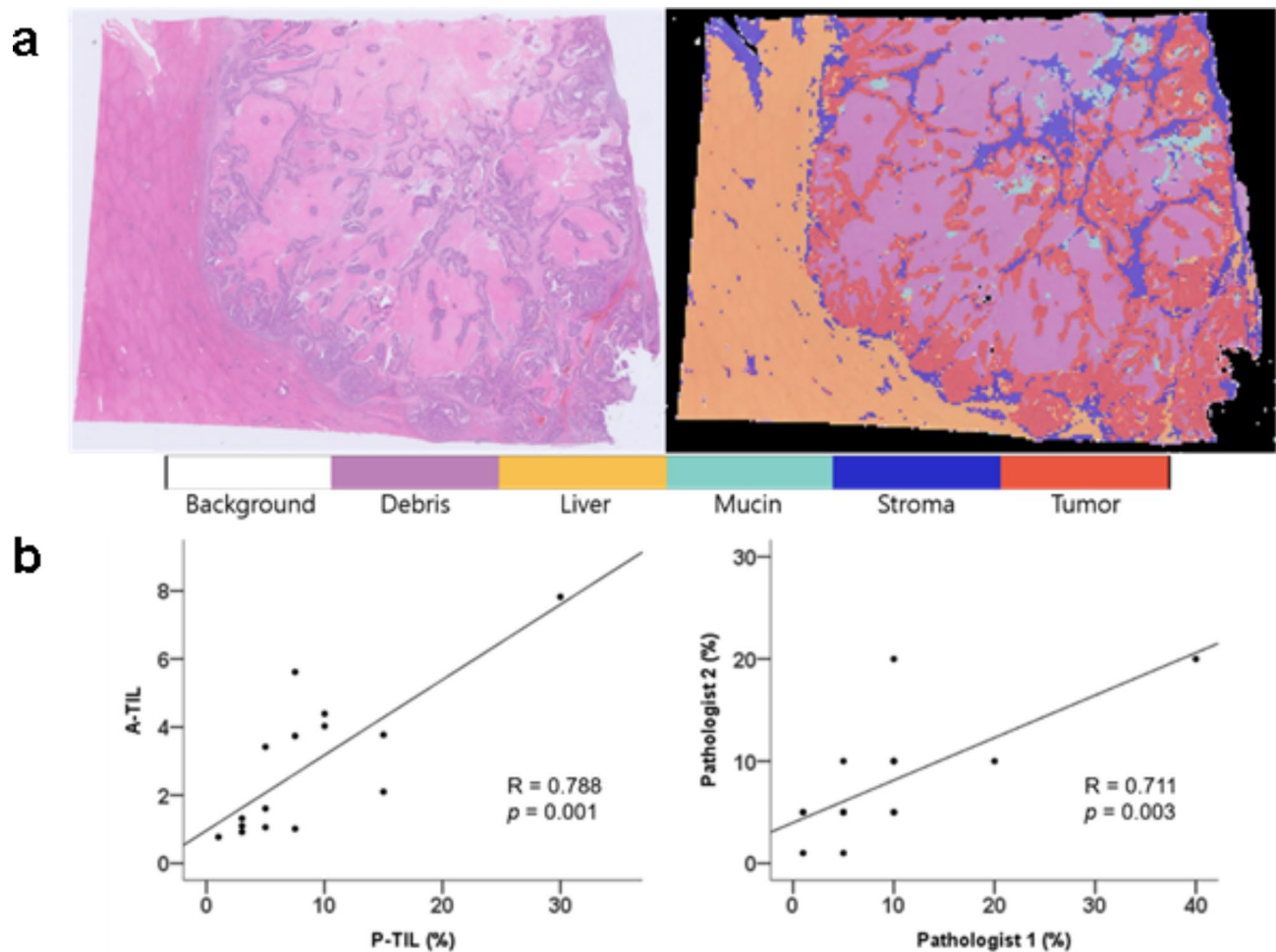


Fig. 2. Artificial intelligence (AI)-assisted quantification of histology slide. **(a)** Representative example of tissue composition analysis. **(b)** The inter-observer correlation of stromal TIL assessments (between pathologists and AI, left; pathologists 1 and 2, right).

