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ORIGINAL ARTICLE

Pilot study of adoptive immunotherapy with sentinel node-derived T cells in muscle-invasive urinary bladder cancer

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Abstract

Objective: The aim of this study was to determine by computed tomography (CT) whether treatment with tumor-draining lymph-node-derived expanded autologous T lymphocytes results in objective responses and/or improved survival in patients with metastatic urinary bladder cancer (UBC) and to record the toxicity of the treatment. *Materials and methods:* Eighteen patients with metastatic UBC were prospectively selected from two centers. The preoperative staging was T2–T4bN1–2 and/or M0–M1 or MX. Tumor-draining lymph nodes were harvested at intended cystectomy for the extraction of T lymphocytes. This was followed by expansion of the T lymphocytes in a cell culture, and subsequent reinfusion of these autologous tumor-specific T lymphocytes. Responses to therapy were evaluated by CT scans according to Response Evaluation Criteria In Solid Tumors (RECIST) and clinical follow-up, according to the research protocol. *Results:* Nine out of 18 patients were treated. Treatment was feasible and safe. In two out of nine immunologically treated patients, objective responses were detected in terms of diminished or obliterated nodal metastases. When excluding three patients with disseminated osseous metastases plus one with a T4b tumor left *in situ*, a success rate of two out of six treated patients was seen. The two responders had survival times of 35 and 11 months, respectively. No toxicity was recorded. *Conclusions:* Infusion of expanded autologous tumor-specific T lymphocytes is feasible and safe, and objective responses according to RECIST were recorded. One objective responder to immunotherapy displayed notably long overall survival.

Keywords

Adoptive cellular immunotherapy, sentinel lymph-node biopsy, urinary bladder neoplasms

History

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Introduction

Urinary bladder cancer (UBC) ranks ninth in worldwide cancer incidence. The worldwide age-standardized incidence rate is 10.1 per 100,000 for males and 2.5 per 100,000 for females [1]. One-quarter of UBCs are muscle invasive at the time of diagnosis, accounting for about 80% of disease-related deaths [2]. Approximately one-third of patients diagnosed with muscle-invasive UBC have undetected dissemination at the time of primary treatment [3], while one-quarter undergoing radical cystectomy present with lymph-node involvement at the time of surgery. Radical surgical intervention only results in a 5 year survival of about 50% [4].

One major reason for early death in patients with muscle-invasive UBC is undetected micrometastatic spread ultimately developing into macrometastatically detectable dissemination and/or local recurrence of disease. The sentinel node (SN) is defined as the first tumor-draining lymph node along the direct drainage route from the tumor, and in case of dissemination it is considered to be the first site of metastasis. Pathological examination of the SN(s) reflects the nodal status of the remaining regional nodes. Detection of the SN was introduced in urology in 1977 to increase accuracy in penile carcinoma staging [5]. SN detection is still experimental in most urological cancers and has previously been described in UBC [6–8]. The immune surveillance hypothesis states that T lymphocytes are continuously sensitized against transformed cells, mediating a first line of defense against tumor development [9]. There are clear indications that urothelial cancers elicit a tumor-specific immune response [10,11]. The present authors have previously detected immune responses against UBC in SNs and performed extraction of tumor-specific lymphocytes [12]. As a modification of tracing lymph nodes draining directly from the

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Table 1. Baseline characteristics for all recruited patients.

Patient no.	Gender	Age at time of cystectomy (years)	Histological cell type	TNM classification
1	M	75	TCC	T4b/N2/MX
2	M	65	TCC	T4a/N2/M1
3	M	74	TCC	T3b/N2/pM1
4	M	67	TCC	T4b/N2/pM1
5	F	73	TCC	T2b/N2/M1
6	M	65	TCC	T3b/N2/MX
7	M	70	TCC	T2b/N2/MX
8	M	76	TCC	T3b/N2/M0
9	M	79	TCC	T4b/N2/M1
10	M	61	TCC	T3a/N2/MX
11	M	71	TCC	T3a/N1/M0
12	M	83	TCC	T2b/N1/M0
13	M	74	TCC	T3/N2/M0
14	F	69	TCC	T2a/N2/M0
15	M	72	TCC	T3/N0/M1
16	M	58	TCC	T3/N2/M1
17	M	73	TCC	T3/N1/M0
18	M	48	TCC	T2/N2/M0

All patients had a preoperatively established diagnosis of metastatic disease, by means of intravenously contrast-enhanced computed tomography of the abdomen/urinary system and thorax. The majority harbored N1–2 disease, with the exception of patient 15, who was staged as N0M1.
M = male; F = female; TNM = tumor, node, metastasis.

primary tumor, lymphatic mapping to investigate the possibility of finding the first lymph node or nodes that drain the metastases has evolved [13]. The starting point, instead of the primary tumor, can thus be a metastatic site, for instance a metastatic node. Downstream draining nodes are then named "metinel nodes" (MNs) instead of SNs. MNs can be either non-metastatic or metastatic.