Case	Pathologist				AI model				Post-IE TILs/fragment (e5)
	Tumor (%)	Stroma (%)	Mucin (%)	Debris (%)	Tumor (%)	Stroma (%)	Mucin (%)	Debris (%)	
1	45	15	0	40	58.69	12.53	2.45	26.35	8.75
2	7.5	16	75	1.5	20.97	14.78	55.45	8.83	4.0625
3	40	26.5	1	32.5	69.68	15.59	0.66	14.09	1.125
4	25	22.5	0	52.5	59.23	13.84	1.7	25.26	0.159375
5	20	12.5	2.5	65	39.83	10.58	3.35	46.26	0.925
6	22.5	35	2.5	40	18.56	33.11	5.18	43.17	0.3643
7	35	45	2.5	17.5	51.38	25.42	2.21	21.01	3.6917
8	27.5	35	1	36.5	51.96	17.02	1.01	30.03	0.50
9	32.5	47.5	0	20	54.74	19.85	0.78	24.66	1.98
10	10	22.5	0	67.5	11.78	7.58	0.33	80.32	8.29
11	27.5	7.5	0	65	33	6.98	1.71	58.32	0.95
12	7.5	12.5	80	0	15.53	12.24	67.5	4.75	0.28
13	37.5	50	0	12.5	23.45	25.17	20.14	31.26	2.73
14	42.5	50	0	7.5	50.33	27.83	9.99	11.88	0.73
15	35	40	0	25	75.84	8.68	0.98	14.51	4.25
p value	0.651	0.501	0.880	0.616	0.113	0.940	0.660	0.286	

Table 2. Pathologist and AI evaluation of intratumoral proportions of tumor cells, stroma, mucin, and necrotic debris.