The authors have previously developed and described an adoptive immunotherapy method based on T cells collected from SNs or MNs [14,15]. The method expands effector memory T lymphocytes which, when reinfused, may result in a sustained response against tumor cells and, possibly, induce a state of vaccination [16–18]. The feasibility and safety of the approach in muscle-invasive UBC was evaluated in a previous publication, describing the technical aspects in the first 12 patients of the present report [15]. The current article presents the long-term follow-up of 18 patients with muscle-invasive UBC, who have been included to receive this method as a stand-alone palliative therapy. The primary objectives were to investigate the effects on tumor growth and to evaluate toxicity.

Materials and methods

Patients

Eighteen patients, 16 males and two females, with metastatic UBC [transitional cell carcinoma (TCC)] were included in the study. Sixteen patients were prospectively enrolled between 2007 and 2009, from a single tertiary academic center (Karolinska University Hospital, Stockholm, Sweden), and two patients from a secondary urological referral center (Mälarsjukhuset, Eskilstuna, Sweden). The mean age was 69.6 years and the median age 71.5 years (range 48–83 years) at the time of diagnosis (Table 1).

Inclusion criteria were metastatic UBC (T2–T4bN0–2 and/or M0–M1,MX), TCC, expected survival longer than 4 months, WHO performance status 0–1 and measurable tumor manifestation. Exclusion criteria were blue-dye allergy, aplastic anemia or myelofibrosis, treatment with chemotherapy and/or radiotherapy in the past 3 months, ongoing cortisone treatment or any other medications with known effects on the immune system. History of another active malignancy in the past 5 years, except for adequately treated basal cell carcinoma or squamous cell carcinoma of the skin, was also a reason for exclusion [15]. In patients no. 11–18, bone scintigrams were introduced in the preoperative work-up. From patient no. 11 onwards, patients with verified skeletal metastases were intentionally excluded, owing to presumably low treatment efficacy. According to the research protocol, all patients were offered, at regular intervals, referral to a medical uro-oncologist for standard oncological therapy.

The study was approved by the local ethics committee in Stockholm (dnr: 2006/1269-31/4). Informed consent was obtained from all patients. The Swedish Medical Products Agency (Läkemedelsverket), promoting good manufacturing practice (GMP), approved the production of autologous cells as a medicinal product for clinical trials.

Preoperative investigations

Patients were investigated preoperatively according to Swedish standard preoperative investigations with transurethral resection of the bladder (TURB), and chest and abdominopelvic computed tomography (CT) scans. Patient no. 8 in the series had, out of protocol, undergone preoperative positron emission tomography (PET)/CT.

Identification of metinel and tumor-draining lymph nodes

The metastatic node or nodes were identified preoperatively by CT. The intended identification of MNs was performed intraoperatively by injecting Patent Blue® in a palpable

lymph node suspected to be metastatic. The amount of blue dye varied between 0.2 ml and 1.0 ml. One to two minutes after injection, the spread of dye, in optimal cases, could be followed visually to the nearest draining node(s), i.e. to the MN(s). When MNs failed to be identified, lymph nodes in the ordinary draining area were utilized.

Surgical technique and lymphadenectomy

One urologist (AS) was the main surgeon for all 17 operations (patient no. 5 had no surgery). To safeguard the primary tumor as an antigen source, a repeat TURB in the same session was performed before attempted cystectomy. Lymphadenectomy was intentionally restricted to suspicious metastatic nodes and corresponding MNs in this cohort of patients with known metastatic disease. Other nodes, non-pathological and suspected pathological but non-injected, were mostly left *in situ*.

Preparation of the specimens

Both metastatic nodes and MNs underwent histopathology, and slices about 1 mm thick were cut from central and peripheral parts for flow cytometry. Remaining parts of the nodes underwent routine histopathology. One piece of the primary tumor was extracted from the primary tumor specimen for flow cytometry analyses and as a source of antigens.

In vitro culture of lymphocytes

Handling of peripheral blood leukocytes and draining lymph-node specimens has been described in detail previously [15]. Cell cultures were kept under approved GMP conditions. Autologous tumor extract was prepared as described and added at a dilution of 1/100 (v/v) [19]. After approximately 3 weeks of cell culture *in vitro*, a second round of antigen stimulation was performed using irradiated autologous peripheral blood mononuclear cells as antigen-presenting cells (APCs). For handling of the cells on the day of transfusion, see the previous description [15]. In the nine patients receiving immunological treatment, lymphocytes were expanded against an autologous tumor homogenate. The interleukin-2 content in the *in vivo* expansion protocol was 120 U/ml. The amount of infused T lymphocytes ranged from 29×10^6 to 704×10^6 (average 313×10^6). Patient no. 8. received a second infusion of 40×10^6 cells (Table 2). Because of the issue of T-cell exhaustion during prolonged *in vitro* culture, the expansions were allowed to proceed for a maximum of approximately 30 days, after which the cells were transfused back, after phenotypic characterization. The transfusions took place after checking for pathogens by specific testing for contaminating microbes, endotoxins and tumor cells.

Flow cytometry

Cells were released for immunotherapy based on phenotyping by fluorescence-activated cell sorting (FACS) with regard to CD4, CD8, CD19, C56CD3$^-$ (natural killer cells) and CD4CD25hi/CD127lo (Treg). In addition, FACS for the presence of tumor cells was carried out using specific antibodies at the start of cell culture and before transfusion, as previously described [12].

Table 2. Infusion data.

	No. of infused lymphocytes ($\times 10^6$)		
Patient no.	1st infusion	2nd infusion	Total
1	–	–	–
2	99	–	99
3	220	–	220
4	–	–	–
5	No surgery	No surgery	No surgery
6	122	–	122
7	–	–	–
8	704 + 68	40	812
9	29 + 600	–	629
10	–	–	–
11	40	–	40
12	–	–	–
13	–	–	–
14	41.2 + 12.8	–	54
15	–	–	–
16	15	33.6	48.6
17	—	–	–
18	32.4	–	32.4

Nine out of eighteen patients were treated postoperatively with 40–812 $\times$ 10^6 autologous T lymphocytes after expansion. The original sets of T lymphocytes were extracted from regional tumor-draining lymph nodes.

Blinded computed tomography evaluations from two independent radiologists

Objective responses to therapy was evaluated by contrast-enhanced CT scans of the chest, abdomen and pelvis. Two independent and experienced uroradiologists assessed each patient, blinded to the treatment protocol of the patients and to each other. Evaluations were performed according to Response Evaluation Criteria In Solid Tumors (RECIST 1.1) (Table 3).

Results

Technical outcomes

The patients were stratified into two groups, technical successes and technical difficulties. Patients in the technical success group were substratified into two groups, objective non-responders and objective responders, in terms of detectable objective responses according to RECIST (Table 4). In nine patients the whole procedure was performed as planned (Figure 1). In the remaining nine patients technical difficulties were encountered, as listed in Table 5. None of the nine patients who received infusions encountered any adverse events or long-term complications.

Computed tomography evaluations and progression-free survival

Patient no. 8 showed progressive disease according to RECIST in the first evaluation, 2 months after treatment. In the subsequent two controls 6 and 9 months later, partial

Table 3. Results of blinded computed tomography (CT) evaluations from two independent radiologists.

		Date				
		17/08/07	05/02/08	22/05/08	16/09/08	26/03/09
Patient 8						
Radiologist 1						
	Localization					
1	Right paracaval	22	16	13	13	17
2	Left paraaortic	11	10	10	10	5
3	Right paraaortic	13	12	12	12	20
4	Porta hepatis	25	25	22	22	25
	Sum diameter	71	63	57	57	67
Radiologist 2						
	Localization					
1	Right paracaval	12	5	5	5	5
2	Left paraaortic	10	6	7	7	13
3	Right paraaortic	16	9	6	8	8
4	Porta hepatis	17	17	15	14	14
5	Celiac axis	17	17	15	14	14
6	Right common iliac	12	9	5	5	5
7	Left obturator fossa		10	10	16	26
8	Left periaortic					11
9	Left periaortic					13
	Sum diameter	84	73	63	69	109

		Date			
		07/07/09	17/09/09	05/01/10	27/01/10
Patient 14					
Radiologist 1					
	Localization				
1	Right paraaortic	14	9	3	3
2	Left external iliac	20	7	4	4
3	Right retrocrural	15	12	11	7
	Sum diameter	49	28	18	14
Radiologist 2					
	Localization				
1	Right paraaortic	24	5	5	5
2	Left external iliac			18	18
3					
	Sum diameter	24	5	23	23

The two radiologists evaluated the radiographic examinations independently of each other and blinded to each other. Both radiologists detected objective responses in patients 8 and 14. In patient 8, radiologist 2 calculated partial response (PR) in the second CT series (–35%), PR in the third (–60%), progressive disease (PD) due to an additional lesion in localization [7] in the fourth series and PD in the fifth. In patient 14, radiologist 2 calculated PD in the first CT series, complete response (CR) in the second, PD in the third and stable disease (SD) in the fourth.

responses were detected, with decreases in the sum of diameters of target lesions of 35% and 60%, respectively (Figure 2). Two years after treatment, disease progression was noticed; hence, the progression-free survival (PFS) was 26 months.

Patient no. 14 showed a complete response according to RECIST 2 months after treatment administration (Figure 3). Four months later, new pelvic adenopathies appeared and a metastatic renal pelvis cancer was diagnosed, causing death. From the localization of the recurrent malignant nodes, the new pelvic lesions were most probably secondary to the UBC. PFS was 6 months. No objective responses were found in the remaining seven patients receiving treatment (Figure 4).