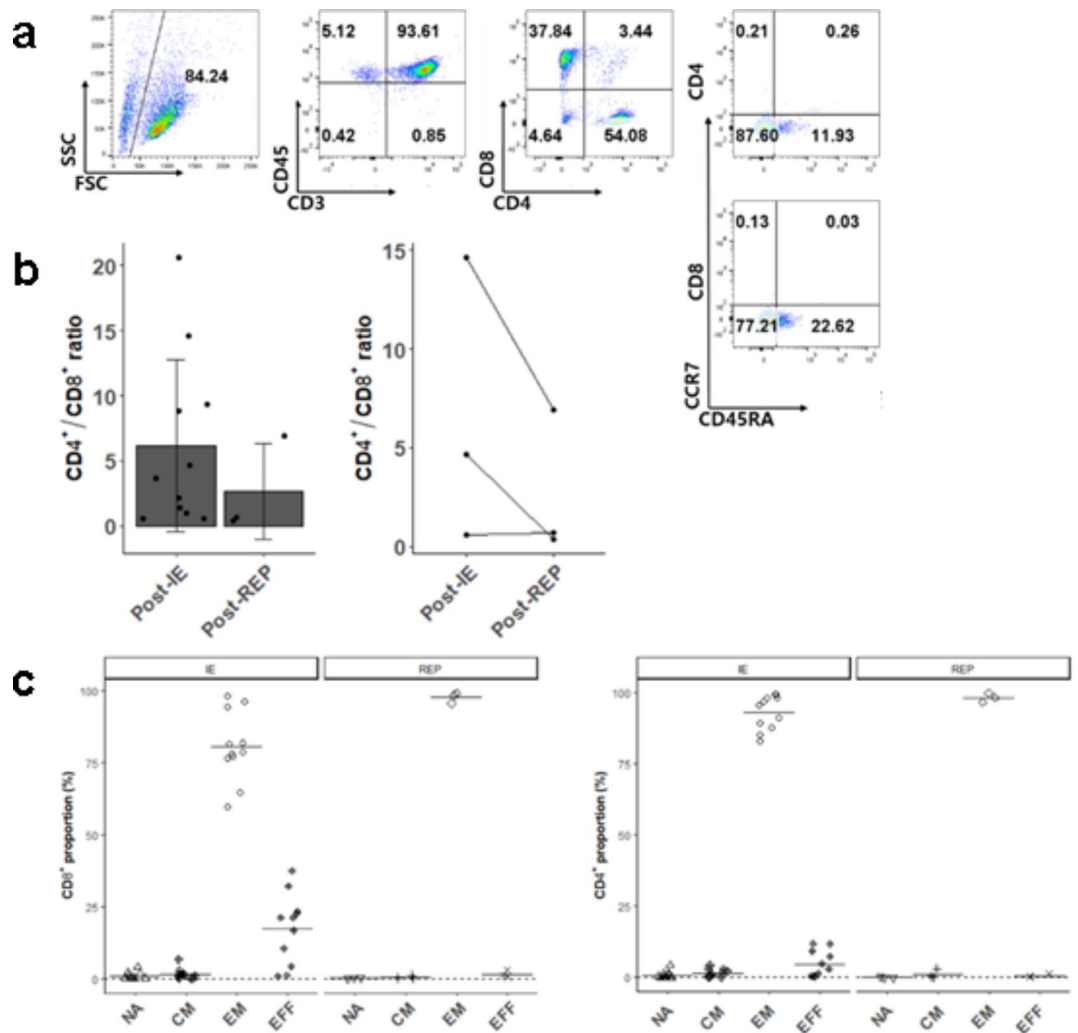


Fig. 3. Composition of expanded tumor-infiltrating lymphocytes (TILs) in initially expanded (IE) TILs and rapid expansion (REP) TILs. (a) Flow cytometry plot. (b) CD4⁺/CD8⁺ ratio in IE- and REP-TILs (left) and post-REP change (right). (c) Composition of CD8⁺ (left) and CD4⁺ (right) TILs in IE and REP samples.

Correlation of expanded TILs and clinicopathologic characteristics

Multiple clinicopathologic factors and their correlation with successful TIL expansion were evaluated. In both A-TIL and P-TIL, a positive correlation was observed between higher sTIL and the number of post-IE TILs per fragment, with a slightly higher correlation in A-TIL, although statistical significance was not reached (Fig. 4a, A-TIL, Pearson $R=0.371$, $p=0.173$; Fig. 4b, P-TIL, Pearson $R=0.206$, $p=0.462$). A higher KM score was significantly associated with a larger number of cultured TIL (Fig. 4c, Pearson $R=0.701$, $p=0.004$). However, the proportion of each histologic component (tumor, stroma, mucin, and necrotic debris) failed to show a significant association with the amount of post IE-TILs in both assessments by pathologists and the AI model (Table 2). In the only MSI-H case, Case 14, the number of IE TILs per fragment was 0.73×10^5 cells, showing no significant difference compared to the overall average (Mann-Whitney U test, $p=0.933$). Also, there was no statistically significant difference in Post-IE TILs between those who did not receive preoperative chemotherapy (mean: 2.86×10^5) and those who did (mean 1.82×10^5) (Mann-Whitney U test, $p=1.000$).

Discussion

In this study, all cases demonstrated successful initial TIL expansion in CRC liver metastasis patients. The recommended count of expanded TILs for ACT is typically considered to be 1×10^9 cells¹⁷. However, a pilot study illustrated a complete response of metastatic CRC with 8×10^7 expanded T cells¹⁸. In our study, the minimum number of TILs per tissue fragment was 0.16×10^6 . Considering an approximate 1,000-fold change during the REP process and the use of large specimen volumes (approximately 250 fragments from 1 cm^3), we anticipate obtaining a sufficient number of expanded TILs for inclusion in a clinical trial. Therefore, all cases in this study present as potential candidates for ACT.

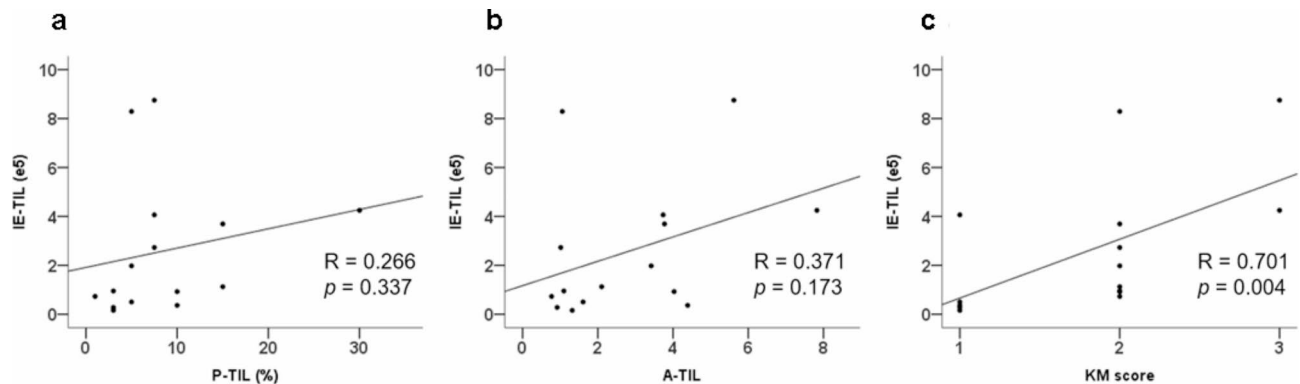


Fig. 4. The correlation between the amount of cultured initially expanded tumor-infiltrating lymphocytes (IE-TILs) and pathologically assessed TIL metrics. **(a)** Stromal TILs assessed by pathologists. **(b)** Stromal TILs assessed by AI model. **(c)** Klintrup–Mäkinen assessment.