Overall survival in nine treated patients and nine untreated patients

Survival of all patients is displayed in two categories: survival from date of attempted cystectomy and survival from date of first cell infusion. Patient no. 8 survived with cystectomy and T-cell based immunotherapy as sole treatment for 35 months and 3 days from cystectomy. Patient no. 14 survived with cystectomy and T-cell based immunotherapy as the only treatment for 11 months and 7 days from cystectomy (Table 4). In the remaining seven immunotherapy-treated non-responding patients, the median overall survival from time of attempted cystectomy was 150 days (range 68–220 days). For technical reasons, nine patients did not receive immunotherapy (Table 5). Patient no. 5 never reached attempted cystectomy. The median overall survival was 286 days (range 58–925 days) for the eight patients undergoing attempted cystectomy, none of whom had osseous metastases, which was the case in three of the immunotherapy-treated patients. In patients no. 1, 4 and 9 the bladder was not removable and remained *in situ* (Table 6). The immunologically untreated patient (no. 10) also received systemic chemotherapy (two cycles of gemcitabin and cisplatin) postoperatively, and was the only patient in the trial undergoing traditional palliative oncological treatment (Table 7).

Table 4. Successes and survival.

Patient no.	Technical success	Survival from date of attempted cystectomy	Survival from date of first cell infusion	Objective non-responder	Objective responder	PFS in the two responders
1	No	17 months, 5 days	No cell infusion	–	–	No cell infusion
2	Yes	2 months, 8 days	1 month, 10 days	Yes	No	–
3	Yes	5 months, 1 day	4 months, 1 day	Yes	No	–
4	No	1 month, 28 days	No cell infusion	–	–	No cell infusion
5	No	No surgery	No cell infusion	–	–	No cell infusion
6	Yes	3 months	2 months	No	No	–
7	No	18 months, 21 days	No cell infusion	–	–	No cell infusion
8	Yes	35 months, 3 days	34 months, 19 days	No	Yes	26 months
9	Yes	5 months	4 months, 8 days	Yes	No	–
10	No	30 months 25 days	No cell infusion	–	–	No cell infusion
11	Yes	6 months, 13 days	5 months, 21 days	Yes	No	–
12	No	8 months, 8 days	No cell infusion	–	–	No cell infusion
13	No	10 months, 23 days	No cell infusion	–	–	No cell infusion
14	Yes	11 months, 7 days	10 months, 9 days	No	Yes	6 months
15	No	4 months, 29 days	No cell infusion	–	–	No cell infusion
16	Yes	7 months, 10 days	5 months, 29 days	Yes	No	–
17	No	3 months, 6 days	No cell infusion	–	–	No cell infusion
18	Yes	2 months, 17 days	1 month, 19 days	Yes	No	–

Two patients in the series, nos 8 and 14, who had undergone successful expansion of autologous T lymphocytes, originally collected from tumor-draining regional lymph nodes, were evaluated as objective responders. Patient no. 8. survived solely with cystectomy and T-cell based immunotherapy, for 35 months and 3 days; patient no. 14 survived for 11 months and 7 days after only cystectomy followed by the described immunotherapy. Patient no. 14 was one of five patients with non-radical soft tissue margins, but this was not the reason for her demise: she had a pan-urothelial cancer and ultimately died from a renal pelvic cancer with regional node dissemination.

PFS = progression-free survival.

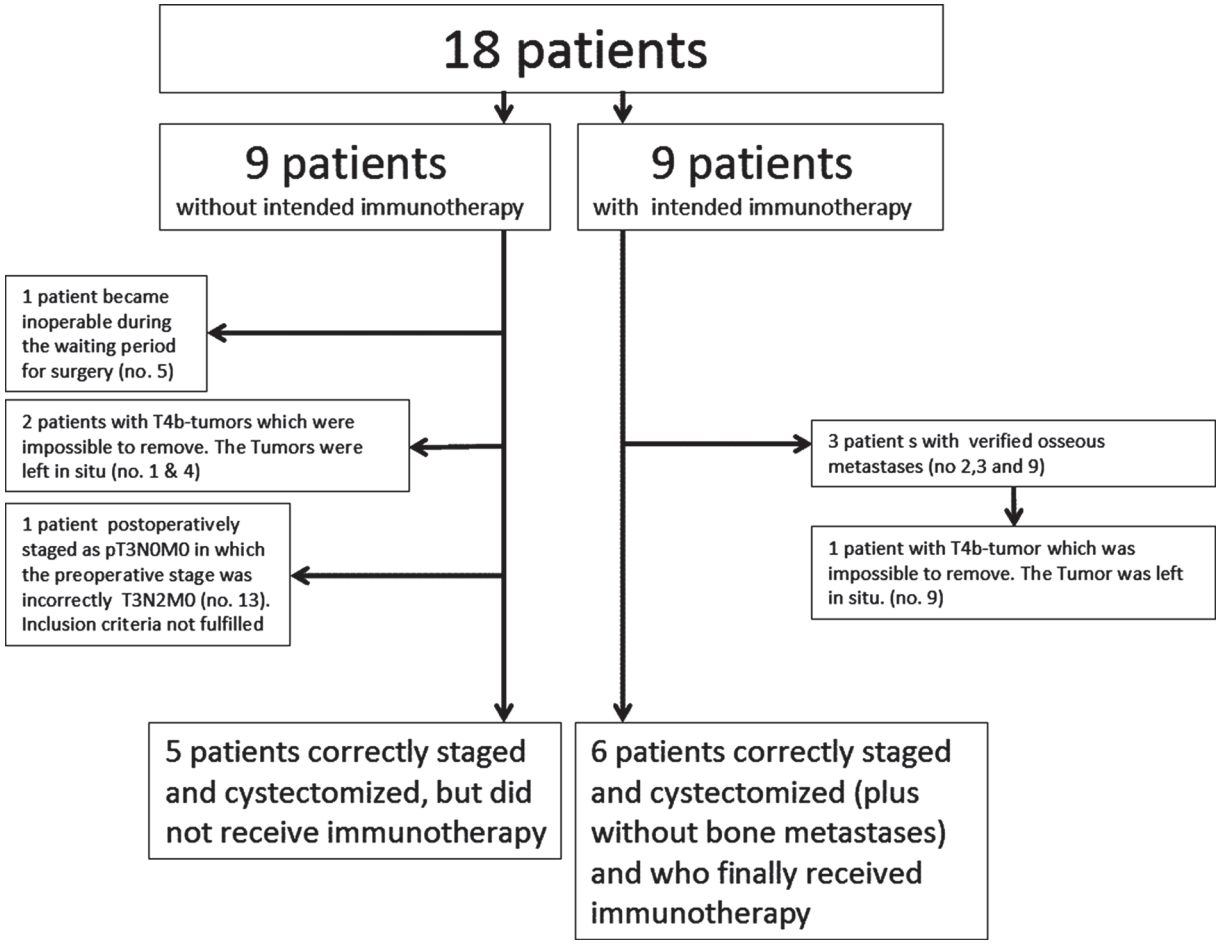

Figure 1. CONSORT diagram. Illustration of the patient flow in the trial in conjunction with treatment results, clinical development and pathological outcomes. Success rate 1 can be calculated as two out of six fully eligible, immunotreated patients not displaying bone metastases. Success rate 2 can be calculated as two out of 11 fully eligible patients for adoptive immunotherapy not displaying bone metastases, five of whom never received immunotherapy.

Table 5. Details of technical difficulties.

Patient no.	Technical difficulty	Cause of technical difficulty
1	Yes	Detection difficulties of metinel nodes
2	No	–
3	No	–
4	Yes	Detection difficulties of metinel nodes
5	–	Patient non-operable
6	No	–
7	Yes	Contamination of cell culture with tumor cells
8	No	–
9	No	–
10	Yes	Extraction of too few lymphocytes primarily
11	No	–
12	Yes	No proliferation of T lymphocytes
13	Yes	Proliferation of very few T lymphocytes
14	No	–
15	Yes	Detection difficulties of metinel nodes
16	No	–
17	Yes	Contamination of cell culture with tumor cells
18	No	–

Detection of metinel nodes can be a problem with the described method, and in this series caused technical difficulties in three patients originally included in the study. The strict threshold for contamination with tumor cells in the cell culture meant that infusion and immunotherapy had to be avoided in patients no. 7 and 17. The advanced tumor stages that the protocol envisaged may have caused very low quantities of viable lymphocytes in tumor-draining nodes. This caused technical difficulty in harvesting sufficient amounts of viable T lymphocytes in patients no. 10 and 15. In a similar fashion, different tumor escape mechanisms in conjunction with the tumors' ability to elicit anti-immunological responses could be the cause of anergic and non-proliferative T lymphocytes in patients no. 12 and 13.

Discussion

Cancer dissemination in muscle-invasive UBC is a major problem in terms of local control and overall survival. Intended local control with extended nodal dissection may not affect the outcome of long-term survival, and the European Association of Urology guidelines has yet not ascribed any specific template for nodal dissection [20]. Attempts to address the generalization of the cancer have been made, with neoadjuvant chemotherapy (NAC) showing survival benefits of 5–8% in 5 years of observation [21,22], provided that preoperative staging is N0M0. Pathological responders to NAC seem to have even better survival, with a 31% absolute risk reduction comparing NAC patients with pT0N0 to no-NAC pT0N0 patients [23]. However, dissemination may be more frequent than expected, and even patients with full NAC and ensuing pathological response (pT0N0) have an elevated risk of early death [23]. In an autopsy study from 1999, metastases were found in 68% of 367 patients with pT2–4 UBC. The most frequent sites were regional lymph nodes (90%), liver (47%), lung (45%) and bone (32%) [24]. Thus, generalized disease is more prevalent than usually predicted by initial preoperative and postoperative assessments. Preoperative investigations relating to lymph-node staging are still suboptimal. Magnetic resonance imaging and CT/PET have not yet shown any major advantages [25]. The standard Swedish preoperative nodal staging method, i.e. contrast-enhanced CT scan, was chosen for this trial.