Approximately 50% of patients with CRC develop liver metastasis, the primary cause of CRC-related mortality. Metastatectomy, the preferred treatment for metastatic tumors, leads to a remission rate of 20% and achieves over 50% survival for at least 5 years. This marks a significant improvement compared with patients without metastatectomy, who typically exhibit a median survival of 5 to 9 months during 2 years, with no survivors at 5-year follow-up. However, adjuvant chemotherapy after metastatectomy has not demonstrated significant benefit in many randomized clinical trials^{19,20}. Additionally, more than 80% of patients with CRC with liver metastasis initially present with unresectable tumors, necessitating chemotherapy, which often results in substantial liver toxicity²¹. In this context, TIL therapy emerges as a promising alternative treatment. Our study indicates that successful initial TIL expansion is achievable using specimens obtained from the liver metastasis of patients with CRC.

For successful trials of TIL therapy, obtaining abundant expanded TILs is crucial. Harvesting primary tumor samples for TIL expansion, particularly from the gastrointestinal tract, poses a challenge due to the difficulty in avoiding contamination. This challenge often results in the outgrowth of fecal bacterial colonies in the TIL culture bottle. Sampling from hepatic metastatic lesions can overcome this contamination, facilitating the collection of pure tumor samples. However, the liver, unlike soft tissue, the gastrointestinal tract, or the breast, serves as the first organ encountering gut-derived and systemic antigens¹⁴. Therefore, the liver maintains an immunosuppressive microenvironment to protect hepatocytes from tissue damage, which could hinder florid lymphocytic infiltration of tumors. Despite this immunosuppressive microenvironment, our study demonstrated successful TIL expansion in all cases involving CRC liver metastasis.

CD8+ T cells, recognized as the most important immune subtype in ACT^{22,23}, are complemented by tumor-specific CD4+ T cells, which can increase the anti-tumor response²⁴. Unlike melanoma, wherein CD8+ T cells dominate, CRC exhibits a CD4+ T cell dominance during TIL expansion^{23,25–27}. However, in our study, the median CD8+ T cells increased from 19.8 to 44.7% and the CD4+/CD8+ ratio decreased during REP step. Hendrik et al. also reported a predominance of CD8+ T cells in expanded TILs in metastatic CRC²⁸. This suggests that the metastatic process may exert an influence on the TIL population. Further studies comparing the TIL populations in primary and metastatic tumors are needed.

To predict successful TIL expansion in hepatic metastasis samples, we assessed various candidates as predictive markers, including the histologic composition of the tumor, stroma, necrosis, and mucin, size of the metastatic tumor, level of stromal TILs, and KM score of the metastatic tumor. Regarding histologic characteristics, KM score exhibited a significant correlation with post-IE TILs ($p = 0.004$). KM scoring, recognized for its convenience and cost-effectiveness, is a valuable method to evaluate tumor microenvironment in CRCs, serving as a useful marker for predicting post-IE TILs. In this study, sTIL and number of post-IE TILs showed a positive correlation for both P-TIL and A-TIL, although statistical significance was not achieved. The correlation between sTILs and post-IE TILs has been controversial. Our previous study demonstrated an association between sTIL and post-IE TILs in triple-negative breast cancer²⁹, whereas Hendrik et al. reported no correlation between sTIL and the number of expanded cells in colon cancer²⁸. Metastatic adenocarcinoma from CRCs typically contains large areas of necrosis and mucin, which could be confounding factors for the evaluation of sTILs by pathologists. Recently, the application of AI has become increasingly common in clinical practice, particularly in the field of pathology. Levering deep learning algorithms, we analyzed TILs and tissue composition of whole-slide images from 15 patients. Compared with the labor-intensive conventional histologic evaluation by pathologists, AI-based automated tissue classification efficiently processes large workloads in a short amount of time, minimizing inter/intra-observer variability. Our model not only showed discrepancies in predicting histologic composition compared to pathologist assessments but also failed to accurately predict post-IE-TIL levels. Instead of the four categories we used—tumor, stroma, mucin, and debris—alternative categorization strategies, such as histologic patterns within the tumor, could be explored. The limited number of cases, heterogeneity of the tumors, representativeness of the slide, MSI status and preoperative chemotherapy could have also contributed to the limitation of model's performance. Further enhancements of performance are anticipated through improvements in model design along with the acquisition of additional training data.