Since cystectomy and extended node dissection in muscle-invasive UBC are obviously far from sufficient in offering all patients improved options for long-term survival, additional palliative and even adjuvant therapies are warranted. Adoptive T-cell based immunotherapy is an appealing alternative, which has been demonstrated to be able to induce tumor regressions in advanced malignant melanoma [26] and, more recently, in other solid tumors [27]. However, the approach has hitherto not been explored in UBC. A previous study demonstrated the presence of a regional immune response against the autologous tumor in patients with metastatic UBC [12]. In the present trial, lymphocytes from attempted identification of tumor-draining (sentinel) lymph nodes were used to enrich tumor-reactive cells. The harvested lymphocytes were expanded *in vitro* against autologous tumor extract, resulting in a mixed T-cell population, composed of both $CD4^+$ (helper) and $CD8^+$ (cytotoxic) T cells, as described in the previous publication [15]. After exclusion of residual tumor cells in the *in vitro* cultures, the patients safely received the expanded lymphocytes as an autologous infusion. The number of T cells given back varied between patients. In part, this probably reflects the intrinsic properties of the tumors, with divergent expression of immunogenic tumor antigens and varying use of immune escape

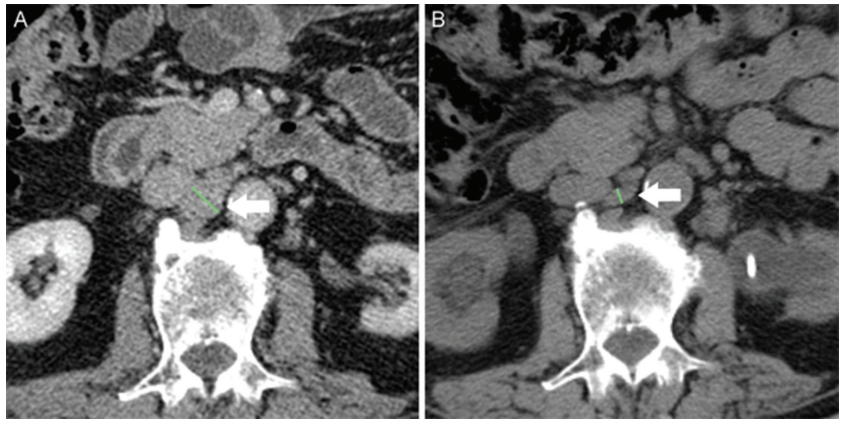

Figure 2. Partial response in patient no. 8 (by RECIST 1.1). (A) Contrast-enhanced computed tomography (CT) scan showing 16 mm periaortic lymphadenopathy (diagonal green line, indicated by white arrow); (B) non-enhanced CT scan 2 years after treatment showing a 50% decrease in the short axis of the lesion, which had become too small to be considered pathological.

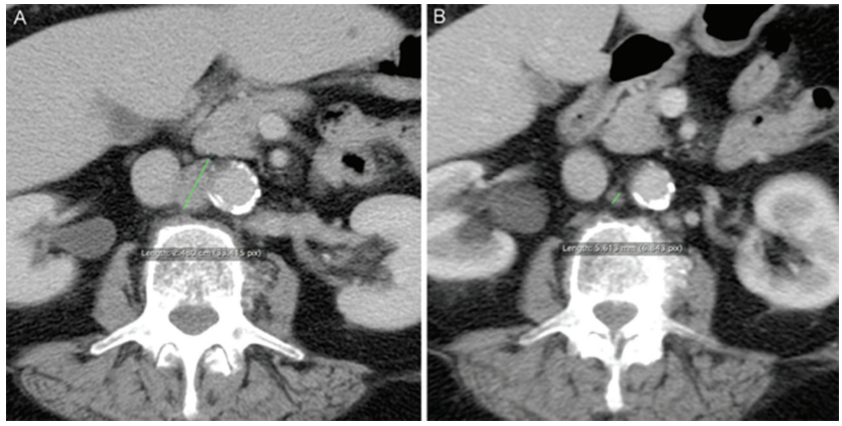

Figure 3. Complete response in patient no. 14 (by RECIST 1.1). (A) Contrast-enhanced computed tomography scan showing periaortic confluent lymphadenopathies (diagonal green line); (b) 2 months later, the lesions became too small to be considered pathological (diagonal green line).

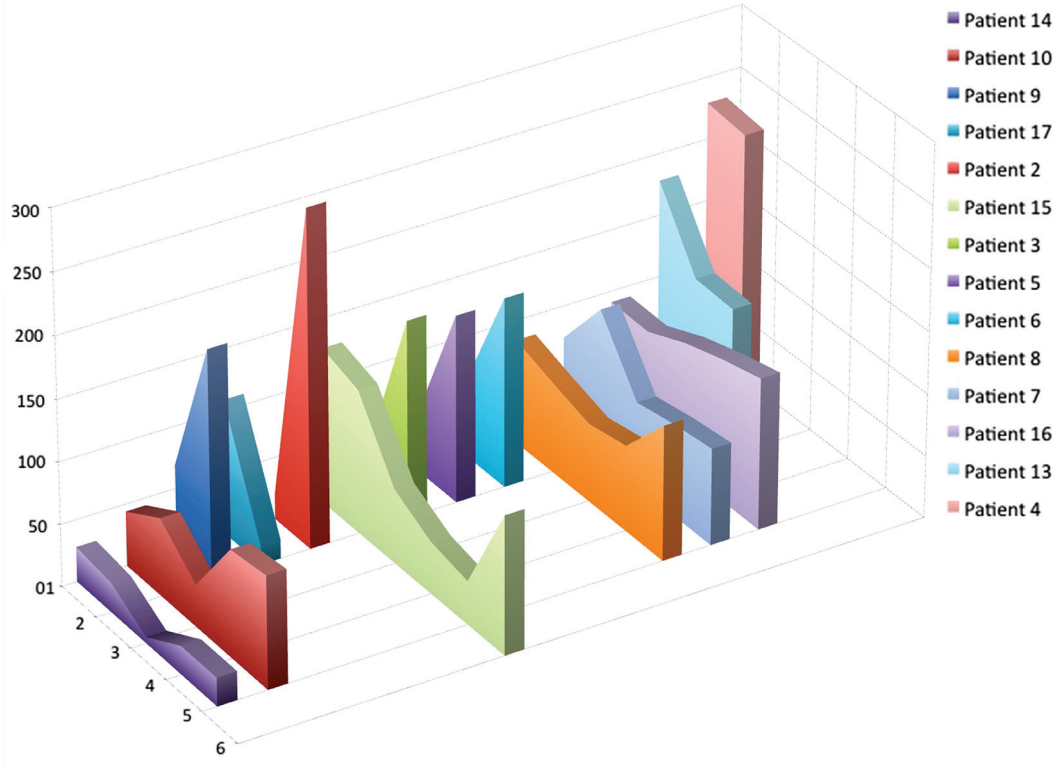

Figure 4. Tumor burden chart. Tumor burdens in all patients are illustrated on a timeline (from patient no. 14 on the left of the chart to patient no. 4 on the right); for the sake of visibility they are not in numerical order. The details of objectively responding patients no. 8 and 14 are also recorded in Table 3.

mechanisms. This experimental treatment was beforehand approved and classified as a cell-based medicinal product (CMP) by the Swedish Medical Products Agency (Läkemedelsverket), being evaluated on the level of pharmaceutical products. As such, and as being an intended oncological treatment, it was necessary to perform this first pilot trial in patients having a seriously disseminated disease with few other treatment options.

In this trial, two responders presented according to RECIST, of whom patient no. 8, in particular, displayed both objective responses and exceptionally long overall survival (Table 4). The infusion data show that patient no. 8 received a total number of 812 million expanded T lymphocytes, which may play a role in this success (Table 2). However, the objective responses in patient no. 14 cannot be attributed to any exceptional amount of infused T lymphocytes. The described immunotherapy method requires optimization and further improvements. Exclusion of T4b patients and patients with osseous metastases, plus improved preoperative imaging with SN detection with single-photon emission computed tomography (SPECT)/CT and additional intraoperative detection with transurethral injection of radioactive technetium, could improve treatment selection. This, in turn, may increase success rates through both identification of the optimal target population and increased yields of autologous T-lymphocyte containing nodes. Increased yields of original

Table 6. Postcystectomy pathological data.

Patient no.	Patients with osseus dissemination	pTNM classification	Radicality
1	–	pT4b, no cystectomy	No, left *in situ*
2	Yes	pT4aN2M1	No
3	Yes	pT3bN2pM1	Yes
4	–	pT4b, no cystectomy	No, left *in situ*
5	–	No surgery	No surgery
6	–	pT3bN2MX	No
7	–	pTcisN2MX	Yes
8	–	pT3bN2M0	Yes
9	Yes	pT4b, no cystectomy	No, left *in situ*
10	–	pT3bN2MX	Yes
11	–	pT3aN1M0	Yes
12	–	pT2bN2	Yes
13	–	pT3N0M0	Yes
14	–	pT4bN2M0	No
15	–	pT3bNXM1	No
16	–	pT3aN2M1	Yes
17	–	pT3bN1M0	Yes
18	–	pT4aN2M0	No

Apart from three patients in whom the urinary bladder could not be removed owing to advanced cancer, seven patients (nos 2, 6, 14, 15 and 18) had non-radical soft tissue margins.

amounts of harvested T lymphocytes would presumably lead to much greater amounts of expanded T lymphocytes for therapeutic infusion.