In chemorefractory cancers, immune checkpoint inhibitors are a viable treatment option. Immunotherapy demonstrates an effective response to high mutational burdens, such as MSI-high (MSI-H) colorectal cancers³⁰. However, MSI-H tumors represent only 15% of CRCs^{31,32}. In our study, we achieved successful TIL expansion from liver metastatic CRCs, irrespective of MSI status. Therefore, ACT utilizing TILs can be a potential treatment option for MSS tumors, which constitute most cases of CRC. While MSI and preoperative chemotherapy are known to influence the tumor microenvironment (TME), neither showed a direct association with cultured TIL amounts in this study. MSI-H tumors are typically associated with an immunogenic TME³², and preoperative chemotherapy can enhance immune activity³³. In contrast, our previous study on triple-negative breast cancer (TNBC) found that patients who received neoadjuvant chemotherapy had lower cultured TIL amounts²⁹. Although differences in cancer type and treatment regimens may explain the lack of significant association with cultured TIL amounts in this study, further research with a larger cohort may yield different results. In this study, the MSI-H case exhibited relatively low stromal TIL levels (P-TIL 1%), and the amount of cultured TILs was also lower than average. Anuja et al. reported that interactions between macrophages and fibroblasts in liver metastases of CRC can contribute to the formation of an immunosuppressed niche³⁴. There is a possibility that MSI- and chemotherapy-associated TME interactions may induce different niche in metastatic CRCs from primary CRCs.

In cases of advanced CRC with unresectable metastasis, confirmation of metastasis can be achieved through a needle biopsy. An 18-gauge needle biopsy typically yields specimens of approximately $20 \times 1.3 \times 1.3$ mm per core, and multiple cores are commonly obtained in general practice. Thus, at least 20 fragments can be derived from a needle biopsy. Considering the minimal threshold of 1×10^9 cells for effective treatment, we require 0.5×10^5 TILs per fragment of biopsy tissue, given the anticipated 1,000-fold change of REP. Among the cases in our study, 12 (80%) exhibited over 0.5×10^5 IE TILs per fragment. Therefore, core biopsy specimens prove sufficient for expanding TILs for potential use in ACT. This approach holds promise for ACT in advanced CRC cases with multiple metastases that are not candidates for curative metastatectomy.

A notable limitation of this study is the lack of in vitro or in vivo functional studies of expanded TILs, primarily attributed to the absence of matched tumor cells. However, previous research has demonstrated tumor regression in stage IV colorectal cancers through adoptive immunotherapy¹⁸. Additionally, successful outcomes have been observed with TILs targeting mutant KRAS³⁵. TIL therapy in metastatic CRC can be expected to be an effective treatment modality in chemorefractory or MSS tumors.

In conclusion, successful TIL expansion from CRC liver metastasis was achieved, encompassing cases with MSS. Notably, the assessment of inflammatory cell infiltrate at the invasive margin and TIL evaluation utilizing an AI model may offer valuable insights for predicting the quantity of expanded TILs. Our findings indicate that expanded TILs from CRC liver metastasis could serve as a promising source for further studies to establish the effective ACT in patients with CRC. Considering the absence of established predictive markers for TIL expansion in metastatic CRC, our study holds potential significance for the development of ACT for patients with advanced CRC.

Data availability

All data generated or analysed during this study are included in this published article.

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Author contributions

Conceptualization; Joo Young Kim, Hee Jin Lee Data curation; Gyungyub Gong, Hye Seon Park, Chae-Lyul Lim-Formal analysis; GunHee Lee, Hyun Lee Methodology; GunHee Lee, Hyun Lee Supervision; Hee Jin Lee Writing - original draft; Jina Baek, Gyuheon Choi, GunHee Lee Writing - review & editing; Joo Young Kim, and Hee Jin Lee.

Declarations

Competing interests

HL, HSP, and CLL were employees of NeogenTC during the conduct of this study. CLL and HJL are cofounders of NeogenTC. GG received research support from NeogenTC.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. This study was approved by the Institutional Review Board of Asan Medical Center (IRB No. 2020 – 0980). Informed written consent was obtained from all patients.

Additional information

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