Future perspectives entail main two options. One option would be to include patients according to the described protocol, but excluding patients with skeletal metastases, as in three of the patients with verified osseous dissemination (patients 2, 3 and 9) (Table 6). The authors' retrospective assessment is that autologous MN- and/or SN-derived T lymphocytes from the minor pelvis do not display any immunological functions, relating to transformed tumor cells in distant bone tissue. For a cancer cell to enter bone, multiple steps occur, including the expression of very late activation antigen-4 (VLA-4) and chemokine receptors such as CXCR4, CXCR6 and CXCR7 enabling binding to the endothelium. Following binding to the endothelium, cancer cells expressing appropriate chemokine receptors transmigrate in the direction of a chemokine gradient of stromal cell-derived factor-1 (SDF-1) and CXCL16 produced by bone marrow cells [28]. Circulating T lymphocytes do not normally express CXCR4, CXCR6 and CXCR7 (O. Winqvist, unpublished observation), and therefore their entry into bone marrow is impeded. The authors note that the three patients with skeletal metastases did not respond to the T-lymphocyte

Table 7. Oncological therapy.

Patient no.	Preoperative chemotherapy or radiotherapy	Palliative oncological treatment in patients who did not receive immunotherapy	Palliative oncological treatment in patients who received immunotherapy
1	No	Local radiation, 39 Gy	–
2	No	–	Local radiation, 8 Gy × 2
3	No	–	Local radiation, 4 Gy × 5
4	No	No	–
5	No	No	–
6	No	–	No
7	No	No	–
8	No	–	No
9	No	–	No
10	No	2 cycles: gemcitabin and cisplatin	–
11	No	–	No
12	No	No	–
13	No	No	–
14	No	–	No
15	No	No	–
16	No	–	No
17	No	No	–
18	No	–	No

Patient no. 1, who did not receive immunotherapy, received palliative local radiation. Patient no.10, the only patient in the series who received systemic oncological therapy, also did not receive immunotherapy; his chemotherapy was delivered with palliative intent. Two patients (nos 2 and 3) in the immunotherapy group, who also were considered non-responders to that experimental treatment, also received palliative local radiation.

therapy and consider that the expressions of appropriate chemokine receptors were not induced by using APCs from abdominal lymph nodes or from the restimulation by monocytes from peripheral blood. Future experimental T-lymphocyte expansion protocols may need to include specific stimuli to provide expression of appropriate chemokine receptors.

A second developmental line would be to explore the described immunotherapy in a target population considered at high risk of micrometastatic disease, yet preoperatively staged as N0M0. The focus would be on a target population of T2b–T3bN0M0 with or without NAC. Until recently, cisplatin and other chemotherapeutic drugs were believed to have a general immunosuppressive effect owing to their myelosuppressive nature [29]. However, the opposite may be true, as recent findings suggest that cisplatin is a positive inductor of the immune system through different mechanisms. One immunorelated mechanism entails preconditioning chemotherapy with cisplatin, as in a described murine colon adenocarcinoma model. Cisplatin augmented the infiltration of $CD3^+$ T lymphocytes into the tumor mass and reduced the percentage of both intratumoral and splenic Treg cells (the Treg cells naturally act as a negative feedback mechanism on antitumor immune responses) [30]. Another mechanism is cisplatin inhibition of STAT6 phosphorylation leading to a downregulation of programmed death receptor ligand-2 (PD-L2) expression, resulting in increased activation and proliferation of T lymphocytes by dendritic cells and enhanced T-cell recognition of tumor cells, followed by increased tumor apoptosis [31]. The present group has also recently shown that cisplatin enhances the immune-stimulatory ability of human $CD14^+$ monocytes, acting as APCs, a mechanism that is likely to be mediated by increased production of interferon-gamma [32].

With this background, the authors intend further to focus on both high-risk patients receiving NAC and chemotherapy-naïve patients, but without overt dissemination (T2b–T3bN0M0), exploring possible survival benefits of adjuvant T-cell based autologous immunotherapy.

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Declaration of interest: For the author Ola Winqvist, two patents have been submitted and accepted; Cancer immunotherapy (prevention of cancer recurrence) and Method for treating urinary bladder cancer. Both patents are owned by Sentoclone International, in which he has no shares, and from which he receives no consultancy fees or any economic support. Ola Winqvist was also cofounder of the start-up company Sentoclone AB, which is now liquidated. All other authors declare no conflicts of interest.

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